SECOND EDITION

VETERINARY HEMATOLOGY AND CLINICAL CHEMISTRY

MARY ANNA THRALL, GLADE WEISER,
ROBIN W. ALLISON, AND TERRY W. CAMPBELL

with website

WILEY-BLACKWELL

Veterinary Hematology and Clinical Chemistry

Second Edition

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The authors wish to dedicate this book to their mentors, the pioneers in veterinary clinical pathology. In particular, the book is dedicated to Drs. Maxine Benjamin, Oscar Schalm, and J.J. Kaneko for their respective first-generation discovery and textbooks addressing veterinary clinical pathology, hematology, and clinical chemistry and for their inspiration to the many subsequent careers in veterinary clinical pathology.

Dr. Mary Anna Thrall wishes to thank and remember Dr. Maxine Benjamin for her generosity, patience, and friendship. The authors acknowledge and remember Dr. E. Duane Lassen for his important contributions to the first edition of this textbook. He has since lost a hard-fought battle with cancer. He was an outstanding teacher, excellent clinical pathologist, and dear friend across much of the veterinary clinical pathology community.

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Extra PowerPoint presentations available online at www.wiley.com/go/thrall.

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Preface

On behalf of the contributing authors and Wiley-Blackwell we are pleased to introduce the Second Edition of *Veterinary Hematology and Clinical Chemistry*. Our goal is to provide an image-rich, readable resource addressing routine laboratory diagnostics in veterinary practice. The theme of the presentation is applied clinical pathology for veterinary students and veterinary health professional teams in the practice setting. We aimed to maintain our intended target audience and original organizational structure.

Audience

A continuing trend in frontline veterinary medicine is the movement of laboratory diagnostics into the veterinary facility. Evolving technological advancements in point-of-care diagnostic capability drives this trend, which increases the need for education in veterinary clinical pathology. Although this book was written primarily for veterinary students and practitioners, it has applications for a broader audience, serving as a useful adjunct for the educational and reference needs of a variety of other users. The following audiences may benefit from this resource:

- students in professional veterinary medical education programs;
- health professional teams in veterinary care facilities;
- clinical pathologists and clinical pathologists in training;
- product development groups utilizing veterinary clinical pathology.

Organization

Veterinary Hematology and Clinical Chemistry is organized into six sections, arranged as follows:

- I: presents principles of laboratory technology and test procedures used in veterinary laboratories to generate laboratory results. It also presents perspectives on how laboratory data interpretation is used in diagnosis and overall clinical case management.
- II: presents hematology and hemopathology of common domestic species. This includes all aspects of the hemogram or complete blood count, bone marrow, hemostasis, and transfusion medicine.
- III: presents hematology of common nondomestic species encountered in veterinary practice.
- IV: presents clinical chemistry of common domestic species and is organized primarily by organ system.
- V: presents clinical chemistry of common nondomestic species.
- VI: is a compilation of clinical cases. Each case includes a signalment, brief history, and pertinent physical examination findings. Then, relevant laboratory data are presented in tables followed by a narrative interpretation of the data.

Revisions and additions

Some of the more important revisions and additions include the following.

The overview of laboratory technology has been updated to reflect continued advances in and adoption of in-clinic diagnostic instrumentation and capabilities. Some of the historical laboratory procedures that are no longer used have been removed. Next, we comment on data interpretation skills. Our experience indicates that veterinarians are reasonably adept at understanding how laboratory tests relate to pathophysiology, but then don't think probabilistically about the magnitude of data abnormalities and often struggle interpreting complex data sets. Rules for interpreting diagnostic tests assume homogeneity of pathophysiologic responses, or that our animal friends have "read the book"; as imagined in Figure P.1.

However, we know that there are many variables that create considerable biologic variability in expected responses. Chapter 3, Perspectives in Data Interpretation, has been revised to provide introductory guidance to build the skill set required for adroit interpretation of laboratory data. This involves development of flexible, probabilistic thinking skills when solving the complex puzzle formed by the array of clinical findings and laboratory data.

Extensive revision and some additions have been made possible for selected chapters by incorporation of content from new authors. Examples include:

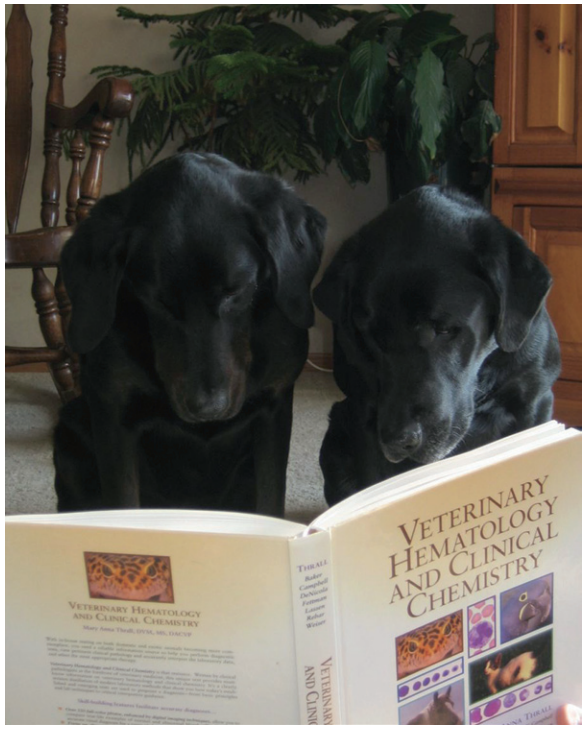

Figure P.1 Dogs caught 'reading the book' in an attempt to make their disease responses predictable. Not all will read the book. (Courtesy of Dr. Sara Hill.)

• Wayne Jensen, Morris Animal Foundation, provides an update of the rapidly evolving area of immunodiagnostics.
• Advances have continued to be made in the diagnosis and classification of hematopoietic cell neoplasia. A new chapter from Anne Avery, Colorado State University, provides an overview of evolving molecular and flow cytometric diagnostics applied to hematopoietic neoplasia. This complements the other chapters detailing leukocyte responses, bone marrow evaluation, and hematopoietic proliferative disorders.
• Robin Allison, Oklahoma State University, has made extensive revision of laboratory diagnostics related to pathology involving liver, pancreas, blood proteins, and muscle.
• Andrea Bohn and Glade Weiser, Colorado State University, have revised the often-dreaded subjects of electrolyte and acid-base pathology, with an attempt to simplify clinical understanding of these laboratory tests.
• Judy Radin, The Ohio State University, has provided a new chapter covering lipid pathology.
• Last, but not least, Don Meuten, North Carolina State University, brings his extensive experience and expertise cultivated from too many years in both anatomical and clinical pathology. He contributes new treatment of renal, endocrine, and calcium metabolic pathologies.

The Clinical Case Presentations were a separately bound supplement to the 1st Edition. In the 2nd Edition, the Clinical Case Presentations are incorporated into this single-bound textbook. These presentations are intended to provide students "practice" to develop interpretive skills by seeing examples of how data are interpreted into pathologic processes and how pathologic processes may culminate in a diagnostic scenario. The original cases are retained because their classical usefulness does not change. In addition, a number of new cases have been added by some of the new contributing authors.

It is our wish that readers not only learn principles and skills from this work, but also enjoy interacting with it. As veterinarians and specialists in bioanalytical pathology, we share our passion for the art and science of laboratory diagnostics applied to animal health.

Respectfully submitted,

Glade Weiser
Mary Anna Thrall
Robin Allison
Terry Campbell

Preface to the First Edition

The publication of Veterinary Hematology and Clinical Chemistry marks a new and unique contribution to veterinary clinical pathology. The product of a collaborative effort by a team of experts in the field, this text combines critical information about performing diagnostic tests, viewing pertinent clinical pathology, and interpreting laboratory data with an innovative approach to incorporating color visual content.

Audience

A current trend in the field is the movement of laboratory diagnostics into the veterinary facility, enabled by technological advancements in point-of-care diagnostic capability. This movement to in-house testing increases the need for education in veterinary clinical pathology. Although this book was written primarily for veterinary students and practitioners, it has applications for a much broader audience, serving as a useful adjunct for the educational and reference needs of a variety of other users. The following audiences will benefit from this resource:

- Students in professional veterinary medical education programs
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- Part III presents hematology of common nondomestic species encountered in veterinary practice.
- Part IV presents clinical chemistry of common domestic species and is organized primarily by organ system.
- Part V presents clinical chemistry of common nondomestic species.

Unique art program

Many aspects of veterinary clinical pathology are highly visual. The most unique feature of this book is the quantity and quality of color artwork. This was facilitated by digital image acquisition and processing performed by the authors. Optimization and standardization of images was performed by digital image engineering techniques to achieve an improvement in imagery over what is possible with conventional photomicrography. Our goal was to bring a new level of realism to the visual communication of concepts pertaining to microscopy. In some instances, visual content has been amplified by combining images from multiple microscopic fields into a single figure or showing different levels of magnification within the same figure. Digital image engineering also allows for image manipulation; an example is arrangement of cells that are randomized on a microscope field into a specific order to convey a concept such as cell maturation. We believe that the fidelity of visual imagery, as well as its liberal integration with text content, makes this work the first of its kind.

Author team

Contributing content and expertise to this project are a number of recognized authorities in the field of veterinary clinical pathology. These individuals have helped shape the existing curriculum, train the existing faculty, and create the disciplines of comparative laboratory medicine and diagnostic cytology as we know them today. It is through the combined efforts of so many experts in the field that this book was made possible.

We hope you find this publication to be an excellent resource in the clinical laboratory and for laboratory data interpretation.

M. G. Weiser and M. A. Thrall
Fort Collins, Colorado

I General Principles of Laboratory Testing and Diagnosis

1 Laboratory Technology for Veterinary Medicine

Glade Weiser
Colorado State University

This chapter presents an overview of the laboratory technology used to generate data for hematology and clinical biochemistry. For the procedures and technologies likely to be employed within veterinary hospitals, general instructions and descriptions provide a review of the principles previously learned in laboratory courses. This, in conjunction with the instructions accompanying different devices and consumables, should enable users to reproduce the procedures to a satisfactory performance standard. For technologies more likely to be used only in large commercial or research laboratories, the overview provides familiarity with the basic principles.

Hematologic techniques

Basic techniques applicable for any veterinary hospital

The procedures outlined here are most appropriate for the in-house veterinary laboratory in most practice settings. These procedures, with the exception of a cell counting hematology system, require minimal investment in instrumentation and technical training. These basic hematologic procedures include:

- Blood mixing—for all hematologic measurements
- Packed cell volume or hematocrit by centrifugation
- Plasma protein estimation by refractometry
- Cell counting instrumentation
- Preparation of blood films
- Differential leukocyte count and blood film examination

Blood mixing

The blood sample is assumed to have been freshly and properly collected into an ethylenediaminetetraacetic acid (EDTA) tube (as described in Chapter 2). When performing any hematologic procedure, it is important that the blood is thoroughly mixed. Cellular components may settle rapidly while the tube sits on a counter or in a tube rack (Fig. 1.1). As a result, failure to mix the sample before removing an aliquot for hematologic measurement may result in a serious error. Mixing can be performed by manually tipping the tube back and forth a minimum of 10–15 times (Fig. 1.1). Alternatively, the tube may be placed on a rotating wheel or tilting rack designed specifically to mix blood (Fig. 1.2).

Packed cell volume

The packed cell volume value is the percentage of whole blood composed of erythrocytes. It is measured in a column of blood after centrifugation that results in maximal packing of the erythrocytes. Tools for performing the packed cell volume include 75×1.5-mm tubes (i.e., microhematocrit tubes), tube sealant, a microhematocrit centrifuge, and a tube-reading device.

The procedure is performed using the following steps. First, the microhematocrit tube is filled via capillary action by holding it horizontally or slightly downward and then touching the upper end to the blood of the opened EDTA tube (Fig. 1.3).

Next, allow the tube to fill to approximately 70–90% of its length. Hold the tube horizontally to prevent blood from dripping out of the tube, and seal one end by pressing the tube into the tube sealant once or twice (Fig. 1.4). Note that air may be present between the sealant and the blood (Fig. 1.4). This is not a problem, however, because the trapped air is removed during centrifugation.

The tube is then loaded into the microhematocrit centrifuge according to the manufacturer's instructions (Figs. 1.5 and 1.6). The microhematocrit centrifuge is designed to spin the lightweight tube at very high speeds to generate sufficient centrifugal force to completely pack the red cells within 2–3 minutes. With such centrifugal force, most (or all) of the plasma is removed from the layers of packed cells.

Veterinary Hematology and Clinical Chemistry, Second Edition. Edited by Mary Anna Thrall, Glade Weiser, Robin W. Allison, and Terry W. Campbell.

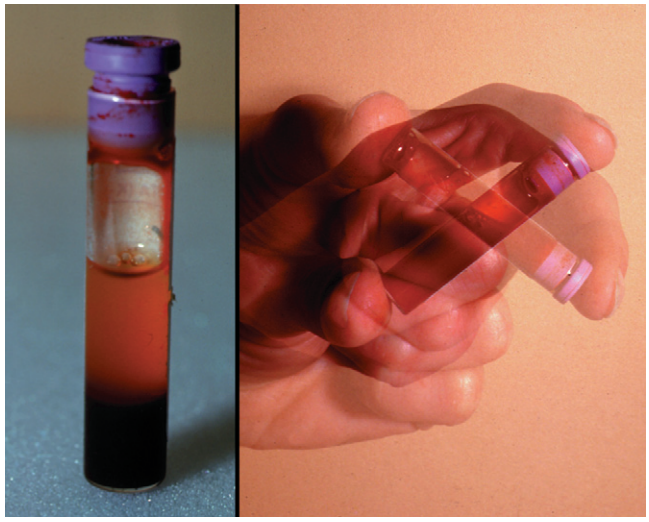


Figure 1.1 Left. Gravity sedimentation of whole blood. Right. A gentle, repetitive, back-and-forth tube inversion technique used to manually mix blood before removing aliquots for hematologic procedures.

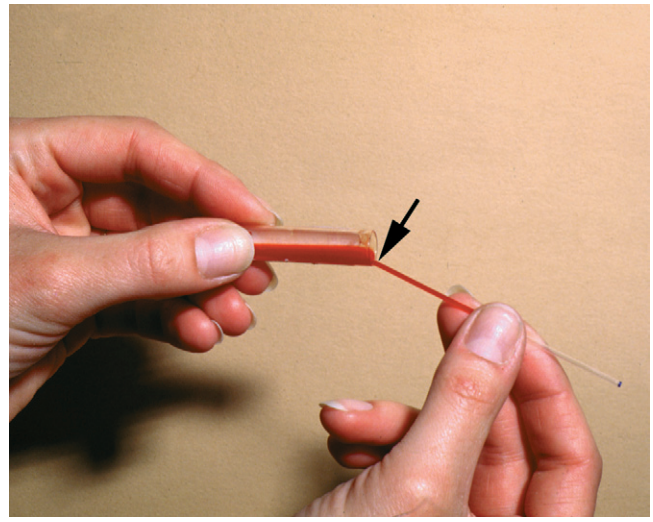


Figure 1.3 Proper technique for filling a microhematocrit tube. The tube should be positioned horizontally or tilted slightly downward to facilitate filling by capillary action. Capillary action is established by touching the upper end of the tube to the blood (arrow).

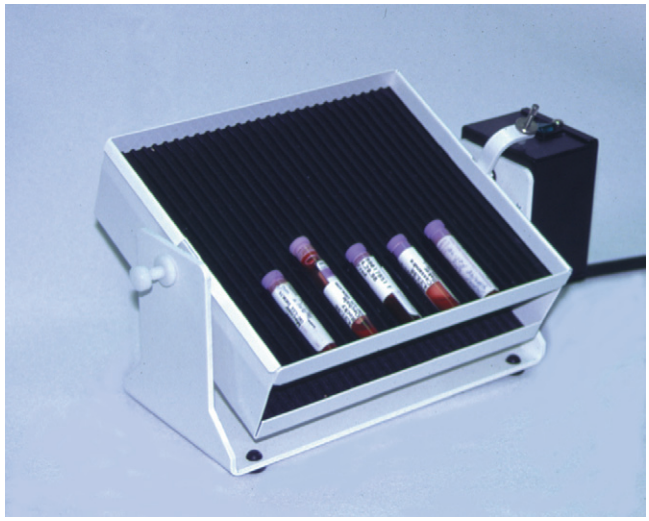


Figure 1.2 Representative mechanical blood-mixing table. The surface holds several tubes on a ribbed rubber surface and tilts back and forth at the rate of 20–30 oscillations per minute.

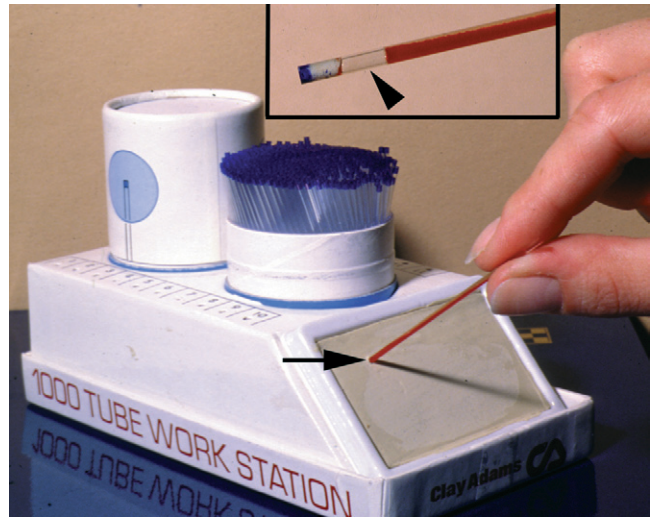


Figure 1.4 A microhematocrit tube is sealed by pressing two to three times into the clay sealant (arrow). Note that a small amount of air trapped between the blood and white clay is not a problem (arrowhead in the inset).

Three distinct layers may be observed in the tube after removal from the centrifuge: the plasma column at the top, the packed erythrocytes at the bottom, and a small, middle white band known as the buffy coat (Fig. 1.7). The buffy coat consists of nucleated cells (predominantly leukocytes) and platelets, and it may be discolored red when the nucleated erythrocyte concentration is prominently increased. Observations of any abnormalities in the plasma column above the red cells should be recorded. Common abnormalities such as icterus, lipemia, and hemolysis are shown in Figure 1.7. Icterus is excessively yellow pigmentation of the plasma column that suggests hyperbilirubinemia; the magnitude of this hyperbilirubinemia should be confirmed by a biochemical determination of serum bilirubin concentration (see Chapter 26). The observation of an icteric coloration to the plasma is diagnostically useful in small animals. It is not reliable in large animal species, however, because their serum usually has a yellow coloration from the normal carotene pigments associated with their herbivorous diet. Lipemia is a white, opaque coloration of the plasma column

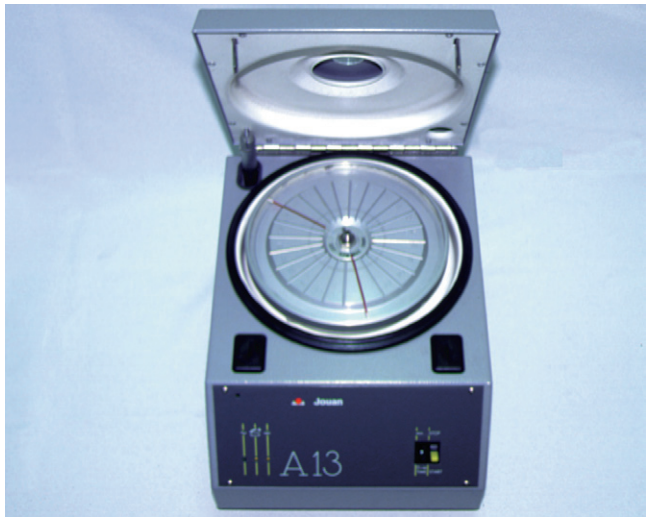


Figure 1.5 Representative microhematocrit centrifuge. The head and motor are designed to spin the tubes at very high speeds to achieve maximal erythrocyte packing.

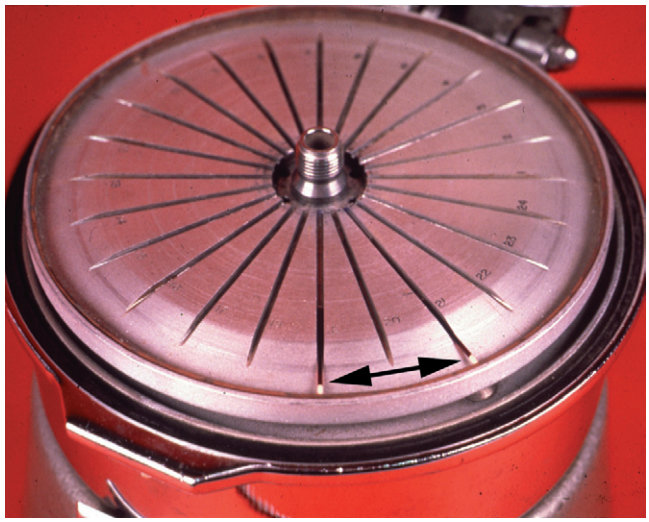


Figure 1.6 Placement of microhematocrit tubes on a microhematocrit centrifuge head. Note the proper orientation of two microhematocrit tubes, with the clay-sealed end positioned at the outer ring of the centrifuge head (double arrow).

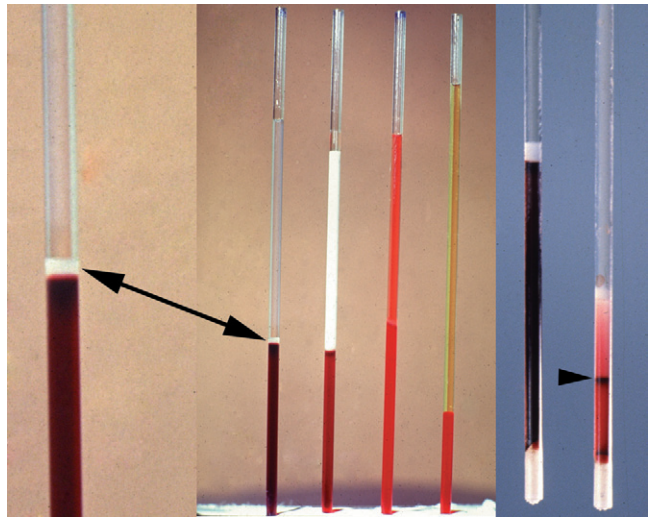


Figure 1.7 Normal and abnormal spun microhematocrit tubes (4 tubes in middle panel). The tube on the left is normal. Note the packed erythrocytes at the bottom, plasma layer at the top, and buffy coat in the middle (arrow; enlarged at left). The second tube illustrates lipemia, the third hemolysis, and the fourth icterus. Note also that the hematocrit is considerably decreased in the fourth tube. Two additional tubes illustrate buffy-coat abnormalities (enlarged at right). The first of these tubes has an increased buffy coat that correlates with an increased leukocyte concentration. The second (right) is from a sheep with leukemia and has a dramatically increased buffy coat. The leukocyte concentration was greater than 400,000 cells/μL. There is also severe anemia. With such major abnormalities in cell concentration, separation of erythrocytes and leukocytes is not complete, and division may be blurred. What is interpreted as being the "top" of the erythrocyte column is indicated by the arrowhead. The red discoloration of the buffy coat may be caused by a prominent increase in nucleated erythrocytes.

because of the presence of chylomicrons. Lipemia most commonly is associated with the postprandial collection of blood, but it also may be associated with disorders involving lipid metabolism (see Chapter 31). Hemolysis is a red discoloration of the plasma column, which usually results from artifactual lysis of red cells induced during the collection of blood. A small quantity of lysed erythrocytes is sufficient to impart visual hemolysis. Therefore, if the hematocrit is normal, one may assume it is an artifact. Less commonly, causes of anemia that result in intravascular hemolysis give rise to observable hemolysis in the plasma fraction, which also is known as hemoglobinemia (see Chapter 8). This will typically also be associated with hemoglobinuria.

The packed cell volume is measured on a reading device, such as a microhematocrit card reader (Fig. 1.8). The procedure is performed by positioning the erythrocyte–clay interface on the 0 line and the top of the plasma column on the 100 line. The position of the top of the erythrocyte column is then read on the scale as the packed cell volume.

Plasma proteins by refractometry

After measurement and observation of the microhematocrit tube, the plasma column may be used to estimate the plasma protein concentration on the refractometer (Fig. 1.9). This instrument may be used to estimate the concentration of any solute in fluid according to the principle that the solute refracts (or bends) light passing through the fluid to a degree that is proportional to the solute concentration. The principle or property being measured is the refractive index relative to distilled water. The scale for a particular solute can

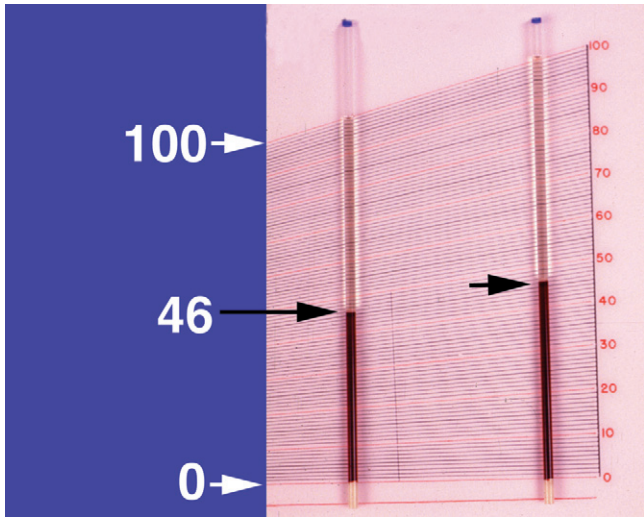


Figure 1.8 Determination of packed cell volume on a microhematocrit tube card reader using two tubes of blood from the same patient sample. Note that the scale allows the tube to be read over a considerable range of filling levels. The steps are to line up the erythrocyte–clay interface with the 0 line, line up the top of the plasma column with the 100 line, and then read the top of the erythrocyte column on the scale. The positions of these steps are indicated by the arrows. Note in this example that the packed cell volume is 46%.

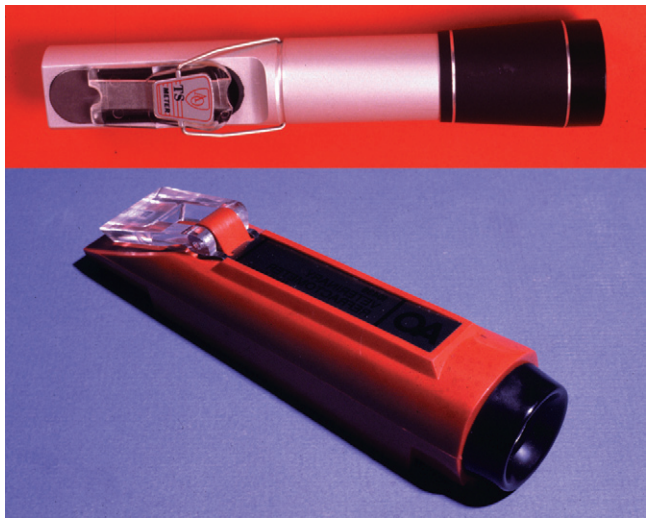


Figure 1.9 Refractometers. The lower refractometer is more rugged, because it is encased in rubber. It is known as a veterinary refractometer, and it has a canine and feline urine specific gravity scale that calibrates for minor differences between species during this determination.

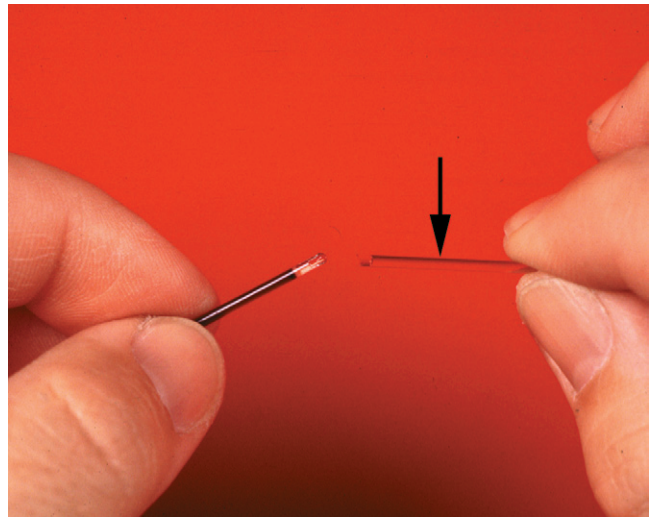


Figure 1.10 Preparation of the microhematocrit tube for measuring plasma protein concentration. The tube is broken just above the buffy coat to yield a column of plasma (arrow).

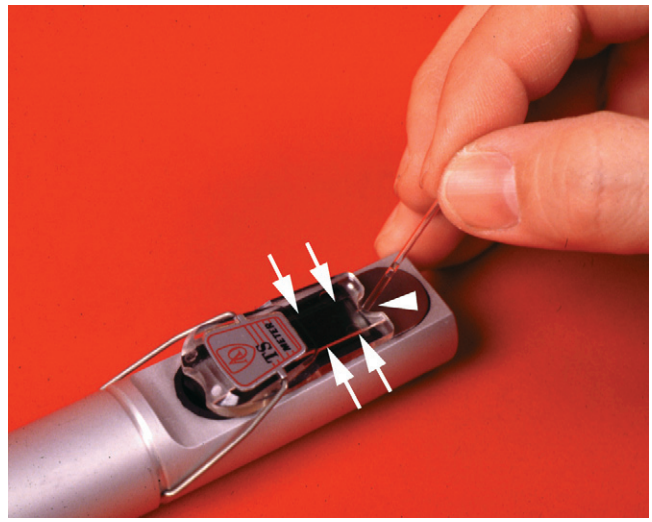


Figure 1.11 Loading plasma from the microhematocrit tube to the refractometer. To wick plasma onto the refractometer, capillary action is established by touching the end of the plasma tube at the notch of the prism cover (arrowhead). Flow should establish a thin layer of plasma under the plastic cover to fill the area delineated by arrows. After reading, the plastic cover is flipped back and wiped clean with a laboratory tissue.

be developed from refractive index measurements calibrated to solutions with known solute concentrations. In clinical diagnostics, refractometry is used to estimate the plasma protein concentration and urine specific gravity.

Plasma protein is measured using the plasma column in the microhematocrit tube. The tube is broken above the buffy coat layer (Fig. 1.10), and the portion of the tube containing the plasma is used to load the refractometer (Fig. 1.11). The instrument then is held so that an ambient light source can pass through the prism wetted with plasma, and the light refraction is read on a scale through an eyepiece (Fig. 1.12).

The protein measurement is regarded as being an estimate based on calibration, assuming that other solutes in the serum are present in normal concentrations. The measure-

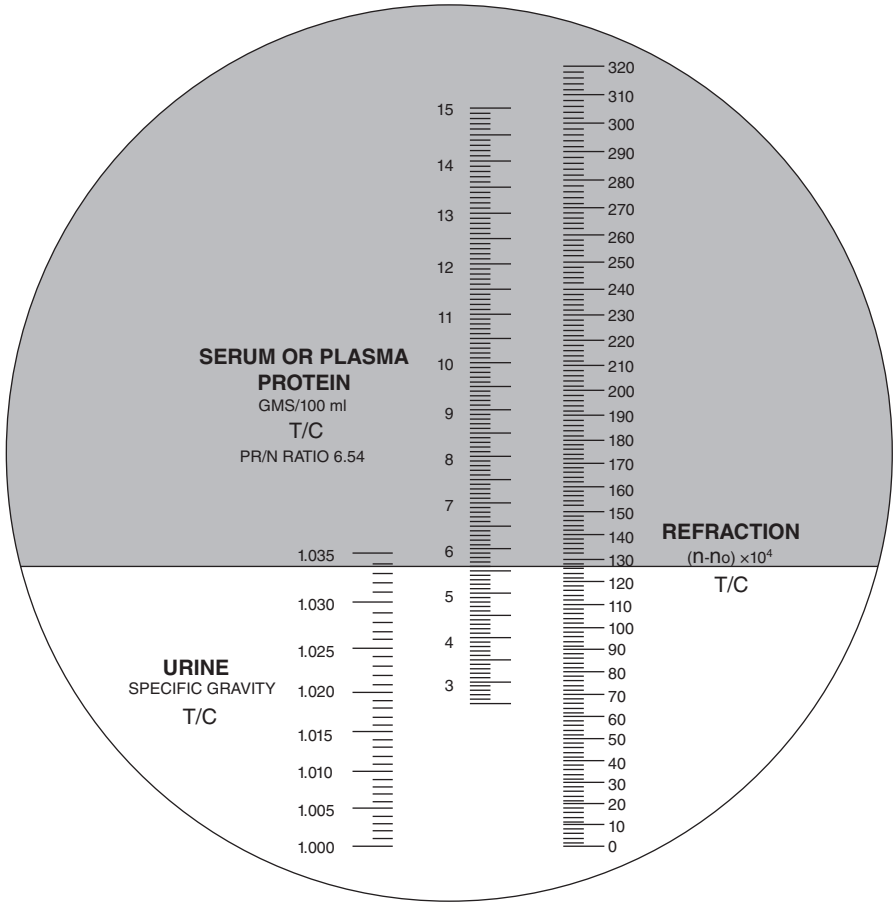


Figure 1.12 Representative refractometer scale as seen through the eyepiece. Light refraction creates a shadow–bright area interface that is read on the appropriate scale.

ment may be influenced by alterations in other solutes. Most notably, lipemia may artificially increase the protein estimate by as much as 2 g/dL. Other alterations of solutes such as urea and glucose influence the protein estimate to a much lesser, and usually negligible, degree.

Determination of total leukocyte concentration

Two general approaches are available to determine the leukocyte concentration. Historically, cell concentrations were measured manually using a blood dilution placed onto a hemocytometer and counted while observing by microscopy. This procedure, and associated consumables, is regarded as obsolete for the veterinary practice setting. Over the past 30 years this procedure has been progressively replaced by automated cell counting hematology systems or alternatively expanded buffy coat analysis technology in which cellular estimates are made from layers in a specialized hematocrit tube. The total leukocyte count is the concentration of nucleated cells, because the techniques detect all the nuclei in solutions from which erythrocytes have been removed by lysis or centrifugation. Therefore, nucleated erythrocytes typically are included in this count. In most cases the concentration of NRBC is negligible, but on rare occasion they may make up an appreciable fraction of the total nucleated cell concentration.

A variety of electronic cell counters operate by enumerating nuclear particles in an isotonic dilution in which a detergent is used to lyse the erythrocytes. These systems must be engineered for animal blood, however, to generate accurate measurements of cell concentrations. There are also continued advances in these hematology systems for performing leukocyte differentiation. Three-, four-, and five-part differential systems exist. The differential capability works best with normal blood, but there are individual exceptions. All systems may produce questionable results when there is leukocyte pathology and none properly detects abnormalities such as left shift, toxic change, and cell types outside the routine five normal cell types (see Chapters 10 and 12). (For principles of hematology system operation, see the discussion of advanced hematologic procedures later in this chapter.) The quantitative buffy coat analysis system (QBC, Becton Dickinson) estimates the leukocyte concentration by

measurement of the buffy coat layer in a specialized microhematocrit tube, in which a float is present to expand the buffy coat region for optical scanning.

In isolation, the total leukocyte count is not particularly useful for interpretive purposes; this measurement is used to determine the concentration of various leukocyte types that make up the differential count. The concentration of individual leukocytes is the most useful value for the interpretation of disease processes. This information is determined by evaluating the stained blood film (discussed below). Because of the limitations in automated leukocyte differentiation described above, it is important to utilize blood film examination in conjunction with automated hematology systems. This is essential not only for leukocyte characterization, but also for evaluation of erythrocytes in cases of anemia and platelets when the instrument produces a decreased platelet concentration value.

Preparation of blood films

The stained blood film is an essential tool for determining the concentrations of individual leukocyte types (i.e., differential count) and for evaluating important pathologic abnormalities involving leukocytes, erythrocytes, and platelets. Successful derivation of information from the blood film requires a proper technique, which both creates a monolayer of individually dispersed cells and a minimal disturbance of relative cell distributions that reflect the cell concentrations in mixed blood. A poorly prepared film presents confusing artifacts and may result in cell distributions on the slide that lead to serious errors in the differential count.

Preparation of a good-quality blood film requires mastery of a specific technique (Figs. 1.13–1.15). The most common procedure is known as the wedge or push technique and uses two glass microscope slides. A drop of blood is placed near one end of the first slide supported on the counter. The second slide is placed on the first in a way that forms a "wedge" consisting of a 30–45° angle in front of the drop of blood. The second slide, which is known as the pusher slide, then is backed into the drop of blood and advanced forward to the end. This should be accomplished in one rapid motion that involves a flip of the wrist holding the pusher slide. Downward pressure on the pusher slide should be minimal.

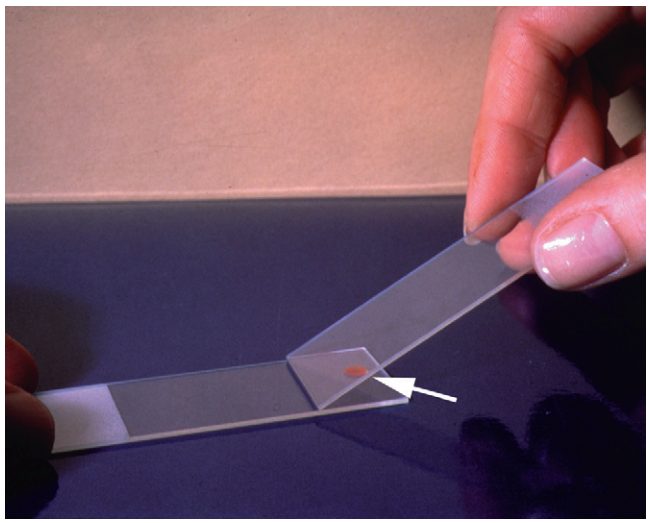


Figure 1.13 Blood film preparation. The blood slide is held on a firm surface, and a drop of blood is placed near the end (arrow). The pusher slide then is placed on the blood slide in front of the drop of blood to form an angle of approximately 30°.

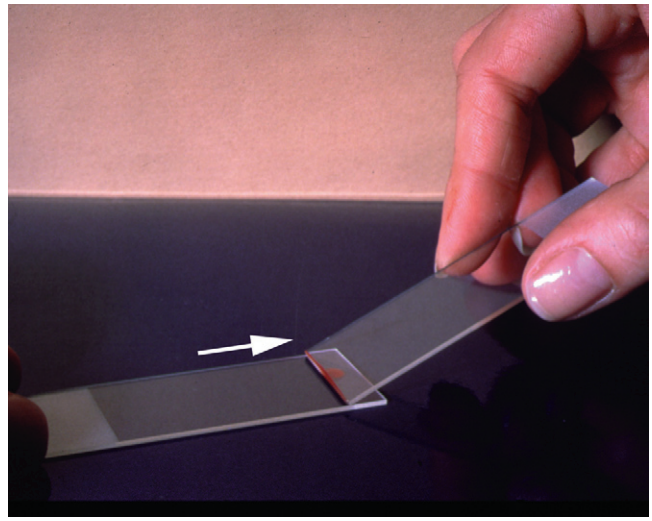


Figure 1.14 Blood film preparation. The pusher slide is backed into the drop of blood with a directional movement (arrow).

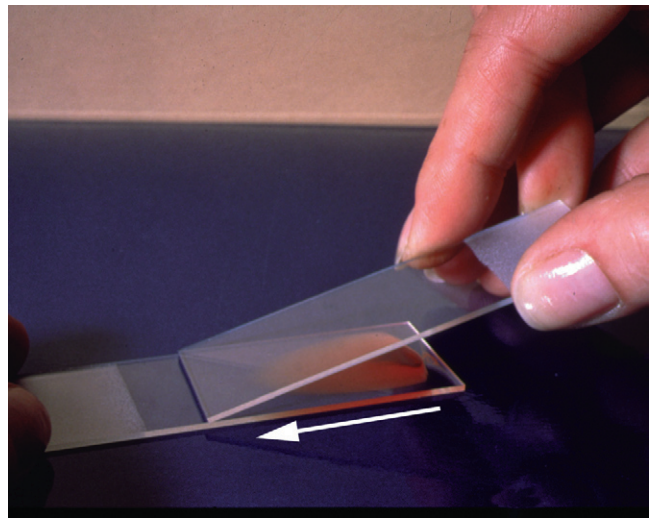


Figure 1.15 Blood film preparation. The pusher slide is pushed forward with a rapid directional movement (arrow). It is important that the movements shown in Figures 1.13 through 1.15 are a single, rapid procedure involving a flip of the wrist. Considerable practice is required to develop this skill. The result should be a uniform film of blood that gets progressively thinner (see Fig. 1.17).

Learning this technique in the presence of someone experienced with making good films is helpful, and considerable practice is advised. A common poor technique is to push the pusher slide too slowly, thereby creating a film that is too thin. This results in very poor distribution of leukocytes at the end of the film and artifacts in the evaluation of erythrocytes. In blood with reduced viscosity, such as that from patients with severe anemia, increasing the angle to avoid a slide that is too thin is useful.

Staining

After preparation, the blood film is usually stained within minutes. However, it may be stained within hours to days if it is being sent to a diagnostic laboratory. The staining system used for microscopic evaluation of cellular elements is the Wright stain, or a Wright stain modified by the addition of Giemsa. This is a relatively complex procedure that requires care and maintenance, thus often being limited to larger laboratory facilities. Quick-stain procedures that mimic the classical Wright stain are available, however, and for convenience, these are the most commonly used stains in the veterinary practice setting. The best-known stain kit is Diff-Quick (Dade Behring Inc., Newark, DE). Quick stains may result in nuclear overstaining and blurring of chromatin detail, but they provide sufficient quality for differential leukocyte counting and screening for morphologic abnormalities. Examples of manual to automated staining systems are shown in Figure 1.16.

Figure 1.16 Blood film and cytology staining apparatus. Top. Manual staining jars containing Diff-Quick stain. Slides are manually moved from one jar to the next according to the manufacturer's instructions. Bottom. An automated stainer used for higher-throughput situations. Note the mechanical arm that moves a rack of slides (not shown) through the sequence of staining procedure baths (arrow). The stainer may be programmed to control the timing in each bath. Most such machines provide the ability to stain as many as 20–25 slides per cycle.

Expertise for examination of blood films

Once stained, the anatomy of a blood film must be known to properly orient the slide for microscopic viewing (Fig. 1.17). The largest part of the film is the thick area or body, in which cells are superimposed and leukocytes are rounded up, thereby making microscopic evaluation of all components difficult. The feathered edge occurs at the end of the film. Artifacts in this area include broken leukocytes and the inability to evaluate the erythrocyte central pallor. The counting area is a small area between the thick portion and the feathered edge, and it consists of a monolayer of cells in which microscopy is optimal. Leukocytes are flattened out so that the internal detail is most evident.

The amount of interpretive disease relevance that can be gained from examination of the blood film is proportional to the expertise available for the examination. Success in dealing with all components of such examination depends on the quality of film making, stain maintenance, ability to look in the correct place, ability to differentiate preparation artifacts from morphologic abnormalities, and experience with interpretive blood film pathology. To the extent that the user cannot make these distinctions, abnormal blood films should be referred to a specialist for examination and/or second opinion.

It is important to examine the gross appearance of blood films as a correlate to artifact recognition. Improper preparation can be recognized, thereby alerting the observer to

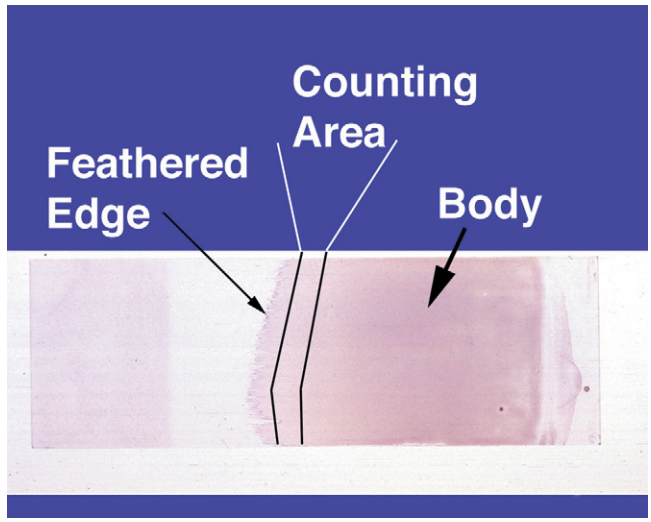


Figure 1.17 Anatomy of a stained blood film. Note the feathered edge (thin arrow) and the thick area or body of the slide (thick arrow). The counting area containing a monolayer of cells is present in a relatively small area, which is delineated approximately by the lines across the slide. This gross examination of the slide is very helpful in orienting the observer before placing the slide on the microscope stage. This facilitates alignment of the optics over the proper area of the slide, making it easier and faster to perform low-magnification observations and to find the counting area.

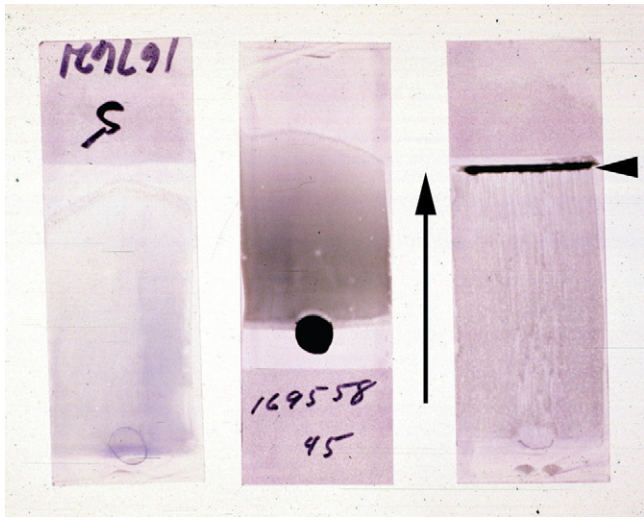


Figure 1.18 Gross appearance of blood films. All three of these films are oriented the same way. The drop of blood was placed near the bottom of the picture, and the film was made by pushing in the direction of the arrow. The middle film has a normal appearance and intensity of color. The appearance is homogeneous but gets progressively thinner as one approaches the feathered edge. The film on the left is very pale; this is the appearance when severe anemia is present. With severe anemia, blood viscosity is reduced, resulting in a much thinner film. The film on the right is made improperly and does not yield accurate information. The pusher slide was pushed too slowly, making a thin film with streaks. Note the streaking and irregularity over most of the slide. Blood was still present at the end of the slide as well, resulting in a line of densely concentrated cells (arrowhead). It is not possible to find a good monolayer for evaluation of erythrocyte morphology on this slide. In addition, the leukocytes are disproportionately concentrated at the end of the slide, which ordinarily has a feathered edge. Performing a differential count will be difficult in this case—and likely not accurate. A thin slide as a result of pushing too slowly is the most common problem in technique found at veterinary facilities.

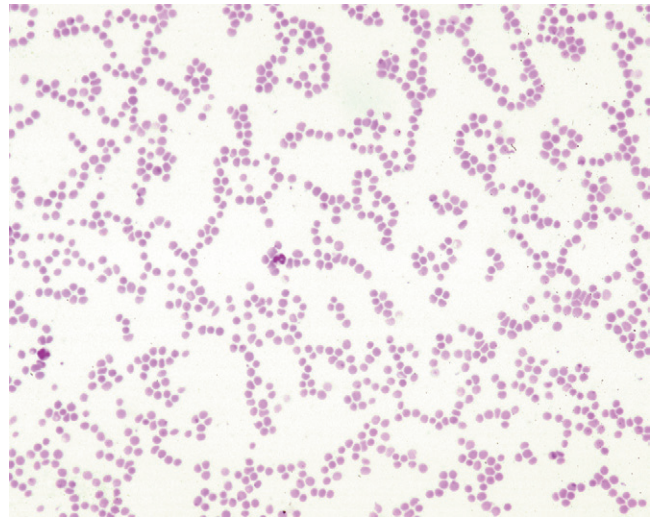


Figure 1.19 Low-magnification appearance of the feathered edge. Note the reticulated pattern of erythrocyte distribution. Artifactual loss of central pallor makes evaluation of erythrocyte morphology difficult, and false interpretation of pathologic abnormalities is likely to occur in this area.

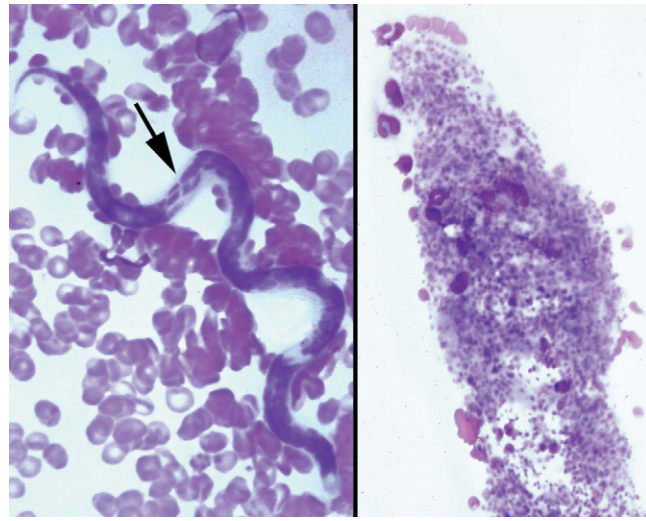


Figure 1.20 Large items pushed to the feathered edge. Left. Microfilaria (arrow) in an animal with heartworm disease. Right. A large clump of platelets with trapped leukocytes. Several hundred platelets are contained in this microclot.

artifacts that can be avoided and preventing any associated, errant interpretations. Common abnormalities that may be recognized grossly are presented in Figure 1.18. The most common and important abnormality is a slide that is too thin, which can be recognized by streaks progressing toward the feathered edge. This results in a leukocyte distribution that presents major errors in the differential count. In addition, there is not an area adequate for the evaluation of erythrocyte abnormalities.

The observer should locate the counting area using the 10× objective. The feathered edge is recognized by a loss of erythrocyte central pallor and a reticulated pattern of erythrocyte distribution on the film (Fig. 1.19). Quick, low-power examination of the feathered edge is useful for the detection and identification of abnormalities such as microfilaria, platelet clumps, and unusual, large cells that are preferentially deposited here (Fig. 1.20). The thick area is recognized by a progressive superimposition of erythrocytes as the observer moves further into the thick area of the slide. In very thick areas, the evaluation of cells is severely compromised (Fig. 1.21). The counting area is recognized by a monolayer of evenly dispersed cells (Figs 1.22 and 1.23).

Once the counting area is located, the experienced observer can estimate the leukocyte concentration on a well-prepared blood film. This is useful as a gross quality-control measure, and it is recommended that the observer

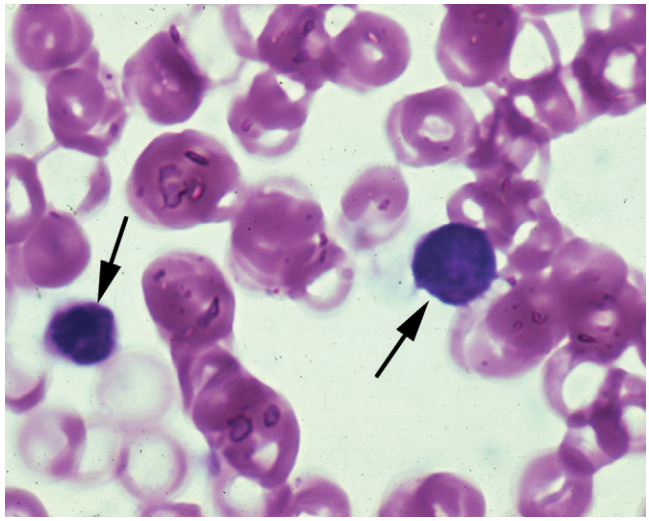


Figure 1.21 High-magnification appearance of cells in the thick area or body of slide. Note the superimposition of erythrocytes, thus making evaluation of erythrocyte morphology difficult. In addition, specifically identifying leukocytes (arrows) is difficult to impossible. In this area, leukocytes are spherical or rounded-up rather than flattened. It is not possible to see intracellular detail or even the delineation between the cytoplasm and the nucleus.

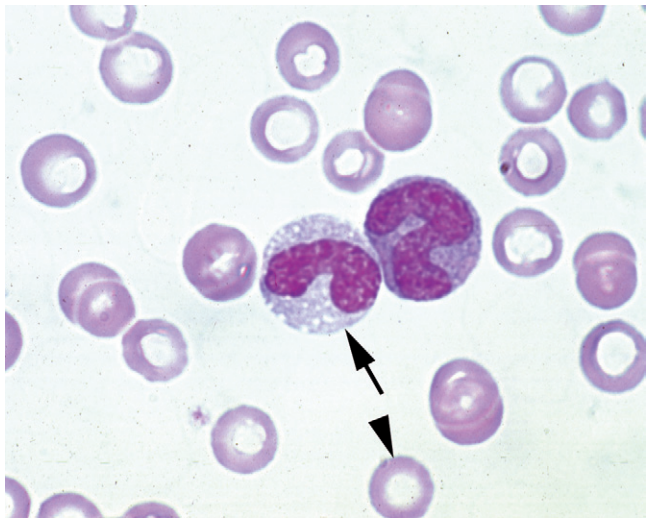


Figure 1.22 High-magnification appearance of cells in the counting area or monolayer. Note the minimal superimposition of erythrocytes, which facilitates evaluation of erythrocyte morphology (arrowhead). Leukocytes (arrow) are flattened on the slide, which makes it possible to see details of the cytoplasm and nucleus. Note that the nuclear borders are sharply delineated from the surrounding cytoplasm.

gain experience at this by repetitive comparison of leukocyte density on well-prepared blood films with total leukocyte counts from a cell counter. The low-power appearances of a leukocyte count in the normal range, marked leukopenia, and marked leukocytosis are shown in Figures 1.23, 1.24, and 1.25, respectively.

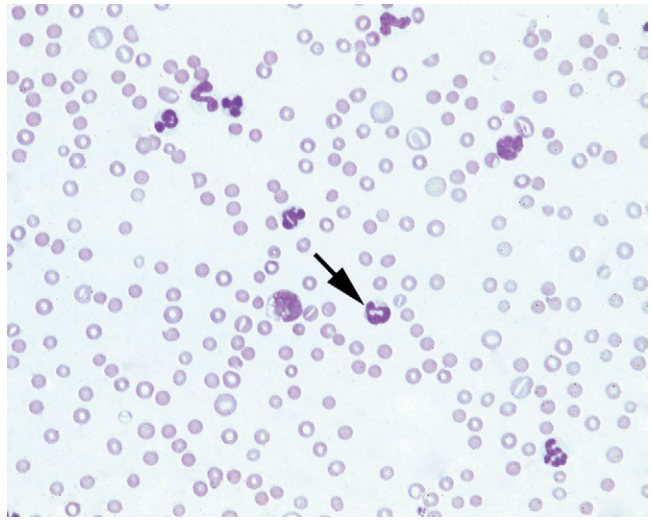


Figure 1.23 Low-magnification appearance of the counting area. Note the evenly dispersed cells and the ability to visualize the erythrocyte central pallor. The density of leukocytes (arrow) is that expected with a leukocyte concentration in the normal range.

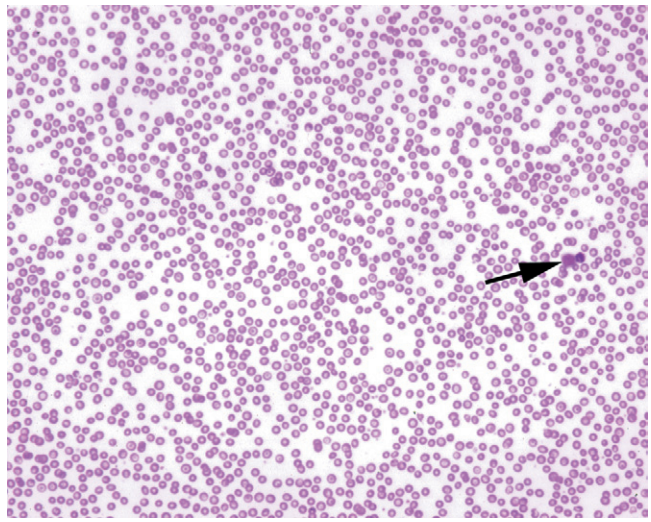


Figure 1.24 Low-magnification appearance of the counting area with a marked decrease in the leukocyte concentration. A rare leukocyte per field is present (arrow).

Procedures using the 100×, oil-immersion objective

Once the counting area is located and these assessments are completed the microscope should be adjusted for oil immersion, high magnification observation. The observer will then perform a systematic evaluation of the three major cell lines. This includes a differential count for leukocytes with notation about any abnormal cells, evaluation of erythrocyte morphology, and evaluation of platelets.

Within the counting area, the observer will move across fields and obtain the differential leukocyte count by classifying a

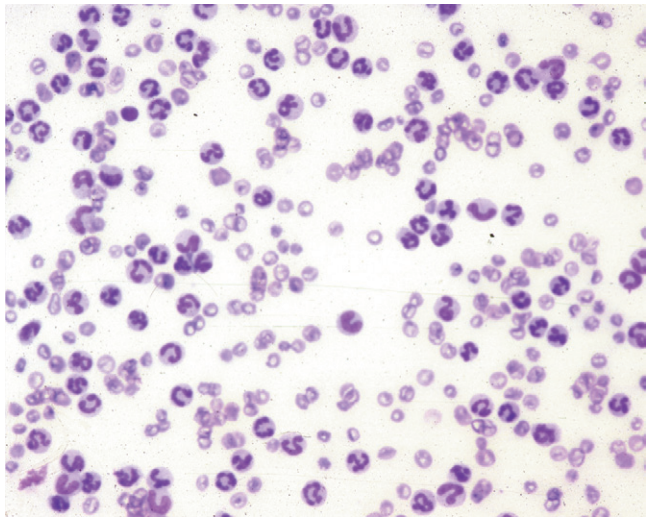


Figure 1.25 Low-magnification appearance of the counting area with a marked increase in leukocyte concentration. The density of leukocytes is considerably greater than that seen in Figure 1.23.

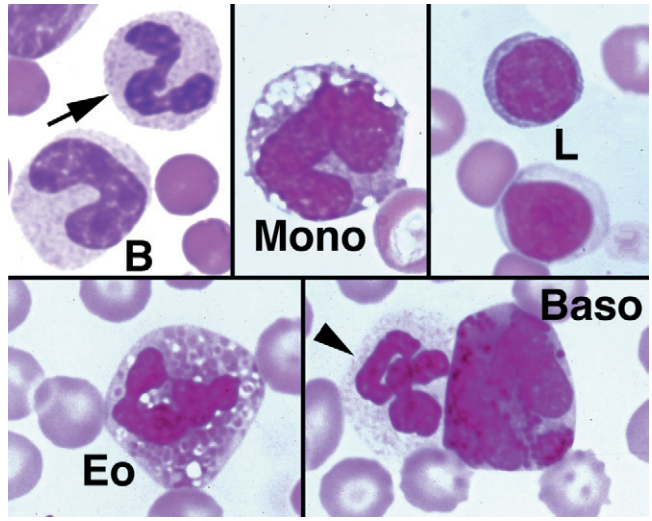


Figure 1.26 Basic leukocytes encountered in the differential count. Upper left. Neutrophils. Note the segmented neutrophil (arrow) and the constrictions in the nuclear contour. The band neutrophil (B) has smooth, parallel nuclear contours. Upper middle. Monocyte (Mono). The nucleus may have any shape, from round to bean-shaped to ameboid and band-shaped, as in this example. The cytoplasm is blue-gray and may variably contain vacuoles. Upper right. Two lymphocytes (L). Lower left. An eosinophil (Eo). Note that granules stain similar to the surrounding erythrocytes. Occasionally, granules may wash out in the staining procedure, leaving vacuoles. Lower right. Basophil (B) with dark granules that stain similar to nuclear chromatin. Note the adjacent neutrophil (arrowhead) and that neutrophils may have small, poorly staining granules that are much smaller than those of eosinophils or basophils.

minimum of 100 consecutively encountered cells. Cells are classified into a minimum of five to six categories, with the presence of abnormal cells being recorded into a category of "other," in which a specification is made for the individual sample. The common six categories of normal cells—neutrophil, band neutrophil, lymphocyte, monocyte, eosinophil, and basophil—are shown in Figure 1.26. (See Chapter 10 for additional visual details regarding leukocyte identification that may be helpful in differential counts.)

The result of counting 100 cells is that the number of each leukocyte type is a fraction of 100, or a percentage of the leukocyte population. Once cells are categorized into percentages, they must be converted to absolute numbers for interpretation purposes. This is done by multiplying the total leukocyte concentration by the percentage of each leukocyte type, which yields the absolute number or concentration of each leukocyte in the blood sample. The following example illustrates the conversion of percentages to absolute numbers:

Example 1.1. Conversion of Percentage Counts to Absolute Concentrations

Total white-blood-cell count = 10,000/μL
Differential white-blood-cell count:

	Percentages	Absolute Numbers/μL
Neutrophils	60%	(6000)
Lymphocytes	30%	(3000)
Monocytes	5%	(500)
Eosinophils	5%	(500)

Any abnormalities in leukocyte morphology also should be noted. Important morphologic abnormalities are detailed in Chapter 12.

Erythrocyte morphology then is systematically evaluated. The observer should note any important erythrocyte shape or color abnormalities; this is particularly important for evaluating anemias. (See Chapter 5 for a review of morphologic erythrocyte abnormalities.)

The presence of platelet adequacy may be interpreted from a properly prepared blood film. A minimum of 8–12 platelets per oil immersion high-power (1000×) field may be interpreted as adequate. The number seen may be considerably greater than described, however, because of the wide range of normal platelet concentrations. This number is only a guideline for most microscopes with a wide field of view. It should be adjusted downward when using a microscope with a narrow field of view and upward if using one with a superwide field of view. If the platelets appear to be decreased, a search for platelet clumps on a low-power setting at the feathered edge should be performed. The ability to look for platelet clumps is also important when a cell counter produces a decreased platelet concentration value; this is a frequent problem in cats. Morphology of platelets also may be noted. Platelets that approach the

diameter of erythrocytes or larger are referred to as macroplatelets or giant platelets. In dogs, these suggest accelerated platelet regeneration, but this interpretation usually is not applied to macroplatelets in cats.

Advanced hematologic techniques

Historically, these capabilities were limited to central laboratories. Over the past 20 years there has been rapid technological evolution resulting in reduced cost and complexity such that these capabilities are now available to the common veterinary facility. Currently, the predominant differences of the larger, more expensive systems used by commercial laboratories are higher throughput rate, automated tube handling, and more sophisticated differential counting technology. (See Chapter 2 for additional discussion of equipment and laboratories.) Hemograms performed on modern hematologic instrumentation provide the following additional measurements.

Items determined by spectrophotometry or calculation:

- Hemoglobin concentration of blood, g/dL
- Mean cell hemoglobin content, pg
- Mean cell hemoglobin concentration (MCHC), g/dL

Items determined by cell (particle) counting and sizing:

- Erythrocyte concentration of blood, $\times 10^6$ cells/μL
- Mean cell volume (the average size of erythrocytes; MCV), fL
- Hematocrit (equivalent to the packed cell volume), %
- Platelet concentration of blood, $\times 10^3$ cells/μL
- Mean platelet volume (MPV), fL
- Total and differential leukocyte concentrations, $\times 10^3$ cells/μL
- Reticulocyte concentration, $\times 10^3$ cells/μL

The method and applicability for each of these measurements are now described.

Items determined by spectrophotometry or calculation

Hemoglobin concentration

This measurement of the quantity of hemoglobin per unit volume, expressed as g/dL, is performed in conjunction with the total leukocyte count. Briefly, a blood sample is diluted, and a chemical agent is added to rapidly lyse cells, thereby liberating hemoglobin into the fluid phase. Nucleated cells remain present in the form of a nucleus with organelles collapsed around it. The absorbance of light at a specific wavelength then may be measured by spectrophotometry in a small flow cell known as a hemoglobinometer. The absorbance of light is proportional to the concentration of hemoglobin. The system is calibrated with material of known hemoglobin concentration using reference techniques.

Interpretation of the hemoglobin concentration is the same as that of the packed cell volume, or hematocrit. It is an index of the red cell mass per unit volume of blood in the patient. Because it is roughly equivalent to the packed cell volume, however, it is not particularly useful for clinical interpretations. Most clinicians are more familiar or experienced with interpreting packed cell volumes. The hemoglobin value is always proportional to hematocrit and is a separate, independent measurement. Therefore, the hemoglobin value may serve as a quality-control adjunct for laboratory personnel when used to calculate the MCHC.

Mean cell hemoglobin

The mean cell hemoglobin is calculated from the hemoglobin concentration and erythrocyte concentration. It is regarded as being redundant to other measurements and, therefore, is not useful.

Mean cell hemoglobin concentration

The MCHC is calculated from the hemoglobin concentration and the hematocrit. It provides an index for the quantity of hemoglobin (HGB) relative to the volume of packed erythrocytes (expressed as g/dL):

$$\frac{\text{HGB (g/dL)}}{\text{PCV (\%)}} \times 100 = \text{MCHC (g/dL)}$$

where PCV is the packed cell volume. An example calculation is

$$\frac{10\ \text{g/dL}}{30\%} \times 100 = 33.3\ \text{g/dL}$$

A universal relationship among mammalian species, other than the camel family, is that the hemoglobin value normally is approximately one-third of the hematocrit value. Thus, from the relationship described, the MCHC for all mammalian species ranges from approximately 33 to 38 g/dL. Because members of the camel family (camel, llama, alpaca, vicuna) have relatively more hemoglobin within their cells, their MCHCs are expected to range from 41 to 45 g/dL.

The MCHC is not particularly useful for clinical interpretations; however, it is useful to laboratorians for monitoring instrument performance. The rationale is that the hematocrit and hemoglobin are determined on different blood aliquots, which are diluted in two different subsystems of the instrument. A malfunction in either of these subsystems may result in a mismatch between the hemoglobin and the packed cell volume, which is reflected by a deviation from the reference interval. In addition, some abnormalities of blood can result in an artifactually increased MCHC, and these can include any factor that causes a false increase in the spectrophotometric determination of hemoglobin relative to the hematocrit. Severe hemolysis in the sample is a common cause of an increased MCHC. Alternatively, common examples of increased turbidity that interfere with

light transmittance are lipemia and a very large number of Heinz bodies (see Chapter 8) in cats. Erythrocyte agglutination, as may occur in immune-mediated hemolytic anemia, may result in a false high MCHC. In this situation, the hemoglobin measurement is accurate, but the hematocrit is falsely low because the agglutinated erythrocytes are out of the system's measuring range and are therefore not counted or sized in derivation of hematocrit.

Two erythrocyte responses related to anemia may be associated with a slightly decreased MCHC. The first is marked regenerative anemia. Reticulocytes or polychromatophilic cells are still synthesizing hemoglobin and, therefore, have not yet attained the cellular hemoglobin concentration of a mature erythrocyte. A very high fraction of reticulocytes is required, however, such as greater than 20%, to develop a detectable decrease in MCHC. The second is severe iron deficiency, in which cells have a reduction in hemoglobin content because they are smaller (i.e., microcytic) but also may have a minor reduction in cellular hemoglobin concentration. There are no causes of a dramatically decreased MCHC (<28 g/dL) other than an analytic instrument error.

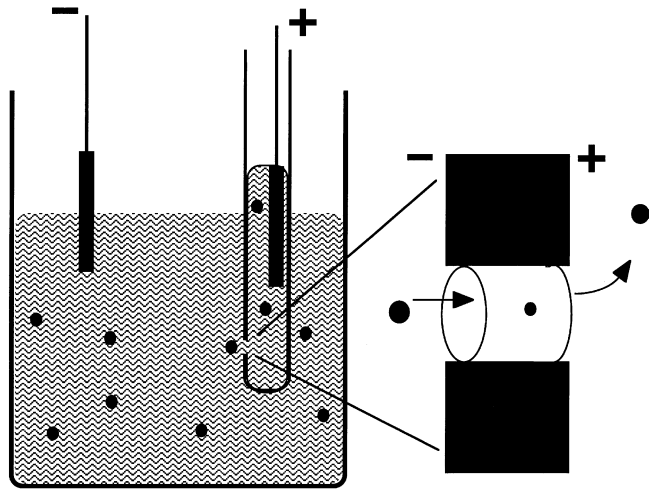


Figure 1.27 Principle of electronic impedance cell counting. Left. Overview of the fluidic chamber. Cells (dots) are diluted in an isotonic fluid (wavy lines). Two electrodes (+ and –) are separated by a glass tube containing a small opening or aperture. Electric current is conducted by the isotonic fluid across the electrodes via the aperture. Vacuum is applied to move the fluid and cells through the aperture. Right. Magnified, diagrammatic view of the aperture. Cells flow through the aperture (arrows). The aperture is a cylindric shape with a volume called the sensing zone. While occupying space within the aperture, cells transiently impede the flow of current. Cell passages are counted as deflections in the current voltage. In addition, the magnitude of voltage deflection is proportional to the volume of the cell.

Items determined by cell (particle) counting and sizing

Cell counting and sizing technologies

A brief overview of cell counting and sizing technology common to all of these measurements is appropriate. One of two technologies is used by most hematology instrument systems.

The first is light-scatter measurement of cells passing through a light source. Cells are passed through a flow cell that is intersected by a focused laser beam. The physical properties of the cell scatter light to different degrees and at different angles relative to the light source. Cell passages eliciting scatter events may be counted to derive the cell concentration. The degree of scatter in the direction of the light beam, which is known as forward-angle scatter, is proportional to the size of the cell. In addition, measurement of light scattered to different angles may be correlated with cellular properties, which leads to the ability to differentiate nucleated cell types.

The second is more common and incorporated into a wider range of instrument designs and may also be used as a second measuring principle in light-scatter systems. This is electronic cell counting, which is also known as impedance technology or Coulter technology (after the original inventor). It is based on the principle that cells are suspended in an electrolyte medium, such as saline, that is a good conductor of electricity. The suspended cells, however, are relatively poor conductors of electricity. Thus, these cells impede the ability of the medium to conduct current in a sensing zone known as an aperture. By simultaneously passing current and cells through a small space or aperture, deflections in current can be measured (Fig. 1.27). In addition, the size of the cell is proportional to the resultant deflection in current. This volumetric size discrimination may be used to measure the size distribution of erythrocytes, to discriminate platelets from erythrocytes, and to partially differentiate leukocytes. Cells within a given population are counted and assigned to a size distribution by particle-size analyzer circuitry (Fig. 1.28). The particle-size analyzer assigns each cell to a size scale that is divided into a large number of discrete size "bins" of equal size. The size scale is calibrated with particles of known size. By rapidly accumulating several thousand cells, a frequency distribution of the sizes of the cell population may be constructed (Fig. 1.29).

The size distribution curve is most useful for the evaluation of erythrocytes in the laboratory. It also may be used to derive leukocyte differential and platelet information.

The following measurements derive from the described cell counting and sizing technology. Because of the considerable differences in erythrocyte and platelet sizes between species, instrument systems require careful design and/or adjustment to accurately obtain the various measurements. For example, instruments manufactured for the analysis of human blood do not perform accurately for most animal species without modification.

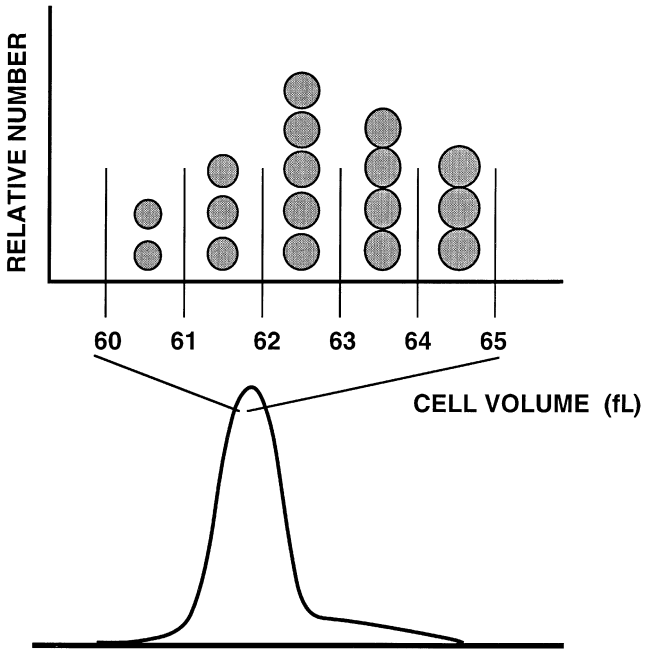


Figure 1.28 Cell volumes assigned to size bins. In the case of erythrocytes, a cell volume scale of approximately 30 to 250 fL is divided into a large number of discrete size bins (e.g., 60–61 fL, 61–62 fL). As the cells are counted, they are assigned to size bins (circles). Accumulation of many cells allows the construction of a size distribution histogram on the cell-volume scale (curve tracing at bottom). The drawing of bins at the top would represent a small area of the total curve.

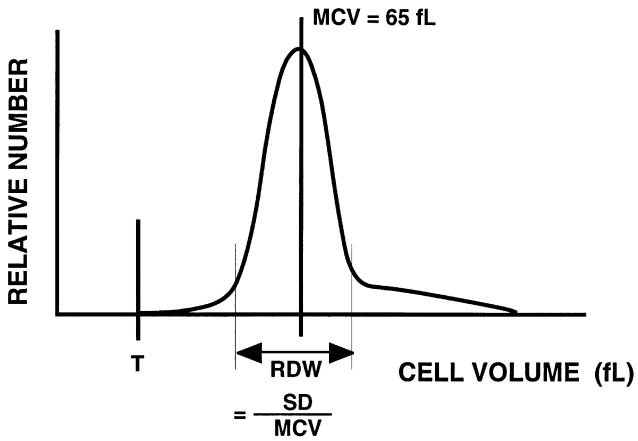


Figure 1.29 Histogram of erythrocyte size distribution. The x-axis is the cell volume, and the y-axis is the relative number of cells at each volume. Only cells above a specified volume or threshold are included in the analysis; this is indicated by the vertical bar (T). The mean cell volume (MCV) is indicated by the large vertical bar. The RDW (red-cell distribution width) value, an index of volume heterogeneity, is the standard deviation (SD) divided by the MCV, with the SD being that of the volumes of erythrocytes within the region indicated by the fine lines marked by the double arrow.

Erythrocyte concentration

The erythrocyte concentration is measured directly by counting the erythrocyte particles in an isotonic dilution of blood. This value is not useful for purposes of clinical interpretation. It generally parallels the packed cell volume and hemoglobin concentration, but the packed cell volume is the preferred value for the interpretation of erythrocyte mass. The erythrocyte concentration is used by the instrument to calculate the packed cell volume (described later).

Mean cell volume, erythrocyte histogram, and red cell distribution width

As the erythrocytes are counted, their size distribution is simultaneously constructed (Fig. 1.29), and from this size distribution, the MCV is easily calculated. The red cell distribution width (RDW) is a mathematic index describing the relative width of the size distribution curve. It is the standard deviation of most the erythrocytes divided by the MCV. The tails of the erythrocyte distribution usually are excluded from this mathematic treatment.

These values are useful for the evaluation of anemia. Iron deficiency results in the production of microcytic erythrocytes, and accelerated erythrocyte regeneration results in the production of macrocytic erythrocytes. Early in these responses, a widening of the erythrocyte size distribution and RDW value may be observed (Fig. 1.29). As a larger proportion of these cells accumulate during the response, the curve shifts in the respective direction, and eventually, the MCV may fall out of the reference interval. The RDW is more useful in the laboratory, in conjunction with the examination of blood films, whereas the laboratorian and the clinician both may interpret the MCV. Examples of interspecies variation and representative reference intervals for MCV are

Humans	80–100 fL
Dogs	60–72 fL
Cats, horses, and cows	39–50 fL
Sheep	25–35 fL
Llama	21–29 fL
Goat	15–25 fL

For additional detail on microcytic and macrocytic anemias and other breed-specific information regarding erythrocyte size, see Chapter 6.

Hematocrit

One of the advantages of hematology instrumentation is that the hematocrit may be determined by calculation, thereby avoiding the need for microhematocrit centrifugation. The instrument calculates hematocrit (HCT) using the erythrocyte concentration (RBC) and the MCV:

$$(\text{MCV} \times 10^{-15}\ \text{L}) \times (\text{RBC} \times 10^{12}\ \text{L}) = \text{HCT}$$

Or, simplified:

$$\frac{\text{MCV} \times \text{RBC}}{10} = \text{HCT}$$

Thus, for example:

$$\frac{\text{MCV 70 fL} \times 7.00\ \text{RBC}}{10} = \text{HCT 49\%}$$

Platelet concentration

Platelets may be counted simultaneously with erythrocytes. Because platelets are considerably smaller than erythrocytes, however, they are analyzed in a separate area of the particle-size analyzer scale. Most species have little or no overlap between platelet and erythrocyte volume, thereby making such analysis both simple and accurate. Cats are an exception, in that their platelets are approximately twice the volume of those in other domestic species. In addition, macroplatelet production is a frequent response during most hematologic disturbances in cats. This response is not specific for any specific disease pattern, but it results in considerable overlap between erythrocyte and platelet size distributions, thus making determination of accurate counts difficult. Therefore, feline platelet counts should be regarded as being estimates only. Because large platelets tend to get counted as erythrocytes, the platelet concentration frequently may be artifactually low. In general, if the platelet concentration falls in the reference interval, it may be regarded as being adequate. If the platelet concentration is decreased, however, the blood film should be examined by a laboratorian to confirm this finding.

White blood cell and differential leukocyte concentrations

To analyze leukocytes, a lytic agent is first added to a dilution of blood. This agent rapidly lyses or dissolves cytoplasmic membranes, thereby making the erythrocytes and platelets "invisible" to the detection technologies. Only nuclear particles of nucleated cells remain, around which is found a "collapse" or condensation of cytoskeletal elements and any attached organelles. These particles are measured by one of the detection technologies previously described to obtain the total leukocyte concentration. Using specially formulated lytic reagents, the degree of collapse may be controlled to different degrees in different leukocyte types. The result is a differential size that can be measured by a particle-size analyzer or light-scatter technology. Automated differential leukocyte counting is not as perfected in domestic animals as in humans; however, the procedure is reasonably accurate for normal blood and, therefore, is very useful in situations such as safety assessment trials, in which most (or all) of the blood samples to be analyzed are normal. When blood is abnormal, however, the frequency of analytic error in the differential count increases considerably. Analytic errors are handled by using the blood film for comparison and the visual differential count whenever an instrument analytic error is either present or suspected. It is essential to monitor instrument performance by visual inspection of the histogram or cytogram display for each sample to know when analytic failure occurs. It is very difficult, if not impossible, to determine this simply by monitoring numeric data from the instrument. Therefore, use of this technology requires considerable training and expertise by the operator to monitor the instrument performance and appropriately intervene with visual inspection of the blood film.

Summary of blood analysis by automated or semiautomated instrumentation

The flow of dilutions, analysis, and calculations within an automated hematology instrument is summarized in Figure 1.30. This flow has two main pathways. In one, an isotonic dilution of blood is made for erythrocyte and platelet analysis. In the other, a dilution is made, into which a lytic agent is added; in this pathway, leukocytes and hemoglobin are measured.

Reticulocyte concentration

Reticulocyte enumeration

The reticulocyte concentration is very useful in the evaluation of anemias. The rate of release of reticulocytes from the bone marrow is the best assessment regarding the function of the erythroid component of bone marrow. (See Chapters 6–8 for a more detailed discussion of the anemias.)

The basis for the reticulocyte count involves the events in the maturation of erythroid cells. The developing erythroid cell is heavily involved in aerobic metabolism and protein (i.e., hemoglobin) synthesis. As it nears the final stages of

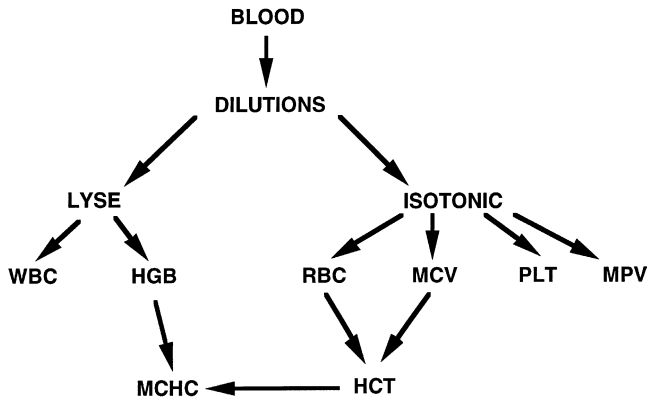


Figure 1.30 Summary of blood analysis pathways in an automated instrument. Two major dilutions are made (see text). In the left pathway, a lytic agent is added, and leukocytes are counted and the hemoglobin concentration measured. In the right pathway, erythrocytes and platelets are counted and sized. From the direct measurements, the hematocrit is calculated. A cross-check between the two pathways is provided by calculation of the mean cell hemoglobin concentration (MCHC).

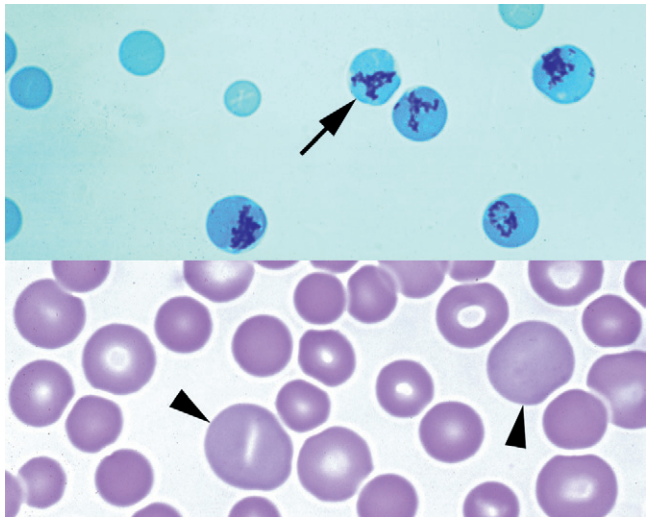


Figure 1.31 Reticulocytes. Top. Representative reticulocyte (arrow) using new methylene blue stain. Note the dark-staining, aggregated organelles in several reticulocytes. Bottom. Blood film stained with Wright-Giemsa stain. Polychromatophilic cells (arrowheads) are roughly equivalent to reticulocytes on the counterpart stain.

Figure 1.32 Examples of reticulocyte stains. Left. New methylene blue in a liquid dropper bottle. Right. Commercial preparation of brilliant cresyl blue. The stain is coated on the bottom of disposable tubes.

maturity, the nucleus undergoes degeneration and is extruded from the cell, and the organelles supporting the synthetic and metabolic events are removed. After denucleation of the metarubricyte, the remaining erythrocyte undergoes its final maturation, which involves the loss of ribosomes and mitochondria during a period of 1–2 days. To enumerate reticulocytes, a stain is applied to erythrocytes, thereby causing aggregation of these residual organelles. This results in visible, clumped granular material that can be seen microscopically (Fig. 1.31). The aggregation is referred to as reticulum, hence the name reticulocyte. Reticulocytes are equivalent to the polychromatophilic cells observed on the Wright-stained blood films (Fig. 1.31). Evaluation of polychromatophilic cells on the Wright-stained blood film can provide an assessment of the bone marrow response to anemia. The appearance of these cells, however, is more subjective, and they are more difficult to quantitate than counting the corresponding cells on the reticulocyte stain.

Stains that can be used are new methylene blue (liquid) and brilliant cresyl blue, which is available in disposable tubes that facilitate the procedure (Fig. 1.32). First, several drops of blood are added to the stain in a tube. The tube then is mixed and incubated for 10 minutes. From this mixture, a conventional blood film is made and air-dried. A total of 1000 erythrocytes are counted and categorized as either reticulocytes or normal cells. From this, the percentage of reticulocytes is derived. Interpretation of the percentage reticulocytes is somewhat misleading, however, because it does not account for the degree of anemia. Thus, for purposes of interpretation, the absolute reticulocyte concentration should be calculated by multiplying the erythrocyte concentration (RBC) by the percentage of erythrocytes that are reticulocytes:

$$\text{RBC}/\mu\text{L} \times \%\ \text{Reticulocyte} = \text{Reticulocytes}/\mu\text{L}$$

Some instrument systems are also capable of reticulocyte enumeration. The method involves staining erythrocytes with a fluorescent dye that binds to residual RNA in the reticulocyte that is not present in the mature erythrocyte. RNA content, proportional to fluorochrome per cell, is measured and gated to differentiate reticulocytes from mature erythrocytes and other nonerythroid cell types. The percent and absolute values are presented as described above.

Interpretation of the reticulocyte concentration

The reticulocyte concentration is most useful in dogs and cats, and it also has some application in cows. It is not used in horses, however. Reticulocyte maturation is confined to the marrow space in the horse, and reticulocytes almost never are released into their circulation. Reticulocyte concentration guidelines for domestic mammals are the concentrations to be expected when the hematocrit is normal:

Dogs and cats	0–60,000 cells/μL
Cows	0 cells/μL
Horses	Do not release reticulocytes

When anemia is present, a greater degree of release from the marrow is to be expected if the marrow can respond to the anemia. This gives rise to the following guidelines for the interpretation of reticulocyte concentrations with respect to the type of anemia present:

Nonregenerative anemia to very poor regeneration	0–10,000 cells/μL
Nonregenerative to poorly regenerative anemia	10,000–60,000 cells/μL
Regenerative anemia with mild to moderate output	60,000–200,000 cells/μL
Maximal regeneration	200,000–500,000 cells/μL

Reticulocyte maturation

In dogs, reticulocyte maturation occurs in 24–48 hours. Maturation involves a continuum of progressive loss of the visible organelles (Fig. 1.33).

Cats are unique in that more than one kind of reticulocyte may be present. These reticulocytes are of the aggregate and the punctate forms (Fig. 1.34). The aggregate reticulocyte has a clumped reticulum that appears to be identical to that of other species. In the punctate reticulocyte, discrete dots are seen without any clumping; other species do not have this reticulocyte counterpart. Only aggregate reticulocytes appear to be polychromatophilic with Wright stain. Punctate reticulocytes are indistinguishable from normal, mature erythrocytes with Wright stain.

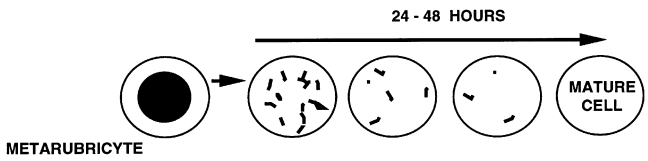


Figure 1.33 Sequential erythroid maturation as related to the reticulocyte stain and interpreted in dogs. The metarubricyte denucleates on leaving the reticulocyte. Reticulum is progressively lost during a 24–48-hour period, resulting in a mature erythrocyte.

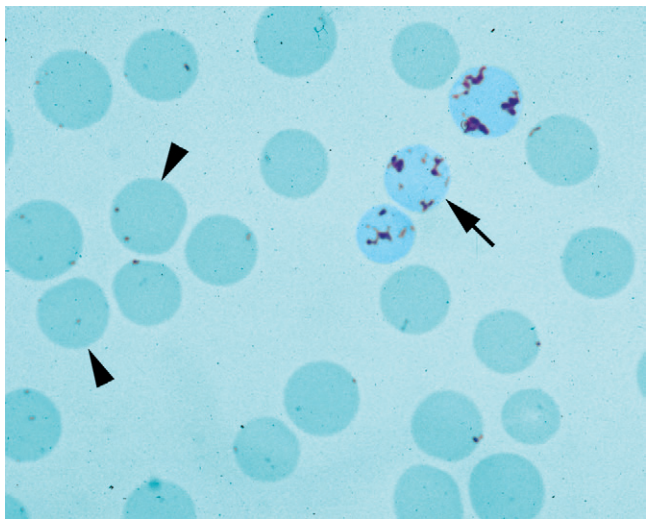


Figure 1.34 Feline reticulocyte morphology with new methylene blue stain. Three aggregate reticulocytes are in the field; note the representative one (arrow). The remainder of the cells are punctate reticulocytes; note the representative cells (arrowheads).

Reticulocyte maturation in cats also may be viewed as a continuum (Fig. 1.35). Aggregate reticulocytes mature to the punctate form in approximately 12 hours; the punctate cells may continue to mature for another 10–12 days. Because of the short maturation time of aggregate reticulocytes, these cells are the best indicator of active marrow release. Therefore, only aggregate cells are counted in cats, and interpretive guidelines apply to this cell type only. Experience is required to exclude punctate cells when performing the reticulocyte count.

Organization of the complete blood count (hemogram)

It is useful to summarize the described basic and advanced determinations in a way that shows the organization of how they are performed and interpreted. This provides a mental framework for simplifying the complexity of this information into an everyday, intuitive tool: the hemogram. The techniques for generating data may be organized conceptually as direct measurements, microscopic procedures, and calculations. The complete blood count may include:

Direct Measurements

- Packed cell volume (by microhematocrit centrifugation)
- Hemoglobin concentration
- Red cell concentration (RBC)
- Mean cell volume (MCV)
- White cell concentration
- Plasma proteins (by refractometer)

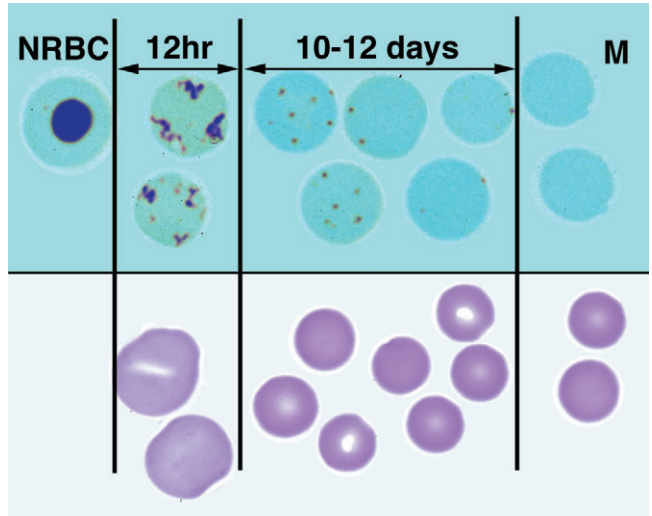


Figure 1.35 Feline reticulocyte maturation, progressing from left to right. Top. Cells stained with new methylene blue. After denucleation of the metarubricyte (NRBC), an aggregate reticulocyte is formed. This cell matures to the punctate form in approximately 12 hours. The punctate forms continue to mature by slow loss of punctate granules during a 10–12-day period. Mature cells (M) on the right have no granularity. Bottom. Corresponding cells stained with Wright-Giemsa stain. Note that polychromatophilic cells correspond to aggregate reticulocytes. Punctate and mature cells are indistinguishable with Wright-Giemsa stain.

- Platelet concentration
- Mean platelet volume (MPV)

Microscopic Procedures

- Differential white cell count
- Red cell morphology
- Platelet morphology and assessment of adequacy
- Microscopic reticulocyte enumeration in patients with anemia

Calculations

- Hematocrit, when instrument derived
- Erythrocyte indices (e.g., MCHC, MCH, and RDW)
- Absolute white blood cell differential values
- Absolute reticulocyte count

These determinations are organized into a report form that aids the clinician in efficiently interpreting the information. The best way for this information to be organized is into banks of data that relate to the three major cell lines (i.e., erythrocytes, leukocytes, and platelets). For each cell line, all pieces of relevant information are organized in one place on the form.

Laboratory tests useful in the diagnosis of immune-mediated hemolytic anemia

Coombs or antiglobulin test

The Coombs or antiglobulin test is used as an aid in establishing the diagnosis of immune-mediated hemolytic anemia by detecting species-specific immunoglobulin that is adsorbed or attached to the surface of erythrocytes. The test uses the Coombs reagent, which is a polyclonal serum (usually prepared in rabbits) to the immunoglobulins of the species of interest. Some reagent manufacturers claim their reagent also detects complement. The procedure involves washing the erythrocytes in saline to remove plasma proteins and immunoglobulin that may be nonspecifically associated with erythrocytes. An aliquot of washed cells then is incubated with the Coombs serum. If appreciable patient immunoglobulin is attached to the erythrocytes, the Coombs serum induces erythrocyte agglutination. By means of two binding sites per molecule, the Coombs reagent immunoglobulin binds the patient immunoglobulin attached to the erythrocytes. The two binding sites result in progressive bridging of erythrocytes, which is visualized as agglutination. The absence of agglutination is interpreted as being a negative result, whereas the presence of agglutination is interpreted as being a positive result. Appropriate controls are performed as well.

False-negative reactions are a common problem with the Coombs test, likely because of the elution of pathologically adsorbed immunoglobulin or immune complexes during washing of the erythrocytes in preparation for the test. The best evidence for this is that prominent autoagglutination may disappear with washing. Autoagglutination, if confirmed microscopically, may be interpreted as being equivalent to a positive Coombs test. False-positive reactions also may occur, but are less well documented because the test is typically only performed when one suspects the disease.

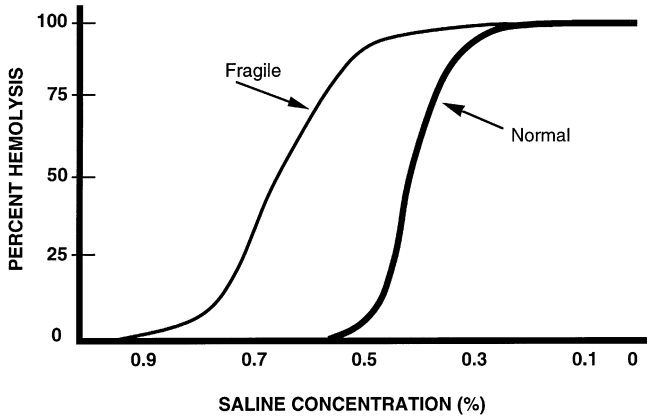


Figure 1.36 Erythrocyte fragility curve. Percentage hemolysis is plotted against decreasing saline concentration. Note the normal curve (arrow marked Normal). Increased erythrocyte fragility is recognized by a shift of the curve to the left (arrow marked Fragile).

Saline fragility test

Resistance of patient erythrocytes to hemolysis is measured in decreasing concentrations of saline. This test is not commonly used because of its complexity and labor intensity. It remains a useful diagnostic aid, however, in occasional cases of immune-mediated hemolytic anemia in which other hallmark pieces of information are not clearly interpretable. An equal aliquot of erythrocytes is added to a series of tubes containing decreasing concentrations of saline. After incubation, the tubes undergo centrifugation, and the hemoglobin concentration then is measured on the supernatant. A tube with distilled water serves as an index for 100% hemolysis. A plot of the percentage hemolysis and the concentration of saline facilitates interpretation, as shown in Figure 1.36.

These tests must not be used or interpreted in isolation. They are to be used in conjunction with analysis of other hematologic data and morphologic evaluation of the blood film by the laboratorian. Because of the frequency of false-negative and -positive results with the Coombs test, interpreting the results of this test in the light of the other available hematologic information is important. (See Chapter 8 for a detailed discussion of the strategy for diagnosing immune-mediated hemolytic anemia.)

Chemistry techniques

A wide variety of techniques, which have been incorporated into many different instrument designs, are used in veterinary clinical chemistry. No attempt is made here to discuss all of these techniques and instruments, but the basic information

on a variety of chemistry techniques used in analyzing samples from animals is provided. A complete understanding of these techniques is not necessary for veterinarians who send clinical chemistry samples to a reference laboratory; however, an increasing number of chemistry instruments are being marketed to veterinarians for in-practice use. Therefore, an understanding of how these instruments work is important for understanding the advantages and disadvantages of the various instruments, the laboratory techniques necessary for their use, the problems that might arise during their use, and the basic principles underlying their variations in design.

The chemistry techniques discussed in this chapter and the substances that may be measured with them are listed in Table 1.1. Absorbance or reflectance photometry is used to measure most of the substances in clinical chemistry profiles. Fluorometry also is used to measure certain analytes in some clinical chemistry analyzers. Blood pH, partial pressures of carbon dioxide and oxygen, and concentrations of electrolytes such as sodium, potassium, and chloride most commonly are measured by electrochemical methods. Atomic absorption spectrophotometers are not commonly used in clinical chemistry laboratories; rather, they are more common in laboratories testing for elements considered nutrients and/or toxicants. Osmometers are common in clinical chemistry laboratories and are used to measure blood and urine osmolality or osmolarity. Protein electrophoresis is used to measure concentrations of the various protein fractions comprising the total serum protein, especially in samples with either decreased or increased protein concentrations. Light-scatter techniques that quantitate turbidity are used less commonly to measure the concentrations of substances such as large protein molecules.

Photometry

Photometry is a general term used to describe an analytical chemistry technique in which the concentrations of substances and the activities of enzymes are determined by measuring the intensity of light passing through or emitted from a test chamber. This test chamber contains the substance to be detected and, in most cases, reagents intended to react with that substance to produce a color reaction. Strictly speaking, the term spectrophotometry should be applied when the instrument being used has the ability to produce light of a variety of wavelengths through some type of light-fractionating device, such as filters, prisms, or diffraction gratings.

Absorbance spectrophotometry

Absorbance spectrophotometry is an analytic technique in which concentrations of substances are determined by directing a beam of light through a solution containing the

Table 1.1 Techniques in veterinary clinical chemistry and substances measured with those techniques.

Technique	Substances Measured
Photometry	
Absorbance photometry	Glucose, BUN, creatine, calcium, phosphorus, magnesium, protein, albumin, bilirubin, bile acids, ammonia, cholesterol, bicarbonate, total CO_2, enzymes
Reflectance photometry	Similar to those measured by absorbance photometry
Atomic absorption spectrophotometry	Many elements including nutrients and toxicants (e.g., calcium,[a] magnesium,[a] lead, arsenic)
Fluorometry	Glucose, bilirubin, bile acids, calcium, magnesium, enzymes, antithrombin III, heparin, plasminogen, hormones, drugs
Light-scatter techniques	
Turbidimetry	Immunoglobulins, antigen–antibody complexes, other large proteins, drugs
Nephalometry	Immunoglobulins, antigen–antibody complexes, other large proteins, drugs
Electrochemical methods	
Potentiometry	Blood pH, PCO_2, sodium,[b] potassium,[b] chloride[b]
Amperometry	PO_2
Coulometry and conductometry[c]	BUN
Osmometry	Osmolality or osmolarity
Protein electrophoresis	Albumin, α-globulin, β-globulin, γ-globulin

BUN, blood urea nitrogen.

[a] May be used to measure the concentration of these substances in solid tissues that have been ashed or digested. Absorbance photometry is more commonly used to measure concentrations of these substances in serum or plasma.

[b] Electrodes used to measure concentrations of these electrolytes are called ion-selective electrodes.

[c] Conductometry also is used to perform cell counts in some hematology analyzers.

substance to be detected (or a product of that substance) and then measuring the amount of light that either of these absorb. The principles described here are incorporated into automated and semiautomated processes on today's chemistry analyzers. Automation, from sample and reagent addition management to calculation of test results to generation of a patient diagnostic report, is made possible by computer control and information processing integral to these systems.

To understand absorbance spectrophotometry, some basic knowledge regarding light is necessary. Typically, light is classified by its wavelength, which is measured in nanometers (nm). Light with the shortest wavelengths (<380 nm) is termed ultraviolet (UV) light (Table 1.2). Light in the visible spectrum has wavelengths of 380–750 nm. Light with the longest wavelengths (>750–2000 nm) is termed infrared (IR) light. The energy of light is inversely proportional to its wavelength; therefore, UV light has the highest energy and IR light the lowest.

The visible spectrum includes a variety of wavelengths that represent the colors with which we are familiar. It is important to remember that color results from the transmittance or reflectance of light. In other words, a green object is that color because it reflects the green area of the visible spectrum and has absorbed the other wavelengths of light in that spectrum. Likewise, a green solution is green because it allows light in the green area of the visible spectrum to be transmitted through it and has absorbed the visible light of other wavelengths. These same principles also apply to light outside the visible spectrum. Different substances absorb and reflect different wavelengths in a pattern that is typical for that substance. The pattern in which a substance absorbs light at various wavelengths is known as its absorption spectrum, and each substance has its own unique absorption spectrum.

Table 1.2 Wavelengths resulting in ultraviolet light, various colors of visible light, and infrared light.

Wavelength (nm)	Color
<380	None (ultraviolet)
380–440	Violet
440–500	Blue
500–580	Green
580–600	Yellow
600–620	Orange
620–750	Red
750–2000	None (infrared)

A basic absorbance spectrophotometer is diagrammed in Figure 1.37. Various sources of light can be used, with the choice being based on the portion of the spectrum desired plus issues such as longevity of the bulb and the basic instrument design. In the application of absorbance spectrophotometry for measuring the concentration of a substance, a wavelength of light that is absorbed by that substance (or by a product of that substance) is used. This wavelength is determined by examining the absorption spectrum of the substance of interest. Usually, the wavelength chosen is the one at which the maximum absorbance occurs. Occasionally, however, some other wavelength may be chosen to avoid interference with substances such as hemoglobin and bilirubin, which may be present in serum samples secondary to hemolysis (in vitro or in vivo) or disease leading to high bilirubin concentration. Hemoglobin and bilirubin have their own absorption spectrums, and methods attempt to avoid using the wavelengths that these substances strongly absorb.

A monochromator is an optical device between the light source and the measuring cuvette. It will narrow the spectrum of light that passes to and through the cuvette. Monochromators can be filters, prisms, or diffraction gratings. When attempting to produce light of a specific wavelength, the actual range of wavelengths produced by a monochromator is called the spectral bandwidth. Each type of monochromator can produce rays of light at certain spectral

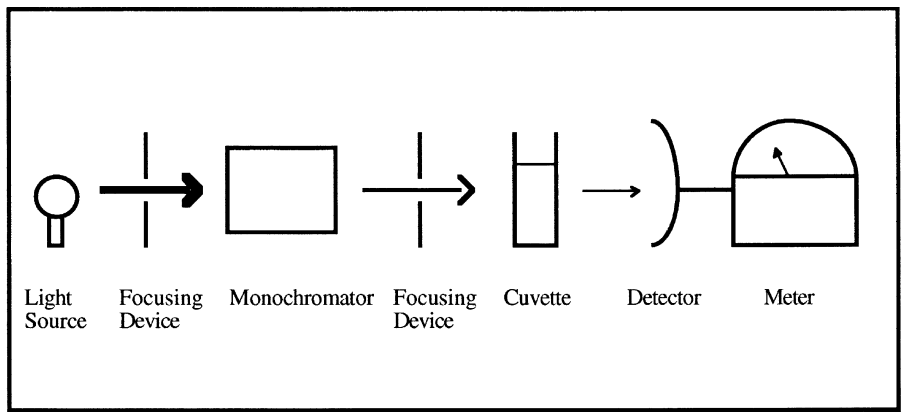


Figure 1.37 Components of a simple absorbance spectrophotometer. Arrows represent light.

bandwidths. Monochromators capable of producing light of a narrow spectral bandwidth have more spectral purity. The importance of spectral purity varies with the type of spectrophotometry, however, and with the substance being analyzed. Filters may be a thin layer of colored glass that transmits light at wavelengths corresponding to the filter's color, or they may be more complex structures, with a layer of dielectric material sandwiched between two pieces of glass coated with a thin layer of silver. The latter type of filter transmits light at wavelengths equal to or at multiples of the thickness of the dielectric layer. In some cases, multiple filters may be placed in series to produce light of greater spectral purity. Prisms separate the wavelengths of white light by refracting this light. As light passes through a prism, shorter wavelengths are bent more than longer wavelengths, thus separating them. The desired wavelength then can be selected from this spectrum for transmission. Diffraction gratings are a metal or glass plate covered with a layer of metal alloy into which multiple parallel grooves have been etched. When the grating is illuminated, each groove separates the light into a spectrum, and light of specific wavelengths is produced as wavelengths that are in phase are reinforced and those that are not in phase are cancelled.

The focusing devices usually are lenses or slits that are inserted before and/or after the monochromator. This placement varies with the instrument. Focusing devices are used to narrow the light beam, to produce parallel light rays, and/or to regulate the intensity of the light reaching the photodetector. In some modern instruments, application of fiber optics has eliminated some of the lens and slits used for narrowing and directing the light beams.

Cuvettes are also known as absorption cells. They have constant dimensions for a given instrument, and they can be made of various materials (e.g., glass, quartz, or plastic) and be of various shapes (e.g., round, square, or rectangular). The materials or shapes used depend on the instrument design and on the portion of the light spectrum being used. During analysis, a solution containing the absorbing substance is placed in the cuvette, and the light rays that have been produced pass through the cuvette walls and the solution. If the correct wavelength has been chosen, the substance absorbs this light in direct proportion to its concentration. In addition to the absorbing substance, the cuvette walls and the solution in which the substance is suspended also absorb small amounts of light. It is, therefore, necessary to "zero" spectrophotometers in order to eliminate the effect of these other factors, and this typically is accomplished by taking an absorbance reading on a cuvette containing only the solution in which the substance is suspended (i.e., the solution contains none of the absorbing substance). The absorbance reading of the instrument typically is set to zero while reading the absorbance of this "blank." Some spectrophotometers are designed to read the absorbance of the test solution and the blank solution simultaneously, which requires splitting the light beam and then shining each beam through either the test or the blank cuvette.

Photodetectors collect the light that has passed through the cuvette (i.e., the light that has not been absorbed). Several different technologies can be used in photodetectors. Factors such as cost, sensitivity, speed of response to changes in light intensity, propensity to fatigue (i.e., decreased response over time despite constant light intensity), and heat sensitivity help to determine which technology is used in a given application. Regardless of the type of photodetector, the underlying mechanism involves the production of electrons and, therefore, an electrical current in response to light striking the detector. This electrical current then is transmitted to a readout device or meter.

Readout devices or meters measure the electrical current produced by the photodetector. This current can be read out directly, but more commonly, this information is converted to a readout that gives either the absorbance or the actual concentration of the substance being measured. This conversion usually requires some type of microprocessor, which can store and use calibration information (discussed later) and also automatically adjust for the reading of the blank sample. The actual readout might be presented as some type of digital display, but it more commonly is printed.

Modern readout devices also incorporate recorders for obtaining multiple absorbance readings on the same sample over time. This is most useful in kinetic assays. In such assays, a reaction is allowed to occur over a period of time, and the production or disappearance of the absorbing substance is evaluated at multiple time points by measuring the absorbance of light normally absorbed by that substance. The change in absorbance over the time period is proportional to the activity of an enzyme or to the concentration of a substance, depending on which is being assayed. Such an assay obviously requires a device that can record and use data produced over time.

In addition to the basic instrumentation of absorbance spectrophotometry, the basic physical chemistry principles used in obtaining measurements via this technology also should be understood. When a light beam of a certain wavelength is projected through a solution containing a substance that absorbs light at that wavelength, the light is absorbed in direct proportion to the concentration of that substance. The intensity of the light leaving the solution, therefore, is less than the intensity of the light entering the solution. If these two intensities are known, the percentage transmittance of light (%T) can be calculated. For instance, if the intensity of light entering the cuvette is designated as I_1 and the intensity of light leaving the cuvette as I_2, then %T is calculated as

$$\%T = \frac{I_2}{I_1}$$

The intensity of light entering the cuvette is measured by projecting light of the appropriate wavelength through a

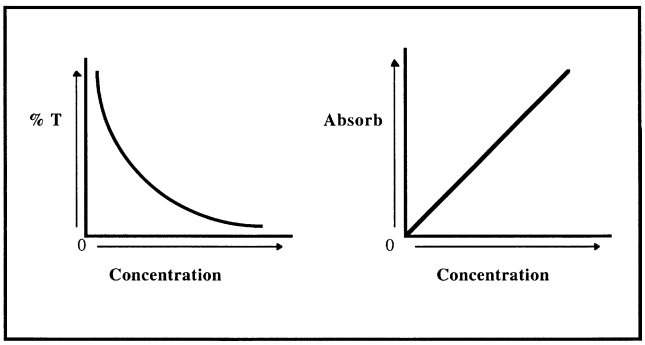


Figure 1.38 The relationships between percentage transmittance (%T), absorbance (Absorb), and concentration of a substance being measured. Note that as the concentration increases, %T decreases logarithmically or nonlinearly and absorbance increases linearly.

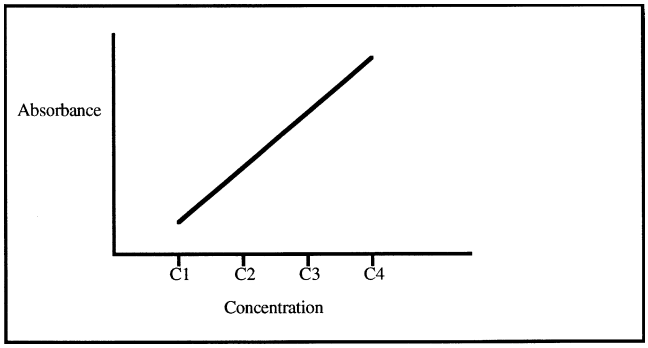


Figure 1.39 Use of calibrators to establish a calibration curve. In this case, four calibrators (C1, C2, C3, C4) were used. Note the linear relationship between concentration of the substance being measured and resulting absorbance.

cuvette containing the solution in which the substance to be measured is suspended. In this case, however, the solution contains none of the substance. Therefore, $\%T$ is set at 100% for this "blank" solution. The solution containing the substance to be measured is then placed in a similar cuvette, and the light is intensity measured, after which the $\%T$ can be assessed.

In the described situation, transmittance varies inversely and logarithmically with the concentration of the substance being measured. If $\%T$ versus the concentration of such a substance is plotted, a curved line results (Fig. 1.38). Light that is not transmitted is absorbed; therefore, transmittance and absorbance are inversely related, as described by the formula:

$$\text{Absorbance} = 2 - \log \%T$$

Because of this relationship, absorbance of light increases linearly with increasing concentration of the substance being measured (Fig. 1.38). This linear relationship between absorbance and concentration makes it more convenient to deal with absorbance than with transmittance during spectrophotometric analysis. Modern spectrophotometers measure transmittance, but then convert transmittance to absorbance. In addition, microprocessors in most spectrophotometers convert absorbance results to concentrations or activities and then report these in a final diagnostic test result format.

The concentration of a substance can be calculated from the absorbance by use of Beer's law:

$$A = abc$$

where A is the absorbance measured, a is the molar absorptivity (also known as the proportionality constant), b is the light path in centimeters (the diameter or width of the cuvette through which the light passes), and c is the concentration of the substance in question. The concentration (c) then can be calculated as

$$\text{Concentration} = \frac{A}{ab}$$

For Beer's law to apply, a linear relationship must exist between concentration and absorbance. In some cases, this might be true only up to certain concentrations or absorbance levels. To assure that Beer's law applies to a given assay, calibration solutions (also known as calibrators), which contain known concentrations of the substance to be measured, are used. The ranges of concentrations used as calibrators should include those that might be measured in samples from patients. Absorbance results for each calibrator are plotted against the concentrations of these calibrators to establish a calibration curve. Ideally, this curve is a straight line rather than an actual curve, showing that a linear relationship exists between absorbance and concentration (Fig. 1.39). In most applications, one or more calibrators are included with each series of sample measurements. It is best, however, to reestablish the calibration curve at frequent intervals (at least daily), because many slight day-to-day changes in the conditions of the test can affect this curve. These changes (e.g., light intensity, temperature, condition of reagents) can occur even in situations when instruments and reagents have been designed to minimize such variation. If a linear relationship does exist between the concentrations of the calibrators and the resulting absorbances, the solutions are said to obey Beer's law, and the calibrators can be used to establish a calibration constant (K):

$$K = \frac{\text{Concentration of the calibration solution}}{\text{Absorbance of the calibration solution}}$$

If K is known, then the concentration of an unknown solution can be calculated as:

Concentration of unknown = (Absorbance of unknown) × K

Microprocessors in instruments can plot absorbance results from calibrators, assure that a linear relationship exists, and calculate the calibration constant. These results are stored, and the concentrations of unknowns are calculated by measuring their absorbances and calibration constant.

A linear relationship between concentration and absorbance over the possible range of unknown concentrations is highly desirable, but a nonlinear calibration curve also can be used to derive unknown concentrations. In such a case, enough calibrators must be used to define the shape of the calibration curve, and as with a linear calibration curve, the range of calibrator concentrations should include the possible range of concentrations that might be found in samples from patients.

In absorbance spectrophotometry, two types of assay methods—endpoint or kinetic—may be used. In both types, the same principles of spectrophotometry described earlier apply. Endpoint assays usually are applied when measuring the concentration of some preexisting substance in serum or plasma. In such an assay, reagent(s) is added to a quantity of serum, and a chemical reaction occurs. The product resulting from this reaction then is measured by spectrophotometry. In other words, the solution in which the reaction has occurred is placed in a cuvette (or the reaction itself might have occurred in the cuvette), a light beam of a wavelength absorbed by the product is projected through a cuvette, and the absorbance is measured. By using a calibration curve and/or a calibration constant, the concentration of the substance being measured then is calculated. An example of an endpoint assay is a method for measuring the concentration of serum calcium:

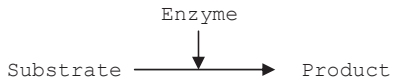
calcium + o-cresolphthalein complexone
→ calcium–cresolphthalein complexone

In this assay, the substance of interest (i.e., calcium) is complexed with cresolphthalein complexone, which has a purple color and absorbs light at a wavelength of 570 nm. This reaction is allowed to occur long enough to allow nearly all of the calcium in the sample to be complexed. More calcium–cresolphthalein complexone results in more light being absorbed, and a higher concentration of calcium reported by the instrument. After the absorbance is determined, it is compared with the absorbance of a calibration solution, and the absorbance of the unknown then is calculated as:

$$\text{Concentration of the unknown} = \text{Absorbance of the unknown} \times \frac{\text{Concentration of the calibration solution}}{\text{Absorbance of the calibration solution}}$$

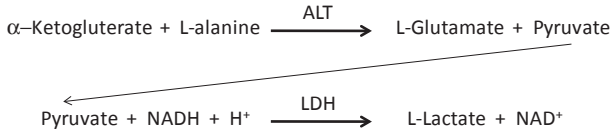
Note that the second portion of this formula is the calibration constant (K).

Kinetic assays typically have been used to measure enzyme activities, but also have been adapted to measure the concentrations of many analytes in blood. Typically, enzyme concentrations are not measured directly in clinical chemistry. Rather, the amount of enzyme in the serum usually is gauged indirectly, by the activity of that enzyme. Enzymes are proteins that catalyze (i.e., speed-up) chemical reactions, with the result that substrate is converted to product more quickly:



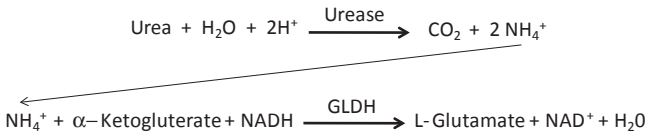
To measure an enzyme's activity, the rate at which it converts a substrate to a product must be assessed. The more quickly conversion occurs, the higher the enzyme activity is assumed to be. To measure the rate of conversion from substrate to product, the rate at which the product is being produced must be assessed, and this requires multiple measurements of the product concentration over time. Because this type of assay is a dynamic process, it is termed a kinetic assay. In a kinetic assay of enzyme activity, a solution containing the substrate of the enzyme of interest is added to the sample serum in a cuvette that already is in a spectrophotometer. When enzyme in this serum begins to convert substrate to product, absorbance is measured periodically by the same methods and using the same principles of spectrophotometry described previously (i.e., using a light beam of a wavelength absorbed by the product). In this process, the conversion rate of substrate to product is monitored. This rate can be converted to enzyme activity by using a formula involving the rate of absorbance change and several constants related to the absorptivity of the product as well as to test characteristics such as sample volume, total sample volume, and light path.

An example of a kinetic enzyme assay is an assay of alanine aminotransferase (ALT) activity:



where LDH is lactate dehydrogenase. In this assay, NADH is converted to NAD^+ at a rate proportional to the activity of ALT in the sample. The NADH absorbs light at 340 nm, and its rate of disappearance is measured by periodically assessing the absorbance of the reaction mixture. The rate of absorbance change in this mixture can be converted to units of ALT activity.

As previously noted, kinetic assays also are used for measuring the concentrations of preexisting substances in the blood. In these assays, the rate of appearance or disappearance of an absorbing substance is monitored by periodically measuring the absorbance of the reaction mixture. An example of a kinetic assay for measuring the concentration of a preexisting substance is an assay of the blood urea nitrogen (BUN) concentration, which uses the chemical reaction



where GLDH is glutamate dehydrogenase. In this reaction, the disappearance rate of NADH is monitored by periodically assessing the absorbance of the reaction mixture at a wavelength of 340 nm. The disappearance rate is proportional to the urea nitrogen concentration in the serum being tested. The BUN concentration is calculated by relating the rate of change in the absorbance of the sample with that of a calibrator.

Enzyme activity also can be measured by endpoint methods, which involve mixing serum with reagent containing substrate for the enzyme and then allowing the conversion of substrate to product to proceed for a specific period of time. At the end of that period, the concentration of substrate or product is measured. The more substrate used or product produced during the time period, the higher the enzyme activity is assumed to be.

Reflectance photometry

The principle of reflectance photometry is used in a few large, automated clinical chemistry analyzers and in several of the smaller clinical chemistry analyzers designed for in-practice use. Most of these instruments use "dry chemistry" systems, in which the fluid to be analyzed is placed on a carrier that contains the reagents for the assay. This carrier can take different forms, including a dry fiber pad or a multilayer of film. After the sample is applied, the chemical reaction occurs in this carrier, and a product is formed in a concentration proportional to that of the substance being measured. The carrier then is illuminated with diffused light, and the intensity of the light reflected from the carrier is measured and compared with that of either the original illuminating light or the intensity of light reflected off a reference surface. Reflectance photometry, therefore, is analogous to absorbance photometry in that the chemical reaction occurring in the carrier results in a product that absorbs a portion of the illuminating light. The remaining light is reflected, analogous to transmittance in absorbance spectrophotometry, to a photodetector that measures its intensity. The intensity of the reflected light is not related linearly to the concentration of the substance being produced. As a result, formulas are required to convert the reflectance results to concentrations. These formulas vary with the type of instrument being used.

Atomic absorption spectrophotometry

Atomic absorption spectrophotometry (AA) is used for measuring the concentrations of many elements. Advantages of AA include its superior sensitivity (i.e., it can detect smaller concentrations) and its ability to measure the concentrations of various elements. AA is typically limited to toxicology laboratories for clinical purposes. Applications include measurement of concentrations of elements such as lead, copper, and selenium in fluids or tissues. As the name implies, AA involves measuring absorption of energy by atoms. This technique involves heating a sample in a flame that is hot enough to cause the element in question to dissociate from its chemical bonds and form neutral atoms—but not hot enough to cause large numbers of electrons to jump to the excited state. These atoms then are in a low-energy (i.e., ground) state and can absorb light of a narrow wavelength that is specific for that element. If a light of this wavelength is projected through the flame, the amount of light absorbed is proportional to the concentration of the element in the sample. Measurement of the amount of light absorbed, therefore, allows the concentration of that element in the sample to be calculated. Focusing devices, photodetectors, meters, and readout devices serve the same purposes in AA as in other types of spectrophotometry.

Fluorometry

Fluorometric techniques can be used in a wide variety of applications, ranging from measurement of the concentrations of substances to assessment of the numbers and other characteristics of larger particles, including cells. This section discusses use of these techniques in measuring concentrations of various substances in body fluids.

Among the substances that can be measured by these techniques are some that commonly are measured in clinical chemistry analysis (e.g., bilirubin, bile acids, glucose, calcium, magnesium, and various enzymes), substances related to coagulation (e.g., antithrombin III, heparin, and plasminogen), drugs, and hormones. Some of these substances are fluorescent; in other cases, measurement of these substances is possible by linking other fluorescent substances to the analyte of interest, either directly or indirectly, as the result of a series of chemical reactions.

The basic principle underlying use of fluorometry is that certain substances, when exposed to light of the proper wavelength, will fluoresce. Fluorescence results when a substance absorbs light at one wavelength and then emits light at a longer (i.e., lower energy) wavelength. The ability to fluoresce varies with a compound's chemical structure; therefore, not all compounds can be readily measured by fluorometry.

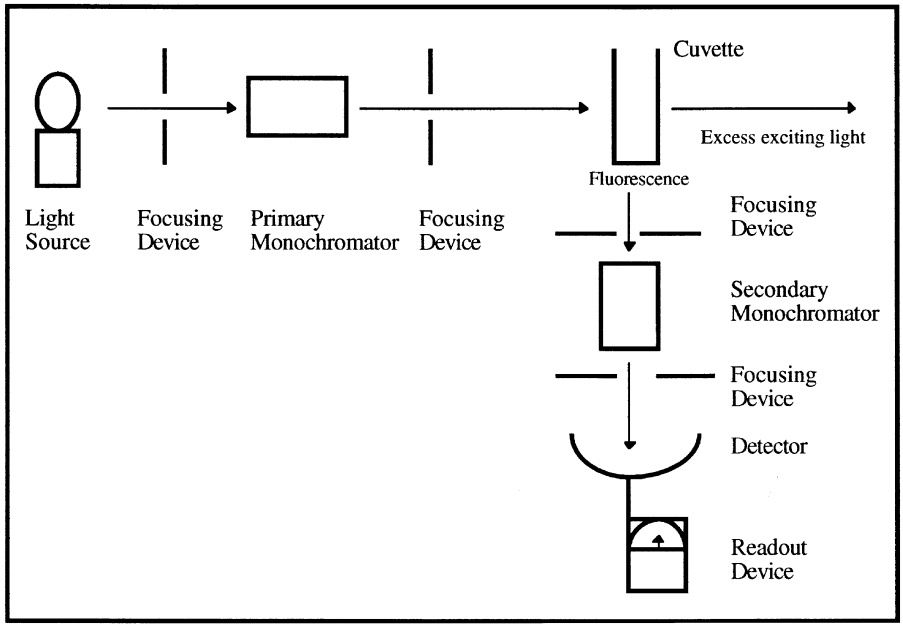


Figure 1.40 The basic design of a fluorometer. Arrows represent light.

The basic design of a fluorometer is shown in Figure 1.40. A variety of light sources, including various types of bulbs and lasers, can be used. Most fluorescent compounds absorb light at 300–550 nm; therefore, light sources must produce light at these wavelengths. The primary monochromator isolates light at the proper wavelength to produce fluorescence in the substance being analyzed. Each compound can best be caused to fluoresce at specific wavelengths, and these wavelengths are known as the apparent excitation spectrum of the compound. Of these wavelengths, a narrow band at which peak fluorescence is caused usually is chosen to be isolated by the primary monochromator and, from there, transmitted to the cuvette. When light strikes the solution in the cuvette, it produces fluorescence in the substance being measured. The detector of this fluorescent energy usually is placed at a 90° angle from the projected (i.e., the exciting) light beam. This placement means that light from the exciting light beam continues straight through the cuvette and does not need to be dealt with by the secondary monochromator or the detector. Because fluorescent energy is projected in all directions, this energy can be measured at 90° without measuring the energy from the exciting light beam. Some fluorometers incorporated into absorbance spectrophotometers measure fluorescence directly in the path of exciting light (i.e., an end-on design), because this is the typical light path for absorbance spectrophotometers. In such cases, mechanisms must be incorporated to exclude excitation light that has passed through the cuvette.

The secondary monochromator excludes light from sources other than the fluorescence itself and allows only a narrow band of wavelengths to pass to the photodetector. Just as each fluorescent compound has an apparent excitation spectrum of light in which optimum fluorescence occurs, each compound also has an emission spectrum, which is the spectrum of wavelengths in which most of the emitted fluorescent energy from that compound is found. To develop a fluorescent assay, the emission spectrum of the compound of interest must be determined. Then, the narrow band of wavelengths in which maximum emission occurs is isolated by the secondary monochromator. Light passing from the monochromator is collected by a photodetector, measured, and processed in a manner similar to that described for spectrophotometry. Various lenses, slits and in some cases, polarizing devices are included in fluorometers to help direct and/or polarize light as well as to reduce stray light in the system.

A wide variety of fluorometer designs are available. Strictly speaking, fluorometers are instruments that can produce light at only a few wavelengths, because their primary monochromator is a filter. Many instruments that use fluorometry have primary monochromators that are diffraction gratings or prisms. These instruments can produce a spectrum of excitation wavelengths and are known as spectrofluorometers. Some fluorometers are designed to compensate for variations in the intensity of the light source and, therefore, decrease the frequency with which calibration is required. Fluorometers also might use a pulsed light source and measure fluorescence only during those periods of time when the source is off. This technique, which is known as time-resolved fluorometry, eliminates the effects of light scatter.

Interference by other molecules is a potential problem when biologic fluids are being analyzed by fluorometry. Some

of these molecules fluoresce (e.g., bilirubin and some proteins), whereas others scatter light (e.g., proteins and lipids). When developing assays on biologic fluids, adjustments must be made to minimize the effects of these molecules.

Although the mechanism of measuring concentrations is different, the basic procedure for performing fluorometry is similar to that for absorbance spectrophotometry. Calibrators are used to establish a calibration curve, and blanks are used to negate any effects other than those attributable to the substance of interest. At low concentrations of fluorescing substances (e.g., resulting in an absorbance of <2% of the exciting light), a direct, linear relationship usually exists between fluorescence and concentration. If the concentration of the fluorescing substance is high (e.g., >2% of the exciting light is absorbed), the relationship between fluorescence and concentration might be nonlinear.

Light-scatter techniques

Light-scatter techniques can be used to measure the concentrations of larger molecules in fluids. When light is projected through solutions containing large molecules such as immunoglobulins and other large proteins, antigen–antibody complexes, and some drugs, these molecules cause light to scatter in all directions. These techniques, therefore, are potentially useful in measuring the concentrations of these substances. With light scattering, the wavelength of the light being scattered is the same as that of the light being projected into the solution. By assessing the degree of light scattering, the concentration of the substance of interest can be measured. Two techniques, turbidimetry and nephelometry, use the principles of light scattering to make such measurements.

In turbidimetry, the decreased intensity of a light beam passing through a turbid solution is measured. The intensity of light decreases, because a portion of it has been scattered by the large molecules of interest. A basic turbidimeter is diagrammed in Figure 1.41. In a turbidimeter, light rays are projected through a cuvette containing the analyte in solution, and the intensity of light leaving the solution (i.e., the transmitted light) is measured in a straight line from the transmitted light. The decrease in transmitted light intensity is proportional to the concentration of the analyte. A turbidimeter, therefore, is similar in principle to an absorbance spectrophotometer.

In nephelometry, a beam of light also is projected through a solution containing the analyte, but the photodetector is placed at a 90° angle to the cuvette (Fig. 1.42). In addition, scattered rather than transmitted light is measured. The intensity of the scattered light is proportional to the concentration of the analyte. Nephelometry, therefore, is analogous to fluorometry in terms of configuration of the light path. If a solution is not visibly turbid, nephelometry is a somewhat better technique than turbidimetry.

A direct relationship exists between the concentrations of light-scattering molecules and the degree of light scattering. A direct relationship also exists between the sizes of the light-scattering molecules and the degree of light scattering. When developing light-scatter techniques, the size of the particles being measured must be considered, because larger particles (e.g., immunoglobulin M, chylomicrons, and antigen–antibody complexes) cause an asymmetric distribution of scattered light. In some cases, the position of the photodetector must be altered to adjust for this. Large molecules or particles other than those of interest can interfere with light-scatter techniques as well.

With light-scatter techniques, the analytic procedures are similar to those of absorbance spectrophotometry. Calibrators are used to establish a calibration curve, and blanks are used to negate the effects of reagents and other light-scattering molecules.

Electrochemical techniques

A variety of electrochemical techniques are used in clinical chemistry and most often are applied in measurements of electrolytes and acid-base status. This includes electrolytes such as sodium (Na^+), potassium (K^+), chloride, (Cl^-), ionized

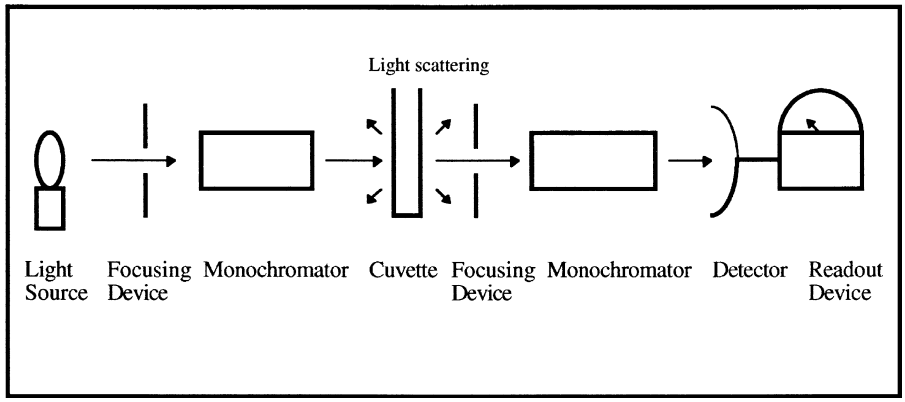


Figure 1.41 A basic turbidimeter. Arrows represent light.

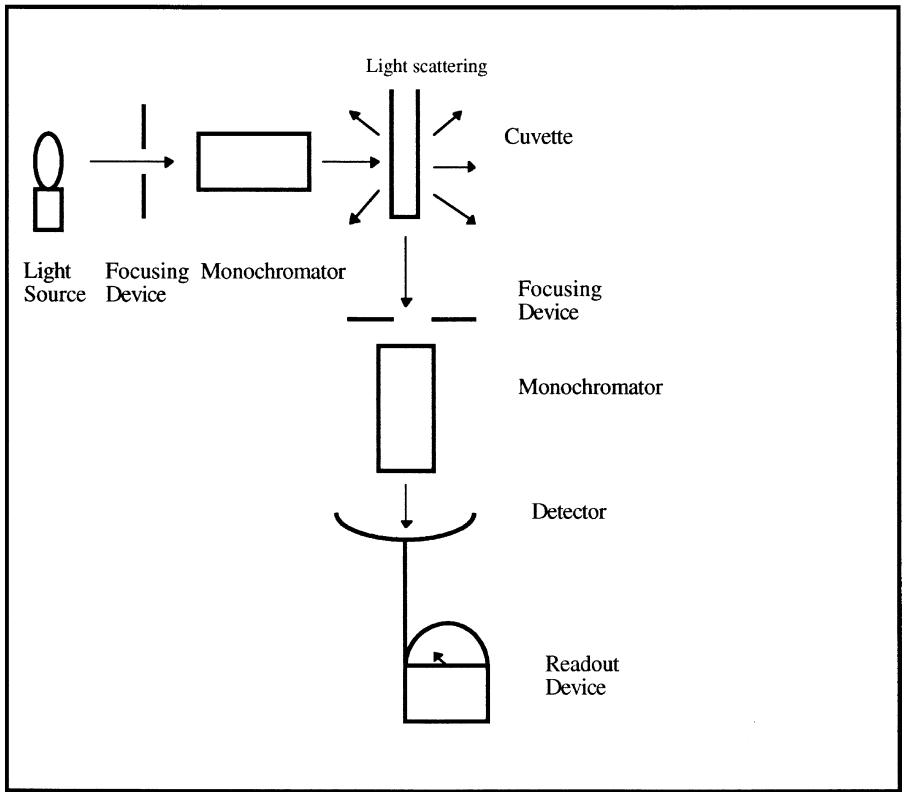


Figure 1.42 A basic nephalometer. Arrows represent light.

calcium (Ca^{+2}), pH (H^{+}), and partial pressures of oxygen (pO_2) and carbon dioxide (pCO_2) in whole blood. These techniques also can be used to measure other substances if the chemical reactions used in the assay system result in production or consumption of an ion. For example, such reactions exist for determination of glucose, urea, and creatinine concentrations. Basic electrochemical techniques and examples of some of their applications are described in this section. Electrochemical methods are applied through a wide variety of electrode and instrument configurations. In recent years several electrochemical systems have had complexity, cost, and applications reduced to practice in point-of-care formats. These systems have rendered blood gas, electrolyte, and selected chemistry capability both affordable and practical in the typical veterinary facility. Some of these devices utilize microfabricated disposable cartridges in which these measurements are made on whole blood. Other systems use small volumes of blood injected into a port leading to sample flow-through fluidics within the analyzer. Regardless of design, these instrument systems typically combine potentiometry, amperometry, and conductometry to provide acid-base and electrolyte panels, as described below.

Potentiometry

Potentiometry is commonly used for measurement of pH (i.e., hydrogen ion concentration), partial pressures of carbon dioxide and oxygen, and concentrations of electrolytes in whole blood or serum. In potentiometry, the electrical potential between two electrodes is measured thereby giving a value that can be used to calculate the concentrations of various electrolytes.

Potentiometry involves the development and measurement of the potential difference between two electrodes. This technique is used to measure electrolyte concentrations using ion-selective membrane electrodes, also known as ion-specific electrodes (ISE). The technique is used to measure ion concentrations in whole blood, plasma, serum, and occasionally other body fluids. The ISE is the variable electrode sensor immersed in the sample of measurement interest; see Figure 1.43. The ISE has a barrier or membrane that isolates the internal electrode from the body fluid. Only the specific ion being measured is allowed to cross or interact with the barrier, leading to accumulation of charge on the internal electrode. At equilibrium, the potential in the ISE will vary depending on the concentration of ionic interaction with the sample. The second electrode is a reference electrode that has constant, fixed potential. The basic principle is that contact of the ion-selective membrane with the body fluid results in ion selective passage or interaction with the ISE membrane leading to development of a potential difference from a reference electrode. A sensitive voltmeter is used to measure the potential difference when the ISE has

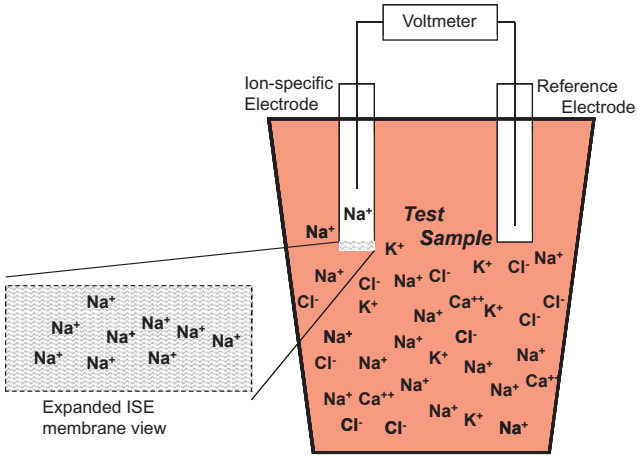


Figure 1.43 Schematic drawing of an ion-selective electrode (ISE) for potentiometric measurement; see text for further explanation. There is a reference electrode, chemically saturated to have fixed potential. The test sample contains differing concentrations of various ions. The ISE selectively allows movement of the ion of interest (e.g. Na^+) into or across the membrane resulting in a potential difference between the two electrodes (expanded view). The potential difference is proportional to the concentration of specific analyte in being measured.

come to equilibrium with the sample. The potential difference that develops is due to the activity of the ion being measured. The potential difference is used to calculate the concentration of ion in the sample. The ISE system is calibrated with solution containing known concentration of the ion of interest.

ISEs are the core technology in most or all modern blood gas and electrolyte analyzers, including those recommended for in-clinic applications. The design and materials used to manufacture these electrodes vary considerably. An important component of each electrode is a membrane that is selective for the ion that the electrode measures. The membrane may be composed of thin glass specially formulated to allow diffusion of a specific ion; glass is used in ISEs for pH and Na^+ measurement. A second type of membrane involves a water insoluble ion exchange chemistry coupled with a barrier membrane matrix. This type of electrode may be used to measure K^+, NH_4^+, and Ca^{2+}. There are also solid-state electrodes consisting of a single crystal of some ion-selective material or salt imbedded in an inert matrix membrane. This type of electrode is typically used to measure chloride (Cl^-).

The partial pressure of carbon dioxide (PCO_2) in the blood also is measured by potentiometry. This method is used in blood-gas analyzers. Whereas CO_2 is not an ion, the CO_2 electrode is designed to produce an ion in proportion to the PCO_2 in the blood. The design of such an electrode is shown in Figure 1.44 as a modified pH electrode. In this electrode, a chamber containing sodium bicarbonate solution is separated from the blood sample by a thin membrane. The CO_2 diffuses through the membrane into the sodium bicarbonate solution, and the following chemical reaction occurs:

$$CO_2 + H_2O \rightarrow H_2CO_3$$
$$H_2CO_3 \rightarrow H^+ + HCO_3^-$$

The amount of CO_2 that diffuses through the membrane affects the H^+ concentration in the sodium bicarbonate solution in direct proportion to the PCO_2. The remainder of this electrode is a pH electrode that senses the change in H^+ concentration of the sodium bicarbonate solution. These changes alter the electrical potential of this electrode, and the instrument then calculates the PCO_2 from these changes.

Amperometry

Amperometry is a technique that measures the electrical current passing between two electrodes in a chemical cell while a constant voltage is applied. This differentiates the technique from potentiometry, in which no electrical current flows and no voltage is applied. The most common application of amperometry in clinical chemistry is electrochemical measurement of the partial pressure of oxygen (PO_2) in blood.

The technique is most easily understood by considering how this electrochemical cell operates. A typical PO_2 electrode is diagrammed in Figure 1.45. An electrical potential of –0.65 V is applied to this electrode, and almost no current passes through this electrode if no oxygen is present. When this electrode is submersed in blood, O_2 from the blood diffuses through the O_2-permeable membrane and comes into contact with the tip of the platinum electrode. The O_2 then is reduced by the reaction:

$$O_2 + 2H_2O + 4 \text{ electrons} \rightarrow 4\ OH^-$$

This process consumes electrons and, therefore, produces an electrical current under these conditions. An ammeter is used to measure this current as amperage. The amount of current produced is proportional to the PO_2 of the blood. Calibration solutions are used to relate the amperage to the PO_2 of the unknown.

Coulometry and conductometry

Coulometry and conductometry are two other electrochemical methods that occasionally are used to measure the concentrations of substances. Coulometry involves measurement of the amount of electrical energy passing between two electrodes in an electrochemical cell. This electrical current is produced by chemical reactions occurring at the surfaces of each of two electrodes, resulting in the loss or gain of electrons by these electrodes. The amount of electrical current produced is directly proportional to the concentration of the substance being measured. This substance is

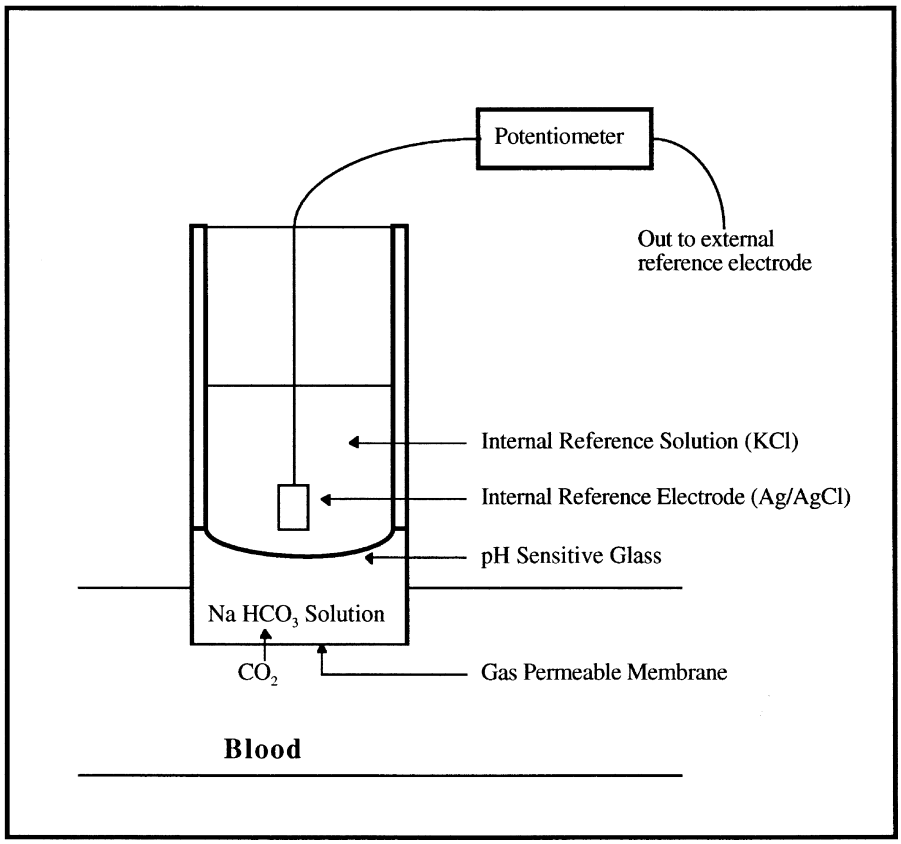


Figure 1.44 An electrode designed to measure the partial pressure of carbon dioxide in the blood.

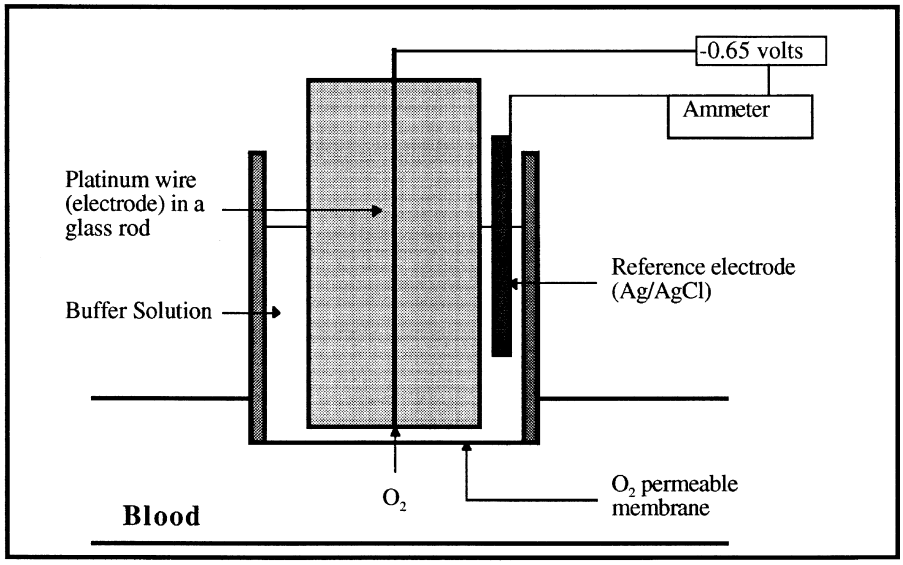


Figure 1.45 An electrochemical cell designed to measure the partial pressure of oxygen in the blood.

consumed in an electron-using or electron-producing process. Unlike potentiometry, the actual current rather than the potential between two electrodes is measured, and unlike amperometry, no outside voltage is applied to the system. This method has been applied to the measurement of serum chloride concentrations.

Conductometry involves measurement of a fluid's ability to conduct an electrical current between two electrodes when a voltage is applied to the sample in the system. This property, which is known as electrolytic conductance, occurs via movement of ions in the fluid. The conductivity of an aqueous fluid depends on the concentration and ionic strength of the electrolytes in that fluid: the higher the electrolyte concentration, the higher the conductivity. Conductometry can be used to measure the production of ions by chemical reactions. Therefore, it is possible to measure the concentration of a substance in a fluid if it is used in a chemical reaction producing ions in numbers proportional to the substance of interest. The increased conductivity resulting from the production of these ions would then be proportional to the original concentration of the substance being measured. It is also possible to measure hematocrit by conductometry on some clinical systems. The plasma fraction readily conducts current while cellular mass acts as an insulator, impeding current. As the hematocrit increases, the ability of the sample to conduct current decreases. This measurement can be calibrated. The calculation factors in electrolyte concentrations simultaneously measured in the same sample.

Osmometry

Osmometry involves measurement of the concentrations of particles in a fluid. The clinical significance of these concentrations, which are reported as osmolality (particles per kilogram of solvent (osmol/kg)) or osmolarity (particles per liter of solvent (osmol/L)), is discussed in Chapter 24. To understand osmometry, the changes that occur in a solution when concentrations of particles (i.e., solute) dissolved in a fluid (i.e., solvent) increase must be understood. These changes, which are known as colligative properties, are increased osmotic pressure, decreased vapor pressure, increased boiling point (because of decreased vapor pressure), and decreased freezing point. Any of these colligative properties could be used to measure osmolality or osmolarity. Among those properties that actually are used to make these measurements are freezing point depression and decreased vapor pressure.

The freezing-point depression technique is the most commonly used. As the name implies, this type of osmometer measures the freezing point of a solution through a number of steps involving freezing, thawing, and freezing again. This process is monitored by a thermistor, which measures temperature, and it determines the freezing point by determining the temperature at equilibrium between freezing and thawing. The osmolality or osmolarity of the fluid then is determined by comparing this temperature with those of various calibration fluids with known osmolality or osmolarity.

Vapor pressure osmometers are less commonly used. These instruments measure the osmolality or osmolarity of a fluid by determining the dew point (i.e., the temperature at the point of equilibrium between vaporization and condensation) of that fluid. The dew point is a gauge of vapor pressure: the higher the osmolality or osmolarity of a fluid, the lower its dew point. In general, vapor pressure osmometers are not considered to be as precise as freezing-point osmometers. In addition, volatile substances such as ethanol are not detected by vapor pressure osmometers, whereas they are detected by the freezing-point depression technique.

Protein electrophoresis

Electrophoresis is an analytic technique based on the movement of charged particles through a solution under the influence of an electrical field. In clinical chemistry, electrophoretic techniques most commonly are used to separate and analyze serum proteins. When serum is placed on or in a supporting substance that allows migration of these proteins and can carry an electrical charge, these proteins move through this material just as other charged particles do. The movement of proteins through such a substance depends on the net charge on the protein molecule, the size and shape of the protein molecule, the strength of the electrical field applied, the type of supporting medium, and the temperature. In a given electrophoresis application, the latter three items are held constant. Therefore, the migration of protein molecules depends on the net charge and on the size and shape of the molecules. As a result, different serum proteins migrate at different rates and, possibly, in different directions in the supporting substance.

A simple electrophoresis chamber is demonstrated in Figure 1.46. Small amounts of serum are placed in specific areas on the surface of the supporting substance or in small depressions cut at one end. Supporting substances commonly used include agarose gel and cellulose acetate. Starch gel is less commonly used in clinical applications. Polyacrylamide gel also can be used for protein electrophoresis and separates more serum protein fractions than the other supporting substances. Polyacrylamide electrophoresis does produce interesting information, but the clinical applications of this information in veterinary medicine are not understood. The common supporting substances usually are in the form of a sheet, and they either have buffer incorporated into them when they are produced or are soaked in buffer before use. The buffer determines the pH at which the process occurs, and the pH determines the type of charge as well as the net charge on each type of protein molecule. Both ends of the supporting substance are in contact with

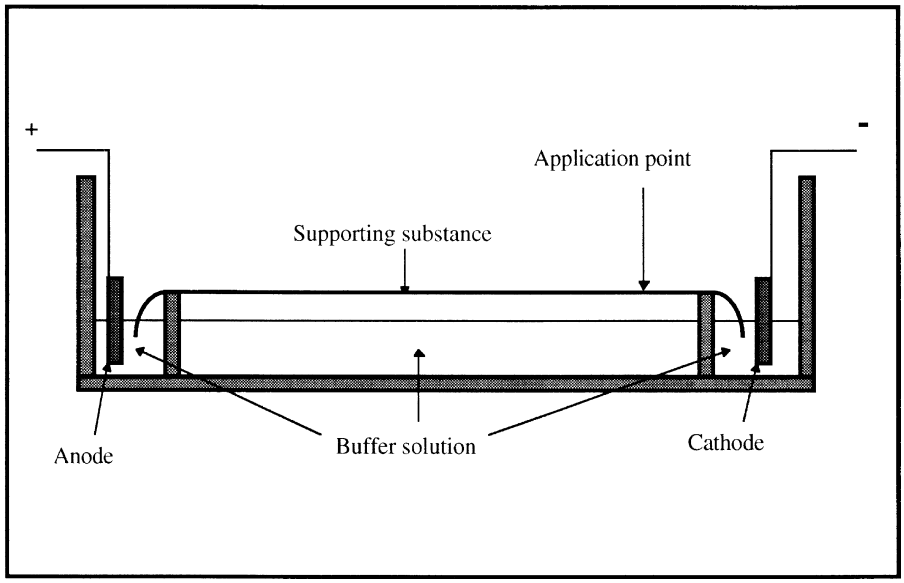


Figure 1.46 A simple electrophoresis chamber.

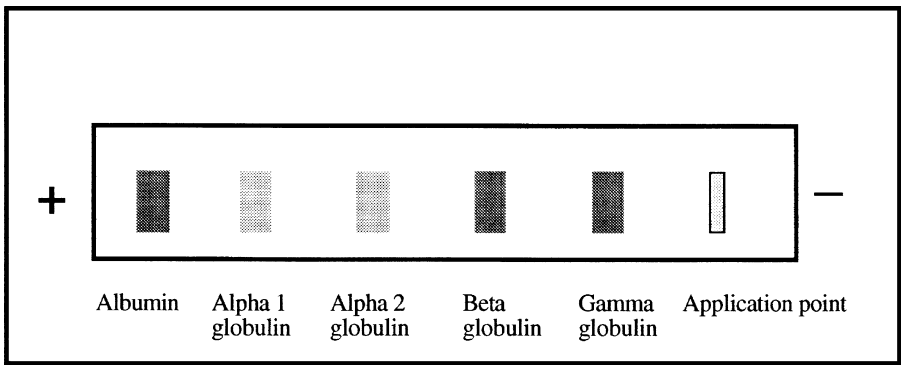


Figure 1.47 Typical electrophoretic separation of serum proteins in a sheet of supporting substance. The type and number of fractions actually separated depends on the type of electrophoresis application and on the species from which the serum was sampled.

buffer solution in an adjacent well. These buffer solutions are not in contact with each other, however, or with the buffer solution in the center well. The electrical current is applied to the system by electrodes placed into each of these wells. A negatively charged cathode is placed in the well at one end, and a positively charged anode is placed in the well at the other end. The serum sample typically is applied at the end near the cathode, because most proteins are negatively charged and migrate toward the anode. When an electrical current is applied to this system, proteins migrate toward either the anode or the cathode, depending on whether they are negatively charged (i.e., toward the anode) or positively charged (i.e., toward the cathode). As noted, the rate of this migration depends on both the net charge of the molecule and its size and shape, and because these vary with the different types of proteins, different proteins migrate at different rates. If this migration is allowed to occur for a fixed period of time, various protein fractions are isolated along a straight line in the supporting substance.

A typical distribution of serum protein fractions in a sheet of supporting substance after electrophoretic separation is shown in Figure 1.47. Albumin is the smallest of the serum proteins and has the highest net negative charge relative to its size. Albumin, therefore, migrates faster than the other proteins, and it advances further toward the anode during the time allowed for separation. The globulins are larger than albumin and therefore do not migrate as far toward the anode. The relative migration distances of the globulins depend on the relationship of their size to their net negative charge. The gamma globulins have the smallest net negative charge relative to their size and, therefore, migrate the shortest distance toward the anode. In some techniques, the application point actually might lie in the gamma-globulin region, with some gamma globulins migrating to the cathode side of this point. The number of fractions separated depends on the electrophoretic technique used and the species being analyzed. (These separations are discussed in more detail in Chapter 29.)

Once electrophoretic separation is completed, the protein fractions usually are identified and quantified. Staining these fractions aids in this process. Various types of dye that stain protein can be used, including amido black, bromphenol blue, Coomassie brilliant blue, nigrosin, and ponceau s. After staining, it is possible, with experience, to visually identify the various proteins fractions based on their order of migration. Visual examination also sometimes reveals apparently increased quantities of some protein fractions. This quantitation is more easily accomplished using a densitometer to scan the protein pattern and calculate the percentages and absolute quantities of protein in each fraction. A densitometer measures the amount of protein in each fraction by projecting light through these fractions as these are mechanically passed over the light source. A photodetector determines the width and density of each fraction. Results are reported as a densitometer scan, which more commonly is known as an electrophoretic pattern or electrophoretogram, as shown in Figure 1.48 and as both a percentage and an absolute value for each protein fraction. The absolute value for each fraction is calculated by the microprocessor in the instrument using the total protein concentration, which is entered by the operator, and the percentage of each fraction as determined by the densitometer:

$$\text{Absolute quantity of each fraction} = \frac{\text{Percentage of each fraction} \times \text{Total serum protein}}{100}$$

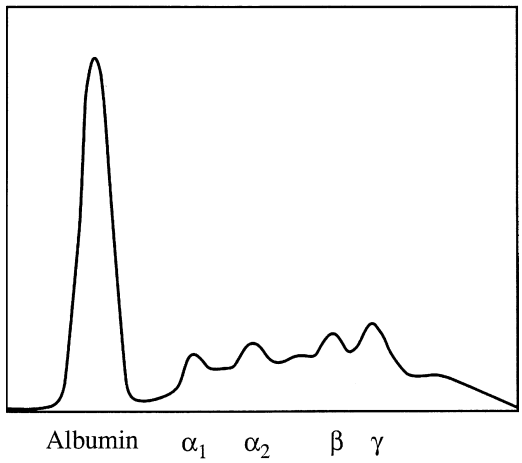


Figure 1.48 A densitometer scan (electrophoretic scan) of a serum protein electrophoresis separation.

Most densitometers automatically identify each fraction as well as the boundaries between these fractions. The operator can and should change these in some cases.

Once the absolute quantities in the various fractions are determined, they can be compared with known reference intervals for that species, and any abnormalities can be identified. Use of such data in clinical chemistry of proteins is discussed in Chapter 29.

Suggested Reading

Hematology

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Chemistry

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CHAPTER 2

2 Sample Collection, Processing, and Analysis of Laboratory Service Options

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In the previous chapter, laboratory technology was reviewed. To take advantage of this technology and its medical diagnostic capability, however, samples for the respective procedures must be properly collected and prepared. The veterinarian must make laboratory diagnostics choices from a vast array of in-clinic and centralized service options. While there is continual growth and improvement in in-clinic diagnostic instrumentation, this capability is not for all facilities. The choices may be influenced by several factors. The important factors include the type of practice (e.g., general, outpatient clinic, emergency facility, specialty referral center), geographic location, expertise of paraprofessional employees, and practice style of the individuals involved. This chapter presents rules for proper sample processing and guidelines for selecting laboratory diagnostics options.

Sample collection and processing

Regardless of the technique or laboratory used for any diagnostic test, obtaining reliable results starts with proper collection and handling of the sample. Sample collection, processing, testing, and interpretation all must be properly performed as a complete, sequential chain of events for a diagnostic result to have its intended value. For example, even the most reliable test, performed in the most reliable facility and interpreted by the most skilled diagnostician, cannot overcome the error introduced by an inappropriate technique used in sample collection or handling. This section provides guidelines for sample collection and handling that will ensure the initial sequence of events are properly performed.

Containers for sample collection

A variety of commercially available tubes are used for blood collection. These tubes contain the appropriate anticoagulant for the various diagnostic procedures and a vacuum for drawing in the appropriate volume of blood. These tubes are commonly known as vacutainer tubes (after the trademark of Becton-Dickinson). The following commonly used vacuum tubes are described in the approximate order of their frequency of use. Tubes are commonly referred to by their stopper color, which is used to identify the type of anticoagulation system the tube contains (Fig. 2.1).

Red-top or serum collection tube

The red-top or serum collection tube contains no anticoagulant. Blood that is placed in this tube is expected to clot so that serum may be harvested. This tube is used to collect serum for common biochemical determinations, such as those tests used in creating biochemical profiles.

Lavender-top tube

The lavender-top tube contains the anticoagulant ethylenediaminetetraacetic acid (EDTA) salt. This tube is used to collect blood for hematologic determinations. The EDTA anticoagulant results in the most consistent preservation of cell volume and morphologic features on stained films. The liquid tripotassium (K3) salt has the most commonly used form of EDTA. A newer formulation is dipotassium (K2) salt that is spray dried into plastic tubes. The tubes are larger and have a recommended visual fill line. Either of these formulations is preferred for use in preservation of cell volumes as measured on automated hematology analyzers. The plastic K2 tubes may be more forgiving of underfilling. It is anticipated that plastic K2 tubes may eventually make the K3 liquid in glass tubes obsolete. Powdered forms are not recommended because of slower, inconsistent mixing with blood that is added to the tube.

Green-top or heparin tube

The green-top tube contains lithium heparin. This anticoagulant is used for certain special biochemistry tests, particu-

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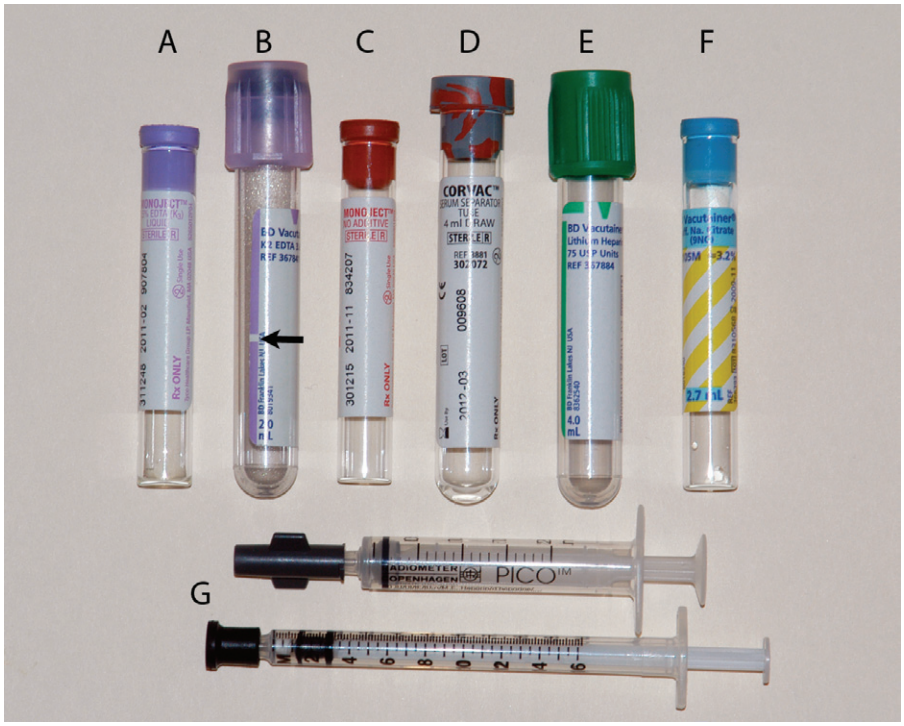

Figure 2.1 Representative collection devices for blood samples submitted for diagnostic tests. A: 3 mL glass lavender-top K3-EDTA tube, B: 2 mL plastic lavender-top K2-EDTA tube, note subtle white fill line indicated by arrow, C: a red-top tube without anticoagulant, D: a serum-separation tube, E: a lithium heparin green-top tube, F: a blue-top citrate tube, G: example balanced heparin syringes with caps for electrochemical diagnostic test collection.

larly those that require a whole-blood aliquot for determination and that might be influenced by the presence of other chemical anticoagulation systems.

Some in-house systems also recommend use of lithium heparin for all common clinical chemistry determinations. The advantage is that time is not required for clotting to completion to yield serum. The plasma may be separated immediately for testing and results for most analytes are equivalent for serum and plasma. There are two exceptions. Total protein will be slightly higher for plasma because it includes fibrinogen. Potassium averages about 0.5 mmol/L higher for serum because of platelet release during clotting.

Lithium heparin is also used for electrochemical determinations. A common sample handling error is overheparinization inherent in manual addition of heparin to collection syringes. The various heparin salts will cause errors to most electrochemical measurements including blood gases, electrolytes, and hematocrit by conductometry. Various in-house electrochemical acid-base and electrolyte analyzers are now available. It is highly recommended that special collection syringes containing "balanced" or "saturated" heparin be used. These are manufactured to contain the minimal amount heparin. Heparin has the ability to weakly bind calcium and cause false low ionized calcium measurement. Balanced heparin is a formulation that has the binding sites saturated with calcium so that binding in the patient sample does not occur. Use of these syringes will minimize sample handling errors for electrochemical measurements.

Blue-top or citrate tube

The blue-top tube contains sodium citrate. It is used for coagulation biochemistry determinations.

Sure-sep tube

The Sure-Sep tube is a variation of the red-top tube containing no anticoagulant. The stopper is red with black mottling. The tube contains a gel that separates packed cell fractions from serum when it undergoes centrifugation. It is convenient for use in situations when centrifugation at the site of collection and transport to the laboratory without the transfer of serum to a separate tube are desirable. The gel physically separates cells from the serum fluid, thus preventing analyte metabolism from occurring at the cell/fluid interface.

Gray-top or fluoride tube

The gray-top tube contains sodium fluoride. Fluoride is not an anticoagulant, however. Rather, it inhibits enzymes in the glycolytic pathway and prevents erythrocytes from metabolizing glucose while whole blood is transported to the laboratory. It is not commonly used.

Microtainers

Very small volume tubes are available for special applications such as very small laboratory animals. These may range from 0.25 to 1 mL. These should be avoided in general veterinary practice because of sample handling error potential. For example, it is very difficult to achieve proper mixing of blood in a 0.5 mL EDTA tube because of surface tension within a very small tube.

Tips for filling vacuum tubes

A few simple habits must be developed for appropriately filling tubes:

1. The ratio of blood to anticoagulant volume is important for hematology and blood coagulation biochemistry tests; therefore, a tube with anticoagulant should be filled to the volume specified for that tube. The amount of vacuum in the tube facilitates this, but the user should watch to ensure that this consistently occurs.

2. Recommendations for the tube filling order after venipuncture vary. Animal applications are different from the human setting because of differences in collection. When collecting blood for several diagnostic procedures, fill the tube(s) containing anticoagulant first and the tube containing no anticoagulant last. The most commonly used combination of tubes is an EDTA and clot/serum tube. The EDTA tube should be filled first so that platelet aggregation and clot formation is minimized. This is unimportant in the tube without anticoagulant because the blood is expected to clot in that tube. This deviates from the recommendation for humans. When filling the EDTA tube first, there is potential to contaminate the blood remaining in the syringe with EDTA. This can severely alter chemistry measurements such as calcium and potassium. Therefore, it is critical when filling an EDTA tube to avoid backflow of blood from the tube to the needle or connected syringe.

3. Vacuum tubes should be filled using minimal positive force, because forceful passage of blood through the needle may cause hemolysis, which in turn may cause an error in the biochemical measurements. Smaller-gauge needles are more likely to cause hemolysis. In particular, use of a 25-G needle, advocated by some, should be avoided because of inherent slow draw and hemolysis in tube transfer. An 18- to 20-G needle is best for most collection procedures.

4. Clean venipunctures with no tissue contamination are important. Tissue contamination may result in unwanted platelet aggregation and clotting in samples collected using anticoagulants. As a result, select venipuncture sites (e.g., the jugular vein) that likely will yield the appropriate volume of blood needed for the diagnostic tests being ordered for a given patient.

5. Select a venipuncture site that will yield the desired amount of blood easily. This means being able to draw the blood with little or no collapse of the vein so that blood may be transferred to the anticoagulant tubes as rapidly as possible. Recommended venipuncture sites for diagnostic screening procedures such as a hemogram and biochemical profile include: the jugular vein for small dogs, cats, horses, and cows; and the cephalic or jugular vein in medium to large dogs. These procedures generally require 4 to 12 mL of blood depending on the laboratory and the complexity of the screening procedures.

General sample handling procedures

Hematologic procedures

Blood collected for a complete blood count (CBC) should be analyzed within 1 hour or be prepared in the proper way for analysis at a later time. If the blood is not analyzed within 1 hour, a blood film should be prepared and the tube refrigerated. Morphologic features of cells may deteriorate rapidly on storage of blood in an EDTA tube; an air-dried blood film preserves the morphology of such cells for later examination. Refrigeration of the blood tube also helps to preserve the cell components that are measured by automated cell-counting systems. For example, cell swelling that could produce artifactual increases in mean cell volume (MCV) and hematocrit occur as blood is stored in a tube at room or higher temperature. For some analytical systems with differential capability, it is recommended by the laboratory that blood be held at room temperature. Blood should never be frozen, however, because this will result in lysis of the cells. In addition, blood films should not be refrigerated, because water condensation on the glass may damage the cellular morphology.

For hematologic measurements, the EDTA tube should be filled to the specified volume, and tissue contamination during venipuncture should be avoided. Underfilling the EDTA tube results in excess EDTA, which osmotically shrinks erythrocytes. In turn, this results in falsely decreased packed cell volume and calculated MCV when the microhematocrit procedure is used. Tissue contamination during venipuncture results in platelet aggregation (Fig. 2.2), and this artifactually decreases the platelet concentration as determined by cell-counting systems and may contribute to fluidic obstruction in hematology instruments.

Clinical biochemistry procedures

Blood collected in the red-top tube is allowed to clot for 15–30 minutes and then centrifuged to separate the cellular components from the resultant serum. The fluid phase of the blood should be separated from the cellular elements, because cells metabolize certain chemical components in the serum. The most notable example is glucose. If left in contact with cellular elements, glucose is metabolized at a rate of approximately 10% per hour. After centrifugation, serum is harvested by a transfer pipette to a second tube or is dispensed directly to devices for biochemical determinations (Fig. 2.3). Harvested serum should be analyzed quickly; otherwise, it can be refrigerated for as long as 24–48 hours. If

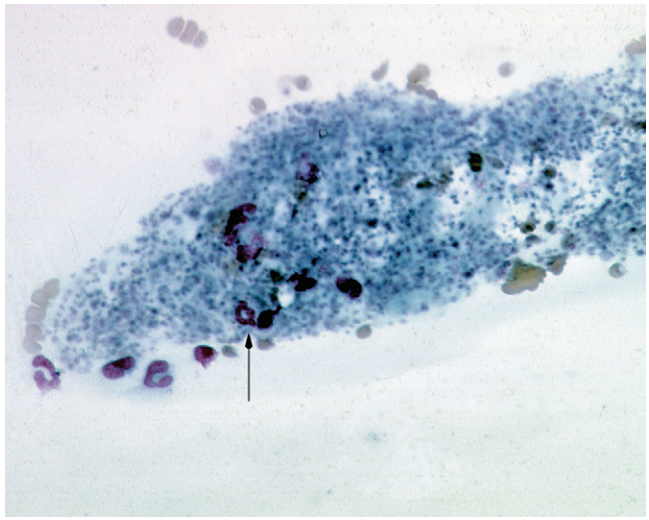

Figure 2.2 Platelet aggregation observed on a stained blood film. Tissue contamination may result in microclots that consist of hundreds of platelets, which falsely decrease the platelet concentration. Microclots also may trap leukocytes. Note the representative leukocyte (arrow); low magnification.

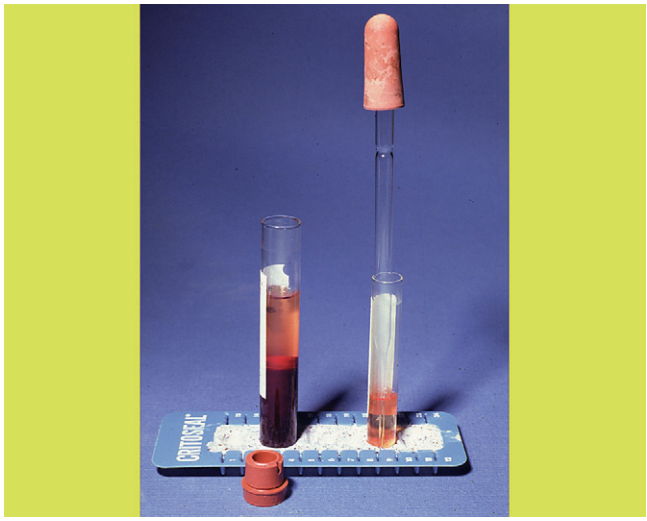

Figure 2.3 Serum preparation for biochemical tests. The tube on the left was allowed to clot and then centrifuged to pack the cells below the serum layer. A transfer pipette is used to transfer serum from the centrifuged sample to the tube on the right.

serum is to be held for longer than 24–48 hours, it should be frozen, and serum that is to be held frozen indefinitely (e.g., for archival purposes) should be stored at –70°C. Most chemical constituents are stable under these conditions. If serum is frozen and then thawed for analysis, the thawed aliquot should be thoroughly mixed before testing.

Serum enzymes require separate consideration regarding storage. A general rule is that for best reliability, serum enzyme activities should be determined within 24 hours of collection. Long-term archival storage of samples for determination of serum enzyme activity is not advised. Data on the exact stability of serum enzyme activity under various storage conditions is difficult to interpret. Knowledge regarding this subject has not been updated in any systematic way in recent years, and historical data were not collected in any consistent manner. Thus, our current understanding of enzyme stability during storage may be summarized as follows: Commonly measured enzymes, including alanine aminotransferase (ALT), aspartate aminotransferase, and alkaline phosphatase, and amylase activities are satisfactorily stable (>70% activity) when stored at 4°C. Freezing, however, may result in considerably accelerated loss of ALT activity. Creatine kinase activity should be measured as soon as possible, because considerable activity is lost after 24 hours regardless of the storage conditions.

Special procedures

Special laboratory diagnostic procedures are usually performed by centralized or commercial laboratories because of the complexity or specialized instrumentation involved. These procedures are performed less frequently, and they are more dependent on unique requirements of the technology employed by the laboratory undertaking the procedure. For example, endocrine assays may vary in measuring principle and reagents used resulting in considerable sample handling and results interpretation differences. As a result, the laboratory protocol for special procedures should be rigorously followed rather than committing these requirements to memory.

Analysis of diagnostic service implementation options

The veterinary facility has several options for obtaining laboratory diagnostic data. These may be generally considered as falling into three categories:

1. In-house (performed on the premises).
2. Commercial veterinary laboratory.
3. Human laboratory or community hospital.

Several factors should be considered when formulating a strategy for using one (or more) of these options. The veterinary facility should self-assess the following:

1. Type of practice (e.g., general practice, outpatient clinic, emergency facility, specialty referral center).
2. Geographic location (proximity to reliable service options).
3. Practice style of the individuals involved.
4. Willingness to implement and evaluate quality-assurance programs.
5. Willingness to invest the time to evaluate and troubleshoot diagnostics systems that have varying degrees of complexity.

6. Willingness to invest in a good microscope and training of personnel regarding basic clinical microscopy.
7. Desired turnaround times.
8. Ability to invest in instrumentation and training for the operators.

Advantages and disadvantages of in-house laboratory testing

It is known that approximately 85% of veterinary facilities utilize instrumentation for hematology and clinical chemistry to some degree. The available instrumentation has been rapidly evolving to increase sophistication and capability that approaches that of the central laboratory.[1] Modern information management allows integration of diagnostic system results into client reports as well as the electronic medical record.

Advantages of in-house laboratory testing include rapid turnaround time and control over when testing is performed relative to when samples are collected in a particular practice setting. In-house testing may also have economic advantages in certain situations.

Disadvantages of in-house laboratory testing include the issue of technical operator expertise for basic laboratory technology, which may not be available or affordable in many veterinary facilities. Attention to detail and quality assurance also must be managed by someone on site, and the investment in instrumentation is required. In addition, access to a clinical pathologist to help with the characterization of abnormal screening tests, particularly blood film analysis for hematology, must be cultivated, and arrangements for specialized testing to supplement in-clinic diagnostic tests must be procured.

Advantages and disadvantages of commercial veterinary laboratories

The major advantages of commercial veterinary laboratories are the cost leveraging of automated instrumentation and centralized testing volume, a complete menu of testing services, professional oversight of technical performance, and pathology support. Because the automated instrumentation is dedicated to animal-specific diagnostics, it is usually already adapted for the proper analysis of animal samples. Quality-control programs are usually implemented as well, but these may be variable.

The major disadvantages of commercial veterinary laboratories include relatively fixed turnaround times, which are dictated by local sample transportation logistics. In addition, sample transportation is a major part of the cost of the service.

Advantages and disadvantages of human laboratory facilities

The advantage of human laboratory facilities is that they may be the only available option in less populated areas. The disadvantages, however, are considerable. The instrumentation, particularly for hematology, is usually not modified for animal-specific diagnostics, and knowledge about the consequences is often lacking. Animal-specific pathology support is usually nonexistent or minimal. The technologists do not have training in veterinary hematology, and nobody on site can provide that training. In addition, turnaround times for animal testing may not receive the appropriate priority relative to the primary purpose of the laboratory.

Factors to consider when committing to in-house testing

Investment in instrumentation

Acquiring diagnostic capability in chemistry and hematology requires an investment of approximately $10,000–$25,000—or more. The cost of instrumentation has somewhat stabilized in this range, but the technical capability for this investment continues to improve. For example, a diagnostic capability in hematology that cost in excess of $80,000 during the 1980s may now be obtained for $10,000–$15,000. The useful technical life span of most instrumentation should be viewed as being from 5 to 7 years. Lease plans may facilitate the acquisition of instrumentation in ways that involve planned replacement at 3- to 7-year intervals. These plans generally pay for themselves during use by their flow of diagnostics revenue generation per month.

Commitment to personnel

Commitment to personnel requires hiring—and retaining—a technologist who is capable of reliable performance in diagnostics. Essential elements include an understanding of the basic laboratory technology, an ability to perform these procedures, a willingness to implement quality control, and a mindset that allows the technologist to seek consultation when he or she is confronted with uncertainty.

Commitment to quality assurance

A commitment to quality assurance involves a willingness to invest in periodic training regarding diagnostic technology for the personnel who perform these procedures as well as in the oversight of a regular quality assurance program.[2] The latter involves regular monitoring of instrumentation accuracy and precision using commercial control materials with known target values. This may cost from $100 to $300 per month for materials.

Establishing a pathology consultation relationship

A working relationship with a veterinary clinical pathologist to provide help with data interpretations and morphologic assessments in difficult cases, as well as microscopy support, is highly desirable. A relationship with an anatomic patholo-

gist is also required for interpretations of surgical biopsy specimens.

The business plan

Veterinarians who are considering in-house testing must have a mindset that allows them to use diagnostics liberally as part of their practice style. Instrument salespersons may make a compelling case for how one or two CBCs per day will pay for the cost of an instrument system. The same occurs for chemistry as well. First and foremost, these schemes are profitable for the seller, but this may or may not be true for the buyer. One should not make this investment without first analyzing the costs of various alternatives, such as the use of external laboratories. Veterinarians who perform only occasional diagnostic workups likely are better off using an external laboratory. Alternatively, diagnostics may be viewed as a source of revenue if the practice style calls for a combination of frequent diagnostic workups, pre-anesthetic testing, and wellness testing programs. Thus, a business plan should be created that projects the number of diagnostic tests to be performed across the practice caseload. Multiplying these numbers by the projected internal charge for laboratory tests will yield the gross revenue of the proposed in-house testing effort. Recommended target values are the charges for similar tests imposed by a veterinary commercial laboratory in the region. The projected gross revenue then should be compared with the projected costs, including instrumentation amortization, consumable supplies, personnel, training, quality assurance, and time for supervision.

For chemistry, one must recognize that most of the currently available systems are not economically favorable for performing complete biochemical profiles in-house. For example, the cost of consumables per test with an in-house system may easily exceed $1–$3 per test, whereas a complete biochemical profile may be obtained from a laboratory for approximately $16. With these circumstances, one is paying a premium for the convenience of in-house profile results, often while the client waits. In-house chemistry is more economically favorable for monitoring single tests or mini-panels after a diagnosis and treatment plan have been implemented.

Factors to consider when selecting external laboratory services

Instrument adaptation

Instrumentation must be suitably adjusted for animal blood testing. This is particularly important regarding hematologic analyses. Such adaptation is most likely to occur in veterinary commercial laboratories, and it is much less likely to be found in human hospital laboratories that analyze animal samples as a secondary priority.

Sample pickup service

Many veterinary laboratories offer once or twice daily sample pickup service to facilitate the shortest possible time from sample collection to the return of results. The tradeoff is that courier services represent a considerable fraction of the cost of the laboratory service. Human laboratory facilities usually rely on users to transport samples to the facility.

Appropriate turnaround time

In general, the rate-limiting step is transporting the sample to the laboratory. The trend toward consolidation of laboratory services, however, often results in very large transportation distances, thus extending the turnaround time. Once a sample arrives at the laboratory, most facilities perform the analyses as rapidly as possible and then electronically report the results. Laboratories that prioritize animal samples behind a busy human diagnostics schedule may not provide convenient timing for the delivery of results.

Species-specific ability

The laboratory should have the ability to recognize and interpret species-specific morphologic and pathologic abnormalities. In addition, the laboratory should be able to provide knowledgeable evaluation of abnormalities in data and morphology on blood films and cytology.

Telephone consultation

The veterinary user must be able to consult with laboratory staff and pathologists regarding abnormal or unusual data generated by the laboratory.

Decision process

The analysis of one's diagnostic options may be summarized as follows: The decision process for implementing diagnostic support is complex, and this complexity is enhanced by rapidly changing technologies and services. It is advisable to run some experiments to facilitate this analysis. To maintain flexibility when uncertainty exists, it is advisable to avoid entering long-term purchase or service agreements.

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CHAPTER 3

3 Perspectives in Laboratory Data Interpretation and Disease Diagnosis

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The ability to interpret laboratory data is based on knowledge regarding the normal physiologic mechanisms underlying each laboratory test and recognition of the effects of diseases on these normal physiologic mechanisms and, therefore, on the test results themselves. With these perspectives, one can assess possible explanations for an alteration in a laboratory test result, and one can sort through these possibilities to identify the most likely explanations. If performed properly, laboratory testing and interpretation of laboratory data can provide significant insights regarding diseases and respective therapeutic options. Most chapters in this book discuss normal physiologic mechanisms and the effects of disease processes on these mechanisms as well as on laboratory test results; this chapter provides basic information that applies to the interpretation of all types of laboratory data.

Introduction

The typical laboratory diagnostic work-up may consist of 30–50 different parameters or pieces of information. Laboratory reports could be more simplified. For example, about half of the values in a routine hematology report are either redundant calculations or are used solely for calculation of more important parameters. These unimportant values are not diagnostically useful and cause time consuming clutter. However, both instrument manufacturers and laboratory service providers are reluctant to remove those parameters for fear of appearing to offer less information than competitors.

The busy clinician is faced with distilling this complex body of information into a summary that, when combined with other historical and physical findings, may diagnose health or potential disease. The veterinary clinician in training often learns this process by trial and error. The purpose here is to provide some basic background and perspective to facilitate that process. This includes an understanding of reference intervals, sensitivity/specificity of laboratory tests, knowledge of factors that may introduce errors in laboratory results, the role of laboratory quality control, and a discussion of how to develop a skilled approach to interpreting laboratory data.

Reference interval background

To recognize laboratory results as being abnormal, the values expected to be obtained from healthy animals must be known. These normal values are correctly termed reference intervals; although they may also be referred to as reference intervals, this is technically incorrect since the term 'range' refers to a single number describing the difference between two values. A reference interval is typically defined as values encompassing the median 95% of a tested population of apparently healthy animals. Inherent in this definition is that 2.5% of the healthy population will have values outside either side of the median 95%, suggesting they are abnormal.

When interpreting patient data, the first interpretive step is to sort data into normal and abnormal values. Flagging each abnormal value on the laboratory report form often starts this process. Information systems can do this by comparing the value against the defined reference interval. However, determination of abnormal is not as simple as it may seem for a couple of reasons. First, reference intervals are usually based on limited population testing and do not account for variation within subpopulations defined by age, sex, breed, or other factors. Second, one must think probabilistically about values that are near the reference limit. An abnormal flag does not necessarily mean the value is abnormal for that animal.

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Different statistical methods can be used to establish reference intervals, but all of them begin with the sampling of animals from an apparently healthy population. In most cases, healthy animals are those that have no apparent illness and have no detectable abnormality in cursory examination. Reference intervals must be established for each species being tested, but such intervals would ideally also be established for subdivisions within that species when some characteristic of a subgroup results in significantly different reference intervals compared with those for the species as a whole. These subdivisions might occur on the basis of age, breed, gender, pregnancy status, or type of husbandry. Because establishing reference intervals is an expensive, time-consuming task, intervals for such subdivisions are usually not established, and veterinarians generally use a single reference interval for all animals of a given species. When this is the case, it is important to consider variations in those test results that could relate to the previously mentioned characteristics (e.g., age, breed, gender) and to consider these characteristics when evaluating the possible causes of values falling outside the reference interval (especially mildly abnormal values). For example, the hematocrit (HCT) reference interval for dogs is usually regarded to be approximately 36–55%. However, it is known that some small breeds, notably the poodle, typically have HCT values in the 50s. A poodle dog with an HCT of 42 may be anemic. Another example is the serum enzyme alkaline phosphatase. Because bone remodeling is a potential source for this enzyme, serum alkaline phosphatase activity is considerably greater in young growing animals than in adults of the same species. Many such interpretive nuances are developed from experience. Refinement of population subset reference intervals may someday be performed in veterinary medicine, but this will require compilation of a huge database.

Adequate numbers of normal animals must be sampled to develop intervals that are valid for healthy animals from the defined population. In general, the more animals that are sampled, the more likely the reference intervals will truly reflect the range of values to be expected from healthy animals. Sampling large numbers of animals to make the results most reflective of the healthy population is desirable, but practical constraints (e.g., availability of apparently healthy animals, costs of obtaining samples and of performing large numbers of tests) dictate limits on the number of animals that can actually be tested. For best reliability, at least 120 samples should be analyzed when establishing reference intervals. The minimum number of samples to establish a crude reference interval is generally considered to be 40.

Several statistical methods exist for establishing reference intervals. The method of using mean ± 2SD was historically used to define the median 95% of the tested population, but this is only valid if the test results have a normal or Gaussian distribution (Fig. 3.1A). This approach is flawed if the test

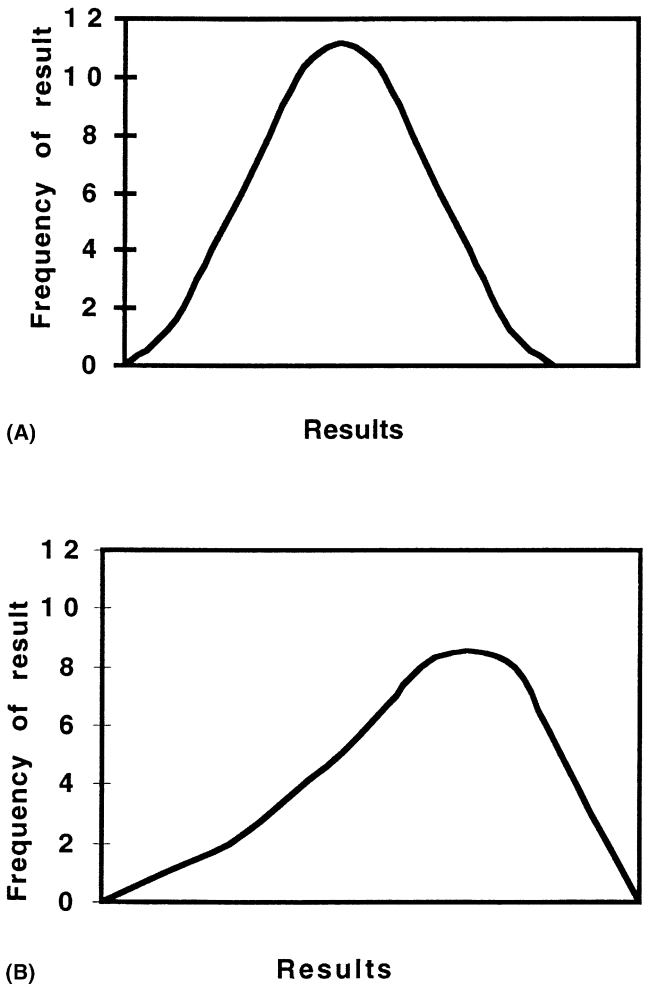

Figure 3.1 Two distributions of values resulting from sampling a large number of apparently healthy animals. A. Plotted by their frequency of occurrence, these values form a symmetric, bell-shaped curve. This is known as a normal or Gaussian distribution. B. Plotted by their frequency of occurrence, these values form an asymmetric distribution that is skewed toward the higher values. This is not a normal distribution (a non-Gaussian distribution).

results are not normally distributed (Fig. 3.1B). It is now thought that most laboratory test data are not normally distributed. A simple solution is to derive reference intervals using a nonparametric technique. With nonparametric methods, all the test values are rank ordered, any outliers are removed, and then the middle 95% of test results define the reference interval. As an example, for a population of 120 rank ordered results, the lowest 3 and highest 3 ($2.5\% \times 120 = 3$) are removed and the remaining results define the median 95% of the population. A few values from the apparently healthy sample population might be markedly higher or lower than most of the other values. These extreme values are known as outliers and are likely indicative of occult disease. If outliers are included in the sampled

values when the intervals are calculated, they will widen the reference intervals, thus making the test less sensitive for the detection of unhealthy animals. One relatively simple rule-of-thumb for defining an outlier is to calculate the difference between the highest (or lowest) value and the second highest (or lowest) value. If this difference exceeds one-third of the range of all values, then consider the highest (or lowest) value to be an outlier, and eliminate it when calculating the reference intervals. Once this value has been eliminated, the same test can be applied to the next highest (or lowest) value. For example, Figure 3.2 presents the blood glucose values obtained from a population of 120 apparently healthy animals plotted in a frequency distribution histogram. One value (30 mg/dL) is obviously much lower than the others. The difference between this value and the next lowest value is 25 mg/dL, and the range of all values is 70 mg/dL (100–30 mg/dL). Because 25 mg/dL is greater than one-third of the range of all values (70 ÷ 3 = 23.3), the lowest value (30 mg/dL) is eliminated as an outlier. If this value is eliminated, the difference between the remaining lowest value (55 mg/dL) and the next lowest value is then 10 mg/dL. This is less than one-third of the range of all remaining values (45 ÷ 3 = 15) and, therefore, should not be eliminated as an outlier.

An example of establishing a reference interval by the rank order nonparametric method is presented in Table 3.1, which uses the data as presented in Figure 3.2. As noted earlier, one value (30 mg/dL) has been eliminated as an outlier; therefore, the range of the remaining 119 values is 55 to 100 mg/dL. Identifying and eliminating those values in the lowest 2.5% and in the highest 2.5% then determines the central 95% of these ranked values.

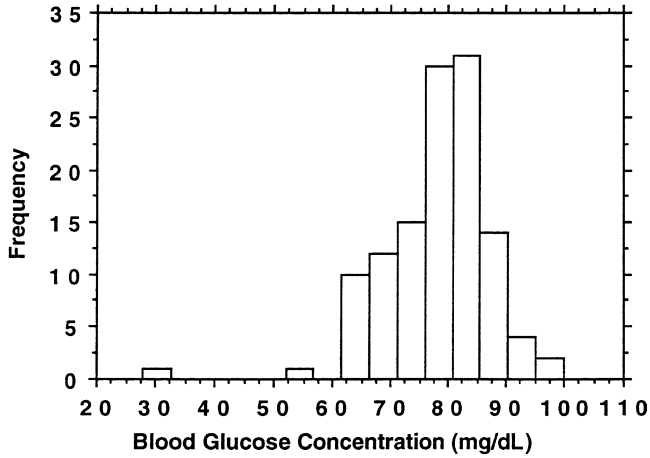

Figure 3.2 Blood glucose values obtained from a population of 120 apparently healthy animals and plotted in a frequency distribution histogram. Frequency represents the total number of samples with that blood glucose concentration.

Table 3.1 An example of nonparametric determination of a reference interval.[a]

Lowest 10 Values and Their Ranks										
Value	30	55	65	65	65	65	65	65	65	65
Rank	1	2	3	4	5	6	7	8	9	10
Highest 10 Values and Their Ranks										
Value	90	90	90	90	95	95	95	95	100	100
Rank	110	111	112	113	114	115	116	117	118	119

Highest value of the lower 2.5% = 0.025 × (number of values + 1)
Highest value of the lower 2.5% = 0.025 × (119 + 1) = **3**

Lowest value of the upper 2.5% = 0.975 × (number of values + 1)
Lowest value of the upper 2.5% = 0.975 × (119 + 1) = **117**

Lower Values Eliminated from Reference Interval			
Value	30	55	65
Rank	1	2	3
Upper Values Eliminated from Reference Interval			
Value	95	100	100
Rank	117	118	119

Resulting reference interval = 65–95

[a] Blood glucose concentrations were obtained from 120 apparently healthy animals, and one of these values was eliminated as an outlier (see Fig. 3.2). The method involves ranking values from lowest to highest, calculation of ranks representing the highest rank of the lower 2.5% of values and the lowest rank of the upper 2.5% of values, and eliminating values corresponding to these ranks as well as values corresponding to lower and higher ranks, respectively. The remaining values are the central 95% and are used as the reference interval.

The statistical method just described is applicable when the sampled population includes 40 or more animals. If fewer than 40 animals are sampled, the lower and upper 2.5% of values cannot be reliably determined. In such a case, the reference interval is considered to be the observed range of values that remains after the outliers have been eliminated. Such a reference interval is less reliable than those determined from a larger population.

Limiting reference intervals to 95% rather than 100% of values obtained from healthy animals is an attempt to maximize detection of diseased animals. As defined by reference intervals, approximately 5% of healthy animals will have values considered to be abnormal for any given test. By extension, if many tests are performed on an individual animal (as is common in biochemical profiles), the likelihood of that individual having at least one abnormal test result increases dramatically. For example, in a 20-test biochemical profile, approximately 64% of healthy animals will have at least one abnormal value. It is also possible that animals with disease may have respective laboratory values just within the reference interval. One must recognize the reality that healthy and unhealthy animals overlap at each end of the reference interval (Fig. 3.3). Thus, the concept of a black and white delineation between normal and abnormal does not exist. The clinician must learn to think probabilistically about laboratory data, particularly for values close to reference limits. Therefore, laboratory values that are close to the reference limits need to be more closely correlated with the patient history, clinical signs, or other laboratory data to assess the likelihood that they represent disease. Test results that are markedly above or below the reference limits, however, are more easily recognized as representing disease.

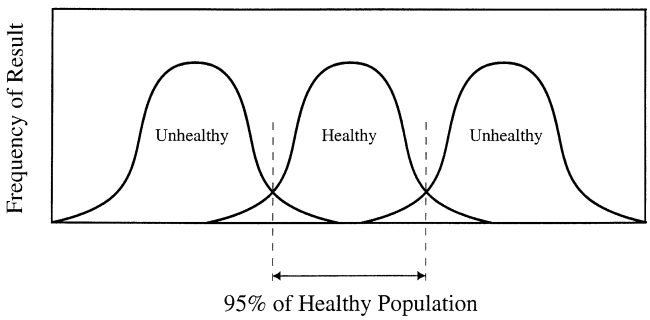

Figure 3.3 The overlap of laboratory values that can be expected from healthy and unhealthy populations (populations with diseases that cause either decreases or increases in the values for a given test). Note that defining the reference interval at 95% of the healthy population excludes the values from some healthy animals, but it also excludes the values from most unhealthy ones (i.e., it allows one to recognize these animals as being potentially unhealthy). If the reference interval were broadened to include more of the potential values from healthy animals, it would also recognize more values from unhealthy animals as being normal (i.e., the unhealthy animal might not be detected). Using a reference interval based on 95% of the healthy population is a compromise that increases the sensitivity of the test for recognizing unhealthy animals while causing only a few healthy animals to be recognized as being potentially unhealthy.

An adjunct to population reference intervals is the individual health database. Ideally, a laboratory value database is established for young adult companion animals or other animals of value. This data may serve to identify more precisely where that animal's values reside relative to the more broad range of the general species population. For example, a dog has an HCT value of 52% defined in its health database. If sometime later the HCT is measured at 39%, there is a high probability of an underlying disease resulting in anemia, even though the laboratory report may not flag the value as abnormal.

Sensitivity, specificity, and predictive values

When interpreting laboratory abnormalities, the concepts of sensitivity, specificity, and predictive values must be considered. Sensitivity is a measure of the frequency with which the test result will be positive or abnormal in animals with the respective disease process. The following formula is used to determine sensitivity:

$$\text{Sensitivity}\,(\%) = \frac{\text{TP}}{\text{TP} + \text{FN}} \times 100$$

where TP (true positive) is the total number of animals that tested positive and actually have the disease process, and FN (false negative) is the total number of animals that tested negative but actually have the disease. For instance, if the sensitivity of a test for a disease is 99%, then 99 of 100 animals with that disease will have a positive (i.e., abnormal) result. One percent of the animals with the disease will have a negative (i.e., normal) result; that is, 1% of the tests would have false-negative results. Specificity is a measure of the frequency with which the test result will be negative or normal in animals without the disease one wishes to detect. The following formula is used to determine specificity:

$$\text{Specificity}\,(\%) = \frac{\text{TN}}{\text{TN} + \text{FP}} \times 100$$

where TN (true negative) is the total number of animals that tested negative and actually do not have the disease, and FP (false positive) is the total number of animals that tested positive but actually do not have the disease. For instance, if the specificity of a test for a disease is 99%, then 99 of 100 nonaffected animals will have negative (i.e., normal) results.

One percent of nonaffected animals will have a positive (i.e., abnormal) result; that is, 1% of the tests would have false-positives results.

Sensitivity and specificity are established by applying the test in question to animals with known disease status (i.e., animals known to have or not have the disease in question). Another diagnostic procedure, often termed the "gold standard," is used to establish which animals do or do not have the disease. This gold standard is often another laboratory test known to be reliable for detecting the disease. Sensitivity and specificity, therefore, do not apply directly to animals of unknown disease status, but they do provide information regarding the reliability of the test in question for detecting that disease.

In practice, one needs to know the reliability of a test for detecting a certain disease in animals with unknown disease status. In other words, how reliable is an abnormal or a normal test result for predicting whether the animal does or does not have the disease in question? In this situation, predictive values define the chances that abnormal or normal test results are reliable indicators of disease status. Predictive values depend on the sensitivity and specificity of a test, but the prevalence or likelihood of the disease in the population being tested affects predictive values as well. Such prevalence or likelihood of disease is established before performing the test, based on the judgment of the veterinarian of the chance (expressed as a percentage) that the animal has the disease in question. This judgment can be based on several other observations, including patient history, clinical signs, other test results, and epidemiologic data. Both positive (i.e., abnormal) and negative (i.e., normal) test results have predictive values. The predictive value of a positive test (positive predictive value) is the probability that a positive (abnormal) test result truly indicates the animal has the disease:

$$\text{Positive Predictive Value} = \frac{TP}{TP + FP} \times 100$$

where TP is the total number of animals that tested positive and actually have the disease, and FP is the total number of animals that tested positive but actually do not have the disease. The higher the predictive value of a positive test, the more likely it is that an animal with a positive (i.e., abnormal) test result actually has the disease in question. Tests with high positive predictive values will produce few false positive results; thus confidence is high in a positive test result.

The predictive value of a negative test (negative predictive value) is the probability that a negative (normal) test result truly indicates the animal does not have the disease:

$$\text{Negative Predictive Value} = \frac{TN}{TN + FN} \times 100$$

where TN is the total number of animals that tested negative and actually do not have the disease, and FN is the total number of animals that tested negative but actually do have the disease. The higher the predictive value of a negative test, the more likely it is that an animal with a negative test result does not have the disease in question. Tests with high negative predictive values will produce few false negative results; thus confidence is high in a negative test result. As stated previously, predictive values are determined from a combination of the sensitivity and specificity of the test and the veterinarian's pretest judgment regarding the likelihood of the disease in that animal. A rather complex formula to estimate predictive values based on these factors does exist, but the roles of sensitivity, specificity, and disease prevalence or likelihood in the interpretation of diagnostic test results can be understood without it. The roles of these three factors are best understood by considering a hypothetic situation in which an excellent diagnostic test is used to detect a specific disease. The heartworm antigen test is a good example of such a diagnostic for which there are abundant data. This test has a sensitivity of 99% (i.e., it will be positive or abnormal in 99 of 100 animals with the disease) and specificity of 99% (i.e., it will be negative or normal in 99 of 100 animals without the disease). This test has excellent performance when applied in areas with reasonable prevalence of heartworm infection. However, if this test is used for screening a population of animals in which you, as the veterinarian, judge there is a 1% chance of the disease being present, the following predictive values result:

Predictive value of a positive test = 50%

Predictive value of a negative test = 100%

In other words, a positive or abnormal test is correct 50% of the time and incorrect 50% of the time. This is equivalent in reliability to flipping a coin, and it might lead one to question the wisdom of performing such a test in a population with a low likelihood of disease. In this situation, however, a negative or normal test result is almost 100% reliable in ruling out the possibility that an animal has the disease (i.e., the predictive value of a negative test is approximately 100%). This combination of excellent test sensitivity and specificity with low prevalence or likelihood of disease is quite common when using serologic tests to screen for various infectious diseases.

Because most diagnostic tests have an inherent sensitivity and specificity, the most easily altered factor that affects the predictive value is the pretest likelihood of the disease. Veterinarians can use this to enhance the predictive values. For instance, in the previous example, a test with excellent sensitivity and specificity was used to screen for a disease in a population with a low prevalence of that disease. This resulted in a low positive predictive value. If, however, a

veterinarian were presented with an animal that had a history, clinical signs, and other features suggesting that disease, such an animal would represent a different population, and the veterinarian would establish a different, higher pretest likelihood for that disease. In such a case, the veterinarian would, perhaps, be 75% certain that the animal had the disease in question. Therefore, the predictive value of a positive test result would be nearly 100%, and the predictive value of a negative test result would be approximately 97%. The test result in this scenario would, in fact, be very reliable for predicting the presence or absence of the disease in question.

In summary, the more likely that an animal has a certain disease before the test is performed, the more reliable a positive or abnormal test result suggesting the presence of that disease will be. The effects of the pretest likelihood of disease on the positive and negative predictive values of a test are demonstrated in Figures 3.4 and 3.5. In practice, most veterinarians incorporate this approach to diagnostic testing instinctively. If the test result is compatible with the disease the veterinarian suspected before conducting the test, this result is considered to be supportive evidence that the animal has the disease; if the result is not compatible with the suspected disease, the veterinarian does not completely rule out that disease but does begin to consider other options more seriously. Biochemical abnormalities that suggest a disease that was not strongly suspected before the profile was completed will occasionally be detected, and in this situation, these abnormalities are not as reliable in predicting that disease as they would be had the disease been previously suspected.

Most routine clinical pathology tests (i.e., hematology, biochemistry, and urinalysis) have sensitivities and specificities for detecting any given disease that are considerably less than the 99% in the previous example. This makes the pretest likelihood of disease an even more important factor in this type of testing. For instance, both the sensitivity and specificity of the pancreatic enzyme amylase for detecting pancreatitis are quite low. Serum amylase activity is routinely measured on some biochemical profiles. Thus, an increased serum amylase activity on a biochemical screen from a dog in which pancreatitis was not previously suspected would have a very low positive predictive value, because the sensitivity, specificity, and pretest likelihood of pancreatitis are all low. On the other hand, an increased serum amylase activity on a biochemical profile from a dog with clinical signs that suggest pancreatitis would have a much higher positive predictive value. This concept is important to remember whenever unexpected abnormalities are detected on any routine clinical pathology test.

Quality control

To obtain reliable laboratory test results, the quality of the results being produced must be monitored so that they are both accurate and precise. Accuracy is a gauge of how close the result is to the true value for that test, and precision is

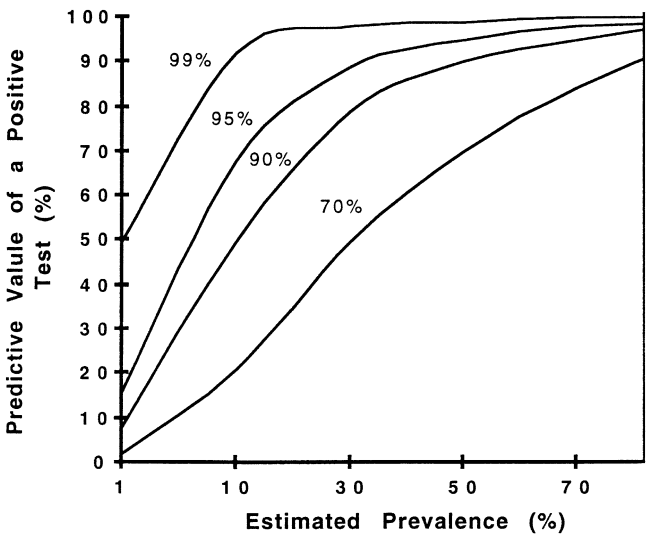

Figure 3.4 The effect of various pretest estimates of disease likelihood on the predictive value of a positive test. Each line represents a different level of sensitivity and specificity (99% = 99% sensitivity and specificity, 95% = 95% sensitivity and specificity, and so on). The predictive value of a positive test decreases as the pretest estimate of disease likelihood decreases.

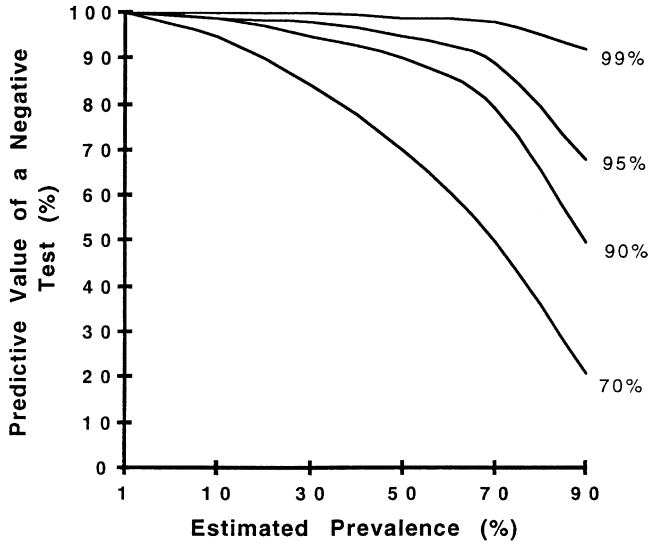

Figure 3.5 The effect of various pretest estimates of disease likelihood on the predictive value of a negative test. Each line represents a different level of sensitivity and specificity (99% = 99% sensitivity and specificity, 95% = 95% sensitivity and specificity, and so on). The predictive value of a negative test increases as the pretest estimate of disease likelihood decreases.

a gauge of how repeatable the result is when assaying the same sample. A single result might be accurate, for instance, but if a similar result cannot be obtained repeatedly using the same sample (i.e., if the test is not precise), then the results for that assay are not reliable. Conversely, one may obtain the same result repeatedly using the same sample, but if that result does not reflect the true value for the substance being measured (i.e., if the test is not accurate), then the results again are not reliable.

Reputable laboratories maintain quality-control programs to ensure the accuracy and precision of their results. This is accomplished by assaying control samples at previously determined intervals along with the samples from patients. These intervals might be daily or several times per day, depending on the workload of the laboratory. The control samples are similar to those from patients (e.g., blood or serum) and are usually obtained from a commercial source. Control samples can be categorized as either assayed (i.e., the probable accurate value for the test in that control sample has been previously determined) or unassayed (i.e., the probable accurate value for the test in that control sample has not been previously determined). If unassayed control samples are obtained, the laboratory then establishes the probable accurate value for that sample using methods similar to those summarized earlier for determining reference intervals. Because establishing such probable accurate values is both time-consuming and expensive, most laboratories today use assayed control samples. Only assayed controls are suitable for in-clinic quality control.

During routine laboratory operation, the result from each control sample is compared with what is documented to be the accurate result for that sample. This tests the accuracy of the assay. In addition, results obtained from the control sample over time are analyzed to determine if the value obtained changes over time, thus establishing the precision of the test. Both accuracy and precision usually are assessed by graphing the values obtained from the control sample on a quality-control chart (Fig. 3.6). Some instrumentation will have on-board software for automated analysis and management of quality control data. If the results obtained from the control sample are outside the previously established acceptable range, which is also known as the control limit (usually ± 2–3 SD from the mean), or if the results drift either up or down over time, then a problem with the analytic instrument, reagents, or operator may exist. Results obtained from patient samples during these "out-of-control" periods are rejected, and the analytic methods used are carefully reviewed to correct the problem.

Quality-control programs are common in large reference laboratories, but they are also important for in-clinic laboratories. Manufacturers may supply quality-control materials with laboratory instruments. These programs should be followed in detail to have some assurance that the results produced by the in-clinic laboratory are reliable.

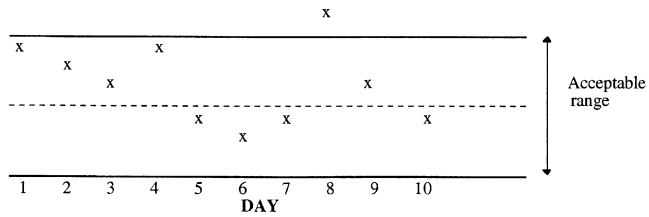

Figure 3.6 An example of a quality control chart used to monitor the accuracy and precision of a laboratory test. To produce this chart, a control sample was analyzed each day along with the patient samples. Daily results from the control sample are plotted (X). The dashed line (- - -) represents the expected mean value for this sample. Solid lines (___) represent acceptable positive and negative variation from the mean value. Note that the result on Day 8 was outside the acceptable range of variation. This would trigger rejection of the results for the test on that day until completion of an assessment of the instrument, reagents, and operator to identify and correct the problem. The chart indicates problem resolution on Day 9 with acceptable control performance.

Common factors that introduce error in laboratory values

There are a number of factors that can cause laboratory test result errors that may affect interpretation of the patient status. These should be considered whenever a laboratory result(s) is either nonsensical or does not match the patient's condition. These factors can be classified as pre-analytical, analytical, and postanalytical errors. Pre-analytical errors are the most common, and may be introduced by a number of problems related to sample collection and handling. Analytical errors occur at the level of the test methodology, and may be due to either an interfering substance or phenomenon within the sample or a problem with the test method performance. The latter is now relatively rare, and is typically recognized and prevented with a quality control program. Postanalytical errors may be due to transcription or other errors related to report generation and distribution. Postanalytical errors are also relatively rare with the current use of automated laboratory information systems and report generation.

Sample handling errors

A number of pre-analytical factors may result in laboratory test errors. Improper handling of samples is the most common cause of gross errors in laboratory test values. These are procedural errors that violate handling rules related to sample stability or other processing variables. Some common sample handling errors in veterinary facilities include:

- Sample labeling and transcription errors, leading to data assigned to the wrong patient
- Use of wrong anticoagulant
- Inappropriate anticoagulant contamination of the sample

- Improper ratio of anticoagulant to sample
- Traumatic transfer of blood to tubes causing hemolysis (see below)
- Improper storage conditions during transportation to a laboratory
- Improper sample storage conditions before analysis
- Lack of or insufficient mixing of blood for hematology measurements.

There are specific sample handling procedures that must be followed to ensure sample quality, and these may vary depending upon the laboratory test being requested. Central laboratories provide these procedures for proper sample submission. Suppliers of in-clinic diagnostic instrumentation also provide these procedures. Failures related to these procedures occur because the person(s) involved are either not aware of them or are not paying attention to detail. It is the responsibility of the veterinary facility to ensure that the respective procedures be followed exactly in order to minimize associated errors in laboratory results. The various personnel in the veterinary facility often have limited training in laboratory technology. For this reason, it is recommended that facilities designate a lead person or key laboratory operator to educate others about and monitor laboratory related procedures.

Interfering substances: Lipemia, hemolysis, and hyperbilirubinemia

An interfering substance is a common source of analytical error that is present in the sample. Hemolysis, lipemia, and increased serum bilirubin (Fig. 3.7) can potentially affect the results of biochemical assays. Hemolysis refers to the lysis of erythrocytes and liberation of hemoglobin, and may occur either in the circulating blood (in vivo) or during or after blood collection (in vitro). Hemolysis in the sample is usually due to improper sample collection or handling. Hemolysis may interfere with assay results by color interference with spectrophotometric assays. Less commonly, hemolysis may cause a false increase in analyte being measured as a result of marked differential concentrations or enzyme activities between serum and erythrocytes. For instance, horses and cattle have high concentrations of potassium within erythrocytes, whereas dogs (with some exceptions) and cats do not. Therefore, marked hemolysis may result in a falsely increased serum potassium concentration in horses and cattle, but not in most dogs or in cats.

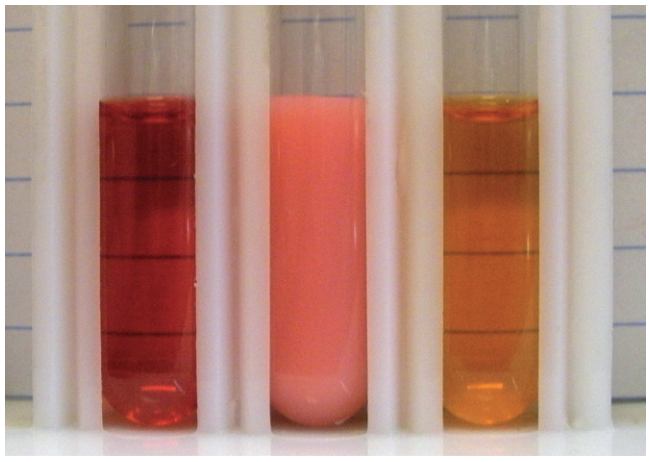

Figure 3.7 Hemolysis, lipemia, and hyperbilirubinemia (left to right) in serum samples. Lipemic serum here is tinged pink as a result of concurrent hemolysis; it may appear white to red-tinged.

Lipemia causes visible turbidity of the serum, often making it opaque to transmitted light. It is expected to occur in small animals when they have not been fasted before blood collection. It may also occur in hyperlipidemic syndromes. This interference with light transmission can interfere with spectrophotometric assays, particularly in liquid or cuvette chemistry systems. It can also result in apparent dilution of normal substances (e.g., electrolytes) in the aqueous component of serum, resulting in falsely decreased concentrations (ion exclusion effect).

Increased serum bilirubin concentrations result in a serum with a darker-yellow color than normal for that species. This increased color can interfere with the results of spectrophotometric assays.

The potential alterations in biochemistry results caused by hemolysis, lipemia, and hyperbilirubinemia vary with the substance being assayed and with the method being used for the assay itself. Reference laboratories usually can provide specific information regarding the effects of hemolysis, lipemia, or hyperbilirubinemia on test results. Likewise, manufacturers may provide this information for in-house diagnostic laboratory instrumentation.

It is also conceivable that drugs and other chemicals may alter laboratory test reactions. Known interferents are typically outlined in reagent application sheets. This information is usually available from the laboratory or in the form of technical briefs from diagnostics suppliers.

Approach to interpreting laboratory data

Comments on general approach

It is important to appreciate that individual diagnostic test results are rarely interpretable into a clinical diagnosis. Abnormal test results typically indicate a relatively nonspecific pathologic process. Grouping of several abnormal results may improve the specificity of the process or processes. It is usually only after integration of history, physical findings, and other diagnostic procedures with laboratory data abnormalities that a more defined clinical diagnosis is achieved. Most laboratory abnormalities have multiple potential causes, and the history and physical examination results should be used to determine which of these potential causes is most likely. Using a combination of history, physical examination results,

and the pattern of laboratory abnormalities, the veterinarian should attempt to summarize the likely operative pathologic processes present. This summary can often be translated into a working clinical diagnosis or diagnoses. Patterns of abnormal test results often suggest which tissue or organ systems are affected, which pathologic processes are occurring, or both. For example, a combination of an increased concentration of blood urea nitrogen (BUN; a test of kidney function) with a urine specific gravity indicative of inadequate urine concentration is very suggestive of renal failure, whereas an increased BUN with concentrated urine (high specific gravity) is more suggestive of conditions such as dehydration or shock.

Of course, not every abnormality will fit neatly into one disease process, nor will every laboratory profile result in a specific diagnosis. In some cases, more than one disease process may be occurring, thereby producing a confusing combination of abnormalities. These are considered difficult cases that may require analysis over time to unravel, and may benefit from consultation or second opinion interaction with associates.

Analysis of sequential changes in laboratory values over time is sometimes helpful in establishing a diagnosis and is important for monitoring progress of the disease or case management. For instance, periodic determinations of BUN in an animal with renal failure may indicate whether treatment to reestablish renal function is succeeding (i.e., BUN should be decreasing) or not. Negative findings in the form of normal test results also have value. These can rule out tentative differential diagnoses that were considered on the basis of history or physical examination findings.

Expectations and skilled diagnostics interpretation

Behind the scenes of the general approach described above, there are a number of nuances that may be described as expectations related to diagnostics. Sometimes clinicians are handicapped by unrealistic expectations for laboratory data. The discussion here is aimed at clarifying some of the more common expectations to aid the clinician-in-training to be more adept at data interpretation. Important considerations include the following:

- How measurement reproducibility affects data interpretation
- Magnitude of change associated with disease(s)
- Relationships or interdependency between diagnostic tests
- Reference intervals and the elusive determination of what is normal vs. abnormal
- Laboratory test results that are inconsistent with preconceived notions

Measurement reproducibility

This is discussed first because it influences other expectations and is important for interpretation of sequential laboratory data. A common misconception is that the numbers on a laboratory report are definitive numbers. The reality is that if an individual test is repeated multiple times on the same sample by the same method in the same laboratory, a range of results will be obtained. If results are produced by two different laboratory methods, even more variation may be encountered. Actual reproducibility will vary, but some guidelines for satisfactory analytical performance for a single method are:

- Most hematology results—±10% of value
- Platelets—±20% of value
- Most clinical chemistry results—±10% of value
- Enzyme activities in clinical chemistry—±15% of value

When comparing results between laboratories or methods, even greater variation should be expected.

A practical understanding of the expected reproducibility results in the following interpretive guidelines:

- Data must be interpreted with some latitude, especially when test results are near reference limits. This is discussed further under defining what is normal.
- When two different laboratories or methods generate results for the same sample, relatively large differences in "numbers" may occur, but usually the interpretation of those numbers is the same. This often occurs when comparing in-house results with commercial laboratory results.
- When a new sample is analyzed to evaluate patient change, only relatively large change should be interpreted as conclusive change in the patient.

Magnitude of change associated with disease

Considerable experience is required to understand the relationship between the *magnitude* of a given laboratory test abnormality and the *severity* of the associated disease condition. It is not practical to communicate detailed guidelines for all laboratory tests in this chapter. Small numerical changes or abnormalities indicate important or severe disease for some laboratory tests. Examples might include pH, potassium (K^+), creatinine, calcium, phosphorus, albumin, and endocrine assays. For most other laboratory tests, it takes a considerably larger numerical change or abnormality to indicate important or severe disease. Examples include enzyme activities, BUN, glucose, and most hematologic measurements.

The desired interpretive experience comes from repeated analysis of clinical case material. A starting point for veterinarians-in-training includes case discussions in various classes. This is narrated to some degree in various chapters in this textbook, and case presentations at the end of this textbook provide some representative examples. This knowledge is then expanded upon with the clinical cases encountered during the first several years of practice.

Relationships between diagnostic tests

Laboratory tests are more meaningful when interpreted in groupings that are interrelated with respect to pathophysiol-

ogy. For example, a moderately abnormal increased BUN interpreted in isolation may define the relatively nonspecific process of decreased glomerular filtration rate. However, when grouped with hematocrit, total protein, creatinine, phosphorus, and urinalysis findings, the integrated interpretation is likely to be much more specific as to the probable cause of decreased glomerular filtration rate. In addition, the other values may corroborate each other when there is a question about the validity of a given value. Ideally, laboratory reports are organized in a way to provide some initial grouping that facilitates this relationship in interpretation. This is often organized by organ system, realizing that some analytes may have secondary relationships with more than one organ system. However, chemistry, hematology, and urinalysis reports are almost always segregated. The user must learn how to cross interpret sections of the report to achieve all the useful groupings. Table 3.2 shows one method of grouping laboratory tests that achieves most of the primary relationships for integrated interpretation. This is a place to start, realizing that secondary relationships will become more apparent with experience.

Interpreting normal versus abnormal

As discussed in the above section "Reference Interval Background," laboratory data is often not conclusively interpretable as normal or abnormal, particularly when values are near the limits of the reference interval. Laboratory reports may contain flags, usually H for high or L for low. This flagging conditions the user to think too strictly about normal versus abnormal. Clinicians should be encouraged to interpret borderline and mildly abnormal values more loosely, in a probabilistic manner. When a laboratory test result is suspiciously abnormal, look for corroboration in other findings. Also factor in possible age and known breed considerations for suspect values.

Table 3.2 Grouping of laboratory tests for interpretation. Hematology is interpreted separately, but abnormalities may be referable to chemical abnormalities in the groups below.

Kidney	Liver	Metabolic	Specialty
BUN	Bilirubin	Glucose	CK
Creatinine	ALT	Calcium	Amylase
Phosphorus	AST	Total Protein	Lipase
Urinalysis:	ALP	Albumin	Endocrine tests
Specific gravity	GGT	Cholesterol	Immunoassays
Chemistry	Bile acids	Sodium	Other special tests
Microscopic		Chloride	
		Potassium	
		pH	
		HCO_3	
		pO_2 (arterial)	

BUN, blood urea nitrogen; ALT, alanine aminotransferase; AST, aspartate aminotransferase; ALP, alkaline phosphatase; GGT, gamma glutamyltransferase; CK, creatine kinase.

Laboratory test results that are inconsistent with preconceived notions

Occasionally the clinician is surprised by an unexpected lab test value that is moderately or markedly abnormal. A common first reaction is to not believe the result is possible. A more appropriate reaction is to reanalyze the clinical situation. One should look for other laboratory values or undetected clinical abnormalities that may corroborate the value(s) in question. The history and physical should be reevaluated for findings that may corroborate the abnormal value; additional questions may need to be asked of the owner. Next, the possibility of a sample or sample handling error should be considered. Lastly, if no corroboration or errors can be found, it may be appropriate to repeat the test in question.

Summary of interpretive considerations

When interpreting laboratory data in conjunction with all other clinical and physical findings, remain aware of the following interpretive factors. With practice, these become habit of the astute clinician.

- Interpret laboratory values in related groups, organized by organ system.
- Interpret laboratory values probabilistically for abnormality, particularly when values are borderline with respect to the reference interval limits.
- Develop a sense of the expected magnitude of change in a value that is associated with important disease.
- Consider that analytical reproducibility is such that only relatively large changes in sequential values are indicative of true change.
- When laboratory values initially do not seemingly fit the clinical condition(s), evaluate for corroborating laboratory and clinical data.
- Train staff to prevent improper sample handling and interfering substances that may lead to erroneous laboratory data. Consider these possibilities when laboratory data is seemingly nonsensical.

Suggested Reading

Reference intervals

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Solberg HE (2001) Establishment and use of reference values. In: Burtis CA, Ashwood ER (eds.), *Tietz Fundamentals of Clinical Chemistry*, 5th ed. Philadelphia: WB Saunders, pp. 251–61.

Solberg HE (1981) Statistical treatment of collected reference values and determination of reference limits. In: Gräsbeck R, Alström T (eds.), *Reference Values in Laboratory Medicine*. London: John Wiley & Sons, Ltd.

Quality control in veterinary laboratories

Weiser MG, Thrall, MA (2007) Quality control recommendations and procedures for in-clinic laboratories. In *Veterinary Clinics of North America: Small Animal Practice*. Issue title: Clinical Pathology and Diagnostic Techniques. Vol. 37 (March), pp. 237–44.

Westgard Rules for Quality Assurance. Available at: http://www.westgard.com.

Reproducibility and data interpretation

Weiser MG, Vap LM, Thrall, MA (2007) Perspectives and advances in in-clinic laboratory diagnostic capabilities: hematology & clinical chemistry. In *Veterinary Clinics of North America: Small Animal Practice*. Issue title: Clinical Pathology and Diagnostic Techniques. Vol. 37 (March), pp. 221–36.

4

Immunodiagnostics: Current Use and Future Trends in Veterinary Medicine

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Introduction

Immunodiagnostics are tests that use antibody-antigen binding to generate a measurable result that assists in the diagnosis of disease. As such, immunodiagnostics are also "immunoassays" (although the reverse is not always true). Antibodies are plasma glycoproteins, called gamma globulins or immunoglobulins (Ig) generated in response to exposure of the immune system to an antigen. Simply defined, an antigen is any substance that stimulates the immune system to produce antibodies. Antigens are usually proteins or polysaccharides. Immunodiagnostics were initially used in the diagnosis of infectious diseases, either indirectly by detection of antibody or directly by detection of antigen. Detection of antibody indicates previous exposure and not necessarily the active presence of the antigen.

Immunodiagnostics take advantage of the specific binding of an antibody to its antigen. An epitope is the portion of an antigen bound by an antibody. Binding between an antibody and its epitope is dependent on noncovalent interactions including ionic interactions, hydrogen bonds, and hydrophobic interactions. The strength of the interaction between a single antigen-binding site on the antibody and its epitope is called its affinity. Most antigens (e.g., viral capsid proteins) have multiple epitopes. Epitopes to which the greatest amount of antibody is produced are called immunodominant epitopes.

Immunodiagnostics are capable of detecting the presence (qualitative tests) or amount (quantitative tests) of an analyte (either antibody or antigen) present in the sample at concentrations below what can be accurately determined by other routine testing methodologies. Detection is usually accomplished by "labeling" either antigen or antibody and then using the labeled reagent to probe samples for the presence of antibody or antigen, respectively. Common labels used in immunoassays include enzymes (e.g., horseradish peroxidase, alkaline phosphatase, glucose oxidase, luciferase), fluorochromes (e.g., fluorescein, phycoerythrin), radioisotopes (e.g., I-125), or microparticles (e.g., colloidal gold, latex beads). For quantitative results, the signal measured from the sample is compared to the signal obtained from standards containing know concentrations of the analyte.

In addition to detection of antibodies or antigens associated with infectious disease, immunodiagnostic tests are also used for measurement of many other analytes including drugs, hormones, tumor markers, and markers of cardiac injury. In the case of drugs and hormones, the analytes measured are frequently haptens. A hapten can only elicit production of antibodies when combined with an antigenic carrier molecule. However, once formed haptens can react with antibodies in the absence of association with the carrier.

Antibody structure

Antibodies or immunoglobulins are proteins produced by differentiated B-lymphocytes. There are five classes of antibodies, namely, immunoglobulin A (IgA), immunoglobulin D (IgD), immunoglobulin E (IgE), immunoglobulin G (IgG), and immunoglobulin M (IgM). Each antibody consists of four polypeptides—two heavy chains and two light chains held together by interchain disulfide bonds to form a "Y" shaped molecule (Fig. 4.1). The IgD, IgE and IgG antibody classes are found as a single structural unit, whereas IgA antibodies may contain either one or two units and IgM antibodies consist of five disulfide-linked units (Fig. 4.2).

Intrachain disulfide bonds support structural "domains" of approximately 110 amino acid residues in length each. Heavy chains are composed of either 3 (IgA, IgD, IgE) or 4 (IgE, IgM) constant domains and a single amino-terminal

Veterinary Hematology and Clinical Chemistry, Second Edition. Edited by Mary Anna Thrall, Glade Weiser, Robin W. Allison, and Terry W. Campbell.

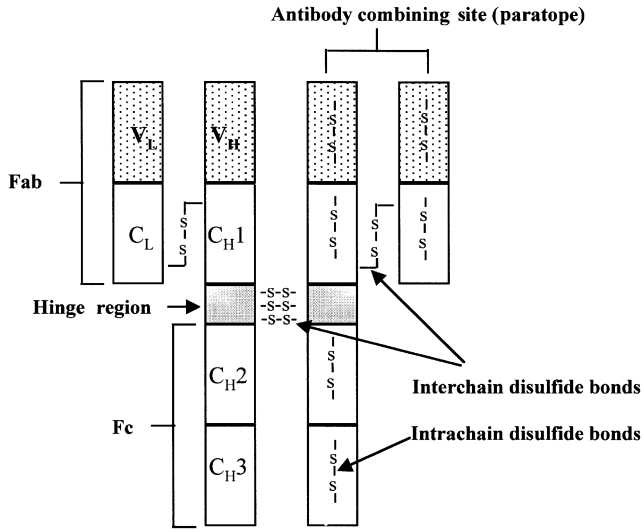

Figure 4.1 Schematic of an antibody molecule illustrating the heavy (H) and light (L) chains held together by interchain disulfide bonds. Intrachain disulfide bonds create structural "domains," each approximately 110 amino acid residues in length. The variable (V) domains of the heavy and light chains form the antigen binding site. The constant (C) domains define the class of the heavy (A, D, E, G, and M) and light (kappa or lambda) chains. Fab fragments contain the entire light chain and the variable and amino-terminal most constant domain of the heavy chain. Fc fragments contain the remaining constant domains of the heavy chain.

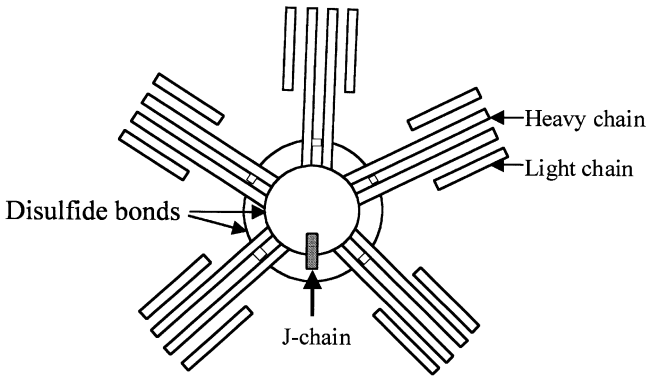

Figure 4.2 Schematic of IgM illustrating its pentameric structure, interchain disulfide bonds, and the J-chain.

variable domain. The constant domains of the heavy chain define the class of each antibody and is responsible for the biological activity of the antibody. Light chains are composed of a single constant domain and a single amino-terminal variable domain. The constant domain of the light chain defines the type of light chain as either kappa or lambda. The combined variable regions of the heavy and light chains form the antigen binding site and are responsible for the specificity of antibody-antigen interaction. The presence of two heavy and two light chains results in two antigen binding sites for each antibody molecule (Fig. 4.1).

Historically, proteolytic enzymes (proteases) have been used to cleave antibody molecules into functional fragments. Antibody molecules are cleaved into three fragments by limited digestion with the protease papain. Two of the fragments are identical and represent the two "arms" of the "Y." These fragments contain the antigen-binding activity and are termed Fab fragments (Fragment antigen binding). Fab fragments contain a complete light chain and the variable and amino-terminal most constant domain of the heavy chain (Fig. 4.1).

The third fragment obtained from digestion with papain contains the remaining constant domains of the heavy chains and does not bind antigen. This fragment is referred to as the Fc fragment (Fig. 4.1) because it was found to be readily "crystallizable." The Fc fragment is the part of the antibody molecule that interacts with effector molecules and cells. An example of this is the interaction of IgE with the FcERI receptor on mast cells. Relative to immunodiagnostics, Fc fragments are used to raise species and class specific antibodies for the detection of antibody responses to many infectious diseases. For example, anti-cat IgM and anti-cat IgG are used to differentially detect an IgM versus IgG immune response, respectively, which has been reported to be useful in the diagnosis of acute *T. gondii* infection in clinically ill cats.[1]

Another protease, pepsin, cleaves on the carboxy-terminal side of the disulfide bonds generating a fragment, referred to as the $F(ab')_2$ fragment, that contains both "arms" of the "Y" (Fig. 4.1). Pepsin cleaves the remaining portion of the heavy chain into several smaller fragments. Since the $F(ab')_2$ fragment contains both antigen-binding sites, it has the same antigen-crosslinking capabilities as the original antibody molecule. $F(ab')_2$ fragments are occasionally used in diagnostic assays since they maintain the specificity of the original antibody but lack the Fc fragment which is sometimes associated with nonspecific binding.

Generation of antibodies used in immunoassays

Immunodiagnostics use antibodies to detect both antigens (e.g., proteins from infectious agents) and antibodies generated in response to foreign proteins. Antibodies used as reagents in immunodiagnostic tests can be either polyclonal or monoclonal. Antibodies are named by the species from which they were obtained and the antigen to which they were produced. For example, rabbit anticanine γ chain is rabbit antibody specific for γ chain of dog IgG. Unless otherwise specified, antibodies are assumed to be polyclonal.

Polyclonal antibodies

Polyclonal antibodies are generated via hyperimmunization of an animal (e.g., rabbit, sheep, goat) with the antigen of

interest. The animal's immune response to the antigen produces antiserum, a heterogeneous mixture of antibodies. Polyclonal antibodies therefore represent a mixture of antibodies derived from many different B lymphocyte clones, each with a unique B-cell receptor and each capable of binding the antigen. As a result, within each pool of polyclonal antibodies will be antibodies that bind to the numerous epitopes present on the antigen. Some of these antibodies will bind to their respective epitope with high affinity and some will have lower affinities.

Monoclonal antibodies

In 1975, Georges Köhler and César Milstein[2] demonstrated that fusion of antibody-producing B-cells with myeloma cells that had lost their ability to secrete antibodies resulted in an immortal cell line or "hybridoma" that secreted a single monospecific antibody. In the fused hybridoma cell, the B-cell supplies the ability to secrete a specific antibody and the myeloma cell gives it immortality. The generation and use of monoclonal antibodies has been an important tool in research and medicine (in 1984, Köhler and Milstein received the Nobel Prize in Physiology or Medicine for their discovery of monoclonal antibodies).

Monoclonal antibodies are typically made using polyethylene glycol to fuse myeloma cells with spleen cells from a mouse that has been immunized with the antigen of interest. The success rate of fusion is low so a selective medium is used that only allows the growth of hybridoma cells. After fusion, the mixture of cells is diluted and aliquoted into 96-well microtiter plates so that only approximately one-third of the wells will contain cells. This increases the chance that each resultant "clone" of cells was generated from a single parent cell.

Cell culture supernatants are then tested for the presence of antibody with the ability to bind to the antigen of interest (usually the same antigen that was used for immunization of the B-cell donor mouse). Immunodiagnostic assays used for the screening process are typically high-throughput assays (e.g., ELISA or immunodot blot) to allow for the screening of hundreds of hybridoma clones. The subcloning process is repeated at least three times to ensure that the final clone was generated from a single parent cell. The most productive and stable clone (some hybridomas lose the ability to produce antibody over time) is then grown in culture medium to a high volume for large scale production of monoclonal antibody.

Immunoassay formats

Immunoassays can be either competitive or noncompetitive. In competitive immunoassays, the analyte (antigen or antibody) in the sample competes with labeled antigen or antibody and the signal generated is inversely proportional to the concentration of analyte in the sample (Figs. 4.3 and 4.4). In noncompetitive immunoassays, the amount of analyte (antigen or antibody) in the sample is directly proportional to the signal generated (Fig. 4.5).

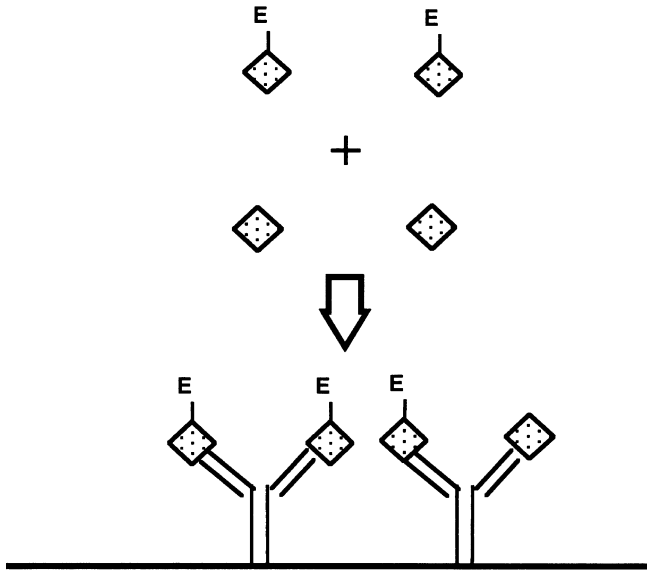

Figure 4.3 Illustration of a competitive immunoassay for the detection of antigen. In this example, antigen in the patient's sample competes with enzyme-labeled antigen for antibody bound to a solid phase. After a wash step to remove unbound enzyme-labeled antigen, addition of chromogenic substrate results in a color change that is inversely proportional to the quantity of antigen in the patient's sample.

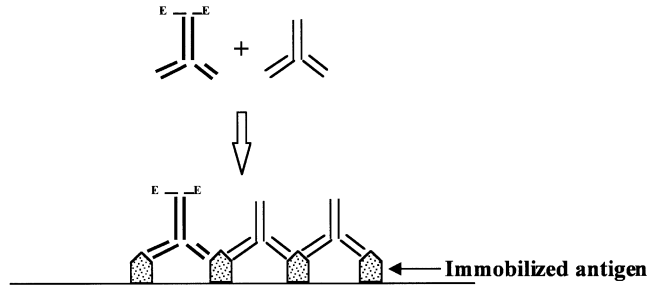

Figure 4.4 Illustration of a competitive immunoassay for the detection of antibody. In this example, antibody in the patient's sample competes with enzyme-labeled antibody for antigen bound to a solid phase. After a wash step to remove unbound enzyme-labeled antibody, addition of chromogenic substrate results in a color change that is inversely proportional to the quantity of antibody in the patient's sample.

Immunodiagnostic assays can also be either homogenous or heterogeneous. Homogenous immunoassays are performed simply by mixing the sample with reagents and measuring the signal generated (or a decrease in signal for competitive immunoassays) by the reaction chemistry that results from antigen-antibody binding. As such, homogenous immunoassays do not require the separation of bound

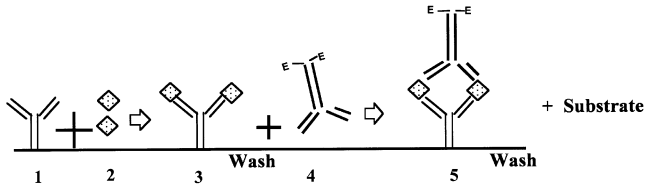

Figure 4.5 Illustration of a noncompetitive immunoassay for the detection of antigen. In this example, antigen in the sample is captured by antibody bound to a solid phase. After a wash step, enzyme-labeled antigen-specific antibody binds to the captured antigen. After another wash to remove unbound enzyme-labeled antibody, addition of chromogenic substrate results in a color change that is proportional to the quantity of antigen in the patient's sample.

antigen-antibody from free antigen (or antibody). For this reason homogeneous immunoassays tend to be easier and faster to perform, however, they are generally less sensitive. An example of a homogenous immunoassay is the detection of antigen-antibody complex formation by measuring the resultant decrease in light transmission through the sample (turbidimetry); see Chapter 2.

Newer homogeneous immunoassay technologies utilize enzyme donor and enzyme acceptor pairs that readily associate to generate active enzyme. In these assays, antibody binding to an antigenic epitope or hapten incorporated into either the enzyme donor or acceptor subunit blocks the association (and therefore, activity) of the enzyme. Analyte present in the sample binds the antibody and prevents it from binding to the enzyme subunit, thereby, allowing the formation of active enzyme. As such, these assays are considered to be competitive immunoassays since the analyte-antibody binding reaction competes with the binding of antibody to the hapten-conjugated enzyme. Another example of a newer competitive homogeneous immunoassay technology utilizes fluorescence energy transfer where a "fluorescer" is conjugated to the hapten and the "quencher" is conjugated to the antibody. In the absence of analyte in the sample the quencher-conjugated antibody binds the hapten-conjugated fluorescer and extinguishes the signal. In the presence of analyte in the sample, a portion of the quencher-conjugated antibodies bind the analyte and are therefore no longer available to bind the hapten-conjugated fluorescer and quench the fluorescence signal.

Unlike homogeneous immunoassays, heterogeneous immunoassays require the separation of antigen-antibody complexes from unbound antigen (or antibody) because the label is not affected by the antigen–antibody binding event. Using enzyme immunoassays (EIA) as an example, unbound enzyme-conjugated reagent must be physically removed from bound enzyme-conjugated reagent prior to the addition of the enzyme substrate. This removal step is usually accomplished by "washing." Another characteristic of heterogeneous immunoassays is the requirement for unconjugated reagent to be fixed to a solid phase (Figs. 4.3, 4.4 and 4.5) to allow for removal of unbound enzyme-conjugated reagent without removal of the bound enzyme-conjugated reagent. Examples of solid phases commonly used in immunoassays include microtiter wells, nitrocellulose and latex or magnetic beads.

Similar to homogeneous immunoassays, heterogeneous immunoassays can also be formatted as either competitive or noncompetitive assays. With competitive assay formats, the presence of analyte in the sample decreases the amount of signal generated. Common competitive immunoassay formats include:

1. antigen in the sample competing with free labeled antigen for a limited quantity of bound unlabeled antibody (Fig. 4.3);

2. antigen in the sample competing with bound unlabeled antigen for a limited quantity of free labeled antibody.

In both of these formats, the binding of antigen in the sample to antibody (either bound or free) blocks binding of the labeled reagent to the solid phase and thus allows the removal of the labeled reagent in the subsequent wash step. Sensitivity can be improved in the first competitive immunoassay format by adding the sample to the bound unlabeled antibody prior to addition of the labeled antigen. Similarly, sensitivity can be improved in the second competitive assay format by adding the sample to the free labeled antibody prior to incubation with the bound unlabeled antigen.

In contrast to competitive immunoassays, noncompetitive immunoassays rely on direct measurement of antibody binding sites occupied by analyte. Another difference between competitive and noncompetitive immunoassays is in the relative concentration of reagents. As mentioned above, competitive assays require limiting the quantities of antigen, antibody, or both. In contrast, reagents are applied in excess for noncompetitive heterogeneous immunoassays in order to maximize sensitivity.

A common noncompetitive heterogeneous immunoassay format for antigen is the capture or sandwich immunoassay (Fig. 4.5). In this format, bound antibody (either polyclonal or monoclonal) specific for the antigen of interest is incubated with sample, washed, then incubated with another labeled antibody (either polyclonal or monoclonal) specific for the antigen of interest. Antigen present in the sample is "captured" (i.e., sandwiched) between the bound antibody and the labeled antibody with the amount of signal generated dependent on the amount of antigen in the sample. Examples of an antigen capture immunoassay frequently utilized in veterinary clinics are heartworm antigen tests manufactured by Abaxis, Heska, Idexx, Synbiotics, and others. Noncompetitive immunoassay formats for the detection of antibody frequently use bound antigen to "capture" specific antibody and anti-Fc labeled antibodies for detection of captured antibody.

Factors influencing immunoassay design

Considerations in the selection of an immunoassay format include; analyte characteristics and concentration, desired endpoint (qualitative or quantitative), and the environment in which the test will be used.

Analyte characteristics and concentration

Small nonprotein analytes and haptens (e.g., thyroxine) are not readily detected in noncompetitive immunoassays due to the inability to "sandwich" the analyte because of a lack of sufficient number of binding sites (epitopes). These analytes are best measured in heterogenous competitive immunoassay or homogeneous immunoassay formats. Homogeneous immunoassay formats are most appropriate for antigens or haptens whose concentrations are relatively high (nmol/L) (e.g., total serum thyroxine) whereas heterogeneous competitive immunoassay formats are capable of detection limits in the picomolar range (e.g., free serum thyroxine). Noncompetitive heterogeneous assays in which reagents are applied in excess are capable of detection limits approaching 1 fmol/L.[3]

Desired endpoint

All immunoassay formats can be used to report a qualitative result simply by identifying an assay "cutoff" (usually arbitrary determined as three standard deviations above the mean of the negative controls) and reporting results as either positive or negative. Visual read test results are usually qualitative but some test formats allow for semiquantitative results without the need for instrumentation (e.g., Heska ERD-HealthScreen®). Most immunoassay formats can also be used to report quantitative results as long as the signal recognition technology is capable of detecting differences in signal magnitudes. Quantitative results usually require the use of calibrators containing known concentrations of analyte to establish a standard curve from which the sample analyte concentration is determined.

Environment

The environment in which the immunoassay is performed has important implications for format selection. As discussed previously, homogeneous immunoassays tend to be easier to perform because there are no washing steps involved. For this reason, homogeneous immunoassay formats are well suited for automation on high throughput clinical analyzers. Due to the requirement for washing between reagents, heterogeneous immunoassays tend to require greater technical skill of the operator. Quantitative immunoassays (both homogeneous and heterogeneous) have historically required sophisticated equipment (e.g., spectrophotometer, spectrofluorometer, luminometer) for detection and quantitation of generated signals and are, therefore, usually performed in centralized laboratories.

Simple to use qualitative heterogeneous immunoassays have been available for use in veterinary practice for many years. These single-use, "in-clinic" tests (sometimes referred to as "point of care tests" or POCT) either have built in wash buffer that is manually activated (e.g., Idexx SNAP®), require the addition of wash buffer after application of sample (e.g., Abaxis VetScan® and Synbiotics Witness®), or do not require wash buffer (Heska SoloStep®). The Idexx SNAP® tests are sandwich ELISA formatted tests for the detection of either antigen or antibody. The Abaxis VetScan®, Synbiotics Witness®, and Heska SoloStep® tests are lateral flow immunoassays (LFIAs). LFIAs are sandwich immunoassays that do not use enzyme/substrate for signal amplification, but rather use antibody (or antigen) conjugated to particles (sometimes referred to as microspheres or beads) made of latex, gold, carbon, or metal for detection of antigen-antibody binding (Fig. 4.6).

In LFIAs, the microsphere-labeled reagent (e.g., antibody) reacts with the analyte (e.g., antigen) as the sample wicks through the pads and membranes making up the test strip. As the mixture migrates through the "test window," antigen-antibody-microsphere complexes are captured at a "test" line by immobilized antibody (either antibody specific for the antigen of interest or an anti-Fc antibody). Excess and antigen-free complexes (e.g., in the case of a negative sample) continue migrating beyond the test line. Both the ELISA and LFIA in-clinic tests have built in procedural controls. These are not true "positive" controls but rather ensure that the enzyme was active and the sample flowed correctly for ELISA and LFIA tests, respectively.

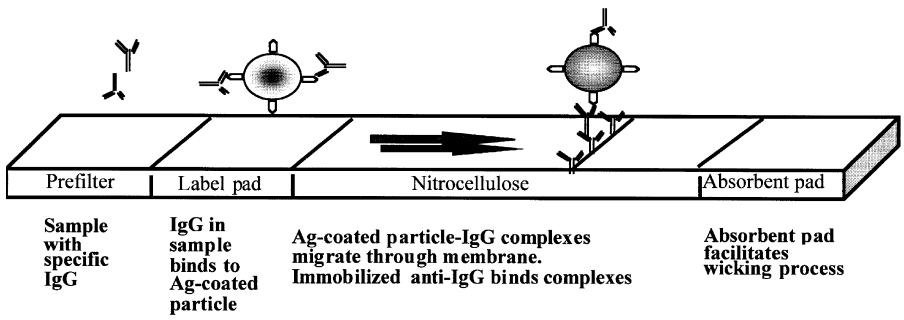

Figure 4.6 Illustration of a lateral flow immunoassay (not drawn to scale).

Factors influencing immunoassay performance

As discussed above, immunoassay format can impact performance. In addition, both the reagents and samples used in immunoassays play a fundamental role in the quality of results provided.

Reagent considerations

Immunoassays for the detection of allergen-specific IgE serve as an example of the impact reagents can have on immunoassay performance. The ideal immunoassay would use high-affinity antibodies that are minimally crossreactive. The specificity of early immunoassays for the detection of allergen-specific IgE was questioned due to the potential crossreactivity of polyclonal anti-IgE antibodies with IgG[4,5] Allergen-specific IgG is found in sera from both atopic and nonatopic animals. Therefore, any crossreactivity of the anti-IgE antibodies will decrease immunoassay specificity. For this reason, specific monoclonal anti-IgE antibodies and Fc epsilon receptor have been used as IgE detection reagents in allergen-specific IgE immunoassays.[4,6]

In addition to crossreactivity of antibody, antigen crossreactivity can also impact immunoassay specificity. In allergen-specific IgE immunoassays, it was found that taxonomically unrelated allergens contained crossreacting carbohydrate epitopes. Specific IgE binding to these crossreacting carbohydrate epitopes results in false positives relative to intradermal skin test results or immunoassay results using deglycosylated allergens.[7,8] Importantly, crossreacting carbohydrate epitopes are also found on horseradish peroxidase, an enzyme frequently used as a label in immunoassays. Under these conditions, non-IgE antibody (e.g., IgG) can simultaneously bind the carbohydrate epitopes found on both the allergen and horseradish peroxidase, generating a false positive result for allergen-specific IgE.[7,9,10] For this reason, many allergen-specific IgE immunoassays now use alkaline phosphatase as the reporter enzyme rather than horseradish peroxidase.[11]

Sample considerations

Relative concentrations of analyte in the sample can impact the quality of immunoassay results. In homogeneous immunoassays that measure antigen-antibody complex formation, excess antigen can saturate antibody binding sites and thereby prevent complex formation. The interference of excess analyte, resulting in a lower than actual measurement of analyte concentration, is referred to as the prozone effect or prozone phenomenon. Similarly, in immunoassays measuring a specific antibody class, excess antibody of a different class can bind the antigen and prevent binding by the antibody class of interest. Examples include interference due to excess IgG in allergen-specific IgE immunoassays resulting in an underestimation of IgE concentration (in atopic animals, the amount of IgG is frequently in great excess relative to the amount of IgE).[12]

Interference in immunoassays can also be caused by the presence of endogenous immunoglobulins that bind to antibodies from other species (heterophilic antibodies). Since mouse antibodies are frequently used as reagents for immunoassays, the detection of interference due to heterophilic antibodies specific for mouse immunoglobulin is not uncommon.[13,14] In people these antibodies are termed human antimouse antibodies (HAMA) and they are thought to originate from either environmental exposure to mice or medical agents containing antibodies derived from mice.[13] Heterophilic antibodies have also been reported in dogs.[15] Heterophilic dog antimouse antibodies cause occasional false positive results in some in-clinic heartworm antigen capture assays. In heartworm antigen tests, false positives can be differentiated from true positives by denaturing the antibodies (using heat or acid treatment of the sample) prior to performing the immunoassay (fortunately, heartworm antigen survives these denaturation processes). For this reason, it is important to verify positive heartworm antigen test results obtained from nondenatured samples prior to treatment for heartworms.

Future trends for use of immunodiagnostics in veterinary medicine

In addition to development of new immunodiagnostic tests for both infectious and metabolic diseases of animals, future immunodiagnostics technology will provide quantitative immunoassays in the in-clinic environment. These instruments will be smaller and less expensive than the current quantitative instruments found in central immunodiagnostic laboratories. Benefits will include improved patient care due to the decreased time to obtain results, lower costs, and alleviation of concerns with shipping of samples to outside laboratories.

The first quantitative in-clinic immunoassay for thyroxine designed for use in veterinary medicine was developed using an EIA format in a single-use cartridge with results measured by a benchtop reader. Initial studies indicated that reproducibility and correlation with the reference standard laboratory-based test were inadequate.[16] However, a more recent comparison demonstrated acceptable reproducibility and better correlation with multiple laboratory-based thyroxine assays.[17] Quantitative immunoassays for cortisol and bile acids have subsequently been introduced for this EIA-based cartridge and reader system.

Additional quantitative in-clinic immunodiagnostic technologies will likely also use "cassette" or "cartridge" based single-use tests similar to the tests discussed above. Quantitative immunoassay formats that do not use enzyme-

dependent signal amplification would have the advantage of not requiring special handling (e.g., refrigeration) to prevent deterioration of the enzyme and substrate. However, current non-EIA based qualitative tests (e.g., LFIAs) lack sufficient sensitivity for routine use in quantitative tests.[18,19]

Efforts are underway to develop quantitative immunoassays which retain the simplicity of non-EIA based qualitative tests (e.g., LFIAs) with improved sensitivity. Following are examples of quantitative immunoassay technologies based on single-use cassettes that have potential for in-clinic use in veterinary medicine.

1. The fluorescence-labeled optical-read immunodipstick assay (FLORIDA) technology which uses a fluorescent dye instead of immunogold particles or latex beads in a LFIA format which results in 100–1000 fold increase in sensitivity.[19] Use of a CCD camera based instrument with densitometric analysis software would allow quantitation of the signal.

2. The Rapid Analyte Measurement Platform (RAMP™) produced by Response Biomedical Corporation (Vancouver, BC, Canada) uses fluorescent-dyed latex particles in a LFIA cassette. Antibody-conjugated latex particles bind to the analyte and are captured at the test line. Unbound antibody-conjugated latex particles are captured at the control line and serve as an internal calibrator. Fluorescence measured at the test and control lines is converted into a ratio which allows for correction of test-to-test variation. The RAMP™ immunoassay system has been shown to provide results comparable to a central laboratory immunoassay platform.[20]

3. Magnetic particles have also been incorporated into LFIAs to increase sensitivity and provide quantitative results.[21,22] To function in LFIAs, the particles must be superparamagnetic, becoming magnetic only when placed in a strong magnetic field. Under these conditions, the magnitude of the change in the magnetic field is directly proportional to the amount of magnetic particles captured at the test line which is proportional to the analyte quantity in the initial sample. Magnetic particle-based immunoassays have a distinct advantage in that the signal is very stable over time.

4. The use of piezofilm in quantitative immunoassays has recently been described.[23] Piezofilm is a polymer film with piezoelectric properties which generates an electric charge when the film is exposed to heat or mechanical strain. In piezofilm-based immunoassays, capture antibody is attached to the surface of the film and the detection antibody is conjugated to carbon colloids which absorb light. In the presence of antigen, the capture antibody-antigen-detection antibody complex is localized on the surface of the piezofilm. Upon stimulation with light the generated heat is transferred to the piezofilm, eliciting an electric charge. An advantage of this technology is the lack of necessity for removal of the unbound carbon-conjugated antibody since heat generated by unbound conjugates is dissipated into the assay medium. The piezofilm immunoassay technology is being developed as a point-of-care system by Vivacta Ltd. located in Kent, UK.[23]

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II

Hematology of Common Domestic Species

5 Erythrocyte Morphology

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The primary function of the erythrocyte is to transport hemoglobin, which carries oxygen to the tissues. The deformable, permeable membrane that encloses the red-cell components is made of lipids, proteins, and carbohydrates. Alterations in the lipid composition (primarily phospholipids and cholesterol) of the membrane may result in abnormal red-cell shapes. Membrane proteins form the cytoskeleton of the membrane, and these proteins also play key roles in maintaining both cell shape and integrity. These membrane proteins have been named according to their relative location from the place of migration when solubilized and subjected to electrophoresis. Bands 1 and 2 (i.e., spectrin) and band 5 (i.e., actin) are the major cytoskeletal proteins. Abnormalities in membrane proteins have also been associated with abnormal red-cell shapes.

Normal erythrocyte morphology varies among different species (Fig. 5.1). Mammalian erythrocytes are anucleate, unlike those of all other vertebrates, which have nuclei. Erythrocytes are round and somewhat biconcave in most mammalian species, except in members of the family Camellidae (e.g., llamas, camels, and alpacas), which have oval erythrocytes. The biconcavity causes stained red blood cells to appear to have a central, pale area, because the observer is looking through less hemoglobin in this area of the cell. This central pallor is most apparent in canine erythrocytes. Species with smaller erythrocytes, such as the cat, horse, cow, sheep, and goat, have less concavity and, thus, little to no central pallor. The biconcave disc shape is efficient for oxygen exchange, and it allows the cell to be deformable as it moves through vasculature with a smaller diameter than that of the erythrocyte itself. Briefly, the significant differences between species are size, shape, amount of central pallor, tendency to form rouleaux, presence of basophilic stippling in regenerative response to anemia, and the presence of reticulocytes in response to anemia (Table 5.1).

Erythrocyte morphology often is an important aid in establishing a diagnosis regarding the cause of anemia, and it sometimes is helpful in establishing the diagnosis of other disorders as well. Critical to blood-cell evaluation is adequate preparation of a blood film (see Chapter 1). The observer should examine the leukocyte counting area to evaluate erythrocyte morphology, because the red blood cells are neither too dense nor too flattened in this area. The interpretation of red-blood-cell morphology should be made in conjunction with other quantitative data from the complete blood count. For example, the degree of polychromasia in erythrocytes usually is more significant when the red-cell mass is decreased.

This chapter concentrates primarily on those morphologic characteristics that are most diagnostically useful. Morphology of erythrocytes is categorized here according to color, size, shape, structures in or on the erythrocytes, and the arrangement of cells on blood films.

Erythrocyte color

Polychromasia

Polychromatophilic cells are young erythrocytes that have been released early. Usually, they are large and more blue in color than mature erythrocytes (Fig. 5.2). The blue color results from organelles (i.e., ribosomes, mitochondria) that are still present in the immature cells. The presence or absence of polychromatophilic erythrocytes is very important when determining the cause of anemia. If immature cells are released, the likely cause of the anemia is blood loss or blood destruction, with the bone marrow attempting to compensate by the early release of cells (see Chapter 8). If the anemia is caused by erythroid hypoplasia or aplasia within the marrow, then the level of polychromatophilic

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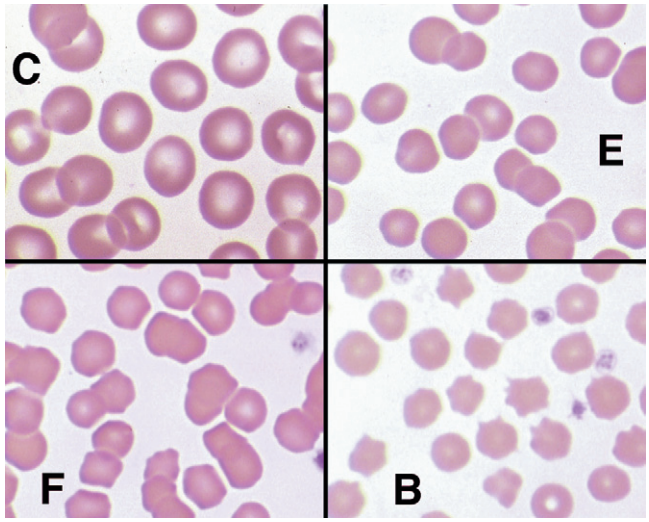

Figure 5.1 Normal canine (C), equine (E), feline (F), and bovine (B) erythrocytes. Note the larger size and marked central pallor of the canine erythrocytes compared to those of the other species. Wright stain.

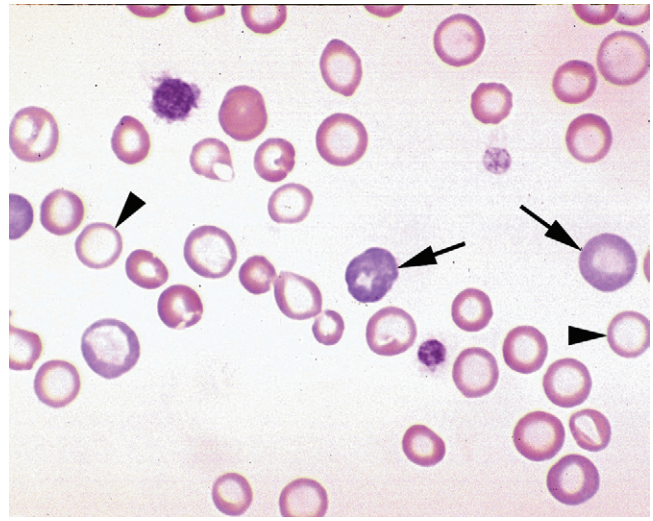

Figure 5.2 Blood film from a dog with iron-deficiency anemia. Note the lack of density of the blood film, suggesting marked anemia. Most of the erythrocytes are small and hypochromic (arrowheads). The anemia is regenerative, and numerous polychromatophilic erythrocytes are present (arrows). Wright stain.

Table 5.1 Significant differences in erythrocytes between species.

Species	Diameter (μm)	Rouleaux	Central Pallor	Basophilic Stippling	Reticulocytes (%)[a]	MCV (fL)
Dog	7.0	+	++++	–	1	60–72
Pig	6.0	++	±	–	1	50–68
Cat	5.8	++	+	±	0.5	39–50
Horse	5.7	++++	–	–	0[b]	36–52
Cow	5.5	–	+	+++	0	37–53
Sheep	4.5	±	+	+++	0	23–48
Goat	3.2	–	–	++	0	15–30

[a]With normal packed cell volume.
[b]Does not increase in response to anemia.

cells will not be increased (see Chapter 7). Horses are unique, however, in that they do not release significant numbers of polychromatophilic cells in the face of anemia.

The degree of polychromasia correlates well with the reticulocyte concentration, but it is more objective to quantify the regenerative response by counting reticulocytes (see Chapter 1). The reticulocyte is analogous to the polychromatophilic erythrocyte, but it is stained with a vital stain (e.g., new methylene blue or brilliant cresyl blue), which causes the ribosomes and other organelles to clump into visible granules (see Fig. 1.37).

Hypochromasia

Hypochromic red blood cells are pale and have increased central pallor as a result of decreased hemoglobin concentration from iron deficiency (Fig. 5.2). Erythrocytes of iron-deficient dogs have more obvious hypochromasia than erythrocytes of other species with iron deficiency; erythrocytes of iron-deficient cats usually are not hypochromic at all. One needs to distinguish hypochromic cells from bowl-shaped (i.e., torocytes) or "punched-out" cells, which are insignificant (Fig. 5.3). Bowl-shaped cells have a sharply defined, central clear area, and they also have a thicker rim of hemoglobin than is seen in true hypochromic cells. Immature polychromatophilic erythrocytes also may appear to be hypochromic, because their hemoglobin concentration is less than normal due to their increased volume. Although hyperchromic states are not thought to exist, spherocytes appear to have increased color intensity because of their lack of concavity.

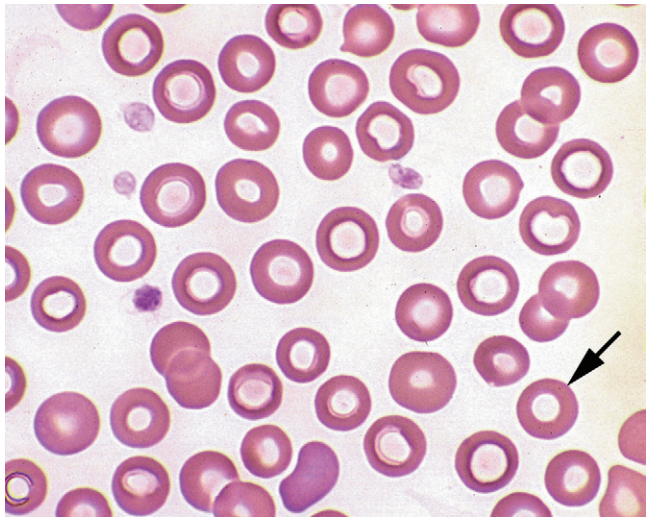

Figure 5.3 Blood film from a dog showing numerous torocytes ("punched-out" erythrocytes). Note the wide rim of hemoglobin and lack of hemoglobinization in the center of the cells (arrow). Torocytes can be mistaken for true hypochromasia. Wright stain.

Erythrocyte size

Variation in erythrocyte size is termed anisocytosis. This variation may result from the presence of large cells (i.e., macrocytes), small cells (i.e., microcytes), or both. In itself, the term does not provide any meaningful information. Red blood cells may appear to be small on the blood film because of decreased diameter, but the cell volume is the true measurement of red-cell size and is determined electronically (see Chapter 1). The best example of this is the spherocyte, which appears to be small because of its spheric shape and subsequent decreased diameter; however, the red-cell volume of spherocytes is almost always within the reference interval. Conversely, hypochromic microcytic iron-deficient red blood cells with an electronically determined decreased volume may have a normal diameter and, thus, not appear to be small on the blood film.

Microcytic erythrocytes

Cells must be markedly small before their decreased diameter can be visually detected (Fig. 5.2). Mean corpuscular volume (MCV) is more valuable than blood film examination in assessing the true size of erythrocytes. Using automated cell-counting systems, a histogram or volume–distribution curve of the erythrocyte population can be generated. Mean cell volume is determined by analysis of the volume–distribution curve, and the hematocrit is then calculated by multiplying the MCV by the erythrocyte concentration (see Chapter 1). The most common cause of microcytosis is iron-deficiency anemia; a decreased MCV is the hallmark of such anemia. In some iron-deficient patients, the MCV may be normal even though the animal has a microcytic population of cells. In these cases, examination of the volume–distribution curve is helpful (see Chapter 1). The pathophysiology of the microcytosis is theorized to involve erythroid precursors continuing to divide until a near-normal complement of hemoglobin concentration is reached, resulting in small erythrocytes. Cells cannot obtain a normal hemoglobin concentration because iron is required to make hemoglobin. If the iron deficiency is severe, microcytosis and hypochromia may be observed on the blood film. In addition, membrane defects are present, which often lead to specific abnormalities in shape and fragmentation (discussed later). Dogs with portocaval shunts may have microcytic anemia that usually is related to abnormal iron metabolism and low serum iron concentration. Some breeds of dogs (i.e., Akitas and Shiba Inus) normally have smaller erythrocytes. Occasionally, erythrocytes of animals with anemia or inflammatory disease may be mildly microcytic.

Macrocytic erythrocytes

Macrocytic erythrocytes are large and have an increased MCV (see Fig. 1.37). The most common cause of macrocytosis is increased numbers of immature erythrocytes that are polychromatophilic on Wright-stained blood films. Unlike other domestic species, horses release macrocytes that are not polychromatophilic. The associated increase in MCV usually is the only evidence of erythroid regeneration in horses. During regeneration, species other than dogs tend to produce regenerative macrocytes that are approximately twice the size of normal erythrocytes, resulting in a marked change in the MCV. Dogs, however, release macrocytes that usually are only slightly larger than normal erythrocytes. Macrocytosis without polychromasia or other evidence of an appropriate regenerative response is a common finding in anemic cats with myelodysplasia and myeloproliferative disease (see Chapter 14). This macrocytosis is associated with feline leukemia virus (FeLV) infection, and it also may be seen in FeLV-infected cats that are not anemic.

Other, more infrequent causes of macrocytosis include macrocytosis of poodles and hereditary stomatocy tosis. Macrocytosis of miniature or toy poodles is rare, is thought to be hereditary, and is usually an incidental finding. Affected dogs are not anemic, but their erythrocyte count may be decreased. The MCV is usually 90–100 fL. Other findings include increased nucleated erythrocytes, increased Howell-Jolly bodies (often multiple), and hypersegmented neutrophils. Numerous abnormalities are seen in erythroid precursors on bone marrow film examination, including megaloblasts with nuclear and cytoplasmic asynchrony of maturation. The cause of the defect is unknown, and no clinical signs are associated with the disorder. Finally, stomatocytes in Alaskan malamutes and miniature schnauzers with hereditary stomatocytosis are macrocytic (discussed later).

Some anticonvulsant drugs, such as phenobarbital, phenytoin, and primidone, have been thought to induce

macrocytosis, but macrocytosis was not experimentally reproduced in dogs receiving long-term anticonvulsant drugs. Vitamin B_{12} (i.e., cobalamin) and folate deficiency do not cause macrocytosis in domestic animals, but these deficiencies are a common cause of macrocytosis in humans. Giant schnauzers with hereditary cobalamin malabsorption are anemic, but this anemia is normocytic rather than macrocytic.

Erythrocyte shape

Abnormally shaped erythrocytes are termed poikilocytes. This terminology is not helpful, however, because it does not suggest a specific change in shape. Thus, no specific interpretation is possible. The most important shape changes include various types of spiculated erythrocytes, spherocytes, and eccentrocytes. Spiculated erythrocytes have one or more surface spicules and include echinocytes, acanthocytes, keratocytes, and schistocytes. One should be as specific as possible when describing shape changes, because certain types of abnormal red-cell shapes are associated with certain diseases. Less significant abnormally shaped red blood cells include leptocytes (i.e., folded or target cells), codocytes (i.e., target cells), dacryocytes (i.e., teardrop-shaped erythrocytes), and torocytes (i.e., bowl-shaped erythrocytes).

A few inherited abnormalities associated with red-cell shape change have been described in animals and include hereditary stomatocytosis in dogs, hereditary elliptocytosis resulting from band 4.1 deficiency in dogs, and hereditary spherocytosis in Japanese black cattle resulting from band 3 deficiency. Hereditary spherocytosis has also been reported in mice. Most inherited abnormalities of red-blood-cell shape are associated with abnormalities of cytoskeletal protein, or plasma or red-cell membrane cholesterol or phospholipid concentration.

Schistocytes and keratocytes

Erythrocyte fragments, also termed schistocytes, usually result from shearing of the red cell by intravascular trauma. This may be observed in animals with disseminated intravascular coagulopathy (DIC) as a result of erythrocytes being broken by fibrin strands, with vascular neoplasms (e.g., hemangiosarcoma), and with iron deficiency. Animals with DIC also may have a concurrent thrombocytopenia (Fig. 5.4). When erythrocyte fragments are observed in blood films from dogs with hemangiosarcoma, acanthocytes usually are present as well. Fragmentation in iron-deficient erythrocytes apparently results from oxidative injury, leading to membrane lesions or increased susceptibility to intravascular trauma. Iron-deficient erythrocytes initially develop an apparent blister or vacuole, which is thought to represent an oxidative injury and in which inner membrane surfaces are crosslinked across the cell. Exclusion of hemoglobin may account for the colorless area. These lesions subsequently enlarge and break open to form cells with one or more spicules. When one spicule is present, these cells are commonly termed apple-stem cells; when two or more spicules are present, they are termed keratocytes (Fig. 5.5). The projections from the keratocytes probably then fragment from the erythrocytes, thereby forming schistocytes.

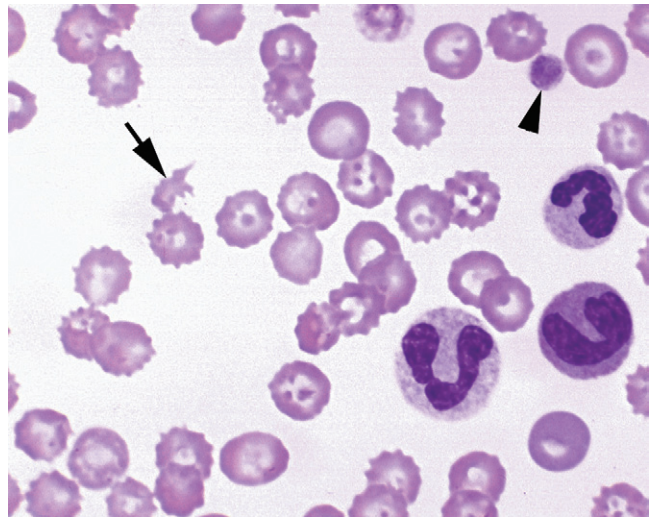

Figure 5.4 Blood film from a dog with splenic hemangiosarcoma and disseminated intravascular coagulopathy. Note the schistocyte (arrow) and single platelet in the field (arrowhead). Wright stain.

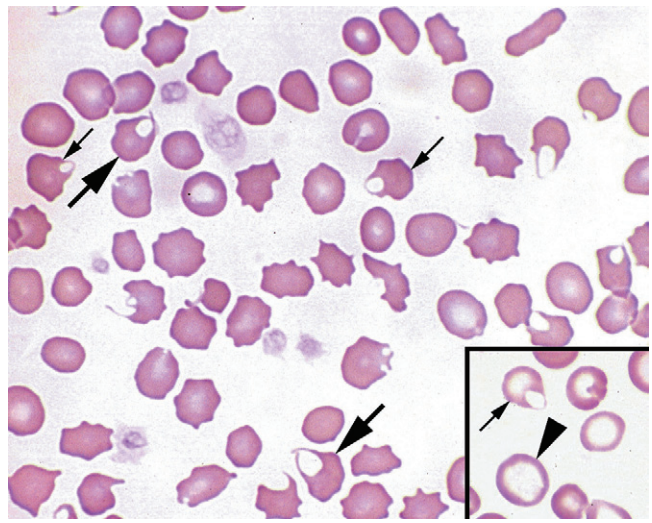

Figure 5.5 Blood film from a cat with iron-deficiency anemia. Note the erythrocyte membrane abnormalities. Lack of hypochromasia is typical for feline iron-deficient erythrocytes. Blister cells (small arrows) and keratocytes (large arrows) also are present. Inset. Blood film from an iron-deficient dog. Note the blister cell (small arrow) and hypochromic erythrocyte (arrowhead). Wright stain.

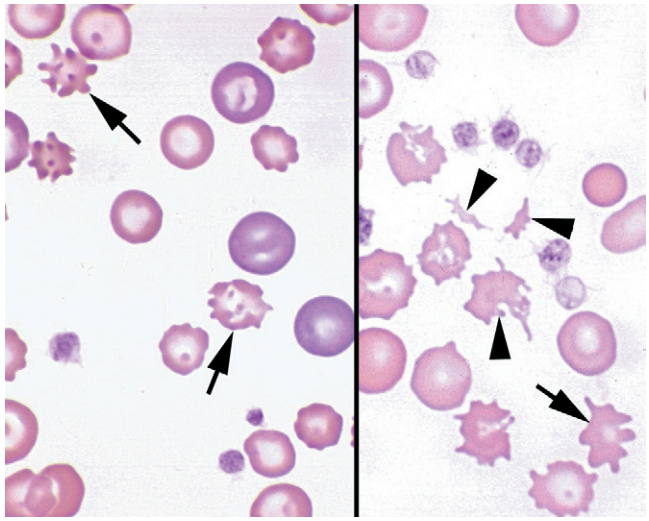

Figure 5.6 Blood film from an anemic dog with a ruptured hemangiosarcoma of the spleen. Left. Numerous acanthocytes are present (arrows). Note the large polychromatophilic cells in the same field, indicating that the anemia is regenerative. Right. Acanthocytes (arrow) and schistocytes (arrowheads) are typical findings in dogs with hemangiosarcoma. Wright stain.

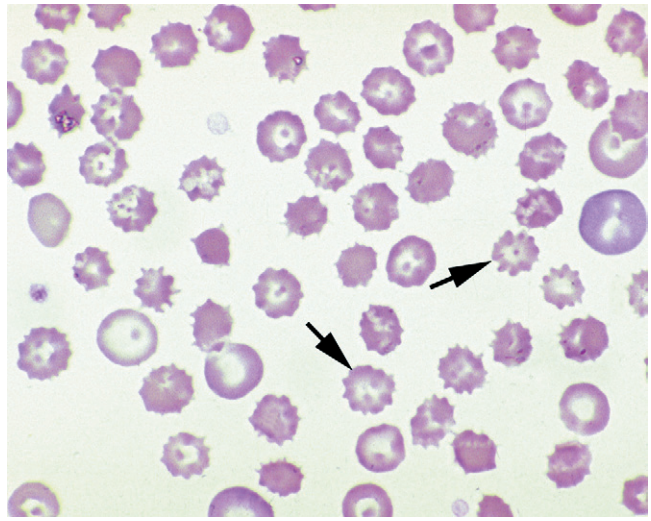

Figure 5.7 Blood film from a dog with lymphoma. Numerous echinocytes are present (arrows). Wright stain.

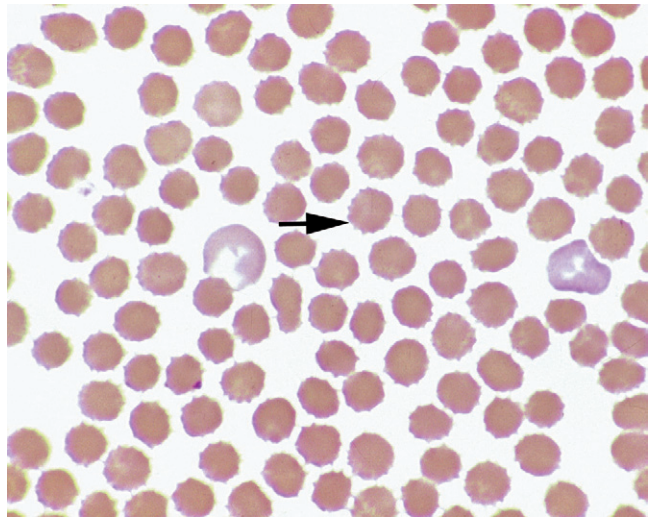

Figure 5.8 Blood film from a dog that was bitten by a rattlesnake approximately 24 hours previously. Almost all the erythrocytes are echinospherocytes (arrow). Note that the polychromatophilic erythrocytes are not affected. Wright stain.

Acanthocytes

Acanthocytes, or spur cells, are irregular, spiculated erythrocytes with few, unevenly distributed surface projections of variable length and diameter (Fig. 5.6). Acanthocytes are thought to result from changes in cholesterol or phospholipid concentrations in the red-cell membrane. They commonly are seen on blood films from humans with altered lipid metabolism, such as may occur with liver disease, but they rarely are observed on blood films from dogs with liver disease. Acanthocytes, however, are generally observed on blood films from cats with hepatic lipidosis and are often seen on those from dogs with hemangiosarcoma. The pathogenesis of this shape change in dogs with hemangiosarcoma is not known, but the presence of acanthocytes in middle-aged to old large-breed dogs with a concurrent regenerative anemia is suggestive of hemangiosarcoma.

Echinocytes

Echinocytes (i.e., burr cells) are spiculated cells with numerous short, evenly spaced, blunt to sharp surface projections that are quite uniform in size and shape (Fig. 5.7). Echinocyte formation can be an artifactual result (i.e., crenation) of a change in pH from slow drying of blood films, but it also has been associated with renal disease, lymphoma, rattlesnake envenomation, and chemotherapy in dogs and after exercise in horses. The echinocytes seen with rattlesnake envenomation are termed type 3 echinocytes, and they are quite characteristic, with numerous very fine spicules on all erythrocytes, except those that are polychromatophilic (Fig. 5.8). In some instances of rattlesnake envenomation, spheroechinocytes are formed. These erythrocytes appear to be spherocytes with fine spicules, usually present from 24 to 48 hours after envenomation, and are a reliable indication that envenomation has occurred.

Spherocytes

Spherocytes are darkly staining erythrocytes that lack central pallor (Fig. 5.9). They appear to be small, but their volume is normal. Spherocytes are not easily detected in species other than dogs because of the small size and lack of central pallor in the normal erythrocytes of most other domestic

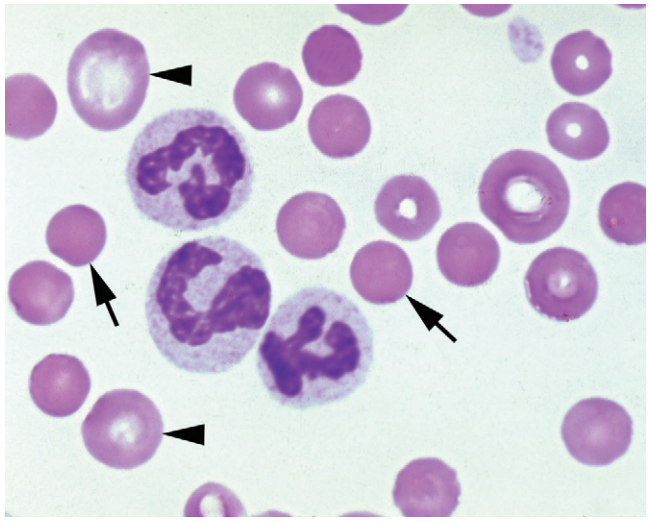

Figure 5.9 Blood film from dog with immune-mediated hemolytic anemia. Note the numerous spherocytes (arrows). The anemia is regenerative, as indicated by the polychromatophilic erythrocytes (arrowheads). Wright stain.

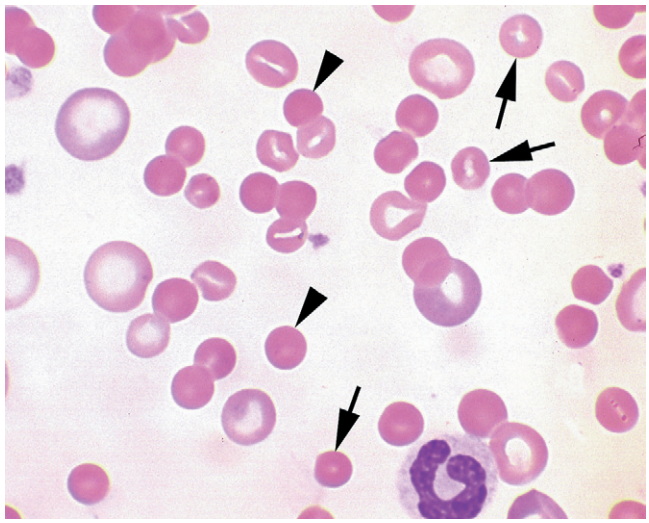

Figure 5.10 Blood film from a dog with immune-mediated hemolytic anemia. Many of the erythrocytes are spherocytes (arrowheads), and several incomplete spheres are present (arrows). Wright stain.

animals. Spherocytes have a reduced amount of membrane as a result of partial phagocytosis, which occurs because antibody or complement is on the surface of the erythrocyte. Spherocytes are very significant, in that their presence suggests immune-mediated hemolytic anemia (see Chapter 8). They also, however, may be seen after blood transfusion with mismatched blood. Spherocyte formation has been reported in dogs with bee stings and zinc toxicosis, but zinc toxicosis also may cause Heinz-body anemia. Sometimes, a small amount of central pallor will remain in a spherocyte, and it then is termed an incomplete spherocyte (Fig. 5.10).

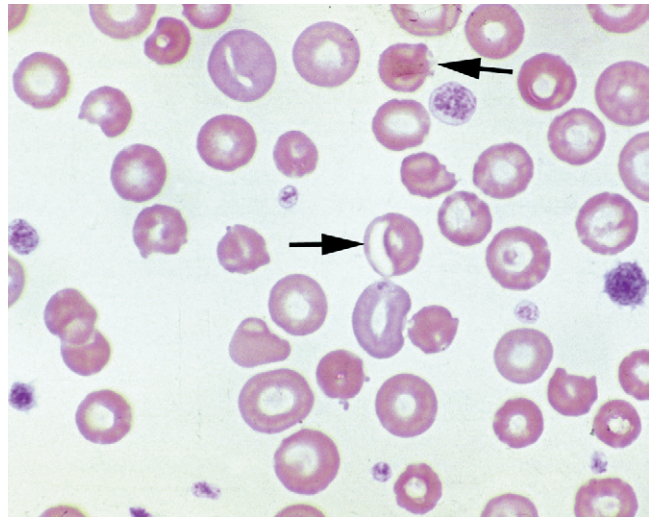

Figure 5.11 Blood film from a dog with Heinz-body anemia after ingestion of onions. Eccentrocytes are present (arrows). Wright stain.

These spherocytes likely represent a continuum of membrane removal that finally results in a complete sphere.

Eccentrocytes

Features of eccentrocytes include shifting of hemoglobin toward one side of the cell, loss of normal central pallor, and a clear zone outlined by a membrane (Fig. 5.11). They are associated with oxidative damage, especially in dogs, and may be found in conjunction with Heinz bodies (discussed later). Animals with an inherited erythrocyte enzyme deficiency, glucose-6-phosphate dehydrogenase deficiency, may show increased susceptibility to oxidant-induced erythrocyte injury, resulting in eccentrocyte formation or increased incidence of Heinz bodies.

Leptocytes and codocytes

Leptocytes are erythrocytes that have undergone a surface-to-volume ratio change in which there is excess membrane relative to the internal contents, resulting in membrane folding and target-cell formation (Fig. 5.12). They have little diagnostic significance, however, and may form in vitro secondary to contact with excess ethylenediaminetetraacetic acid (EDTA) as a result of improperly filling the blood-collection tubes. Target cells also are referred to as codocytes and are thin, bowl-shaped erythrocytes with a dense, central area of hemoglobin that is separated from the peripheral hemoglobinized region by a pale zone. Target cells may be seen in dogs with increased serum cholesterol concentration, but they also are seen in a variety of other conditions and have little significance.

Stomatocytes

Stomatocytes are uniconcave erythrocytes with a mouth-like, clear area near the cell center (Fig. 5.13). A few sto-

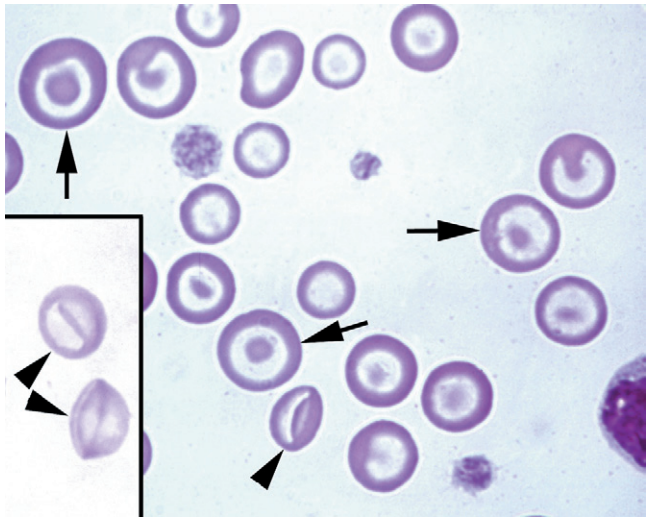

Figure 5.12 Blood film from a dog with numerous leptocytes. Note the numerous target cells (arrows) and folded cells (arrowheads). Wright stain.

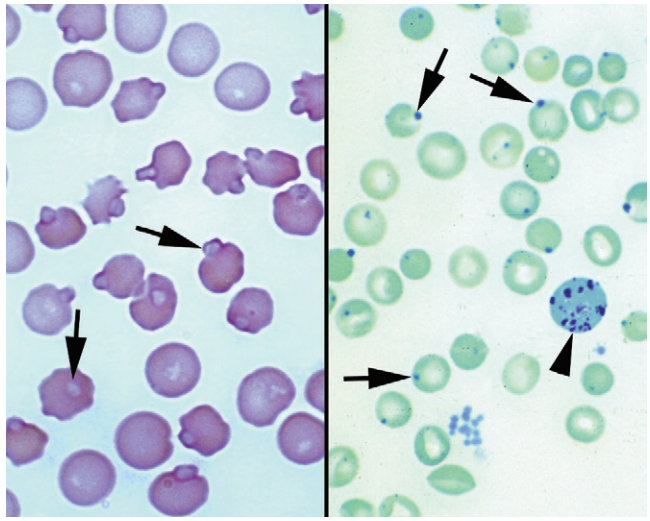

Figure 5.14 Blood films from a cat with acetaminophen toxicosis. Left. Heinz bodies appear as pale, light-blue structures (arrows). Wright stain. Right. Heinz bodies appear as blue structures (arrows). Note the reticulocyte (arrowhead). Brilliant cresyl blue stain.

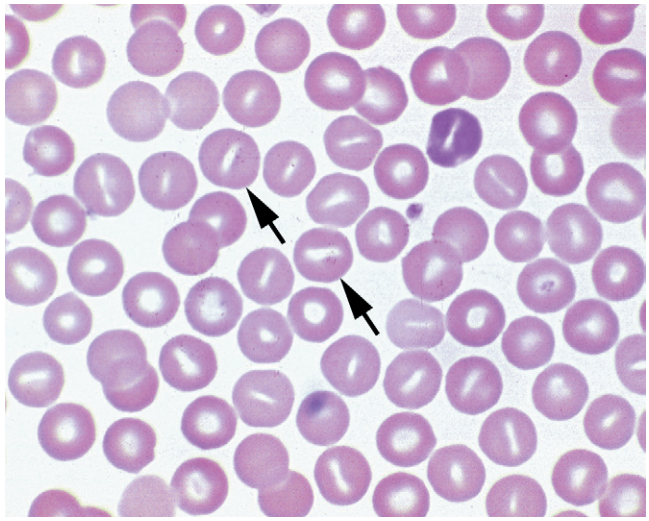

Figure 5.13 Blood film from a miniature schnauzer mix breed dog with hereditary spherocytosis. Note the numerous slit or mouth-shaped clear areas in the stomatocytes (arrows). Wright stain.

matocytes on the blood film usually are insignificant. Hereditary stomatocytosis has been reported in several dog breeds, including Alaskan malamutes, miniature schnauzers, and the Drentse partrijshond. All the disorders are inherited in an autosomal-recessive manner, but stomatocyte formation is caused by different defects in different breeds, involving either cell membranes or regulation of cell volume. Alaskan malamutes with hereditary stomatocytosis also have chondrodysplasia, and only a small percentage of the erythrocytes are stomatocytes. These stomatocytes are thought to form secondary to a membrane defect that allows increased sodium and water content of erythrocytes. Drentse partrijshond dogs with stomatocytosis also have hypertrophic gastritis, retarded growth, diarrhea, renal cysts, and polyneuropathy, and in this breed, the erythrocyte defect is thought to result from an abnormal concentration of phospholipids in the erythrocyte membrane. Miniature schnauzers with stomatocytosis are asymptomatic; the cause of the erythrocyte defect in this breed has not been described.

Structures in or on erythrocytes

Heinz bodies

Oxidative denaturation of hemoglobin results in Heinz-body formation. Approximately 1–2% of erythrocytes from normal cats contain Heinz bodies, presumably because of an unusual propensity for hemoglobin denaturation due to feline hemoglobin molecules containing twice the number of reactive sulfhydryl groups as are in hemoglobin molecules of other species. Heinz bodies appear as small, eccentric, pale structures within the red cell, and they commonly seem to protrude slightly from the red-cell margin on Wright-stained blood films (Fig. 5.14). Heinz bodies usually are 0.5 to 1.0 µm in diameter but may be larger. They usually occur as single, large structures in feline erythrocytes, but in canine erythrocytes, they more commonly are small and multiple. Heinz bodies are difficult to see on Wright-stained blood films, particularly with canine erythrocytes, in which eccentrocyte formation may be more apparent. When stained with vital stains (e.g., new methylene blue or brilliant cresyl blue), Heinz bodies appear as blue structures (Fig. 5.14). The presence of Heinz bodies reduces the deformability of the

cell, making it more susceptible to both intravascular and extravascular hemolysis. If large numbers of erythrocytes are affected, severe hemolytic anemia may result. Oxidative drugs and compounds known to induce Heinz-body formation include onions, garlic, *Brassica* species of plants, wilted or dried leaves from red maple (*Acer rubrum*), benzocaine, zinc, copper, acetaminophen, propofol, phenazopyridine, phenothiazine, phenylhydrazine, naphthalene, vitamin K, methylene blue, and propylene glycol. Ill cats may develop a high concentration of Heinz bodies without being exposed to oxidant chemicals or drugs. The most common disorders associated with an increased concentration of Heinz bodies in cats are diabetes mellitus, lymphoma, and hyperthyroidism, but increased concentrations also may be seen in association with a wide variety of other diseases (see Chapter 8).

Basophilic stippling

In vivo aggregation of ribosomes into small basophilic granules is termed basophilic stippling (Fig. 5.15). Normally, basophilic stippling is associated with immature erythrocytes in ruminants, and it may be seen to a lesser extent in cats and dogs with intensely regenerative anemia. Basophilic stippling not associated with severe anemia is suggestive of lead poisoning, but not all animals with lead poisoning have basophilic stippling. The enzyme pyrimidine 5′-nucleotidase, which is present in reticulocytes, normally catabolizes ribosomes; the activity of this enzyme is reduced in lead toxicosis and normally is low in ruminants.

Nucleated erythrocytes

Increased numbers of erythrocytes in which the nucleus remains (Fig. 5.15) are associated with regenerative anemias and early release of these cells in response to hypoxia. Increased concentrations of nucleated erythrocytes also may be seen in animals with a nonfunctioning spleen and with increased levels of endogenous or exogenous corticosteroids. An increase in nucleated erythrocytes out of proportion to the degree of anemia frequently is associated with lead poisoning, but not all animals with lead poisoning have increased nucleated erythrocytes. In cats, the presence of nucleated erythrocytes in the absence of significant polychromasia is usually an indication of myelodysplasia or myeloproliferative disease.

Howell-Jolly bodies

Nuclear remnants in erythrocytes are termed Howell-Jolly bodies. An increased concentration of Howell-Jolly bodies is associated with regenerative anemia, splenectomy, and suppressed splenic function. These bodies are small, round, dark-blue inclusions of variable size (Fig. 5.15).

Siderotic granules

Siderotic granules are stainable iron granules within mitochondria and lysosomes. These siderotic inclusions are also referred to as Pappenheimer bodies, and their presence is thought to be associated with impaired heme synthesis. Erythrocytes containing these inclusions are termed siderocytes (Fig. 5.16). Siderocytes in domestic animals are rare,

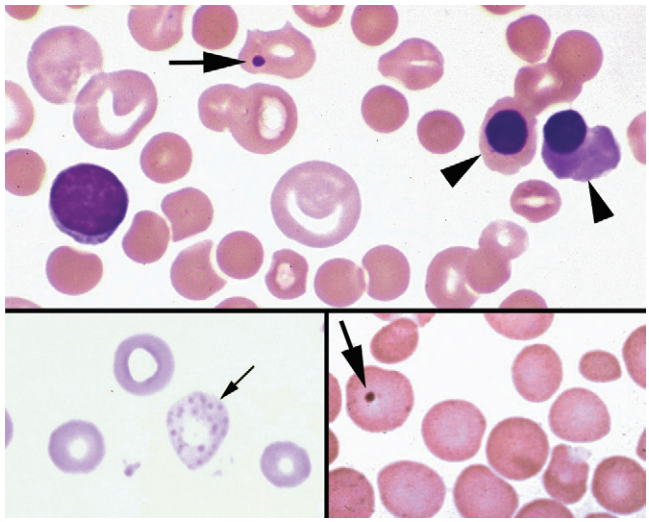

Figure 5.15 Top. Blood film from dog with immune-mediated hemolytic anemia. The anemia is very regenerative, and polychromatophilic erythrocytes, nucleated erythrocytes (arrowheads), and a Howell-Jolly body (arrow) are present. Note that the nucleated erythrocytes (metarubricytes) have variably colored cytoplasm. The one on the left has mature cytoplasm, whereas the one on the right has polychromatophilic cytoplasm. Lower Right. A nuclear remnant, or Howell-Jolly body, is indicated by the arrow. Lower Left. Basophilic stippling (small arrow) in a blood film from a dog with lead poisoning. Wright stain.

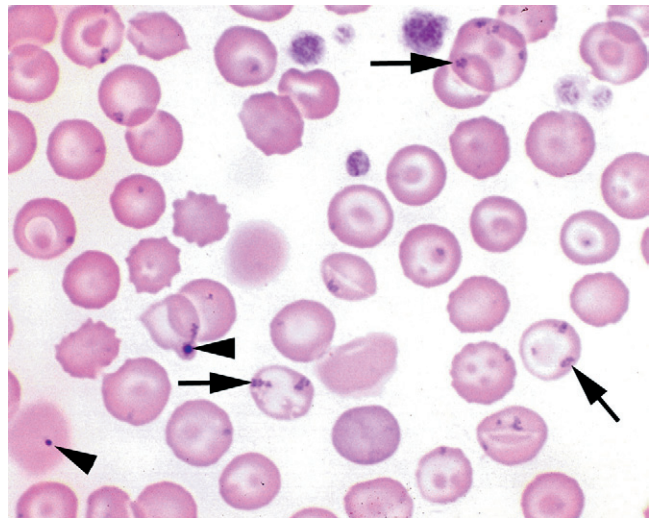

Figure 5.16 Blood film from a dog. Numerous erythrocytes (siderocytes) containing siderotic granules are present (arrows). Note the Howell-Jolly bodies (arrowheads). Wright stain.

but they have been associated with chloramphenicol therapy, myelodysplasia, and ineffective erythropoiesis of unknown cause.

Parasites

Erythrocyte parasites are discussed in more detail in Chapter 8. Spherocyte formation and agglutination may be observed on blood films from animals with erythrocyte parasites, because the organisms induce an immune-mediated anemia.

The primary parasitic disease of feline erythrocytes is infection with *Hemobartonella felis* (Fig. 5.17), which is a mycoplasmal organism that is the causative agent of feline infectious anemia. These organisms are attached to the external erythrocyte membrane and appear as rod-shaped organisms on the periphery of the erythrocyte or as a delicate, basophilic ring on the cell. A less common erythrocyte parasite in cats is the protozoan *Cytauxzoon felis*, which appears as a ring (diameter, 0.5–1.5 µm) and contains a small, basophilic nucleus (Fig. 5.18).

In dogs, erythrocyte parasites are rare. *Hemobartonella canis* usually only occurs in dogs that have been splenectomized or that have nonfunctional spleens. The organisms appear as small dots that chain across the surface of the erythrocyte (Fig. 5.19). *Babesia canis* and *B. gibsoni* are protozoal red-cell parasites in the dog that produce severe hemolytic anemia. Usually, *B. canis* appears as a teardrop–shaped structure (Fig. 5.20), but *B. gibsoni* is smaller and varies considerably in both size and shape (Fig. 5.20). Other erythrocyte parasites include *B. bigemina*, *Eperythrozoon* sp. (Fig. 5.21) and *Anaplasma* sp. (Fig. 5.22).

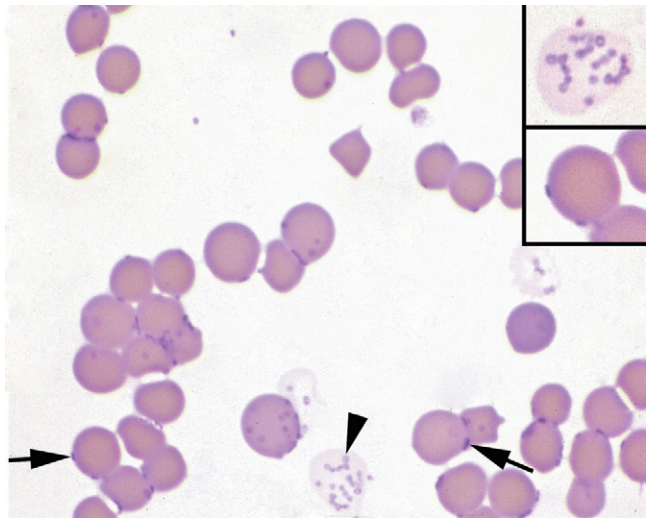

Figure 5.17 Blood film from an anemic cat. Note the numerous *Hemobartonella felis* organisms. Some of these appear as small, ring-shaped organisms on the surface of a "ghost" erythrocyte that has lysed (arrowhead). Others appear as rod-shaped structures on the edge of erythrocytes (arrows). Insets. Higher magnification of both the ring and the rod-shaped forms. Wright stain.

Viral inclusions

Viral inclusions are occasionally seen in erythrocytes from dogs with distemper. Distemper inclusions are variable in size (~1.0–2.0 µm), number, and color (faint blue to magenta) and are more frequently seen in polychromatophilic erythrocytes (Fig. 5.23).

Erythrocyte arrangement on blood films

Rouleaux formation

Rouleaux formation is the spontaneous association of erythrocytes in linear stacks, and its appearance is similar to a

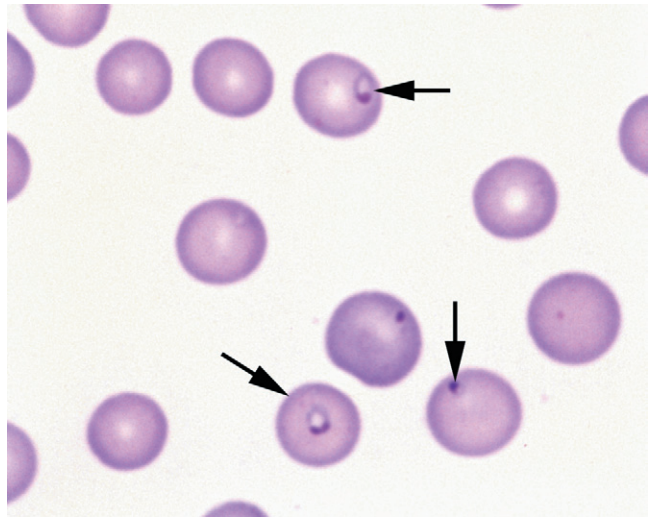

Figure 5.18 Blood film from a cat with *Cytauxzoon* organisms (arrows). Wright stain.

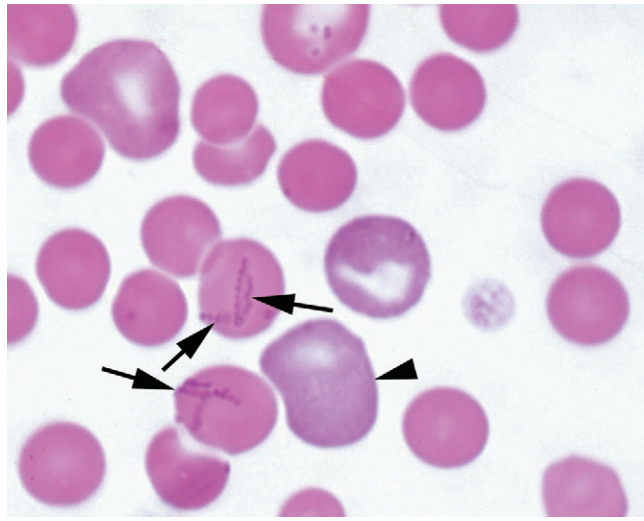

Figure 5.19 Blood film from a splenectomized dog with *Hemobartonella canis*. Note the dot-like organisms that chain across the surface of the erythrocyte (arrows). The anemia is regenerative, as indicated by the polychromatophilic cell (arrowhead). Wright stain.

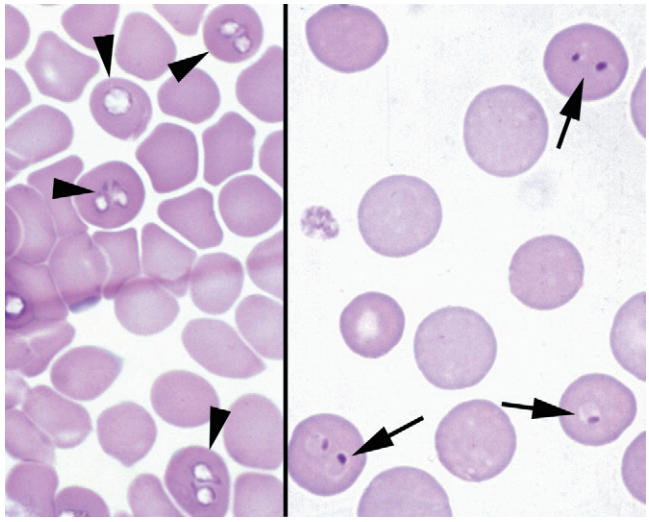

Figure 5.20 Blood film from dogs with babesiosis. Left. *Babesia canis* organisms appear as poorly staining, teardrop–shaped structures (arrowheads). Right. Blood film from a dog with *Babesia gibsoni* (arrows). Wright stain.

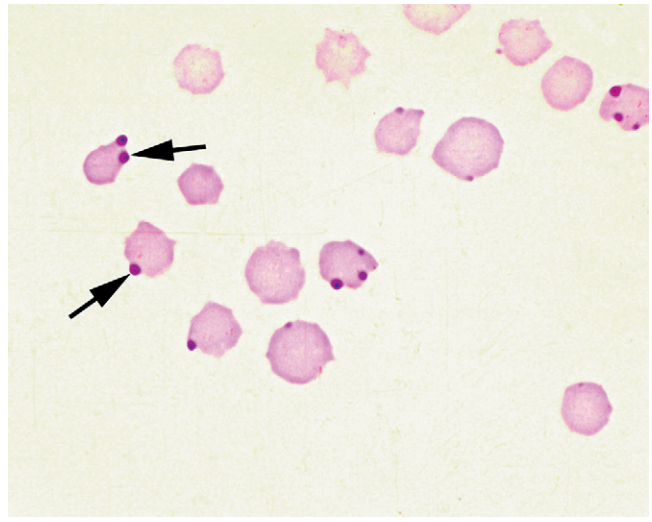

Figure 5.22 Blood film from an anemic cow with anaplasmosis. Note the numerous *Anaplasma marginale* organisms on the periphery of the erythrocytes (arrows). Wright stain.

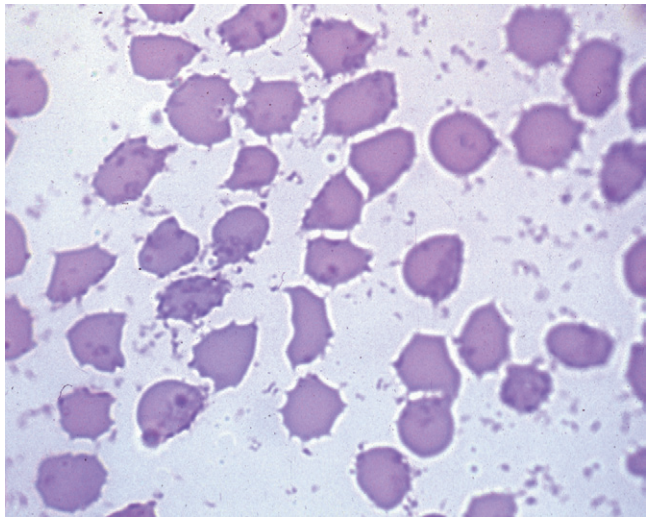

Figure 5.21 Blood film from a cow with *Eperythrozoon wenyoni*. Note the many free organisms in the plasma. Wright stain.

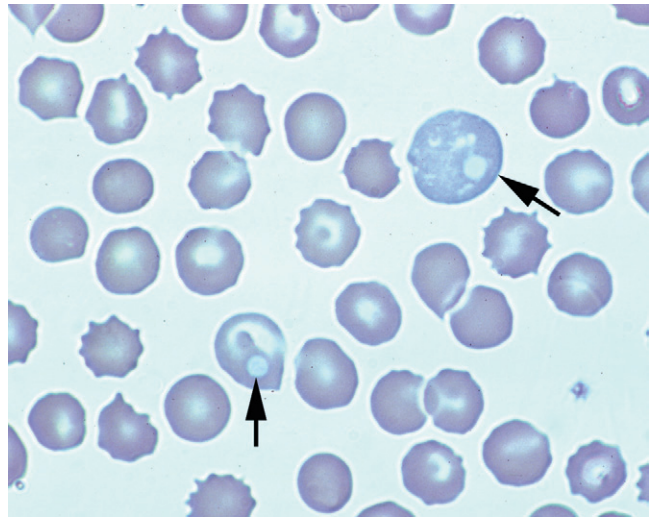

Figure 5.23 Blood film from a dog with distemper. Note the pale-blue viral inclusions of distemper with the erythrocytes (arrows). These inclusions may stain pale blue to dark magenta in color. Wright stain.

stack of coins (Fig. 5.24). Marked rouleaux formation is normal in horses, and a slight amount also is normal in dogs and cats. Rouleaux formation is enhanced, however, when the concentration of plasma proteins such as fibrinogen or immunoglobulins is increased. Increased rouleaux formation often is suggestive of a gammopathy; animals with multiple myeloma almost always have increased rouleaux formation.

Agglutination

Agglutination of erythrocytes results in irregular, spheric clumps of cells because of antibody-related bridging (Fig. 5.25). Agglutination is very suggestive of immune-mediated hemolytic anemia, but it also may be seen after a mismatched blood transfusion. To confirm that agglutination is present, mix a small quantity of blood with a drop of isotonic saline. Agglutination will persist in the presence of saline

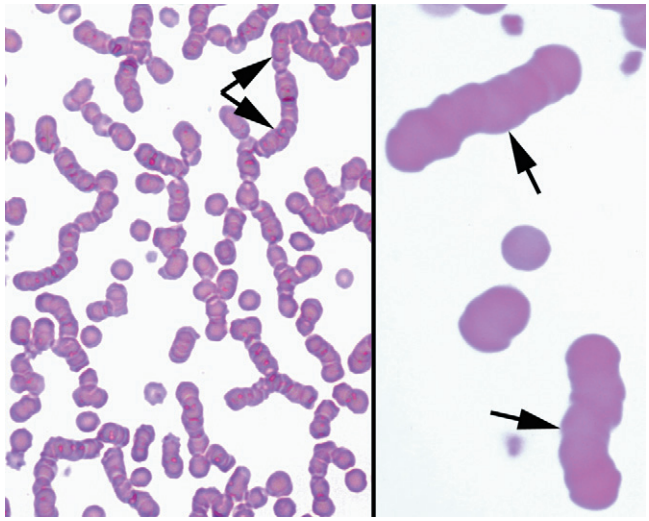

Figure 5.24 Blood film from a normal horse, illustrating rouleaux formation (arrows). Wright stain.

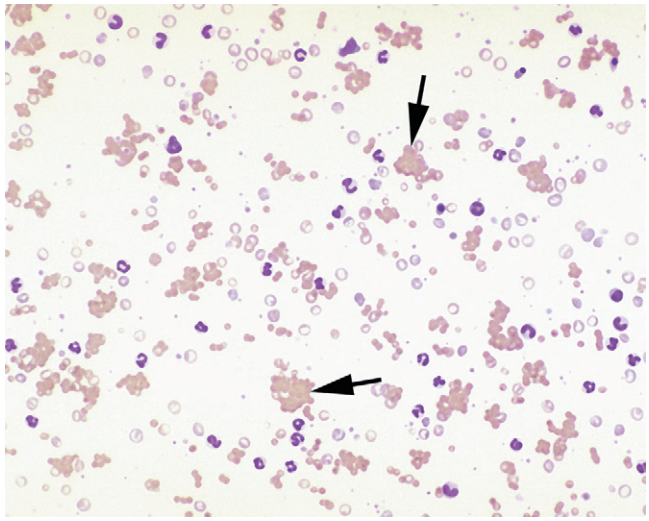

Figure 5.25 Blood film from an anemic dog with immune-mediated hemolytic anemia and marked agglutination. Note the large aggregates of spherocytes (arrows). Wright stain, low magnification.

(Fig. 5.26), whereas rouleaux formation will disperse. Agglutination may be so marked that it can be seen grossly on blood films and on the side of EDTA tubes (Fig. 5.26). Agglutination may result in a falsely increased MCV and a falsely decreased red-blood-cell count, because agglutinated red cells (i.e., doublets and triplets) may be counted as large cells (see Chapter 1).

Erythroid dysplasia and neoplasia in peripheral blood

Dysplasia and leukemia of red blood cells is covered in more detail in Chapter 14. Briefly, erythroid dysplasia, which is

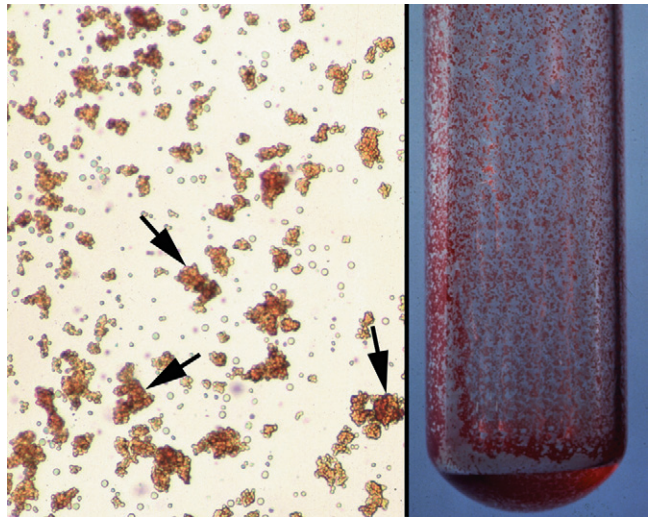

Figure 5.26 Blood from a dog with immune-mediated hemolytic anemia. Left. Blood has been mixed with isotonic saline, and agglutination persists (arrows). Right. Agglutination is so severe that it can be visualized grossly on the side of the EDTA blood-collection tube.

commonly seen in cats associated with FeLV, is characterized by a nonregenerative anemia in conjunction with macrocytosis and megaloblastic erythroid precursors, in which there is advanced cell hemoglobinization with incomplete nuclear maturation. Red-cell leukemia (i.e., erythremic myelosis, M6) is relatively rare in dogs, but in cats, it usually is associated with FeLV. In these patients, an increased concentration of quite immature nucleated erythrocytes typically is present in the face of a severe, nonregenerative anemia (see Chapter 13).

Suggested Reading

Erythrocyte size

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6 Classification of and Diagnostic Approach to Anemia

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Anemia is a decrease in the red blood cell (RBC) mass that results in decreased oxygenation of tissues. The RBC mass is determined by measuring the packed cell volume (PCV; i.e., hematocrit), the amount of hemoglobin in the blood, and the erythrocyte count (see Chapter 1). Of these three, PCV is used most commonly as the primary value for interpretation in North America, although when the hematocrit is calculated by automated cell counters, hemoglobin concentration is more accurate.

Anemia is a manifestation of an underlying disease that has produced increased erythrocyte destruction, increased erythrocyte loss through hemorrhage, decreased production of erythrocytes, or some combination of these events. Clinical signs usually relate to decreased oxygenation or associated compensatory mechanisms and may include pale mucous membranes, lethargy, reduced exercise tolerance, increased respiratory rate or dyspnea, increased heart rate, and murmurs caused by increased blood turbulence. Nonspecific clinical signs, such as weight loss, anorexia, fever, or lymphadenopathy, may be present if the animal has an underlying systemic illness. Specific clinical signs that are associated with blood destruction may include splenomegaly, icterus, and darkly pigmented urine resulting from hemoglobinuria or bilirubinuria.

The severity of clinical signs usually relates to the duration of onset, because animals with a slow onset, resulting from chronic blood loss or bone marrow dysfunction, usually compensate to some extent for the hypoxemia. Compensatory mechanisms include increased concentration of erythrocyte 2,3-diphosphoglycerate, which decreases the oxygen–hemoglobin affinity and, thus, enhances the delivery of oxygen to tissues, increases cardiac output, and aids in the redistribution of blood flow to vital organs. Death may occur in animals that experience severe acute blood loss or blood destruction. Appropriate therapy and prognosis is facilitated by determining whether the anemia is a result of erythrocyte destruction, blood loss, or lack of erythrocyte production, followed by establishing the diagnosis of the underlying disease. This chapter addresses the classification of and diagnostic approach to anemia.

Classification of anemia

Three general schemes are used to classify anemia: erythrocyte size and hemoglobin concentration, bone marrow response, and classification by pathophysiologic mechanism. The classification by erythrocyte size and bone marrow response are the most useful for clinical purposes, because they are tools that allow veterinarians to follow a mental pathway to a differential diagnosis. The pathophysiologic classification merely provides a conceptual framework for a diagnostic library of disorders that cause anemia.

Erythrocyte size and hemoglobin concentration

Traditionally, anemia has been classified by erythrocyte volume (i.e., mean cell volume (MCV)) and the amount of hemoglobin within erythrocytes (i.e., mean corpuscular hemoglobin concentration (MCHC)). An anemia is referred to as being microcytic when the erythrocytes are small, normocytic when they are of normal volume, and macrocytic when they are larger than the reference interval. Moreover, anemia is referred to as being hypochromic when the cells contain a less-than-normal hemoglobin concentration and as normochromic when they contain a normal hemoglobin concentration. Hyperchromic anemias do not occur, but the MCHC is falsely increased when the hemoglobin determination is falsely increased because of intravascular hemolysis, lipemia, or the presence of Heinz bodies. The MCHC is also falsely increased if the erythrocyte size falls below the threshold of RBC detection in the hematology analyzer. This will effectively reduce the hematocrit and increase the MCHC. Although spherocytes appear to be hyperchromic on blood films because of their shape, the

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hemoglobin concentration is normal in these erythrocytes. The MCHC, however, may be falsely increased in patients with immune-mediated hemolytic anemia because of intra vascular hemolysis or agglutination, which causes errors in measurement of the RBC mass.

This classification system is useful, particularly as it relates to cell volume, in that microcytic anemias almost always result from iron deficiency. Other causes of microcytosis include hepatic portocaval vascular shunts in dogs and cats, and normal canine breed variations in Akitas and Shiba Inus. A macrocytic anemia usually indicates that the marrow is functional and is releasing immature cells that are larger than normal in size. Macrocytosis without polychromasia or reticulocytosis should be evaluated further, because a regenerative response likely is not the cause in these patients. The MCV is of particular value in horses, because reticulocytes are almost never released into the circulation in significant numbers. Other causes of macrocytosis include feline leukemia virus, myelodysplasia, poodle macrocytosis, and hereditary stomatocytosis (see Chapter 5). Animals with a normocytic anemia usually have a nonregenerative or a preregenerative anemia. (Preregenerative refers to anemia in animals with blood loss or blood destruction, but in which evidence of regeneration in the peripheral blood is not yet evident.) Animals with a regenerative anemia, however, may have an MCV within the reference interval and, thus, be classified as having a normocytic anemia. The generated histogram or computer graphic is valuable in these patients, in that the subpopulation of macrocytic cells can be observed even though the MCV is normal (discussed later).

The MCHC is less useful in the classification of anemia, in that hypochromia usually is simply associated with an increased concentration of large, immature cells (i.e., regenerative anemia). Reticulocytes are still synthesizing hemoglobin; therefore, their hemoglobin concentration is less than that of mature erythrocytes. Occasionally, animals with iron deficiency may have a hypochromic as well as a microcytic anemia, but in most iron-deficient animals, the MCHC is within the reference interval.

Historically, MCV and MCHC were derived by calculations based on the PCV, hemoglobin concentration, and erythrocyte count. The MCV was calculated by dividing the PCV by the erythrocyte (RBC) count. For example, if the patient's PCV is 42% and its RBC count is 6.0×10^6, then the PCV divided by the RBC count is 70 fL (i.e., 42/6 = 7). In terms of mathematical logic, 1 µL = 10^9 fL, and 42% of 10^9 fL is 420,000,000 fL. Therefore, the MCV = 70 fL (i.e., 420,000,000 ÷ 6,000,000). The MCHC, which is the ratio of the weight of hemoglobin to the volume of erythrocytes in grams per deciliter, can be calculated by the following equation:

$$\text{MCHC (g/dl)} = \frac{\text{HGB}}{\text{HCT}} \times 100$$

For example, if the hemoglobin is 14 g/dL and the PCV is 42%, then the MCHC is 33.3 g/dL.

Electronic cell counters have made calculation of the MCV obsolete, because the cell volume can be measured electronically. Thus, the MCV and RBC are used to calculate the PCV (see Chapter 1). This technology has improved the usefulness of this classification of anemia, because subpopulations of microcytic or macrocytic erythrocytes can be observed in histograms or computer graphics, even when the MCV is within the reference interval (Fig. 6.1). The RBC distribution width, which describes the width of the RBC size distribution, increases when subpopulations of either microcytic or macrocytic erythrocytes are present and often is increased before the MCV value falls out of the reference interval. The MCHC is still derived using the hemoglobin and PCV determinations; however, laser-detection technology using light scatter now allows for direct determination of the amount of hemoglobin within cells. Hemoglobin concentration using this type of technology is reported as corpuscular hemoglobin concentration mean (CHCM). Using this detection system, lipemia or hemolysis will not falsely increase the CHCM. Heinz bodies, however, may, because erythrocytes containing Heinz bodies are more optically dense.

Bone marrow response

Classification of anemia based on responsiveness of the bone marrow is very useful diagnostically. An anemia is classified as either regenerative or nonregenerative based on the number of immature erythrocytes that are circulating. Early release of immature erythrocytes is a normal marrow response to increased erythropoietin production, primarily by renal tissue, secondary to hypoxia. Increased numbers of immature erythrocytes are released into the circulation after blood loss or blood destruction, and they are indicative of a regenerative anemia. An increased concentration of immature erythrocytes usually is seen within 2–4 days after blood loss or destruction. A lack of circulating immature erythrocytes in the face of anemia indicates a nonregenerative anemia and should be considered as evidence of marrow dysfunction.

Immature erythrocytes observed using a Wright-stained blood film are polychromatophilic, and they have a blue-staining reticulum (i.e., reticulocyte) when new methylene blue or brilliant cresyl blue stains are used (see Chapters 1 and 5). In general, an anemia is considered to be regenerative if the reticulocyte concentration is greater than 60,000 cells/µL (see Chapter 1). Reticulocytosis or increased polychromasia is a better indication of bone marrow responsiveness than is an increased mean cell volume (see Chapter 5). Horses almost never release significant numbers of reticulocytes into the circulation.

Pathophysiologic classification

The pathophysiologic classification of anemia essentially is a categorization based on the underlying disorder. Nonre-

generative anemia results from defective or decreased erythropoiesis (see Chapter 7). Decreased erythropoiesis usually is classified according to whether neutrophil and platelet production are also decreased (i.e., aplastic anemia) and whether RBC production is simply decreased (i.e., hypoplasia) or is completely absent (i.e., aplasia). Moreover, impaired erythrocyte production may be caused by an intrinsic (i.e., primary) marrow disorder, such as myelofibrosis, myelodysplasia, or myeloproliferative disorder, or it may be caused by an extrinsic (i.e., secondary) disorder. Secondary disorders include chronic renal disease; some endocrine disorders; inflammatory diseases; infectious agents, such as *Ehrlichia* sp., equine infectious anemia virus, and feline leukemia virus; immune-mediated destruction of erythrocyte precursors; and drug- or chemical-induced damage (see Chapter 14).

Regenerative anemia is caused by blood loss or erythrocyte destruction (see Chapter 8). Blood loss may be external or internal, and it may be acute or chronic. Causes of acute blood loss include trauma, bleeding lesions (e.g., tumors or large ulcers), and hemostatic disorders (e.g., thrombocytopenia or an inherited or acquired coagulopathy such as warfarin toxicosis or disseminated vascular coagulopathy). Common causes of chronic blood loss include bleeding lesions, particularly within the gastrointestinal tract, and gastrointestinal or external parasites. Erythrocyte destruction (i.e., hemolysis) may be either intravascular or extravascular, and it may result from intrinsic (i.e., primary) defects, such as hereditary membrane defects or enzyme deficiencies, or from extrinsic (i.e., secondary) causes, such as erythrocyte parasites or immune-mediated destruction. Intravascular hemolysis is the actual lysis of erythrocytes within the vascular system. Extravascular hemolysis occurs when abnormal erythrocytes are phagocytized by macrophages, usually within the spleen or liver. Common causes of erythrocyte destruction include immune mediated mechanisms, erythrocyte parasites, and drugs and chemicals that produce oxidative damage, resulting in Heinz body formation. Less common causes of hemolysis include hypophosphatemia, water intoxication in young ruminants, bacteria (e.g., *Leptospira* and *Clostridium* sp.), heparin overdose, and hereditary erythrocyte enzyme deficiencies and membrane defects.

Diagnostic approach

When presented with an anemic patient, the ultimate goal is to establish a definitive diagnosis of the underlying disorder so that appropriate therapy can be initiated and a prognosis established. Information can be obtained from the laboratory evaluation, the patient history, and the physical examination. The most clinically useful approach to anemia is based on the classification schemes involving a combination of bone marrow response and erythrocyte size.

Laboratory evaluation

The classification of an anemia based on erythrocyte size and marrow response (discussed earlier) is very important. Essential laboratory data include PCV, MCV, and reticulocyte count. Either blood loss or destruction will result in a regenerative anemia, and marrow dysfunction will result in a nonregenerative anemia. Furthermore, microcytosis usually is evidence of iron-deficiency anemia, and macrocytosis usually is evidence of regeneration. Additional information may be obtained from examination of the blood film; erythrocyte morphology may even reveal a definitive diagnosis (see Chapter 5).

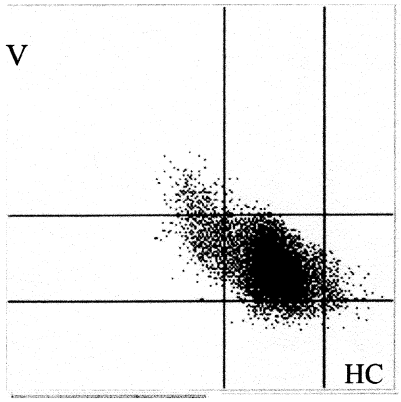
Other laboratory procedures that may provide helpful information include plasma protein estimation by refractometry (see Chapter 1). Blood loss usually results not only in a loss of erythrocytes but also in a loss of other blood components, including protein. Thus, patients with blood loss may be hypoproteinemic. Other causes of hypoproteinemia, however, still must be considered (see Chapter 26). If blood is lost internally, such as within a body cavity, the protein usually is reabsorbed within hours.

Other components of the complete blood count (CBC) also may provide useful information. For example, if a patient is severely thrombocytopenic, anemia may be caused by blood loss secondary to impaired clot formation. On the other hand, if the leukocyte concentration, platelet concentration, and PCV are all decreased and the anemia is nonregenerative, then complete bone marrow failure is the likely cause of the anemia. An animal with a mild, nonregenerative anemia and increased immature neutrophils likely has an anemia of inflammatory disease (see Chapter 7).

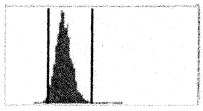
Specific laboratory tests can be performed to help confirm or exclude a suspected diagnosis. If spherocytes are observed on the blood film of an anemic patient, then a Coombs or a saline fragility test (see Chapter 1) can help to confirm immune-mediated hemolytic anemia. In patients with microcytic anemia, serum iron should be measured to determine if the microcytosis is caused by iron deficiency. In addition, the feces should be examined for blood, because chronic blood loss from the gastrointestinal tract is a common cause of iron-deficiency anemia (see Chapter 8). Anemic dogs, particularly those with a concurrent thrombocytopenia and hyperglobulinemia, should be tested for ehrlichiosis, and anemic cats should be tested for feline leukemia virus and feline immunodeficiency virus. Anemic horses should be tested for equine infectious anemia.

The biochemical profile also may provide essential information. Patients with mild to moderate, nonregenerative anemia may have disorders that are extrinsic to the marrow but that affect the marrow function. For example, animals with a nonregenerative anemia that are also azotemic

A RBC V/ HC

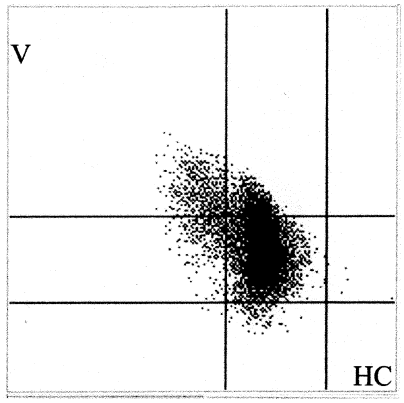


RBC Volume



Dog, PCV 29% (43-58)
MCV 61fL (62-75)
RDW 14.3 (12-14.2)

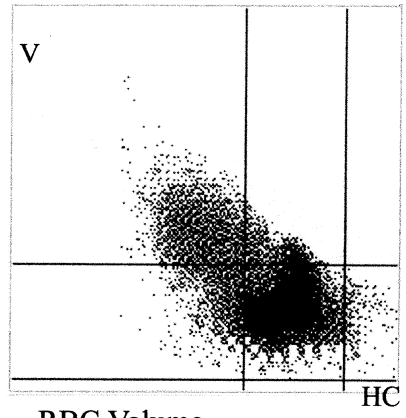
B RBC V/HC



RBC Volume

Dog, PCV 37% (43-58)
MCV 76 fL (62-75)
RDW 14.3 (12-14.2)

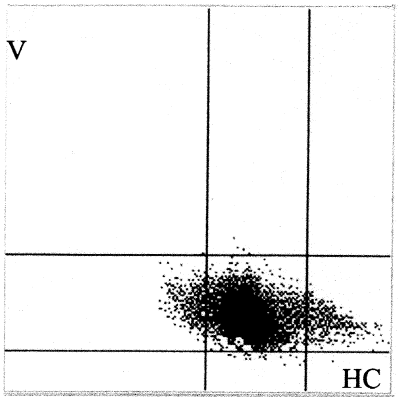
C RBC V/HC



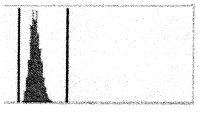
RBC Volume

Cow, PCV 21% (24-34)
MCV 48 fL (38-50)
RDW 31.2 (16-21)

D RBC V/HC

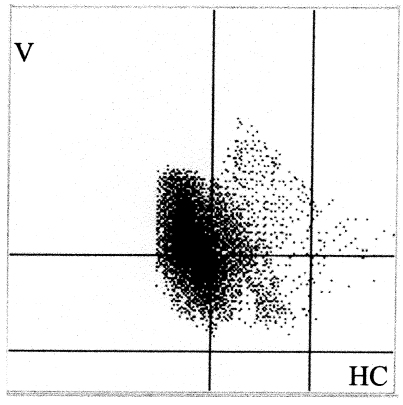


RBC Volume

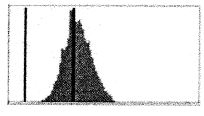


Cat, PCV 30% (33-50)
MCV 38 fL (40-54)
RDW 15.2 (13.5-18.5)

E RBC V/HC

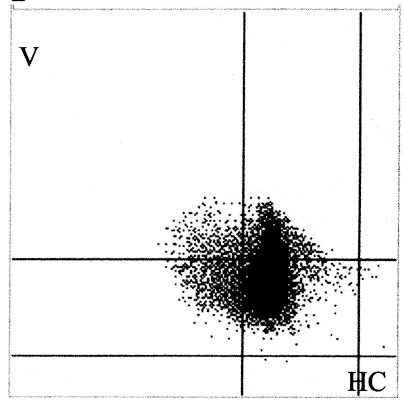


RBC Volume



Cat, PCV 17% (33-50)
MCV 76 fL (40-54)
RDW 18.9 (13.5-18.5)

F RBC V/HC



RBC Volume

Horse, PCV 28% (30-45)
MCV 61fL (40-48)
RDW 17.6 (16.5-19.5)

Figure 6.1 Red-blood-cell volume/hemoglobin concentration (RBC V/HC) cytograms and RBC volume histograms from six anemic animals generated by a Bayer Advia 120 (Bayer Corporation, Tarrytown, NY). On the RBC V/HC cytogram, hemoglobin (Hgb) concentration is plotted along the x (i.e., horizontal) axis, and cell volume is plotted along the y (i.e., vertical) axis. Each RBC is displayed based on volume and Hgb concentration, and normocytic normochromic cells are in the center box of each nine-box cytogram. Larger cells are displayed toward the top of the cytogram and hypochromic cells toward the left; thus, macrocytic hypochromic cells are displayed to the upper left of the cluster of normal erythrocytes. The RBC volume histogram represents the distribution of the RBCs by cell volume; normal samples have a bell-shaped curve. The mean corpuscular volume (MCV) and RBC distribution width (RDW) are determined from this histogram. The MCV is the mean of the RBC volume histogram, and the RDW is the coefficient of variation of the population. Each animal's species, packed cell volume (PCV), MCV, and RDW are provided beneath the RBC V/HC cytogram and RBC volume histogram. Reference intervals are in parentheses.

A. A 12-year-old, mixed-breed dog with a mild anemia, mildly decreased MCV, and mildly increased RDW. The RBC V/HC cytogram shows that many of the erythrocytes are toward the bottom of the middle square, indicating they are microcytic. In addition, a population of hypochromic cells is present, some of which are normocytic and some of which are macrocytic. The RBC volume histogram is shifted toward the left, also indicating that many of the erythrocytes are slightly small. Iron-deficiency anemia was suspected in this patient and was confirmed by decreased serum iron concentration. The dog had a 3-month history of epistaxis associated with a nasal passage chondrosarcoma.

B. A 12-year-old, miniature schnauzer with a very mild anemia, mildly increased MCV, and mildly increased RDW. The RBC V/HC cytogram shows a population of cells above the middle square that represents large cells. A population of macrocytic hypochromic cells is present as well. The RBC volume histogram is shifted slightly toward the right, and a population of macrocytic cells is evident. This is indicative of a regenerative anemia.

C. A 1-week-old anemic calf. Note the population of macrocytic hypochromic cells, even though the MCV is within the reference interval. The RDW is markedly increased. The reticulocyte count is 90,000 cells/μL (2%). The presence of macrocytic cells and reticulocytes indicates that the anemia is regenerative. The calf's umbilical stump had been bleeding since birth, and it also had blood in the feces for 3 days. The PCV on the previous day was 9%, and the calf received a blood transfusion at that time. Many of the normocytic cells probably are donor erythrocytes. The calf responded well to supportive therapy and 1 week later, the PCV was 27%.

D. A 13-year-old cat with a mildly decreased MCV. The RBC V/HC cytogram and RBC volume histogram are similar to those of the dog in panel A, suggesting iron-deficiency anemia. The cat had blood in the feces, as a result of intestinal (primarily colonic) lymphoma, for several weeks before this CBC was performed. The reticulocyte count is 108,000 cells/μL, indicating that the anemia is regenerative, but the immature erythrocytes are also small because of iron deficiency.

E. A 6-year-old cat with a slightly increased RDW. Note that most of the cells are macrocytic and hypochromic. The RBC volume histogram is shifted far toward the right because of numerous large erythrocytes, and the reticulocyte count is 233,260 cells/μL (10.7%), indicating a very regenerative anemia. The cat was Coombs positive, and a diagnosis of immune-mediated hemolytic anemia was made. No *Haemobartonella* organisms were observed on blood films taken during various days, but polymerase chain reaction for *Haemobartonella felis* was not performed. The cat was negative for feline leukemia virus.

F. A 12-year-old horse with a macrocytic anemia. Note the population of large cells, some of which are hypochromic. The RBC volume histogram is shifted toward the right, indicating a subpopulation of large cells. Reticulocytes are not released in horses, but the presence of macrocytic erythrocytes suggests that the anemia is regenerative. The horse was dehydrated, so it likely was more anemic than would be indicated by the PCV. Blood loss or blood destruction should be suspected in this case.

because of kidney dysfunction likely have decreased erythropoietin production. All patients with an unexplained nonregenerative anemia should undergo bone marrow aspiration and examination (see Chapter 14).

Signalment and history

A complete and accurate patient history from the owner may provide valuable information. In some cases, the signalment is also helpful, because certain disorders are more common in certain breeds. For example, immune-mediated hemolytic anemia is relatively common in cocker spaniels. Acute blood loss results in acute onset of clinical signs, whereas both chronic blood loss and marrow dysfunction result in chronic onset of clinical signs. Therefore, determining if the onset of clinical signs was acute or chronic may be helpful. Asking the owner if other clinical signs are present may be useful as well. For example, a dog that is also experiencing polyuria and polydipsia may be anemic as a consequence of renal dysfunction. Alternatively, a dog that is experiencing periodic episodes of weakness may have recurring, intermittent, intra-abdominal hemorrhage secondary to a bleeding lesion (e.g., hemangiosarcoma). One should also determine any history of trauma or recent surgery and if the owner has observed any evidence of blood loss, such as hematuria or epistaxis. (Melena, on the other hand, must be very severe to be obvious by visual examination of feces.) Finally, one should inquire if the patient has had any possible exposure to plants, drugs, or chemicals that might induce blood destruction, marrow dysfunction, or gastrointestinal ulceration and associated blood loss.

Physical examination

A careful, routine physical examination may reveal additional information. For example, if bruising, petechiae, or ecchymoses are present in an anemic patient, the anemia may be secondary to decreased or dysfunctional platelets or to a coagulation disorder (see Chapter 16). If abdominal distension is present, intra-abdominal hemorrhage should

be suspected, and an abdominal paracentesis and fluid evaluation should be performed. If the mucous membranes are icteric as well as pale, erythrocyte destruction should be suspected. If the mucous membranes are cyanotic or brown as well as pale, methemoglobinemia, which may accompany Heinz-body anemia, may be present.

Summary

In summary, the clinical signs, laboratory evaluation, signalment, history, and physical examination are all important in establishing a diagnosis for the underlying cause of anemia. Chronic external blood loss usually results in iron-deficiency anemia, which can be diagnosed on the basis of decreased MCV and serum iron. Acute external blood loss usually can be diagnosed during the physical examination; however, internal blood loss may initially be difficult to differentiate from blood destruction. Significant internal blood loss usually occurs within a body cavity, so careful physical examination, body-cavity aspiration, or other methods of visualization usually are diagnostic. Furthermore, many causes of blood destruction, such as immune-mediated destruction, Heinz bodies, or erythrocyte parasites, can be detected based on examination of blood films and erythrocyte morphology. (Diagnostic procedures for specific causes of anemia are discussed in more detail in Chapters 7 and 8.)

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7 Nonregenerative Anemia

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Anemia is classified as either regenerative or nonregenerative based on the number of circulating immature erythrocytes (polychromatophilic erythrocytes or reticulocytes). A lack of circulating immature erythrocytes indicates a nonregenerative anemia and provides evidence of marrow dysfunction. Most nonregenerative anemias are normocytic.

Nonregenerative anemia is further subclassified based on whether granulopoiesis (neutrophil production) and thrombopoiesis (platelet production) are also affected. Animals with nonregenerative anemia in conjunction with neutropenia and thrombocytopenia (pancytopenia) have either reversible or irreversible stem cell injury. Irreversible stem cell injuries are discussed in Chapter 14 and represent an intrinsic defect in proliferative behavior and/or regulation of stem cell entry into differentiated hematopoiesis. Some irreversible injuries may be induced by drugs, chemicals, viruses (e.g., feline leukemia virus (FeLV)), radiation, and immune-mediated stem cell injury, but the cause often is never discovered. Manifestations of stem cell injury range from dysplasia to lack of cell production (aplastic anemia) to uncontrolled neoplastic proliferation. Reversible stem cell injury is transient but also may be caused by drugs, chemicals, viruses, radiation, and immune-mediated destruction of stem cells. Reversible stem cell injury does not progress to neoplasia; however, both reversible and irreversible stem cell damage may be associated with myelofibrosis in response to the injury.

Pancytopenia also may result from myelophthisic disorders in which nonhematopoietic neoplasms, such as lymphoma and malignant histiocytosis, either metastasize to or originate in the marrow. In addition, pancytopenia may be seen with hemophagocytic syndrome, a rare condition that occurs secondary to infectious, neoplastic, or metabolic diseases and is characterized by the proliferation of benign histiocytic cells that phagocytize hematopoietic precursors.

Animals with nonregenerative anemia in conjunction with normal neutrophil and platelet concentrations may have an intrinsic marrow defect (pure red cell hypoplasia, aplasia, or apparent erythroid maturation defect), or they may have a disorder that is extrinsic to the bone marrow but results in defective or decreased erythropoiesis. Pure red cell aplasia also may be either reversible or irreversible, and it usually is immune mediated or caused by viral (FeLV) damage. Extrinsic causes of nonregenerative anemia include anemia of inflammatory disease, anemia of renal failure, anemias associated with endocrine disorders, and rarely, nutritional deficiencies.

Aplastic anemia (aplastic pancytopenia)

Drugs, chemicals, toxins, and estrogen

Antineoplastic and immunosuppressive drugs, such as doxorubicin, cyclophosphamide, cytosine arabinoside, vincristine, hydroxyurea, and azathioprine, probably are the most commonly used agents that cause reversible stem cell damage in dogs. These drugs are used for brief periods of time, however, and usually result in a neutropenia and thrombocytopenia rather than a significant nonregenerative anemia. Drugs that have been associated with stem cell injury in animals include estrogen (dogs and ferrets), phenylbutazone (dogs and possibly horses), meclofenamic acid (dogs), griseofulvin (cats), phenobarbital (dogs), phenytoin (dogs), colchicine (dogs), azidothymidine (a reverse transcriptase inhibitor; cats), chloramphenicol (dogs and cats), thiacetarsamide (dogs), and albendazole (a broad-spectrum anthelmintic; dogs and cats). Some drugs may induce stem cell destruction by immune-mediated mechanisms. In dogs, trimethoprim-sulfadiazine, cephalosporin, and phenobarbital have been associated with pancytopenia that may be immune-mediated. Drug-induced immune-mediated stem

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Table 7.1 Drugs, chemicals, plants, and hormones associated with nonregenerative anemia in domestic animals.

Dogs
Albendazole
Estrogen
Cephalosporins
Chemotherapeutic agents
Colchicine
Meclofenamic acid
Phenobarbital
Phenylbutazone
Phenytoin
Quinidine
Thiacetarsamide
Cats
Albendazole
Azidothymidine
Griseofulvin
Cattle
Bracken fern
Mycotoxins
Trichlorethylene
Horses
Mycotoxins
Phenylbutazone

cell injury usually responds to discontinuation of the drug. Idiopathic immune-mediated stem cell injury often responds to immunosuppressive therapy, but these injuries may take several weeks to respond and often require long-term treatment for resolution. Table 7.1 summarizes drugs and chemicals that may cause aplastic anemia in domestic animals.

Estrogen toxicosis may occur in bitches given exogenous estrogen for mismating, termination of pseudopregnancy, or urinary incontinence. Myelosuppression may result from the administration of excessive amounts of estrogen or from an idiosyncratic sensitivity to estrogen. Endogenous estrogen, resulting either from Sertoli cell tumors in male dogs or from cystic ovaries or granulosa cell tumors in female dogs, also may result in bone marrow suppression. Because ferrets are induced ovulators, marrow suppression from endogenous estrogen is a common—and potentially fatal—disorder in this species. The mechanism of estrogen toxicosis is unclear, but it is thought to result from the secretion (by thymic stromal cells) of an estrogen-induced substance that inhibits stem cells. Marrow suppression is preceded by an initial thrombocytosis and neutrophilia.

Aplastic anemia in cattle has been associated with grazing on bracken fern and ingestion of soybean meal contaminated with the solvent trichloroethylene. Benzene, a commonly used solvent, may cause aplastic anemia as well as leukemia. Mycotoxins have been associated with bone marrow suppression in horses and cattle, and experimental aflatoxin B_1 toxicity has been reported to cause aplastic anemia in pigs.

Infectious agents

Feline leukemia virus can result in anemia by many mechanisms, one of which is induction of aplastic anemia. In addition, FeLV is associated with anemia that manifests as pure red cell aplasia or hypoplasia, myeloproliferative disorders (see Chapter 15), anemia of inflammatory disease, and hemolysis. Hemolytic anemias that may be associated with FeLV infection include Heinz-body anemia, immune-mediated hemolytic anemia, and feline infectious anemia (see Chapter 8). Before widespread use of the FeLV vaccine, approximately 70% of anemic cats were infected with FeLV. Anemia caused by FeLV often is macrocytic, or a subpopulation of the erythrocytes is macrocytic in the absence of reticulocytosis. This may be caused by prolonged dysplastic erythrocyte production resulting from FeLV-induced myelodysplasia (see Chapter 15).

Ehrlichia canis may result in pancytopenia by two mechanisms: immune-mediated destruction of circulating cells, or aplastic anemia (which also may be an immune-mediated mechanism). In addition, dogs with ehrlichiosis may present with only one decreased cell line (e.g., thrombocytopenia), may have a lymphocytosis, and commonly have hyperglobulinemia. The organism rarely is seen on blood films.

Equine infectious anemia virus (a lentivirus) causes anemia by a number of mechanisms, one of which is bone marrow suppression (possibly immune mediated). Parvovirus infection in dogs and cats causes acute bone marrow necrosis, but these animals usually recover or die before the anemia becomes significant.

Pure red cell aplasia

Pure red cell aplasia is characterized by a markedly decreased concentration of erythroid precursors in the bone marrow in the face of normal granulopoiesis and thrombopoiesis, resulting in a severe nonregenerative anemia with normal neutrophil and platelet concentrations. In dogs, pure red cell aplasia almost always is caused by immune-mediated destruction of erythroid precursors, and it often responds to immunosuppressive therapy. Spherocytes and agglutination may be present, and approximately half the affected dogs are Coombs' positive. Bone marrow examination usually reveals an apparent arrest at some stage of erythroid precursor maturation, ranging from the rubriblast to the metarubricyte stage. Phagocytosis of rubricytes or metarubricytes may be seen. Occasionally, however, erythroid precursors are completely absent.

Some dogs and horses treated with recombinant human erythropoietin developed an immune response against the recombinant as well as endogenous erythropoietin, resulting in a reversible pure red cell aplasia. Recombinant, species-specific erythropoietin does not produce this syndrome.

Finally, certain strains of FeLV virus (subgroup C) cause pure red cell aplasia.

Red cell hypoplasia

Nonregenerative anemia may result from abnormalities that are extrinsic to the marrow, including anemia of inflammatory disease, anemia of chronic renal failure, and anemia associated with endocrine disease, and rarely, anemia associated with nutritional deficiencies. Other laboratory findings, such as an inflammatory leukogram, azotemia, other biochemical profile abnormalities or endocrine panel abnormalities, usually are key to establishing the diagnosis of these types of anemias.

Anemia of inflammatory disease

Anemia of inflammatory disease (anemia of chronic disease) is probably the most common anemia in domestic animals, but it usually is mild and clinically insignificant. This type of anemia is associated with various types of inflammatory processes, including infections, trauma, and neoplasia, and usually is mild to moderate, nonregenerative, and usually normocytic in domestic animals. The pathogenesis of anemia of inflammatory disease is multifactorial, including changes in iron homeostasis, altered proliferation of erythroid progenitor cells and production of erythropoietin, and decreased RBC life span. Immune stimulation results in activation of T cells and monocytes that produce cytokines, such as interferon-γ (IFN-γ), tumor necrosis factor-α (TNF-α), interleukin (IL)-1, IL-6, and IL-10 that affect iron metabolism.

Lipopolysaccharide (LPS) and IL-6 induce hepatic production of hepcidin, which regulates iron homeostasis by repressing intestinal iron absorption as well as iron release from ferritin stores and by mediating other regulators of iron. Specifically, hepcidin inactivates ferroportin, which is responsible for transporting iron out of cells, and LPS can also down-regulate divalent metal transporter 1 (DMT1) and ferroportin expression. These events result in inhibition of duodenal iron absorption and also decrease iron release from stores in macrophages and hepatocytes. Moreover, inflammatory cytokines up-regulate DMT1 expression on macrophages with a resultant increased uptake of iron into these cells. Additionally, IL-10 increases transferrin receptor expression, resulting in increased uptake of iron into cells, and TNF-α, IL-1, IL-6, and IL-10 also up-regulate ferritin expression, promoting intracellular storage and retention of iron. The combined effect of these changes is a relative iron deficiency in both the transport and functional pools, which limits availability of iron for erythropoiesis (see more on iron metabolism under iron deficiency anemia in Chapter 8).

Laboratory findings include a decreased serum iron concentration, normal or decreased total iron-binding capacity, normal or increased serum ferritin, and normal or increased stainable iron stores in the bone marrow. An inflammatory leukogram commonly is present as well. Occasionally animals may have a microcytic anemia, which makes anemia of inflammatory disease difficult to distinguish from iron-deficiency anemia; in these cases, serum ferritin or bone marrow stainable iron must be used to differentiate the two disorders.

A decreased serum iron concentration presumably is advantageous to patients with inflammatory disease, because it reduces the availability of iron for bacterial growth. Diagnosis may be difficult, but assays for hepcidin concentrations are being developed. Treatment is aimed at alleviating the underlying disease. Parenteral iron supplementation may have some benefit, and treatment with recombinant erythropoietin may result in an increased hematocrit. Inhibitors of hepcidin and inflammatory modulators show promise for the future.

Anemia of chronic renal failure

Anemia associated with chronic renal failure usually is moderate to severe, nonregenerative, and normocytic. The severity of the anemia correlates with the severity of the renal failure as evidenced by the degree of azotemia. The primary cause for this anemia is lack of production of erythropoietin by the kidney, and treatment with recombinant canine erythropoietin effectively increases the hematocrit. Other factors, such as increased bleeding tendencies, also may play a role in this type of anemia but likely are comparatively minor in importance. Increases in serum parathyroid hormone and phosphorus concentrations and increased erythrocyte osmotic fragility have not been found to correlate significantly with the degree of anemia. Some patients with anemia of renal disease have concurrent anemia of inflammatory disease.

Anemia associated with endocrine disease

Hypothyroid dogs almost always have a mild, nonregenerative, normocytic anemia, usually with a hematocrit of approximately 30%. This anemia responds to therapy for hypothyroidism and may simply be a manifestation of the lowered metabolic rate. Some dogs with hypoadrenocorticism, particularly those with glucocorticoid deficiency, have a mild, nonregenerative, normocytic anemia that often is masked by dehydration.

Anemia associated with nutritional deficiencies

Iron-deficiency anemia is the most common anemia associated with a nutritional deficiency. This type of anemia

usually is regenerative (unless complicated by anemia of inflammatory disease) and is discussed in Chapter 8. Other types of anemia related to nutritional deficiency are diagnosed very infrequently.

Cobalamin deficiency is observed in dogs and cats as a result of a hereditary absence of intrinsic factor cobalamin receptors in ileal enterocytes, which is inherited as an autosomal recessive trait. This anemia is nonregenerative and usually normocytic (unlike the human counterpart, which is macrocytic) and has been reported in border collies, a beagle, giant schnauzers, and cats. Affected puppies fail to thrive. Other findings include neutropenia with hypersegmentation, anemia with anisocytosis and poikilocytosis, megaloblastic changes of the bone marrow, decreased serum cobalamin concentrations, methylmalonic aciduria, and homocystinemia. Parenteral, but not oral, cyanocobalamin administration eliminates all abnormalities except the decreased serum cobalamin concentration. Chinese Shar Peis have a high prevalence of cobalamin deficiency compared to other breeds and healthy Shar Peis may have subclinical cobalamin deficiency. The disorder is suspected to be hereditary; the hematologic findings have not been reported to date.

Hematologic findings in animals with acquired cobalamin deficiency as a result of gastrointestinal or pancreatic disease have not been well characterized, although some animals with acquired cobalamin deficiency have been reported to have a mild normocytic anemia. Some cats with hyperthyroidism are hypocobalaminemic but not anemic. Cobalt deficiency in ruminants results in a normocytic, nonregenerative anemia and is caused by grazing on cobalt-deficient soil. Cobalt is required for synthesis of cobalamin by rumen bacteria.

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8 Regenerative Anemia

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The term "regenerative anemia" implies that the bone marrow is attempting to compensate for the anemia by increased erythrocyte production, as well as early release of immature red cells. Indications that the anemia is regenerative are increased polychromasia on the Wright's stained blood film, and increased reticulocyte concentration (other than in equine species, which do not release many immature erythrocytes). Mean cell volume (MCV) may be increased, but is a less reliable indication of early release of cells than is the presence of reticulocytes or polycromasia. Regenerative anemia is caused by either blood loss or blood destruction or may be seen in the recovery phase of marrow dysfunction. Blood loss may be external or internal, and may be acute or chronic. Causes of acute blood loss include trauma; bleeding lesions, such as tumors or large ulcers; and hemostatic disorders. Examples of hemostatic disorders include thrombocytopenia, inherited coagulopathies, and acquired coagulopathies, such as warfarin toxicosis or disseminated vascular coagulopathy. Common causes of chronic blood loss include bleeding lesions, particularly within the gastrointestinal tract, and gastrointestinal or external parasites.

Blood destruction (hemolysis) may be either intravascular or extravascular, and may be due to intrinsic (primary) defects, such as hereditary membrane defects or enzyme deficiencies, or extrinsic (secondary) causes, such as erythrocyte parasites or immune-mediated destruction. Intravascular hemolysis is the actual lysis of erythrocytes within the vascular system. Extravascular hemolysis occurs when abnormal erythrocytes are phagocytized by macrophages, usually within the spleen or liver. Common causes of erythrocyte destruction include immune mediated mechanisms, erythrocyte parasites, and drugs and chemicals that produce oxidative damage resulting in Heinz body formation. Less common causes include hypophosphophatemia, water intoxication in young ruminants, bacteria (*Leptospira*, *Clostridium*), heparin overdose, and hereditary erythrocyte enzyme deficiencies and membrane defects.

Blood loss

If blood is lost outside of the body, including loss into GI tract, components of the blood such as iron and plasma protein are lost. On the other hand, if bleeding occurs within a body cavity, the protein is reabsorbed within hours, and most of the erythrocytes are reabsorbed by lymphatics within a few days. The remaining cells are lysed or phagocytized, and iron is reutilized.

Acute blood loss

If blood loss is acute, the PCV initially remains normal because both cells and plasma are lost. However, within a few hours the PCV and plasma protein decrease as a result of dilution, as interstitial fluid is added to blood. By 72 hours postbleed, polychromatophilic erythrocytes (reticulocytes) should begin to appear in blood, and their concentration usually peaks within approximately one week. Plasma protein should return to normal within about one week, unless blood loss is recurrent or ongoing. Examples of disorders causing acute blood loss include trauma and surgical procedures, coagulation disorders, thrombocytopenia, and bleeding tumors.

Thrombocytopenia may result in bleeding when the platelet concentration is less than 25,000/μL; blood loss does not cause platelet concentrations to drop below 10,000/μL. Platelet concentration can usually be estimated from the blood film. The combination of reticulocytosis (or increased polychromasia) and hypoproteinemia is indicative of blood loss anemia, unless hypoproteinemia is coincidental to a regenerative anemia. Causes of hypoproteinemia other than blood loss include decreased intake (malabsorption,

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maldigestion, starvation), decreased production (liver failure), or other types of protein loss (glomerulonephropathy, protein losing enteropathy).

Blood loss outside of the body is usually easy to diagnose, since the source of blood loss is usually apparent, unless it is being lost via the gastrointestinal tract. Blood loss within a body cavity is more difficult to diagnose, and thoracic or abdominal fluid evaluation may be necessary to confirm the diagnosis.

Erythrocyte morphology is usually normal with acute blood loss, with the exception of blood loss from hemangiosarcoma, one of the most common tumors of middle-aged to older dogs, especially large breeds such as German shepherds and golden retrievers. Hemangiosarcomas have been reported in cats, but are rare. They are malignant vascular tumors typically found in the spleen, liver, and right atrium of the heart, and most have metastasized to the lungs or other organs by the time the diagnosis is made. Many dogs present due to acute signs associated with anemia as a result of rupture of the tumor, with blood loss into the abdominal cavity. Some affected dogs have a history of intermittent weakness, as a result of multiple events involving tumor rupturing and bleeding, followed by absorption of blood from the abdominal cavity.

Acanthocytes and schistocytes are seen in some dogs with hemangiosarcoma (Fig. 8.1); these morphologic changes are helpful in making the diagnosis (see Chapter 5), and may also be observed in the erythrocytes in blood aspirated from the abdominal cavity (Fig. 8.2). Other common laboratory findings include reticulocytosis (increased polychromasia), transient hypoproteinemia, and thrombocytopenia, usually mild to moderate, as a result of localized microangiopathy within the tumor, or disseminated intravascular coagulation. Dogs that are treated with surgical resection alone have a mean survival time of approximately 2–3 months, and dogs that are treated with a combination of surgical resection and chemotherapy have a mean survival time of approximately 4–10 months, depending on the protocol used.

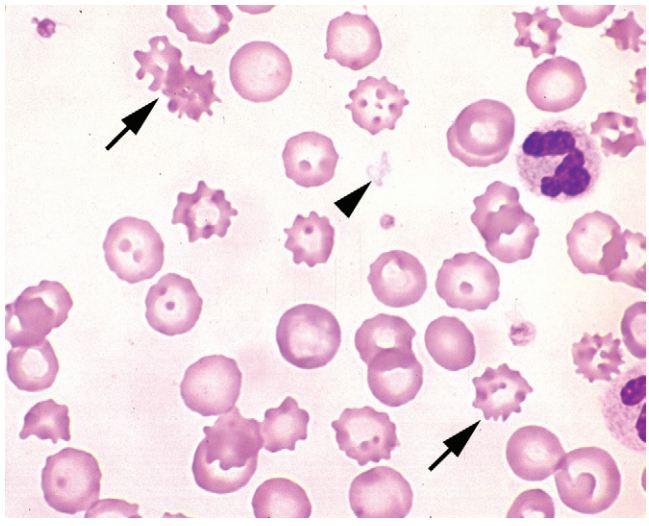

Figure 8.1 Blood film from a dog with hemangiosarcoma of the spleen. Note the acanthocytes (arrows) and schistocyte (arrowhead). Wright stain.

Chronic blood loss (iron deficiency anemia)

Chronic blood loss results in iron deficiency anemia. Iron deficiency anemia in adults is almost always due to chronic blood loss. Conversely, iron deficiency anemia commonly occurs in neonates of all domestic animal species due to inadequate iron intake, since milk contains little iron and growth rates are high. Anemia is particularly severe in baby pigs that have no access to iron-containing soil, but also occurs in kittens, puppies, foals, and calves. When blood loss is ongoing, iron stores are depleted relatively quickly. One mL of blood contains 0.5 mg of iron; normally 1 mg of iron is absorbed and excreted daily. Iron deficiency anemia is quite common in dogs, less common in ruminants, and relatively rare in cats and horses.

Gastrointestinal bleeding is the most common cause of chronic blood loss. Causes of chronic gastrointestinal blood loss include neoplasms such as leiomyomas, leiomysaromas, and carcinomas; gastrointestinal ulcers, usually as a result of the use of ulcerogenic drugs such as glucocorticoids, nonsteroidal anti-inflammatory drugs and salicylates; inflammatory bowel disease; and parasites such as hookworms. Heavy infestations of ectoparasites that utilize blood, such as fleas and some lice, can also lead to iron deficiency anemia. Overuse of blood donors may also lead to features of severe iron deficiency anemia, although the degree of anemia may

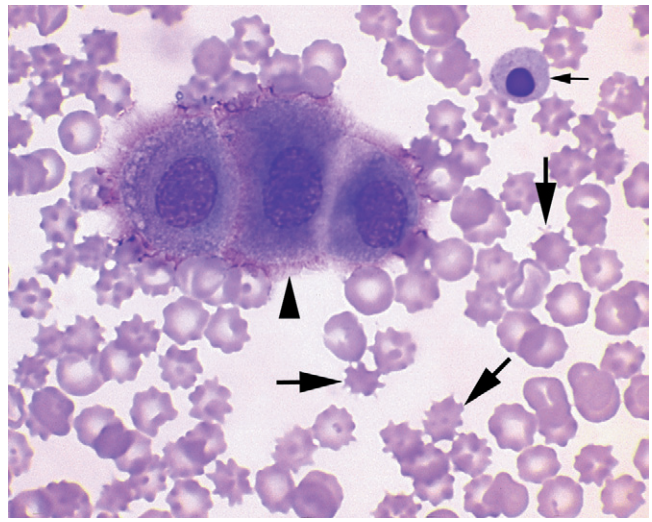

Figure 8.2 Abdominal fluid from a dog with ruptured splenic hemangiosarcoma and resultant hemoabdomen. Although morphology of erythrocytes is usually insignificant in body cavity effusions, animals with hemoabdomen resulting from hemangiosarcoma may have acanthocytes (large arrows) that are diagnostically useful. Mesothelial cells (arrowhead) and a nucleated erythrocyte are also present (small arrow). Wright stain.

be very mild. Rarely, thrombocytopenia or inherited hemostatic defects can lead to chronic blood loss. Clinical signs of chronic blood loss include those of anemia, such as pallor, lethargy, and weakness, and are somewhat variable, depending on the underlying cause of the blood loss.

Laboratory findings

The hallmark of iron deficiency anemia is a decreased MCV or a subpopulation of microcytic cells (see Chapters 1 and 6). Microcytosis occurs because erythrocyte precursors continue to divide in an attempt to reach their full hemoglobin content. Additional divisions result in smaller than normal erythrocytes. Examination of the erythrocyte histogram or computer graphic generated by the electronic cell counter is often useful, because subpopulations of microcytic erythrocytes can be observed, even when the MCV is within the reference interval (see Chapter 6). The MCV of reticulocytes is also decreased, since even immature iron deficient erythrocytes are smaller than normal. The red cell distribution width (RDW), which describes the width of the size distribution, is usually increased when subpopulations of microcytic erythrocytes are present, and will often be increased before the MCV decreases below the reference interval. Although one might expect the MCHC to be decreased in these patients, since the cells contain less hemoglobin than normal, it is commonly within the reference interval.

Reticulocyte indices can be determined with flow-cytometry type electronic cell counters. These indices, especially reticulocyte hemoglobin content (rCH) and reticulocyte volume (rMCV) are excellent indicators of iron deficiency anemia, as both are decreased quite early in patients with iron deficiency, usually before changes in conventional hematologic and biochemical indices.

Blood film examination is diagnostically useful, particularly in the late stages of iron deficiency anemia. Erythrocytes of most species, other than cats, may appear pale, with increased central pallor, and sometimes only a thin rim of hemoglobin is present (Fig. 8.3). Membrane abnormalities are common, including keratocyte and schistocyte formation, presumably due to increased susceptibility to oxidative damage (see Chapter 5). Initially the RBC develops what appears to be a blister or vacuole where inner membrane surfaces are crosslinked across the cell. These lesions subsequently enlarge, break open to form "apple-stem cells" and keratocytes, spiculated red cells with two or more pointed projections. The projections from the keratocytes then fragment from the cell, forming schistocytes. Erythrocytes are thin, and folded cells may be seen, particularly in llamas (Fig. 8.4).

The anemia is usually regenerative, but may become nonregenerative in the late stages. Occasionally, the bone marrow response may be inappropriate due to underlying anemia of inflammatory disease, since many of these animals have concurrent inflammation related to bleeding lesions.

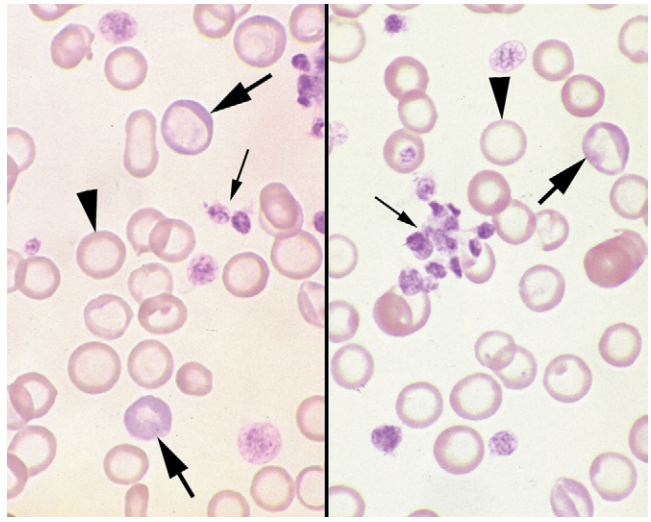

Figure 8.3 Blood film from a dog with iron deficiency anemia and hypochromic erythrocytes (arrowheads). Note the presence of polychromatophilic erythrocytes (large arrows), indicating that the anemia is regenerative. Animals with iron deficiency anemia commonly have increased platelets (small arrows), some of which may be large. Wright stain.

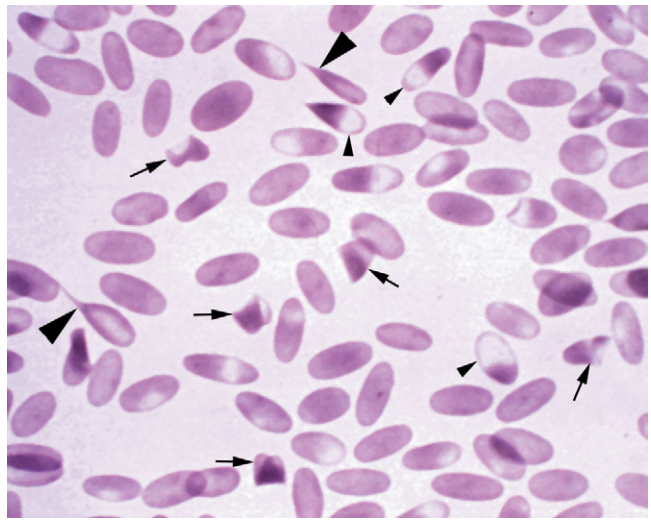

Figure 8.4 Blood film from a llama with iron deficiency anemia. Typical morphologic abnormalities associated with iron deficiency in llamas include dacryocytes (large arrowhead), folded erythrocytes (arrows), and eccentric pallor (small arrowheads). Wright stain.

Thrombocytosis is present in approximately 50% of iron deficient patients. The mechanism for the increased platelet concentration is not well understood, but may be due to increased erythropoietin or other cytokines. Approximately one-third of animals with chronic blood loss become hypoproteinemic, as protein production sometimes cannot keep pace with blood loss.

Other laboratory findings in patients with iron deficiency include decreased serum iron concentration, decreased transferrin (a glycoprotein in plasma that transports iron between compartments) saturation, and low storage iron. Total iron binding capacity, a test for measuring the amount of transferrin available to transport iron, is usually normal in iron deficient dogs and cats, although it is usually increased in other species with iron deficiency. Iron is stored as either ferritin or hemosiderin. Although ferritin is primarily an intracellular iron storage compound, it can be detected in serum. Hemosiderin, on the other hand, is insoluble, and can only be detected by staining cells and tissues. Thus, storage iron can be evaluated by measuring serum ferritin, or by examining a bone marrow aspirate and noting lack of hemosiderin in macrophages. Serum ferritin is difficult to measure, is species-specific, and since it is an acute phase reactant protein, tends to increase when inflammation or liver disease are present. Special iron stains, such as Prussian blue, are not necessary in order to visualize hemosiderin in the bone marrow (see Chapter 14). The absence of hemosiderin in feline bone marrow aspirates is not significant, since hemosiderin is rarely seen in aspirates of bone marrow from normal cats.

For practical purposes, low serum iron in a patient with a decreased MCV and anemia is usually adequate to diagnose iron deficiency anemia, and to trigger additional diagnostic procedures to determine the source of blood loss, such as testing the feces for occult blood.

Therapy

Treatment consists of finding and treating the source of blood loss. Iron supplementation with intramuscular injectable iron in iron-deficient neonates is useful, especially baby pigs, which are usually given 200 mg iron as iron dextran. Although oral iron supplementation is commonly used to treat iron deficiency, it is likely of little value, particularly in dogs and cats, because commercial pet food usually contains more iron than can be absorbed by the intestine. However, intestinal absorption of iron increases dramatically when animals are iron deficient. Oral iron should not be given to neonatal animals, especially kittens, since it can be toxic.

Differential diagnoses

Other causes of microcytosis include portosystemic shunts, which are vascular connections between the portal and systemic circulation that divert portal blood around the liver. The cause of the microcytosis in these animals is not well understood, but is associated with abnormal iron metabolism, and some of these patients may actually have iron deficiency anemia, usually as a result of gastrointestinal hemorrhaging secondary to pressure changes in the liver. The anemia, if present, is usually mild, and although serum iron may be decreased, storage iron is usually normal to slightly increased. Approximately two-thirds of dogs and one-third of cats with portosystemic shunts have microcytosis.

Animals with anemia of inflammatory disease usually have normocytic anemias, but occasionally the MCV will fall below the reference interval. While serum iron is decreased in these animals, storage iron is normal to increased.

Finally, some dogs of the Japanese Shiba and Akita breeds normally have microcytosis. These animals are not anemic, and their iron metabolism is normal.

Blood destruction (intravascular or extravascular hemolysis)

Immune-mediated hemolytic anemia

Immune-mediated hemolytic anemia (IMHA) is a consequence of increased red cell destruction, either as a result of antibody directed against erythrocytes, or immune complexes attaching to erythrocytes. Immune mediated hemolytic anemia is usually a markedly regenerative anemia, with increased polychromasia (reticulocytosis). However, in some instances, the anemia is nonregenerative as a result of antibody formation against RBC precursors, with destruction of polychromatophilic erythrocytes or earlier red cell precursors. The onset may be acute or gradual. Immune mediated hemolytic anemia is sometimes classified as primary (idiopathic), or secondary, if concurrent disease is present. However, this classification is somewhat meaningless, since "secondary" immune mediated hemolytic anemia may be coincidental to the concurrent disorder. Often the cause is never determined, but in some instances can be related to other disorders or events, such as infections, other immune-mediated disorders, modified live virus vaccination, neoplasia, particularly of the lymphoid system, bee stings, zinc toxicosis, and administration of drugs. Drugs that have been associated with IMHA are numerous and include penicillin, cephalosporins, trimethoprim-sulfamethoxazole, levamisole, and amiodarone; in these cases, immune mediated destruction occurs due to either the drug binding directly to erythrocytes (penicillin), or by the formation of drug-antibody immune complexes, which also may bind to red blood cells.

Immune mediated hemolytic anemia is the most common cause of hemolytic anemia in the dog, and has been described in horses, cattle, and cats. Breeds of dogs more commonly affected in North American include cocker spaniels, poodles, and collies, and the disorder is slightly more common in females than in males. In horses, IMHA has been associated with penicillin and other antibiotic administration, clostridial infections, and neoplasia. In cats, IMHA has been most commonly associated with *Haemobartonella felis* (*Mycoplasma haemofelis*) infection, feline leukemia virus, and lymphoproliferative and myeloproliferative disease. Immune mediated hemolytic anemia has been reported in cattle with anaplas-

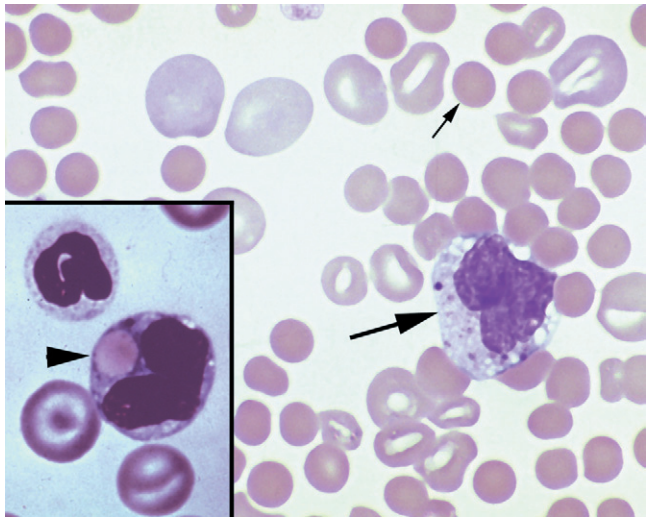

Figure 8.5 Blood film from a dog with immune mediated hemolytic anemia. Numerous spherocytes (small arrow) are present. Rarely, monocytes may be observed that contain hemosiderin (large arrow) or phagocytized erythrocytes (inset, arrowhead). Wright stain.

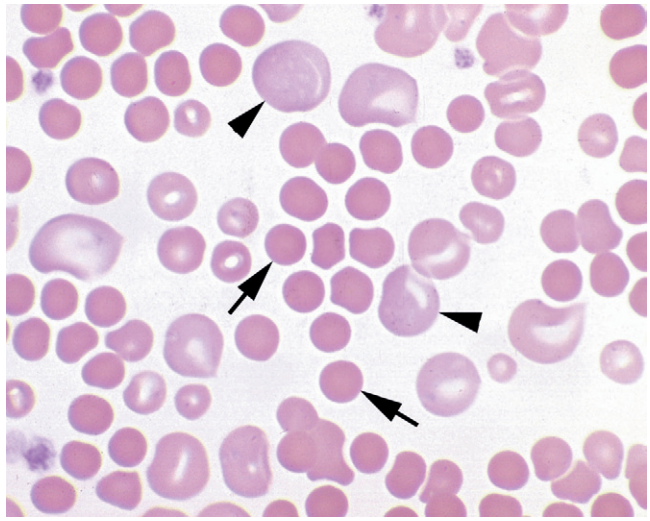

Figure 8.6 Blood film from a dog with immune mediated hemolytic anemia. The polychromatophilic erythrocytes (arrowheads) indicate that the anemia is regenerative; numerous spherocytes (arrows) are present, as is agglutination. Wright stain.

mosis, which is not surprising, since antibody is likely to be directed against the erythrocyte parasite.

Mechanisms of red cell destruction can be due to either erythrophagocytosis or intravascular hemolysis. Macrophages have receptors for antibody as well as complement (C_3b), and removal of erythrocytes by macrophages occurs in multiple organs, including the spleen, bone marrow, and liver. Rarely, monocytes that have phagocytized erythrocytes may be observed on blood films (Fig. 8.5). Partial erythrophagocytosis by macrophages results in the formation of spherocytes, the hallmark of IMHA. Spherocytes appear small, although their volume is normal, because they are sphere-shaped, lack central pallor and appear to be dense (Fig. 8.6). They have a shortened half-life because they are not as deformable as normal biconcave disk-shaped erythrocytes. They exhibit increased saline fragility, which may be diagnostically useful. Spherocytes are difficult to detect in species in which the red cells normally lack central pallor. They are, however, readily detectable in dogs, although imperfect spherocytes, which have a small amount of central pallor, are sometimes missed. If complement fixation goes to completion, resulting in membrane attack complex formation, intravascular lysis occurs. In these instances, ghost erythrocytes are occasionally observed on blood films (Fig. 8.7). Hemoglobinemia, hemoglobinuria, hyperbilirubinemia, and bilirubinuria are often present.

Antibodies associated with IMHA are usually IgG or IgM, but IgA has also been reported to bind to erythrocytes. Usually the antibody is attached to erythrocyte membrane glycoproteins. If IgM is involved, agglutination of erythrocytes can usually be observed on the blood film, and may be

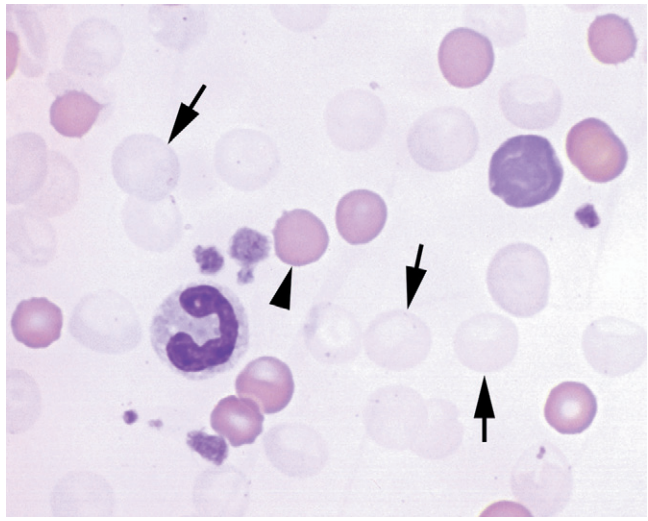

Figure 8.7 Blood film from dog with intravascular hemolysis secondary to immune mediated hemolytic anemia. Numerous spherocytes (arrowhead) and lysed "ghost" erythrocytes (arrows) are present. Wright stain.

grossly evident in the blood tube. IgG is sometimes referred to as an incomplete antibody, since it usually does not result in intravascular hemolysis or agglutination, but rather predisposes to erythrocyte phagocytosis by macrophages. The presence of antibody can be detected by performing a Coombs' test (see Chapter 1). A species-specific antiglobulin reagent (Coombs' serum) is added to a saline-washed suspension of the patient's erythrocytes. Agglutination results if

the red cells are coated with autoantibody. However, if agglutination is already present, a Coombs test is not indicated. In some cases in which agglutination is observed, the Coombs test is falsely negative, presumably because the IgM antibody is eluted from the erythrocytes during the washing process. The Coombs test was first developed for use in humans in 1945 by R.R.A. Coombs, a veterinary immunologist in the Department of Pathology at Cambridge University, who hypothesized that antibody to human globulin could be synthesized by rabbits inoculated with human globulin, and this sera could then attach to globulin binding to erythrocytes, resulting in agglutination. This test is also known as the direct antiglobulin test (DAT). The Coombs test has numerous limitations in domestic animals because of false-negative and false-positive results, both of which are common. False-negative results occur due to the following: low concentration of antibody bound to erythrocytes, improper antiglobulin to antibody ratio, not incorporating the drug that is suspected of inducing the antibody response, and improper temperature. False positive results occur when various types of disease cause immune complexes or complement to bind to erythrocytes, without resulting in anemia. False positives are particularly common in cats. Previous treatment with glucocorticosteroids may cause a negative result, and previous blood transfusion may cause a positive result. A more sensitive enzyme linked immunosorbent assay (ELISA) to detect immunoglobulins bound to erythrocytes has fewer false-negative results. However, this direct enzyme-linked antiglobulin test (DELAT) may also be falsely positive, is laborious, and not available in most laboratories. Direct immunofluorescence (DIF) flow cytometry is more sensitive (but less specific) than the Coombs test, can be used to determine the class of antibody present, detects the percentage of erythrocytes bound with antibody, and can thus be used to monitor response to therapy.

Antibodies against erythrocytes are sometimes classified as either warm, which is common, or cold reactive, which is rare. Warm antibodies react most strongly at body temperature, and cold antibodies react more strongly at cold temperatures. Cold agglutinin disease may result in red blood cell agglutination in distal extremities such as the tips of the ear pinnae, tail tip, nose, and digits, with subsequent obstruction of small vessels and necrosis. Hemolytic anemia is sometimes associated with this syndrome, which has been described in the dog and cat.

Clinical signs and laboratory findings

Clinical signs are variable and often include lethargy, splenomegaly, fever, and icterus, as well as other general signs associated with anemia, such as pale mucous membranes, dyspnea, tachycardia, and systolic heart murmur if the anemia is severe. If the anemia is acute, animals may present in a state of collapse, whereas animals with a more chronic onset may accommodate to the anemia, and show much less severe clinical signs.

Laboratory findings vary, but always include a decreased packed cell volume, red blood cell count, and hemoglobin concentration. If intravascular hemolysis is present, hemoglobinemia, hemoglobinuria, hyperbilirubinemia, and bilirubinuria may be present. Additionally, the hemoglobin concentration may be falsely increased relative to the packed cell volume, thus falsely increasing the MCHC. Blood film examination almost always reveals spherocytosis, which is the most diagnostically useful laboratory finding in these patients.

Agglutination may be present, and platelet concentration is commonly decreased because of concurrent immune mediated destruction (Evans Syndrome) or secondary disseminated intravascular coagulopathy (DIC). Agglutination may be differentiated from rouleaux formation by mixing a small quantity of blood with a drop of isotonic saline; agglutination will persist in the presence of saline while rouleaux formation will disperse. Agglutination may be so marked that it can be seen grossly on the blood film or on the side of the EDTA tube. If agglutination is present, the MCV may be falsely increased, since agglutinated red cells (doublets and triplets) may be counted as large cells (see Chapter 1). The MCV may also be increased if reticulocytosis is present.

The leukogram is almost always inflammatory, with a mature neutrophilia, increased bands, and monocytosis. This inflammatory response was once thought to be due to release of colony stimulating factors from activated macrophages. More recently, the degree of neutrophilia, as well as increased immature neutrophils, has been found to correlate with the amount of tissue damage secondary to hypoxia and thromboembolic disease.

Azotemia may be present, either prerenal, or if intravascular hemolysis is severe, renal. Free hemoglobin binds to haptoglobin, but when the available haptoglobin is saturated, hemoglobinuria secondary to hemoglobinemia occurs. Acute renal failure may be due to either erythrocyte membrane antigen-antibody complex deposition or direct toxicity of free hemoglobin to renal tubular cells.

Bone marrow aspiration is usually not indicated in IMHA, but may be performed in patients in which the anemia is nonregenerative. In these cases, an apparent maturation arrest of the erythroid series, often at the rubricyte stage, may be present, presumably due to destruction of more mature forms of erythrocytes. Metarubricytes and polychromatophilic erythrocytes are often decreased to absent in the marrow from such patients, and occasionally, increased erythrophagocytosis and phagocytosis of nucleated erythrocytes may be observed.

Because both subclinical and clinical DIC are commonly associated with IMHA, other laboratory tests that may be abnormal are those that are used to diagnose DIC, including a prolonged activated partial thromboplastin time, pro-

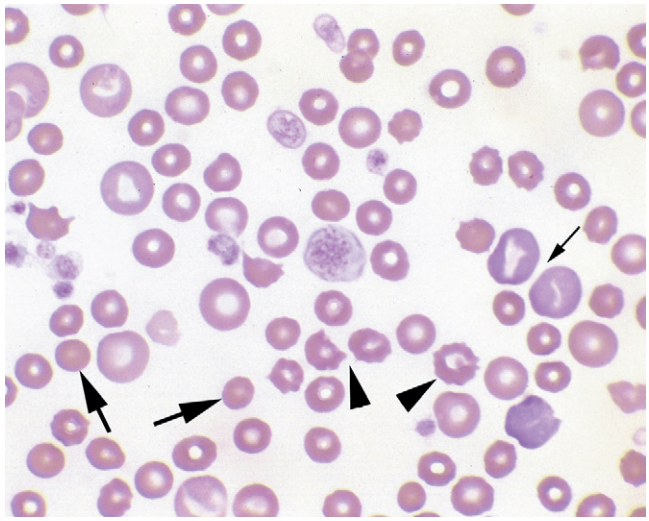

Figure 8.8 Spherocytes (large arrows) in a blood film taken from a dog several days following rattlesnake envenomation. The dog previously had echinospherocytes and some spiculated erythrocytes remain (arrowheads). The anemia is regenerative, as indicated by the polychromatophilic erythrocytes (small arrow). The dog is recovering from thrombocytopenia; a giant "young" platelet is in the center of the field. Wright stain.

longed one-stage prothrombin time, decreased antithrombin activity, increased fibrin(ogen) degradation products concentration, and increased D-dimer concentration.

Differential diagnoses

Immune mediated hemolytic anemia can usually be easily differentiated from other types of hemolytic anemia by the presence of spherocytes in IMHA. However, spherocytes occasionally may be seen in dogs with rattlesnake envenomation (Fig. 8.8). Although spheroechinocytes and type III echinocytes are seen commonly in dogs with rattlesnake envenomation (see Chapter 5), spherocytes may be present after the echinocytic changes have disappeared. It is unclear whether the rattlesnake envenomated dogs with spherocytes have immune mediated hemolytic anemia, or if the spherocyte formation is simply a result of membrane alterations secondary to the phospholipase present in the snake venom. Spherocytes, along with spheroechinocytes and type III echinocytes, may also be observed in horses with clostridial infections presumably as a result of the bacterial phospholipase hydrolyzing erythrocyte membrane phospholipids (sphingomyelin and lecithin), producing lysolecithin, an echinogenic agent. These cases may be confusing, as clostridial infections in horses have been associated with immune mediated hemolytic anemia, diagnosed by the presence of spherocytes, autoagglutination, and positive Coombs' test. However, clostridial organisms also can directly induce hemolysis through the release of toxins. It is also possible that phospholipases may be able to induce immune-mediated hemolysis, likely as a result of attachment of antibody to altered erythrocyte membranes.

Immune mediated hemolytic anemia may be mistakenly diagnosed in horses with Heinz body anemia, possibly because collapse of the erythrocyte membrane following eccentrocyte formation results in erythrocytes that appear similar to spherocytes. However, an alternative explanation is that immune mediated destruction of erythrocytes with spherocyte formation may actually occur, since Heinz body formation may result in band-3 clustering with secondary antibody attachment. Spherocyte formation in cases of bee sting envenomation may be due to mellitin, a band-3 clustering agent, or phospholipase, both of which are present in bee venom. Band-3 clustering probably plays a significant role in immune mediated destruction of erythrocytes and spherocyte formation in these patients. Spherocyte formation secondary to band-3 clustering is also seen in dogs with zinc toxicosis. Interestingly, dogs with zinc toxicosis are Coombs' negative, and it has been hypothesized that during the erythrocyte washing process, zinc is removed, band 3 is returned to a dispersed distribution, and antibodies are eluted, resulting in a negative test. Finally, animals that have had incompatible blood transfusions may develop some degree of IMHA and spherocytosis, and animals that have fragmentation of erythrocytes may have spherocytosis, as the fragments may "round-up" and appear to be small spherocytes. Spectrin deficiency has been reported in Dutch golden retrievers; some, but not all affected dogs had spherocytosis and IMHA.

Prognosis

Mortality rates vary, and are reported to range from 25% to 50%. Although some reports suggest that dogs that are autoagglutinating or have intravascular hemolysis have the highest mortality, this is controversial. Thromboembolism is a common finding in dogs that die. Recurrence of IMHA, or other immune mediated disorders such as immune mediated thrombocytopenia, is relatively common.

Therapy

Treatment of dogs consists of glucocorticosteroids (usually prednisone, 1–2 mg/kg per os every 12 hours), which decreases antibody production, T-cell activity, and diminishes macrophage function. Dexamethasone is often used in horses, and has been reported to be effective in cattle. Disadvantages of glucocorticoids include predisposing patients to infection, thromboembolic disease, and polyuria and polydipsia. Combination treatment may be warranted in dogs that are not responsive to or are intolerant of glucocorticoids. Therapeutic modalities may include azathioprine, danazol, cyclosporine, cyclophosphamide, bovine hemoglobin solution, or human immunoglobulin. However, in one retrospective study, no difference in mortality was detected between the use of multiple immunosuppressive agents and

the use of glucocorticoids alone, and in fact the risk of death was slightly lower (30%) with glucocorticoids alone than the overall mortality rate of 50%. In addition, the use of cyclophosphamide and bovine hemoglobin solution has been associated with increased risk of death, and may be contraindicated. Danzol, a synthetic androgen, and cyclosporine, an immune response inhibitor, have been reported to be of no benefit with respect to reducing mortality. Some immunosuppressive drugs, other than the glucocorticoids, may injure marrow, resulting in a transient loss of regenerative response, and some drugs may not be effectively metabolized with severe anemia, making them more toxic than usual. Fluid therapy is indicated, particularly in patients with intravascular hemolysis, and lactic acidosis secondary to anemia should be corrected. Dogs usually respond to glucocorticoid therapy within one week, although anecdotal information suggests that dogs with antibody directed against erythrocyte precursors may take longer to respond. The dosage of glucocorticoids is gradually decreased once the PCV increases, and can sometimes be discontinued two or three months after the PCV returns to normal. In some cases, however, low dose therapy (0.5 mg/kg per os every other day) with prednisone or prednisolone may be required indefinitely. Blood transfusions should be given only when absolutely necessary, due to a life-threatening anemia. Splenectomy has been thought to not be helpful long term, as removal of the spleen results in decreased erythropoietic tissue and may predispose dogs to *Mycoplasma haemocanis* infection. However, this is controversial, and in one recent study, splenectomy was thought to be associated with improved outcome in dogs.

Neonatal isoerythrolysis

Neonatal isoerythrolysis (NI) is a form of immune mediated hemolytic anemia that occurs in newborn animals secondary to maternal antibodies against the neonate's bloodgroup antigen attaching to the neonate's erythrocytes, with subsequent erythrocyte hemolysis. The maternal antibodies are usually produced after sensitization of the mother with bloodgroup-incompatible erythrocytes, usually from the blood of a previous fetus gaining access to maternal circulation, but sometimes from vaccinations that contain erythrocytes or from mismatched blood transfusions. The disorder is most common in horse and mule foals, but occurs in less than 1% of thoroughbreds. The disorder rarely occurs in puppies, kittens, piglets, and calves. Cats are unique, in that antibodies against kitten erythrocytes can be produced with no previous exposure of the queen to incompatible erythrocytes. In domestic animals, the maternal antibody gains access to the neonate's blood following ingestion of anti body containing colostrum. Hemolytic anemia has been reported in lambs fed bovine colostrum during the first few days of life, and the anemia appears to be immune-mediated.

Affected animals are normal at birth, but within 24–48 hours they become weak, lethargic, pale, and anemic, with icterus and dyspnea. Hemoglobinemia and hemoglobinuria may be present, as well as splenomegaly and hepatomegaly. Thrombocytopenia and DIC may also occur.

In foals, approximately 90% of all cases of NI are attributable to the Aa or Qa antigen, but other antigens may be involved. The occurrence in mule foals may be due to a xenoantigen. It is possible that all mule pregnancies (donkey sire × horse dam) are incompatible with regard to this factor and a potential for NI exists in all cases.

Laboratory diagnosis

Diagnosis is usually made by confirming the presence of maternal antibodies on the neonate's erythrocytes by a Coombs' or a hemolytic test. Blood from pregnant mares can be tested 2 weeks prior to foaling for the presence of antibodies in order to predict the likelihood of neonatal isoerythrolysis in the foal. If the dam is sensitized, then her colostrum can be withheld from the foal for the first 48 hours of life, substituting another mare's colostrum.

Treatment

Treatment consists of blood transfusion if the animal is severely anemic. If the mare's blood is used, the erythrocytes must be washed extensively to remove antibody-containing plasma. Glucocorticoids may be helpful in reducing the rate of clearance of antibody coated erythrocytes.

Erythrocyte parasites

Microorganisms that directly infect erythrocytes may result in intravascular hemolysis or extravascular hemolysis, and some may not cause hemolytic anemia. Traditionally, hemoparasites have been detected by examination of blood films. However, the development of highly sensitive and specific polymerase chain reaction (PCR) assays to detect small quantities of organisms has made diagnosis much more accurate for many of these diseases, in some cases even before the onset of clinical signs. The majority of the hemoparasites cause anemia by immune mediated extravascular hemolysis. Antibody against the organism, immune complexes, or complement bind to erythrocytes resulting in phagocytosis by macrophages. However, *Babesia* and *Theileria* species cause intravascular hemolysis. Specific hemoparasites are discussed below.

Hemotropic mycoplasmas

Hemotropic mycoplasmas are pleomorphic bacteria that parasitize erythrocytes of many domestic animal species. These organisms are small (approximately 0.3 µm in diameter), lack a cell wall, and stain gram-negatively. They adhere loosely to the surface of the erythrocyte membrane, and in many species, fall off easily, therefore appearing in the plasma. They were originally assigned either to the

genus *Haemobartonella* or *Eperythrozoon* on the basis of whether they occurred more commonly as "ring forms," and whether they were found free in the plasma. If they fulfilled both of the previous criteria, they were assigned to the genus *Eperythrozoon*. These characteristics are now considered insignificant. These organisms were formerly classified as rickettsia, but based on sequence analysis of the 16S rRNA gene, they have been reclassified as members of the genera *Mycoplasma*.

Three strains of the organisms previously called *Haemobartonella felis* have been recognized. The Ohio strain has been renamed *Mycoplasma haemofelis*, and the California strain has been named *Candidatus Mycoplasma haemominutum*. A third strain, *Candidatus Mycoplasma turicensis*, was originally identified in a Swiss cat, but is now known to also have a worldwide distribution. Assays based on polymerase chain reaction technology are the most sensitive and specific diagnostic tests available for these organisms. Of the feline mycoplasmas, *M. haemofelis* is the most pathogenic species, and causes hemolytic anemia in immunocompetent cats. The presence of *Candidatus Mycoplasma turicensis* and *Candidatus Mycoplasma haemominutum* is not always associated with anemia. However, *Candidatus Mycoplasma haemominutum* has been associated with fever, anorexia, lethargy, and anemia and is likely a primary pathogen. *Candidatus Mycoplasma turicensis* has reportedly not been seen by light microscopy, likely due to the small numbers of parasites present but possibly due to its smaller size (0.25 µm). It has been morphologically characterized using electron microscopy.

Haemobartonella canis has been renamed *Mycoplasma haemocanis*. *Eperythrozoon suis*, *E. wenyoni*, and *E. ovis* have been renamed *Mycoplasma haemosuis*, *M. wenyonii*, and *Candidatus Mycoplasma ovis*, respectively. The eperythrozoon in alpacas and llamas, previously not named, has been named *Candidatus Mycoplasma haemolamae*. The designation *Candidatus* is reserved for incompletely described members of taxa, to give them provisional status, and is eventually dropped.

Mycoplasma haemofelis

Mycoplasma haemofelis, formerly known as *Haemobartonella felis*, appears as small (0.3 µm) dark blue rods or ring forms on the surface of erythrocytes; it is more easily seen at the feathered edge of the blood film where the erythrocytes are flattened (Fig. 8.9). Agglutination of erythrocytes may be present, as the presence of the organism on erythrocytes results in an immune mediated hemolytic anemia. *Mycoplasma haemofelis* is quite pathogenic, and can cause severe, sometimes fatal, hemolytic anemia. It is transmitted through infected blood, presumably by blood feeding arthropods such as fleas and ticks, cat bites, and iatrogenic exposure, and is present throughout the world. The organism is also transmitted from queens to kittens, either in utero, at birth, or by nursing. The parasitemia is intermittent, making diagnosis by blood film examination sometimes difficult. A PCR assay is available that is more diagnostically sensitive than blood film examination.

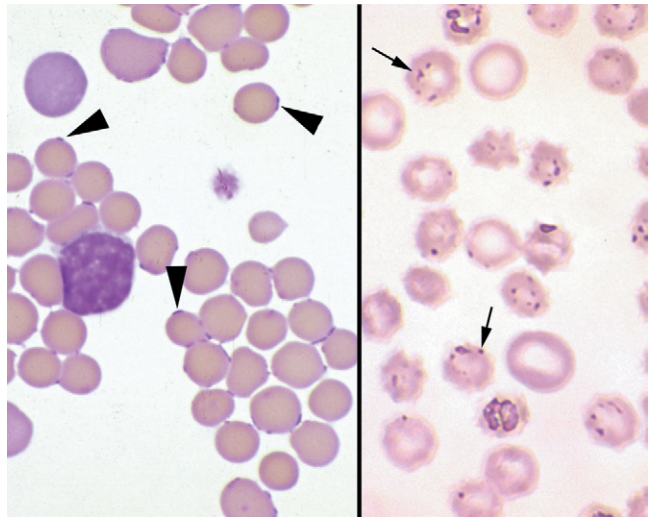

Figure 8.9 Left panel: Blood film from an anemic cat with *Mycoplasma haemofelis* (arrowheads), previously known as *Haemobartonella felis*. Right panel: Erythrocyte parasites are sometimes mistakenly diagnosed when artifacts are on erythrocytes (arrows). Artifacts may be caused by stain precipitate or staining the blood film before it is dry. Wright stain.

Clinical signs include those of anemia, splenomegaly, fever, lethargy, and sometimes icterus. Concurrent disease, immunosuppression, or splenectomy may predispose animals to acute infection. The anemia is regenerative unless underlying disease, often related to feline leukemia virus, is present that would inhibit erythropoiesis. Infected cats should be examined for the presence of feline leukemia virus and feline immunodeficiency virus.

Treatment consists of blood transfusion if the anemia is severe. Prednisone (2 mg/kg per os every 12 hours) will suppress the immune mediated destruction of erythrocytes. Doxycycline (2–5 mg/kg per os every 12 hours for three weeks) is effective against the organism, but cats that recover often become latent carriers. Toxicity of doxycycline may include fever, gastrointestinal disturbances, and rarely, esophageal stricture formation. Enrofloxacin (5–10 mg/kg per os every 24 hours) a fluoroquinolone anti-Mycoplasma antibiotic, has been shown to be effective against *Mycoplasma haemofelis*, but a rare complication is acute blindness.

Mycoplasma haemocanis

Mycoplasma haemocanis, formerly known as *Haemobartonella canis*, is an opportunistic organism, usually causing disease only in splenectomized or severely immunosuppressed dogs. It is closely related phylogenetically to *Mycoplasma haemofelis*, with 99% homology of the 16SrRNA gene. Dogs that are splenectomized develop active infections if they are transfused with infected blood, or if they have latent infections.

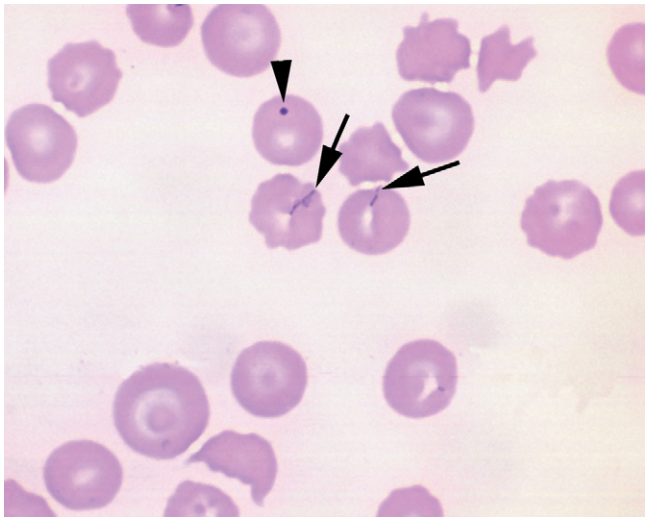

Figure 8.10 Blood film from an anemic splenectomized dog. Note the presence of *Mycoplasma haemocanis* (arrows) (previously *Haemobartonella canis*). Howell-Jolly bodies (arrowhead) are usually increased in splenectomized animals. Wright stain.

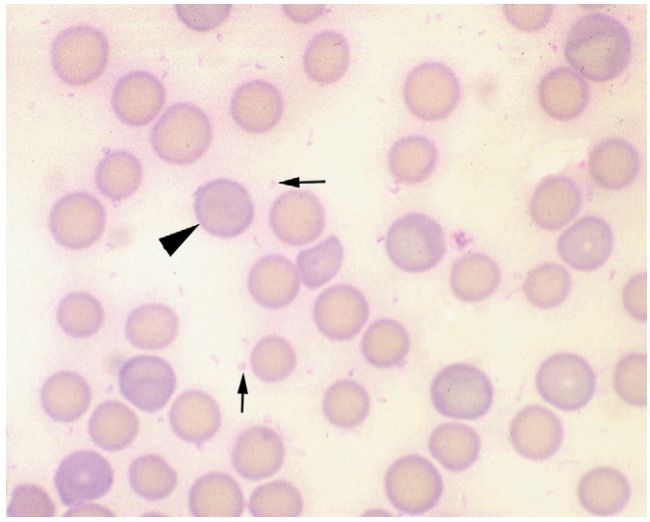

Figure 8.11 Blood film from a cow with hind limb and teat edema. Many *Mycoplasma wenyonii* (previously *Eperythrozoon wenyonii*) organisms are present in the background (small arrows). *Polychromasia* (arrowhead) is present, indicating regeneration. Wright stain.

Active infection may manifest days to weeks after splenectomy. The microorganism appears somewhat different than *Mycoplasma haemofelis*, in that they appear as small chains of cocci across the surface of the erythrocyte. The chain commonly branches, and appears Y-shaped (Fig. 8.10). Clinical signs include those of anemia, and icterus is rarely present. Treatment consists of 5 mg/kg doxycycline orally twice daily for three weeks. *Candidatus Mycoplasma haemominutum* and *Candidatus Mycoplasma haematoparvum* have been reported in dogs, as well.

Haemoplasmas of ruminants

Mycoplasma wenyonii, formerly known as *Eperythrozoon wenyonii*, also occurs worldwide, and similar to *Mycoplasma haemocanis* in dogs, usually only causes severe anemia in immunosuppressed or splenectomized cattle. The organism may be transmitted iatrogenically, by using the same syringe and needle in multiple animals in feedlot situations. Very large numbers of organisms can be seen on blood films, many of which are free in the plasma, in cattle that are not anemic (Fig. 8.11). However, a syndrome has been recognized in cattle that are heavily parasitized, which includes dependent edema and lymphadenopathy. Although the haemoplasma of sheep and goats, formerly known as *Eperythrozoon ovis* (Fig. 8.12) is generally considered nonpathogenic in adults, its role as a cause of anemia in lambs is controversial. It will be renamed *Mycoplasma ovis*.

Mycoplasma haemosuis

Mycoplasma haemosuis, formerly known as *Eperythrozoon suis*, is pathogenic in very young pigs, as well as pigs that have been splenectomized, causing severe hemolytic anemia and sometimes death. In older animals, infection is associated with poor weight gain. The organisms appear similar to those in cattle, with many free organisms present on blood films (Fig. 8.13). Baby pigs are usually treated with a single dose of long acting oxytetracycline (25 mg). Tetracycline is sometimes added to hog food to prevent the acute form of the disease.

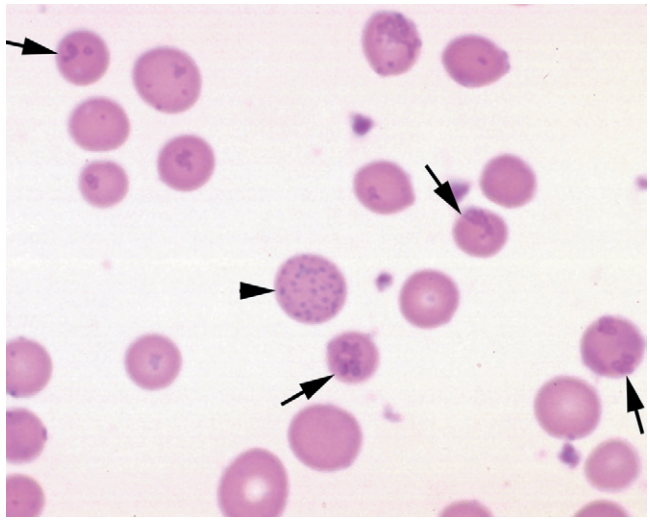

Figure 8.12 Blood film from a sheep with *Eperythrozoon ovis* (arrows). This organism will be renamed *Mycoplasma ovis*. Wright stain.

Candidatus Mycoplasma haemolamae

Haemoplasmas in llamas and alpacas appear to be opportunists that proliferate in animals doing poorly, and usually only

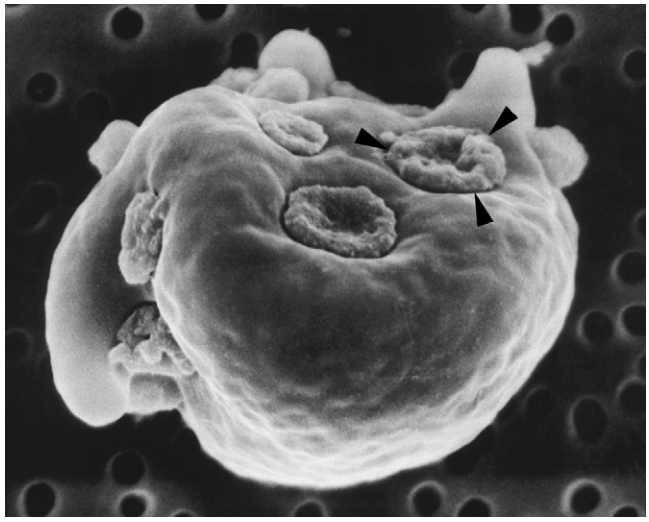

Figure 8.13 Electron micrograph of *Mycoplasma haemosuis* (arrowheads), formerly *Eperythrozoon suis*. Photograph provided by Dr. Joanne Messick.

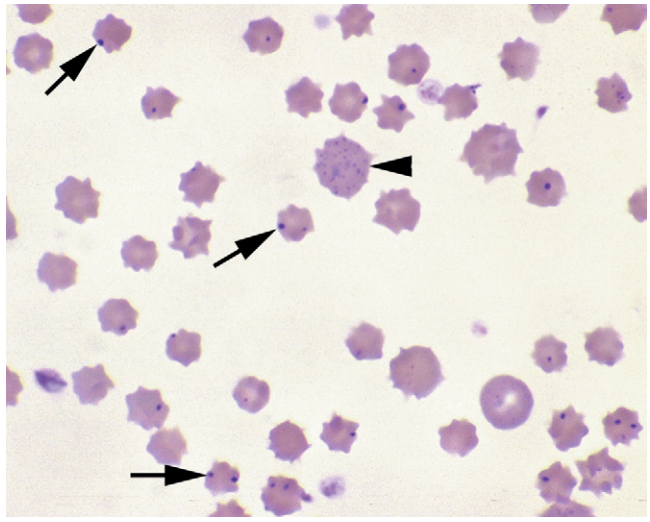

Figure 8.15 Blood film from an anemic cow with *Anaplasma marginale* (arrows). Note the basophilic stippling in the large polychromatophilic erythrocyte (arrowhead). Wright stain.

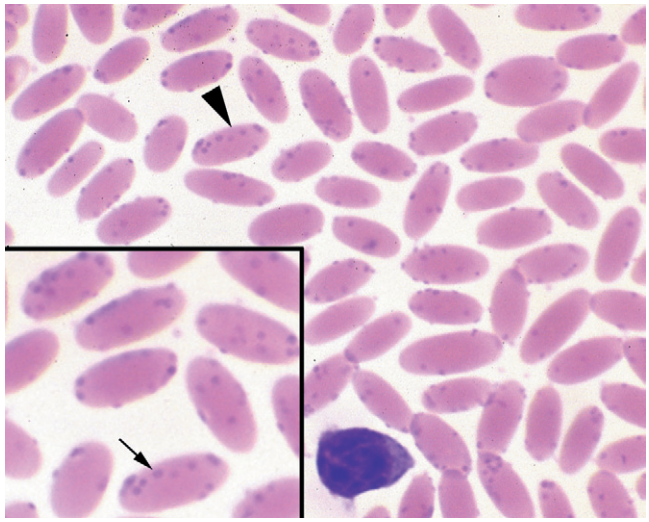

Figure 8.14 Blood film from a poor-doing llama with *Candidatus Mycoplasma haemolamae* (arrowheads), formerly *Eperythrozoon* spp. Higher magnification of the organisms (arrow) is shown in inset. Wright stain.

cause a mild anemia. The organism appears similar to that in cattle (Fig. 8.14).

Anaplasmosis

Bovine anaplasmosis caused by the intraerythrocytic rickettsia *Anaplasma marginale* is the most prevalent tickborne disease of cattle worldwide. *Anaplasma centrale* occurs in south America, the Middle East, and South Africa, and is less pathogenic. *A. marginale* has also been reported in deer, elk, and bison. *Anaplasma ovis* has been reported in goats and sheep, and causes hemolytic anemia. The organism appears similar to *A. marginale*. The organisms are transmitted by ticks, biting flies, and iatrogenically. *Anaplasma marginale* appears as a small (0.5–1 μm) dark blue inclusion on the margin of erythrocytes (Fig. 8.15). *Anaplasma centrale* appears similar, but is located in a more central appearing location on erythrocytes. Infection with the organism can cause a fatal hemolytic anemia; older animals are usually more severely affected. The mechanism of anemia may be immune mediated. Untreated cattle that survive may become chronic carriers. Diagnosis can be made by PCR assays, as well as examination of blood films. Therapy consists of long acting oxytetracycline, but the most efficient method to control anaplasmosis is by vaccination using live *Anaplasma centrale*, which is capable of inducing significant protection against the more virulent *A. marginale*. However these methods of control have numerous limitations and improved approaches are needed. Inactivated or subunit vaccines and alternative pharmacological interventions will likely be developed.

Babesiosis

Several species of babesia cause hemolytic anemia and thrombocytopenia in domestic animals. *Babesia canis* and *B. gibsoni* are pathogenic in dogs, *B. bovis* and *B. bigemina* as well as other less important babesia infect cattle, *B. equi* and *B. caballi* occur in horses, *B. ovis* and *B. motasi* infect sheep, and *B. cati*, *B. felis*, *B. herpailuri*, and *B. pantherae* infect cats. The disease is usually referred to as piroplasmosis in horses. *Babesia* are hemoprotozoan organisms, many of which are in the process of being reclassified based on PCR assays and gene sequencing. Some organisms previously thought to be

babesia appear to be more closely related to *Theileria* spp., including the California isolate of *Babesia gibsoni*, and *B. equi*. Babesia are transmitted by various types of ticks, most cause intravascular and extravascular hemolysis, and pathogenicity is variable. Other mechanisms of transmission include trans placental transmission and blood contamination. *Babesia* spp. vary in appearance; both large forms and small forms have been described. Large forms of babesia include *B. canis*, *B. caballi*, and *B. bigemina*. The other babesia are small forms. Large forms (2–5 µm) appear as single, paired, or tetrad oval inclusions that stain lightly basophilic with an eccentric nucleus (Fig. 8.16). Small forms (1–3 µm) of babesia appear as round organisms (Figs. 8.17 and 8.18). Usually only a few erythrocytes on blood films contain organisms, and they tend to be concentrated at the feathered edge of the blood film.

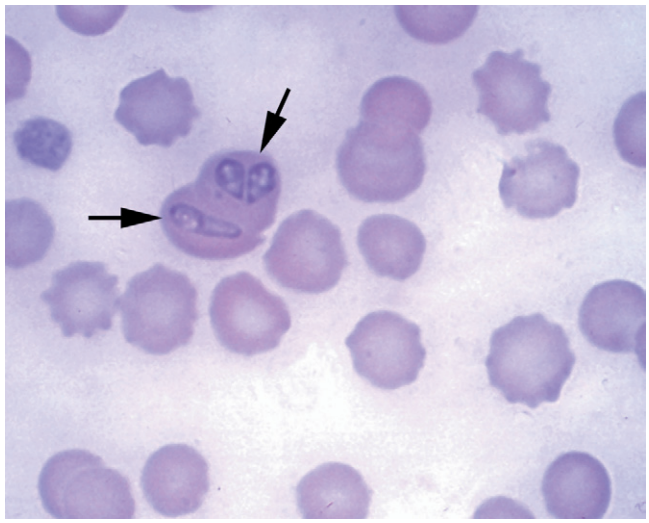

Figure 8.16 Blood film from an anemic dog with *Babesia canis* (arrows). Wright stain.

Canine babesiosis is becoming more common in the United States. *B. canis vogeli* is endemic in the southeastern United States, particularly in greyhounds, but it usually only causes severe hemolytic anemia and life-threatening disease in young dogs or dogs that are heavily parasitized. Another subspecies, *B. canis rossi*, is more pathogenic, and is in South Africa. A third subspecies, *B. canis canis*, is found in Europe and parts of Asia, and is intermediate in pathogenicity. *B. gibsoni* is endemic in northern Africa, the Middle East, southern Asia, and parts of the Caribbean, and is increasingly observed in the United States, particularly in the Southeast and Midwest. A small babesia, originally thought to be *B. gibsoni*, was described in California dogs in 1991. This organism causes severe disease, including hemolytic anemia, icterus, vasculitis, thrombocytopenia, hepatitis, glomerulonephritis, and reactive lymphadenopathy.

Since 1999 *B. gibsoni* has been reported in numerous states east of the Mississippi River. At least some of these organisms have been distinct from the California organism. The disease is primarily seen in American pit bull terriers and Staffordshire terriers. Many dogs survive the acute phase and become chronic carriers. Prevention includes aggressive tick control. The high prevalence in the pit bull breed is now known to be due to direct blood transmission. Babesiosis may be diagnosed by blood film or buffy coat film examination, but PCR is much more sensitive and specific. Because most dogs are Coombs' positive, and many exhibit erythrocyte agglutination, a differential diagnosis is IMHA. Hyperglobulinemia, thrombocytopenia, and neutropenia are commonly observed, therefore ehrlichiosis must also be con-

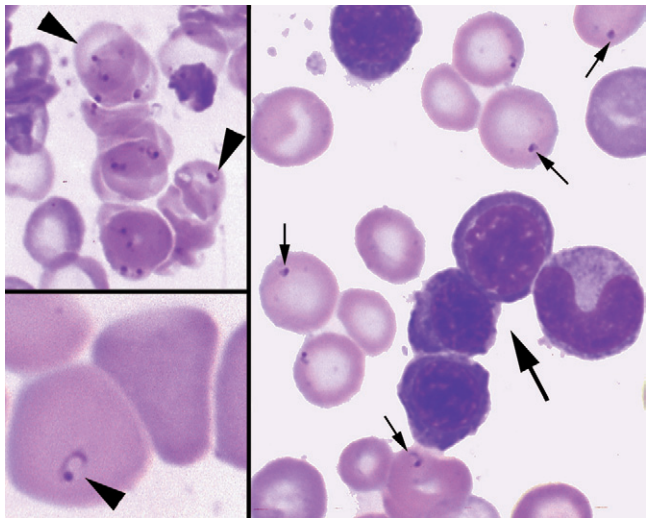

Figure 8.17 *Babesia gibsoni* in a bone marrow aspirate from a severely anemic pit bull terrier from Kentucky. Aspirate provided by Antech Diagnostics, Inc. Wright stain.

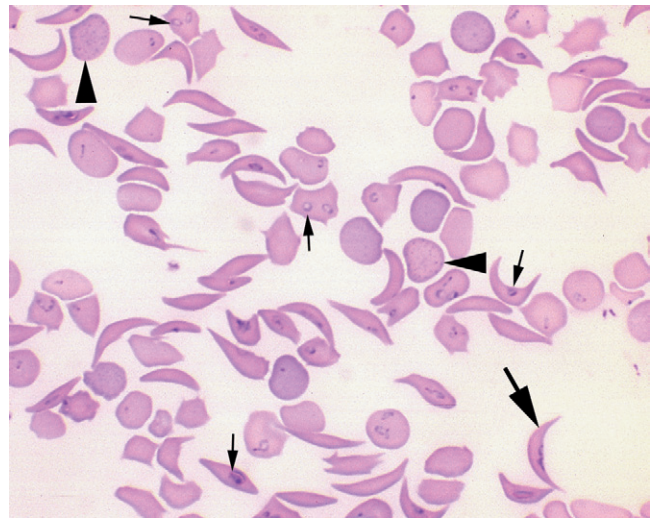

Figure 8.18 Babesia organisms in a deer (small arrows). Note that the erythrocytes have become sickle-shaped, which occurs in vitro (large arrow). Basophilic stippling is also present (arrowhead). Wright stain.

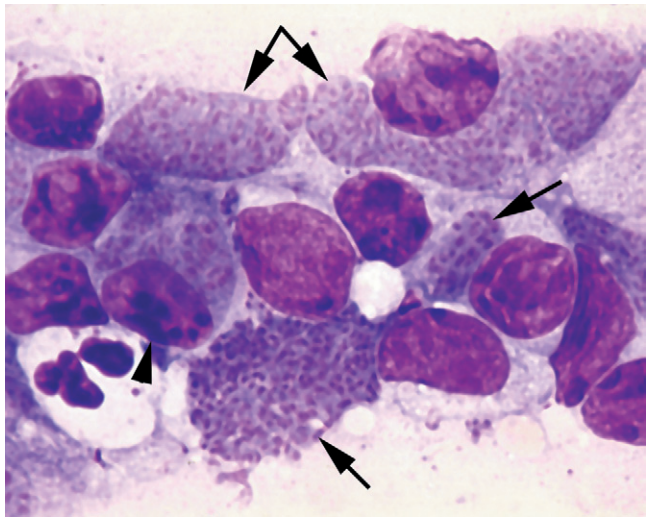

Figure 8.19 Lymph node aspirate from a cow with theileriosis. Lymphocytes are filled with schizonts (arrows). Wright stain.

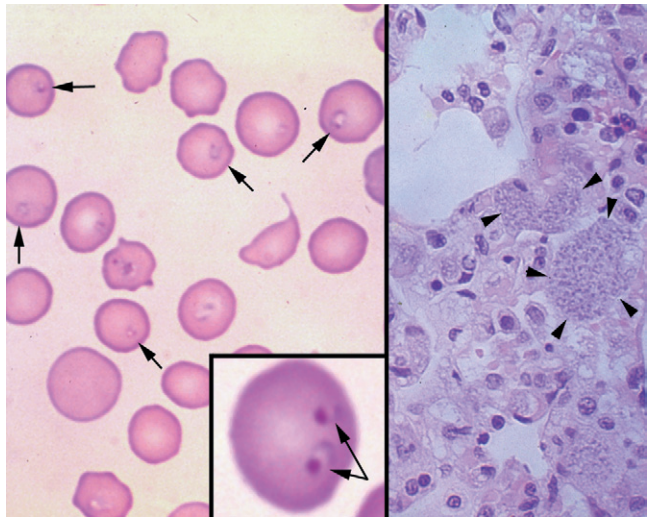

Figure 8.20 Left panel: Feline blood film with *Cytauxzoon* piroplasms in erythrocytes (arrows). Wright stain. Right panel: *Cytauxzoon* schizonts in macrophages of the same cat. H & E stain.

sidered as a differential diagnosis, as these are common laboratory findings in that disease as well.

Treatment consists of imidocarb dipropionate (Imizol, Schering-Plough, Union, New Jersey). The recommended dosage is 6.6 mg/kg IM, repeated in two weeks. Diminazine aceturate is also effective, but is not available in the United States. Most dogs remain chronic carriers after therapy.

Theileriosis

Theileria parva, the cause of East Coast fever in Africa, and *T. annulata* are protozoans that may cause hemolytic anemia in cattle. The organisms are transmitted by ticks. Lymphocytes are first infected by sporozoites, which form schizonts (Fig. 8.19) from which the merozoites are released that infect erythrocytes. The organisms are small (1 µm) and appear signet-ring or comma shaped. *Theileria lestoquardi* causes hemolytic anemia in sheep and goats of southern Europe, the Middle East, and northern Africa. Other, less pathogenic, species of *Theileria* may infect cattle, deer, and elk in North America.

Feline cytauxzoonosis

Cytauxzoon felis is a protozoan that is classified within the same family as theileria. Like theileria, merozoites (piroplasms) infect erythrocytes, while a tissue phase, the schizonts, infect and fill macrophages within and surrounding blood vessels throughout the body. The disease was first described in 1948 in African ungulates, and was initially reported in cats from Missouri in 1976.

The disease is usually fatal, resulting in thrombosis of numerous vessels as a result of distended macrophages occluding vessels. Clinical findings include acute lethargy, anorexia, fever, and icterus. Although the organism causes a hemolytic anemia, the anemia is often nonregenerative, and may be accompanied by leukopenia and thrombocytopenia. Diagnosis is made by finding the signet-ring shaped piroplasms in erythrocytes in blood films relatively late in the course of the disease or by finding the schizonts in macrophages by cytologic or histopathologic examination of spleen, liver, lymph node or bone marrow (Fig. 8.20), or by PCR assay, which is very sensitive and specific. Several cats have survived *C. felis* infection; these cats were from the same geographic area and may have been infected with a less virulent strain.

The organism is transmitted by ticks; although erythroparasitemia may occur following blood inoculation, the tissue phase of the organism and disease do not develop. Bobcats, panthers, and cougars, which serve as natural reservoirs, usually have persistent asymptomatic infections, although bobcats occasionally have fatal disease. Fatal cytauxzoonosis has also been described in a Bengal tiger and white tiger. Antiprotozoal drugs such as dipropionate (Imizol) and diminazine aceturate (Ganaseg, Berenil) are occasionally effective against the organism.

Heinz body anemia

Erythrocytes are particularly susceptible to oxidative damage, both because they carry oxygen and because they may be exposed to various chemicals in plasma. Oxidants that are constantly generated include hydrogen peroxide (H_2O_2), superoxide free radical (O_2^-) and hydroxyl radicals (OH). When oxyhemoglobin is converted to methemoglobin (ferric state to ferrous state), superoxide radicals react with hydrogen peroxide, producing hydroxyl radicals. Formation of reversible and irreversible hemichromes then occurs. Reversible

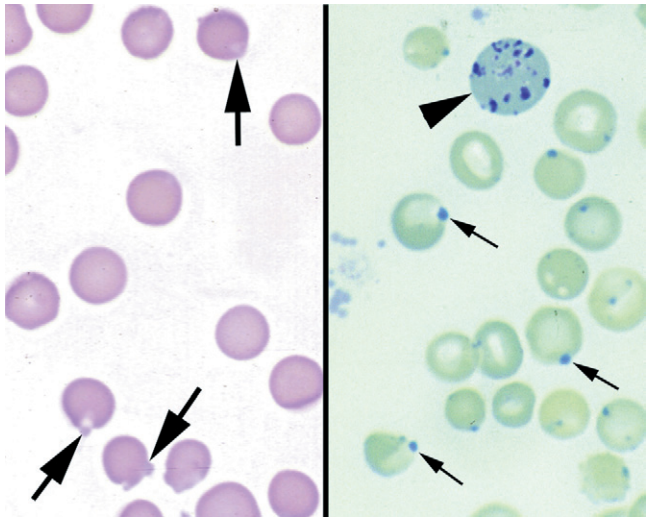

Figure 8.21 Left panel. Blood film from a cat with Heinz body anemia. Heinz bodies appear pale and are more apparent when they protrude from the edges of the erythrocytes (arrows). Wright stain. Right panel: Brilliant cresyl blue-stained blood film. Heinz bodies appear as medium-blue structures on the edges of the erythrocytes (arrows). A reticulocyte is also present (arrowhead).

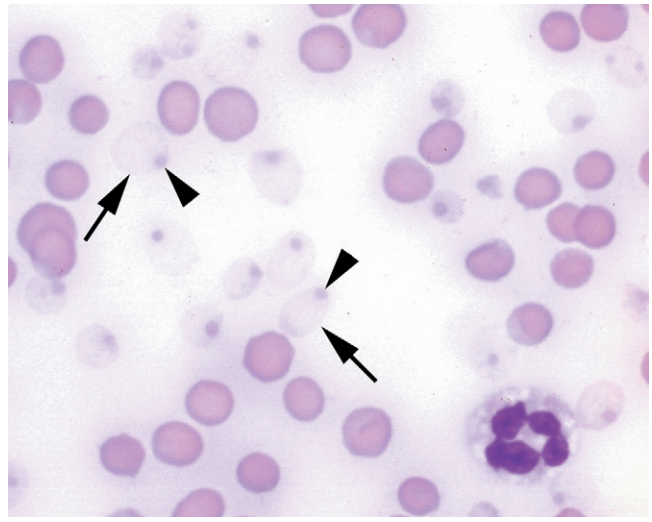

Figure 8.22 Blood film from an anemic cat with acetaminophen toxicosis. Note the lysed "ghost" erythrocytes (arrows). The Heinz bodies (arrowheads) are very apparent in the ghost cells. The pink background is due to hemoglobinemia. Wright stain.

hemichromes include hemoglobin hydroxide and dihistidine ferrihemochrome. These reversible hemichromes can be converted back to methemoglobin and reduced hemoglobin. If irreversible hemichromes are formed, the hemoglobin denaturation continues, and aggregates of irreversible hemichromes are formed. These aggregates are called Heinz bodies, first recognized by Heinz in 1890 in humans and animals exposed to coal-tar drugs. Heinz bodies appear as small eccentric pale structures within the red cell and may protrude slightly from the red cell margin on Wright's stained blood films (Fig. 8.21). They are usually large and single in cat erythrocytes (Fig. 8.22), and small and multiple in dogs. When stained with vital stains such as new methylene blue or brilliant cresyl blue, Heinz bodies appear as blue structures (see Chapter 5).

The sulfhydryl groups on the globin portion of the molecule are also susceptible to oxidative damage, and although Heinz bodies may form by oxidation of these sulfhydryl groups, hemichrome formation is likely more important. Hemichromes have an affinity for membrane protein band 3. The protein band 3-hemichrome complex causes membrane protein band 3 to form clusters, both on the inside and outside of the erythrocyte membrane. This external clustering of protein band 3 creates a recognition site for autoantibodies. Erythrocytes with attached antibody are then phagocytized by macrophages. The clustering of protein band 3 and associated autoantibodies may be the best explanation for why animals with Heinz body formation may also have spherocyte formation and agglutination, such as has been described in zinc toxicosis and methylene blue toxicosis

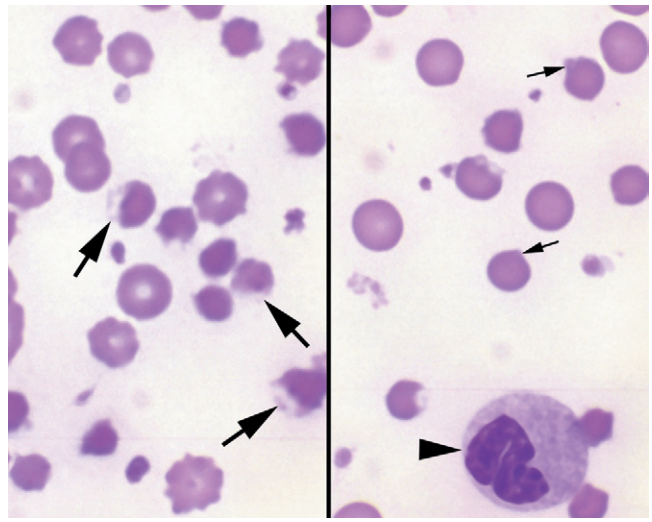

Figure 8.23 Blood film from a cow with oxidant-induced anemia. Note the eccentrocytes (large arrows), and Heinz bodies (small arrows). A neutrophil is present (arrowhead).

in dogs, and red maple leaf toxicosis in horses. Alternately, erythrocytes may have a spherocyte-like appearance because of collapse of the erythrocyte membrane following eccentrocyte formation. Some oxidants may affect the erythrocyte cytoskeleton, resulting in eccentrocyte formation without Heinz body formation. Features of eccentrocytes include shifting of hemoglobin to one side of the cell, loss of normal central pallor, and a clear zone outlined by a membrane (Fig. 8.23).

In addition to formation of protein band 3-hemichrome complexes, spectrin-hemoglobin crosslinking also occurs,

increasing erythrocyte membrane rigidity and decreasing deformability, ultimately making the erythrocyte more susceptible to removal. Heinz bodies may also be removed by the spleen, with the remaining portion of the erythrocyte returning to circulation. Hemichrome binding to the erythrocyte membrane also may stimulate proteolysis, contributing to breakdown of erythrocyte membrane integrity.

Oxidative injury occurs when enzymes and substrates used in the pathway to reverse oxidative processes are depleted, absent, or inhibited. Normally approximately three percent of the hemoglobin is oxidized to methemoglobin daily, but even that small amount is constantly being reduced back to hemoglobin by a reduced nicotinamide-adenine dinucleotide (NADH)-dependent methemoglobin reductase enzyme within erythrocytes. Methemoglobin forms at higher concentrations when oxidative compounds are increased. Other enzymes also protect against oxidative damage to erythrocytes. These include superoxide dismutase (SOD), a zinc and copper containing enzyme that converts superoxide to hydrogen peroxide and water. Nicotinamide-adenine dinucleotide phosphate (NADPH) maintains glutathione in the reduced state, and glucose–6-phosphate dehydrogenase plays an important role in the initial steps of the pathway. Glutathione has an easily oxidizable sulfhydryl group that acts as a free-radical acceptor to counteract oxidant damage. Glutathione peroxidase catalyzes the conversion of hydrogen peroxide to water, producing oxidized glutathione, which is in turn reduced by glutathione reductase. Selenium is an important component of glutathione peroxidase. Finally, catalase is an enzyme that converts hydrogen peroxide to water and O_2 and may be more important than glutathione peroxidase.

Cats are considered to be more susceptible to Heinz body formation than other domestic species for a number of reasons, including differences in their hemoglobin structure, and normal cats commonly have a small percentage of circulating erythrocytes that contain Heinz bodies. Feline hemoglobin has eight sulfhydryl groups, compared with four in dogs and two in most other species. Many causes of oxidative damage to erythrocytes resulting in Heinz body or eccentrocyte formation have been reported, including oxidant drugs and chemicals, oxidant-containing plants, inherited enzyme deficiencies, and nutritional deficiencies. Treatment depends on predisposing cause of Heinz body formation. Most of the oxidative compounds that result in Heinz body formation also cause methemoglobinemia, which when severe is characterized by brown discoloration of blood and cyanosis. These oxidants are discussed in more detail below.

Plants

Allium family (onions, chives, and garlic)

Onion, chive, and garlic ingestion may result in Heinz body anemia and eccentrocyte formation in most species of domestic animals. Sources of onions and garlic include the feeding of cull onions to cattle and sheep, ingestion of wild onions by horses, and ingestion of raw, cooked, dehydrated onions, and baby food containing onion or garlic powder, by dogs and cats. The oxidative compounds in onions and garlic are aliphatic sulfides, specifically allyl and propyl di-, tri-, and tetrasulfides, with the allyl compounds being more potent than the propyl. These compounds decrease glucose-6 phosphate dehydrogenase activity in erythrocytes, which in turn curtails the regeneration of reduced glutathione needed to prevent oxidative denaturation of hemoglobin. Interestingly, the allyl derivatives are also thought to be effective in increasing tissue activities of cancer-protective enzymes such as quinone reductase (QR) and glutathione S-transferase (GST), thus decreasing the risk of cancer in humans who ingest these vegetables. Moreover, aged garlic extract is used to treat sickle cell anemia, because the extract is thought to contain antioxidants that prolong the life of sickle red blood cells.

Although the feeding of cull domestic onions (*Allium cepa*) appears to be reasonably safe in sheep, cattle may develop onion toxicosis. Sheep have been fed an exclusive onion diet, and although they initially developed a Heinz body hemolytic anemia with approximately 25% reduction in packed cell volume, there was no significant decrease in pregnancy or lambing rate, body condition, or fleece weight. Adaptation to an exclusive onion diet in sheep is thought to be due to a strong marrow response to the anemia, as well as modification of rumen metabolism of sulfoxides; one study showed that there was a marked increase in the number of sulfide-metabolizing bacteria (*Desulfovibrio* spp). Conversely, rumen microorganisms that convert sulfur containing amino acids to oxidants have been reported to exacerbate onion- and brassica-induced Heinz body anemia. One study showed that sheep fed onions (50 g/kg body weight/day) for 15 days developed more severe Heinz body hemolytic anemia than did the sheep fed the equivalent amount of onions with 5 g/day ampicillin sodium salt.

Feedlot cattle, on the other hand, can be fed a diet containing up to 25% cull onions on a dry-matter (DM) basis. Although a decrease in PCV occurs due to Heinz body-related hemolysis, the PCV returns to normal within 30 days after onion feeding is discontinued. Average daily gain and feed conversion ratios are not affected. It is thought, however, that the 25% (DM) probably approaches the toxic threshold for onion consumption in cattle. Onions should be mixed in a balanced ration, and cattle should not be allowed free access to the onions, as they may eat them preferentially.

Onion ingestion is the most common cause of Heinz body and eccentrocyte formation in dogs, and is a relatively common cause of clinical and subclinical anemia. In one study in which dogs were fed 5.5 g/kg body weight dehydrated onions, 70% of the erythrocytes contained Heinz bodies at 24 hours, and eccentrocytes were also common.

Packed cell volume dropped approximately 20% by day 5. There appears to be some variation in individual susceptibility to the effects of onion ingestion in dogs. Erythrocytes with high concentrations of reduced glutathione, such as is seen in some Japanese Shiba dogs, may be more susceptible to oxidative damage produced by onions. Garlic will also induce Heinz body and eccentrocyte formation in dogs.

Ingestion of onion soup and baby food containing onion powder has also been shown to produce Heinz body anemia in cats. In one study, as little as 0.3% onion powder significantly increased Heinz body formation; some commercial baby food may contain up to 1.8% onion powder on a dry weight basis.

Brassica (cabbage, kale, rape)

Ingestion of plants belonging to *Brassica* species may result in Heinz body anemia in ruminants. These plants contain S-methyl-L-cysteine sulfoxide, which is metabolized to the oxidant dimethyl disulfide by rumen bacteria. *Brassica* species not only have a high sulfur content, which reduces copper availability, but also are low in copper and zinc concentration. While this copper deficiency may play a role in oxidative hemoglobin damage, copper deficiency has not been shown to exacerbate susceptibility of lambs to brassica anemia. As with onion toxicosis, the severity of the Heinz body anemia is proportional to the quantity of brassica in the diet. A maximum concentration of 30% DM for *Brassica* species consumption is recommended to avoid significant anemia.

Wilted red maple leaves (Acer rubrum)

Severe Heinz body anemia and possibly death in horses, ponies, llamas and zebras may be caused by ingestion of wilted or dried (not fresh) red maple leaves. Eccentrocyte formation and hemolysis may occur without concurrent Heinz body formation. Other findings commonly include methemoglobinemia, hemoglobinuria, hemoglobinuric nephrosis, and hepatic necrosis. The oxidative compound, thought to be gallic acid, causes a rapid depletion of glutathione; leaves are toxic when administered at doses of 1.5 gm/kg of body weight or more. Therapy consists of ascorbic acid, fluids, and blood transfusions, if necessary.

Drugs and chemicals

Acetaminophen (paracetamol)

Acetaminophen (Tylenol) ingestion is probably the most common cause of Heinz body anemia in cats. Owners, unaware of its toxic effects, often give the anti-inflammatory human drug to cats. Acetaminophen is metabolized in part by glucuronide conjugation; cats have limited ability to form acetaminophen glucuronides, probably due to very low activity of the liver enzyme acetaminophen UDP-glucuronosyltransferase, thus resulting in increased oxidant metabolites of acetaminophen. As a result, glutathione concentration is decreased and oxidative damage to erythrocytes occurs. Other findings commonly include methemoglobinemia, with associated brown discoloration of blood and cyanosis, and hepatic necrosis. The toxic dose of acetaminophen in cats is 50 to 60 mg/kg body weight. (One Extra Strength Tylenol Gelcap contains 500 mg acetaminophen, and one Extra Strength Excedrin contains 250 mg acetaminophen.) To confirm the diagnosis, acetaminophen concentrations can be determined on serum. Treatment consists of providing glutathione donors, such as N-acetylcysteine, orally. Acetaminophen-induced Heinz body anemia also occurs in dogs; the toxic dose is approximately 150 mg/kg body weight.

Propylene glycol

Propylene glycol, sometimes used as an additive in semi-moist pet food, causes Heinz body formation in cats, but does not cause an anemia when ingested in those small quantities. However, cats eating such diets may be more susceptible to other additional causes of oxidative injury. Even though overt anemia may not occur, red cells with Heinz bodies have a reduced life span.

Zinc

Ingestion of zinc-containing materials, including pennies, which are 98% zinc by weight, other metal objects such as nuts and bolts in animal carriers, zinc toys, and zinc oxide containing ointments, have been reported to cause Heinz body anemia in dogs. The mechanisms by which zinc results in oxidative damage and Heinz body formation are unclear, but zinc is known to play a role in band-3 clustering. As a result of this clustering, opsonization of antibody and spherocyte formation may occur, resulting in the misdiagnosis of immune mediated hemolytic anemia.

Copper

Copper toxicosis in ruminants, especially sheep, results in Heinz body hemolytic anemia (Fig. 8.24). Copper accumulates in the liver of animals ingesting high concentrations of copper. This copper is released following stress, resulting in a hemolytic crisis. Copper deficiency has also been associated with Heinz body formation.

Selenium deficiency

Selenium deficiency in ruminants, associated with grazing on selenium deficient soils in certain parts of the world, including New Zealand and the Florida Everglades, has been associated with Heinz body anemia. Selenium deficiency has also been associated with reduced activity of glutathione peroxidase in erythrocytes of humans that live in selenium deficient areas, including New Zealand and Finland. It is speculated that reduced glutathione peroxidase activity may be the mechanism of the Heinz body anemia in selenium-deficient cattle.

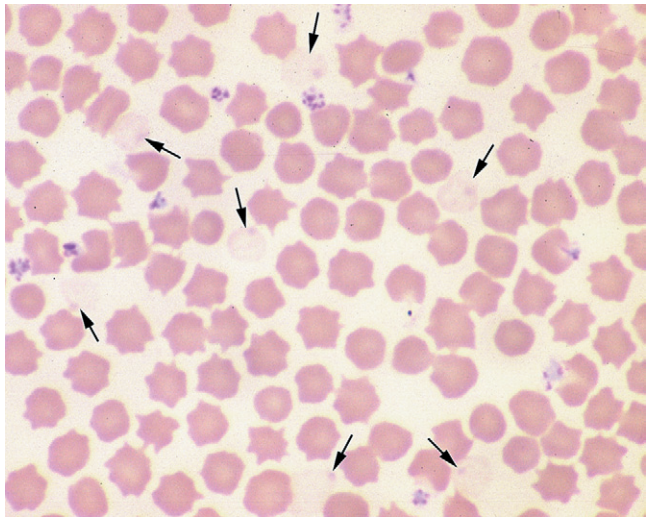

Figure 8.24 Blood film from a sheep with copper toxicosis. Note the Heinz bodies (arrows), which can be seen within "ghost" erythrocytes.

Methylene blue

Methylene blue was historically used as a urinary antiseptic in cats, and commonly resulted in Heinz body anemia with chronic administration. More recently, it has been associated with Heinz body anemia in river otters that were fed bait fish that had been kept in water containing methylene blue, which is used to detoxify ammonia in fish tanks. Interestingly, methylene blue is the drug of choice in the treatment of methemoglobinemia in humans and most domestic animals. There is no evidence to suggest that single therapeutic doses of methylene blue cause hemolytic anemia, even in cats.

Crude oil

Ingestion of crude oil by marine birds results in Heinz body anemia, one of the primary mechanisms of toxicity associated with the ingestion of crude oil by birds.

Other chemicals

Multiple other chemicals, such as naphthalene, a mothball ingredient; propofol, an intravenous anesthetic; phenazopyridine, a urinary analgesic; phenothiazine, an anthelmintic; ecabapide, a gastroprokinetic drug; benzocaine, a local anesthetic; and phenylhydrazine, an oxidative compound commonly used to experimentally induce hemolytic anemia, have been reported to cause Heinz body anemia. Skunk spray, which contains thiols and other oxidizing agents, has also been reported to cause Heinz body anemia in a dog.

Diseases

Heinz body formation is increased in specific disease states in cats and may contribute to anemia. Diabetes mellitus, hyperthyroidism, and lymphoma have been correlated with Heinz body formation. Diabetic cats in particular may have marked Heinz body formation. In one study, these diseases together accounted for nearly 40% of cats with Heinz body formation. Ketoacidotic cats had significantly more Heinz bodies than nonketotic diabetic cats. Percentage of Heinz bodies in diabetic cats is directly correlated with plasma beta-hydroxy-butyrate concentration, suggesting that ketones are associated with oxidative hemoglobin damage in cats. This may be a potential source of in vivo oxygen radical generation in animals with ketosis, such as may be seen in postparturient cattle.

Hypophosphatemia induced hemolysis

Severe hypophosphatemia, usually less than 1 mg/dL, has been reported to induce hemolysis in several species of animals, as well as humans. Erythrocyte glycolysis is inhibited by hypophosphatemia, primarily by decreasing intracellular phosphorus that is required for the enzyme glyceraldehyde phosphate dehydrogenase. This results in decreased glycolysis, leading to decreased erythrocyte ATP concentrations, and subsequent hemolysis. In some cases, this appears to be due to decreased glutathione, and increased susceptibility to oxidative injury. The most well recognized syndrome of hypophosphatemia induced hemolysis is postparturient hemoglobinuria in cattle. Causes in small animals include hypophosphatemia related to diabetes, and enteral alimentation. Severe hypophosphatemia can be life-threatening, not only because of hemolysis, but also owing to depression of myocardial function, rhabdomyopathy, seizures, coma, and acute respiratory failure.

Postparturient hemoglobinuria

Post parturient hemoglobinuria in cattle is a sporadic disease of multiparous, high producing dairy cows characterized by intravascular hemolysis, anemia, and hemoglobinuria. It usually occurs within 4 weeks of calving. Most, but not all, cows with this syndrome are hypophosphatemic at the time of anemia. It is theorized that previous hypophosphatemia predisposes erythrocytes to injury and oxidative damage, primarily by decreasing ATP and glutathione. Experimental hypophosphatemia (1 mg/dL) in postparturient cattle results in a decrease in erythrocyte ATP by 50% and a decrease in glutathione by 30%. The syndrome is complex, because some of the postparturient cattle with hemolytic anemia have Heinz body anemia, and some have ketoacidosis due to their nutritional status prior to and immediately following calving. Ketones are associated with oxidative hemoglobin damage, and may be a potential source of in vivo oxygen radical generation.

Hypophosphatemia in diabetic cats

Hypophosphatemia is sometimes present in diabetic animals, presumably because of phosphorus loss in the urine of polyuric animals. Several instances of hypophosphatemia-induced

hemolysis have been reported in cats. Similar to the situation in cows with postparturient hemoglobinuria, diabetic cats may also be ketotic and have Heinz body anemia; in those cases, the hemolysis may be due to hypophosphatemia, ketosis, or a combination, since hypophosphatemia likely predisposes to Heinz body formation. Hypophosphatemia resulting in hemolytic anemia has also been reported in one cat with hepatic lipidosis.

Enteral alimentation in cats

A retrospective study of cats with hypophosphatemia revealed that hypophosphatemia can occur 12–72 hours after initiation of enteral alimentation. In this study, the nadir for phosphorus concentrations ranged from 0.4 to 2.4 mg/dL. Hemolysis occurred in six of the nine cats that were hypophosphatemic. All cats had normal serum phosphorus concentrations prior to feeding. Hypophosphatemia also has been reported following oral tube feeding of human patients with anorexia nervosa.

Microorganisms (other than erythrocyte parasites)

Bacteria

Clostridial and leptospiral infections may result in hemolytic anemia. *Clostridium perfringens* Type A infection results in a hemolytic anemia in lambs and calves, sometimes referred to as "yellow lamb disease" or "enterotoxemic jaundice." The bacteria produces a phospholipase, which hydrolyses cell membrane phospholipids of erythrocytes, as well as those of other cells. Clinical signs include lethargy, fever, pale mucous membranes, anemia, hemoglobinuria, and icterus. Necropsy findings include evidence of intravascular hemolysis, renal hemoglobin casts, intestinal mucosal necrosis, hepatic necrosis, and petechial and ecchymotic hemorrhages. *Clostridium perfringens* has been associated with immune mediated hemolytic anemia in horses.

Clostridium haemolyticum and *C. novyi* Type D cause hemolytic anemia in cattle that is sometimes referred to as "bacillary hemoglobinuria" or "red water disease," which is acutely fatal. The disease occurs in summer and early fall, is associated with liver fluke migration, and is endemic in swampy areas of numerous countries, including the United States. The disease is rarely recognized ante-mortem. Clinical signs include those of anemia, lethargy, arched back, bloody diarrhea, fever, dyspnea, and occasionally hemoglobinuria. Bacterial spores are ingested and reside in macrophages of the liver. Anaerobic conditions within the liver, resulting from liver fluke migration, result in growth of the bacteria and production of toxic enzymes, including lecithinase, that metabolize lipids and protein in cell walls. Hemolysis and necrosis of other cells, including endothelial cells and hepatocytes, result in death. Necropsy findings include pale and icteric mucous membranes, foci of hepatic necrosis, hemorrhages, thoracic and abdominal effusion, hemoglobinuria, renal hemoglobin casts, and edema.

Leptospirosis (*Leptospira pomona*) may cause hemolytic anemia in young calves and lambs, but is almost never a feature of the disease in adult animals; leptospirosis rarely causes hemolytic anemia in dogs. The mechanism of the anemia may be toxins produced by the bacteria which act as hemolysins, but is more likely an immune mediated hemolytic anemia, probably IgM mediated. Necropsy findings in lambs include icterus, hemoglobinuria, renal tubular necrosis with hemoglobin casts, and hepatocellular necrosis.

Viruses

The equine infectious anemia (EIA) virus may result in hemolytic anemia in the acute stage of the disease. The anemia is likely immune mediated as a result of the virus binding with the erythrocyte membrane and activating complement. Later in the disease the anemia is nonregenerative, and may be similar to anemia of inflammatory disease. Equine infectious anemia is also referred to as "swamp fever." Diagnosis is made by detecting antibody against the EIA virus, using a Coggins test or a competitive ELISA test.

Water intoxication induced hemolysis in calves

Water intoxication resulting in hemolysis, hemoglobinuria, pulmonary edema, brain edema, convulsions, coma, and death may occur in calves that have unlimited access to water following its unavailability. Water intoxication may cause death within two hours, but most calves survive with no permanent ill effects. Cause of hemolysis is decreased osmolality of plasma. It has been theorized that water intoxication induced hemolysis occurs in calves from 4–5 months of age because osmotic fragility of their erythrocytes is greatest at that age, possibly related to the residual presence of iron deficient erythrocytes.

Hereditary membrane defects and metabolic disorders

Either inherited membrane defects or enzyme deficiencies leading to metabolic disorders may result in hemolytic anemia. Inherited erythrocyte membrane defects reported in domestic animals include hereditary spherocytosis, hereditary elliptocytosis, hereditary stomatocytosis, and membrane transport defects. However, hereditary elliptocytosis in dogs, which is caused by a hereditary protein 4.1 deficiency, results in increased osmotic fragility, elliptocytosis, membrane fragmentation, microcytosis, and poikilocytosis, but does not result in anemia.

Membrane defects

Hereditary spherocytosis (HS) results in hemolytic anemia, spherocytosis, and splenomegaly. Hereditary spherocytosis has been reported in people, mice, dogs, and cattle. In cattle, HS is due to hereditary band 3 deficiency, an autosomal dominant trait that has been reported in Japanese black

cattle. Band 3 protein is the most abundant protein in mammalian erythrocyte membranes, and functions include anion exchange across the membrane, as well as maintenance of normal erythrocyte shape. Cattle homozygous for the trait lack band 3 protein in their erythrocyte membranes, have a mild anemia, spherocytosis, hyperbilirubinemia, splenomegaly, and growth impairment. The disease is more severe in calves; adults are relatively normal. Heterozygotes have a partial deficiency of band 3, mild spherocytosis, and compensate for their hemolytic anemia with increased erythrocyte regeneration. Hereditary spherocytosis may also be due to spectrin deficiency. Spectrin is the major constituent of the cytoskeletal network underlying the erythrocyte plasma membrane. It associates with band 4.1 and actin to form the cytoskeletal superstructure of the erythrocyte plasma membrane. This complex is anchored to the cytoplasmic face of the plasma membrane via another protein, ankyrin, which binds to beta-spectrin and mediates the binding of the whole complex to the transmembrane protein band 3. The interaction of erythrocyte spectrin with other proteins through specific binding domains lead to the formation of an extensive subplasmalemmal meshwork which is thought to be responsible for the maintenance of the biconcave shape of erythrocytes, for the regulation of plasma membrane components and for the maintenance of the lipid asymmetry of the plasma membrane. Spectrin deficiency has been reported in a family of Dutch golden retrievers.

Hereditary stomatocytosis has been reported in miniature schnauzers, chondrodysplastic Alaskan malamutes, and in the Drentse partrijshond breed of dogs that also have hypertrophic gastritis (see Chapter 5). These disorders have different underlying causes in these three breeds, and the schnauzers do not have anemia, although their red cell survival time is slightly shortened.

A Coombs' negative chronic intermittent hemolytic anemia has been reported in Abyssinian and Somali cats. Clinical signs and laboratory findings include mild to severe anemia, splenomegaly, increased MCV, and the presence of a few stomatocytes. Osmotic fragility of erythrocytes is markedly increased. Some of the cats improved following splenectomy. The specific cause of the hemolytic anemia is not known, but a membrane defect is suspected.

Animals with erythrocyte membrane transport defects, especially those with defects in transport of amino acids involved in glutathione metabolism, may develop hemolytic anemia (Heinz body anemia) when exposed to oxidants. Some Finnish Landrace sheep have red cell glutathione deficiency, inherited as autosomal recessive. Cysteine uptake and glutathione synthesis are impaired, and glutathione concentration in erythrocytes is only 30% of normal. A similar defect is thought to be common in thoroughbred horses, but does not cause anemia. Some Japanese Shiba and Akita dogs have erythrocytes with high potassium, low sodium concentrations, due to retention of Na,K-ATPase in mature erythrocytes, inherited as an autosomal recessive trait. Some of these dogs have an increased concentration of reduced glutathione in their erythrocytes, which protects the cells against oxidative damage by acetylphenylhydrazine, but increases the risk of oxidative damage by onions (see Heinz body anemia).

Metabolic disorders

Inherited erythrocyte enzyme defects result in abnormalities in metabolic pathways, often resulting in hemolytic anemia. Energy in mature mammalian erythrocytes is generated exclusively by anaerobic glycolysis, also known as the Embden-Meyerhof (EM) pathway, since they have lost their mitochondria, and thus their oxidative phosphorylation capabilities. Briefly, metabolism of glucose produces adenosine triphosphate (ATP), which is used to maintain erythrocyte shape, deformability, membrane transport, and synthesis of purines, pyrimidines, and glutathione. Many enzymes are involved in anaerobic glycolysis, including phosphofructokinase and pyruvate kinase. Deficiencies of both of these enzymes have been described in domestic animals.

Pyruvate kinase deficiency

Pyruvate kinase (PK) deficiency is the most common enzymopathy in humans, and was first recognized in Basenji dogs in 1971. Since that time, it has been reported in beagles, West Highland white terriers, Cairn terriers, miniature poodles, and various other breeds. Clinical signs include those of anemia, such as exercise intolerance. The anemia is very regenerative, and half or more of the erythrocytes on the blood film may be reticulocytes. The MCV may be markedly increased due to the reticulocytosis. Hepatosplenomegaly may be present. Affected dogs die of myelofibrosis or hepatic failure by 3–5 years of age. Myelofibrosis and osteosclerosis are a consistent finding in PK-deficient dogs, but do not develop in PK deficient people or cats. In certain breeds (Basenjis, West Highland white terriers) in which the mutation is specific, diagnosis can be made by PCR-based tests. Bone marrow transplantation has been shown to correct the disorder and prevent the development of osteosclerosis. Pyruvate kinase deficiency in cats has been described in various breeds, including Abyssinian, Somali, and domestic short hair cats. The anemia is mild to moderate, slightly to strongly regenerative; splenectomy reduces the severity of hemolytic anemia. Cats live to advanced age since osteosclerosis does not develop.

Phosphofructokinase deficiency

Phosphofructokinase (PFK) deficiency is a rare genetic disorder in humans, and has been described in English springer spaniels, an American cocker spaniel, and a mixed breed dog. The cocker spaniel had an ancestor that was bred in a

kennel that also had English springer spaniels, and the mixed breed dog was thought to be part English springer spaniel. The mutation in all of these dogs was identical. It is inherited as an autosomal recessive trait, and is also referred to as glycogen storage disease type VII, since the enzyme deficiency also results in a lack of lactate production and accumulation of sugar phosphates and glycogen in muscle. Intermittent severe intravascular hemolysis is triggered by mild alkalemia; even mild respiratory alkalosis caused by hyperventilation and panting may precipitate a hemolytic crisis. Moreover 2,3-diphosphoglycerate (2,3-DPG), a compound that decreases the oxygen affinity for hemoglobin, thus making oxygen more available to tissues, is generated in the EM pathway. PFK deficiency results in a deficiency of 2,3-DPG, which results in tissue hypoxia of affected dogs. However, this tissue hypoxia stimulates erythropoietin production, and thus, except when in hemolytic crisis, these dogs are not anemic. Clinical signs include excitement or exercise-induced hemolytic anemia and occasional mild muscle cramping. Life expectancy can be normal if hemolytic crises are avoided. The disorder can be identified in affected dogs, as well as carriers, by a PCR-based DNA test that is specific for the English springer spaniel mutation.

Glucose-6-phosphate dehydrogenase deficiency

The pentose phosphate pathway (PPP) generates reduced nicotine adenine dinucleotide phosphate (NADPG), which is protective against mechanical and metabolic insults, particularly oxidants. Glucose 6-phosphate dehydrogenase (G6PD) is the rate-limiting enzyme in the PPP. In humans, G6PD deficiency is inherited as an X-linked disorder, which causes hemolytic anemia, particularly following exposure to oxidants. Hemolytic anemia caused by G6PD deficiency has been described in an American saddle bred colt, as well as a dog. Morphologic abnormalities in the colt included eccentrocytosis, and the colt's dam, which was a heterozygote for the disorder, also had eccentrocytes on her blood film.

Hereditary methemoglobinemia

Methemoglobin is not able to bind oxygen because the iron moiety of the heme group has been oxidized to the ferric state (see Heinz body anemia). Approximately 3% of hemoglobin is oxidized to methemoglobin each day, but this methemoglobin is reduced back to hemoglobin, primarily by the enzyme NADH-methemoglobin reductase. Inherited deficiencies of this enzyme have been described in numerous breeds of dogs and cats. This disorder does not cause significant problems in dogs and cats, other than increased risk associated with anesthesia. Glutathione reductase deficiency has been described in horses, and even in the absence of oxidants, resulted in a mild hemolytic anemia with eccentrocyte formation and methemoglobinemia. Horses in one report had normal methemoglobin reductase activity, but activity was reduced in a separate case.

Porphyrias

Hemoglobin synthesis occurs in erythroid precursors, where protoporphyrin, iron, and globin molecules are brought together and assembled into functional hemoglobin. Synthesis of the heme portion of the molecule is complex, and requires numerous enzymes. Inherited deficiencies of these enzymes result in an accumulation of porphyrin precursors, as well as a failure to adequately synthesize hemoglobin, and the disorders are known as erythropoietic porphyrias, which have been described in humans, cattle, swine, and cats. Some of the erythropoietic porphyrias result in hemolytic anemia. Hepatic porphyrias are caused by different enzyme deficiencies, and to date have been discovered only in humans; the liver is the site of synthesis for enzymes containing heme, such as catalase, cytochromes, and peroxidase.

Another inherited disorder, erythropoietic protoporphyria, is due to a defect of the enzyme heme synthetase (ferrochelatase). This disorder has been described in Limousin and Blonde d'Aquitaine cattle, and the only clinical manifestation is severe photosensitivity with intense pruritus. Anemia, porphyrinuria, and discolored teeth are not observed. Inheritance of erythropoietic protoporphyria is recessive in cattle and only occurs in homozygotes, unlike in humans in whom the heterozygous condition results in clinical signs.

Toxins, especially lead, may destroy many of the enzymes involved in the synthesis of heme. These toxicoses lead to a decrease in heme synthesis, as well as an excess of heme precursors, which are eliminated in increased concentration in the urine. These toxicoses are referred to as porphyrinurias.

Clinical signs associated with porphyrias vary, depending on the specific enzyme abnormality, and the amount of residual activity of the affected enzyme. Porphyrins are reddish-brown in color, have a characteristic red fluorescence when exposed to ultraviolet light, and stain various tissues, including teeth and bones; congenital erythropoietic porphyria in cattle was called "pink tooth" at one time. The porphyrins in these animals are excreted excessively in all body fluids, including urine, feces, saliva, sweat, and tears. One of the most common abnormalities is photosensitivity resulting in photodermatitis, particularly evident on light colored areas of the skin. This is due to excitation of porphyrins by ultraviolet light, and subsequent transfer of oxygen to tissues, causing oxidation of cellular lipids, proteins, and organelles.

Bovine congenital erythropoietic porphyria

Bovine congenital erythropoietic porphyria has been reported in Holsteins and Shorthorns, and is caused by a partial deficiency of uroporphyrinogen III cosynthetase (UROgenIII Cosyn), resulting in an accumulation of uroporphyrin I and coproporphyrin I, which accumulate in tissues,

and are excreted in urine and feces in increased quantities. Clinical signs include pigmentation of tissues including teeth, anemia, and photosensitization. The disorder is inherited as an autosomal recessive trait. Affected animals have hemolytic anemia that is regenerative, and blood film findings are those of a regenerative anemia in cattle, including polychromasia, macrocytosis, anisocytosis, basophilic stippling, and increased nucleated erythrocytes. Affected calves have a particularly striking regenerative response, with many nucleated erythrocytes present. Erythrocyte life span is shortened, due to both the heme synthesis disorder, as well as the porphyrin-related damage to erythrocyte membrane lipids. Ultraviolet light may increase severity of hemolysis, due to exposure of erythrocytes while in surface capillaries. The disease has been almost completely eliminated in cattle.

Porphyria of cats

Two forms of porphyria have been described in cats. One type, described in a family of Siamese cats, is due to a partial deficiency of uroporphyrinogen III cosynthetase, and is similar to the disorder in humans and cattle. Affected cats had photosensitivity and severe hemolytic anemia, as well as renal disease. The renal disease was characterized by mesangial hypercellularity and proliferation and ischemic tubular injury. Membrane-enclosed lamellar bodies were present in cytoplasmic and extracellular locations of various tissues, similar to those seen in lysosomal storage disorders.

A second type of porphyria has been described in domestic cats in which the clinical signs are only discoloration of teeth and urine due to the presence of uroporphyrin, coproporphyrin, and porphobilinogen. Anemia and photosensitization are not present. The disorder in domestic cats is inherited as autosomal dominant.

Porphyria of swine

Porphyria has been described in affected swine, which have discoloration of teeth and excessive uroporphyrin in the urine. Affected swine are not anemic and photosensitization is not present. The specific defect is not known, and no animals are currently available for study. The disorder is inherited as autosomal dominant.

Suggested reading

Blood loss

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9

Classification of and Diagnostic Approach to Polycythemia

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Polycythemia refers to an increase in the concentration of erythrocytes in the blood as evidenced by an increased packed cell volume (PCV; or hematocrit), red-blood-cell count, or hemoglobin concentration. Because the term polycythemia implies that all blood cells, including leukocytes, are increased in concentration, the term erythrocytosis is sometimes preferred; in domestic animals with true polycythemia, usually only the erythrocytes are increased in concentration.

Polycythemia may be either relative or absolute. Relative polycythemia may occur due to decreased plasma volume or erythrocyte redistribution. Examples of the former include dehydration and body fluid shifts. The latter is the result of splenic contraction seen most commonly in excitable animals such as cats and horses. Absolute polycythemia is caused by an actual increase in the red cell mass and may be primary or secondary. Secondary absolute polycythemia results from overproduction of erythrocytes secondary to increased erythropoietin concentration, which in turn is secondary to either generalized hypoxia, localized renal hypoxia, or overproduction of erythropoietin by a tumor. Primary absolute polycythemia (i.e., polycythemia vera) is considered to be a well-differentiated myeloproliferative disorder in which erythropoiesis occurs independent of the erythropoietin concentration. Whereas primary polycythemia is rare, it is still more common than secondary polycythemia. Primary polycythemia usually is diagnosed by excluding relative and secondary polycythemia.

Relative polycythemia

Relative polycythemia caused by fluid shifts or dehydration

Patients with relative polycythemia caused by a reduction in plasma volume usually have a concurrent increase in plasma protein. In addition, clinical evidence of dehydration usually is present. Some dehydrated animals, however, may have normal or decreased plasma protein concentration resulting from decreased protein intake, decreased protein production by the liver, or increased protein loss via the kidney, gastrointestinal tract, or cutaneous lesions (see Chapter 26). Moreover, fluid shifts may occur so rapidly, such as in patients with acute gastrointestinal disease or severe acute hyperthermia, that the classic clinical signs of dehydration may not be apparent. Relative polycythemia is treated by diagnosis of and therapy for the underlying disease and by replacement of fluids and electrolytes.

Relative polycythemia caused by transient increase in red cell mass secondary to splenic contraction

Splenic contraction causes only a modest increase in PCV, usually to no greater than 60%. Polycythemia as a result of splenic contraction typically is seen only in animals that normally have a high PCV, such as some poodles, greyhounds, and dachshunds. Splenic contraction may occur secondary to exercise, or it may be a response to epinephrine release in animals that are excited or in pain. Plasma protein concentration is not increased, and the presence of fear, pain, or excitement at the time of blood collection usually is apparent. An excitement leukogram also may be present, as evidenced by a mature neutrophilia and lymphocytosis; occasionally, mild thrombocytosis also is noted. Transient polycythemia has no clinical significance, and the red cell concentration reverts to normal in a short period of time.

Absolute polycythemia

Absolute polycythemia can be either secondary or primary.

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Secondary absolute polycythemia

Secondary absolute polycythemia caused by generalized hypoxia or hypoxemia (physiologically appropriate polycythemia)

Physiologically appropriate polycythemia is observed when inadequate tissue oxygenation triggers an increase in erythropoietin production, which in turn stimulates erythrocyte production and release so that more oxygen can be carried to the tissues. Generalized hypoxia and hypoxemia (reduced Pao_2) may be seen in animals with severe chronic heart or lung disease. Congenital heart disorders that result in shunting of blood away from the lungs are associated more often with polycythemia than in acquired heart disease. Severe lung disease also may result in hypoxemia, but it must be of chronic duration to induce polycythemia. Other causes of hypoxemia include living at very high altitude, alveolar hypoventilation, and severe obesity. Polycythemia associated with hypoxia without hypoxemia occurs in people with certain rare, inherited hemoglobinopathies, but these conditions have not been reported in domestic animals. Acquired chronic hemoglobinopathies (e.g., carboxyhemoglobinemia secondary to carbon monoxide poisoning or methemoglobinemia) may induce polycythemia as well.

Secondary absolute polycythemia caused by hypoxemia is diagnosed by detecting decreased Pao_2 and oxygen saturation. The reference interval for Pao_2 varies somewhat with the altitude. At sea level, the lower end of the reference interval is 80 mm Hg, and oxygen saturation is 92%; at approximately 6000 feet above sea level, the lower end of the reference interval is 74 mm Hg. Usually, the Pao_2 must be less than 60 mm Hg to induce polycythemia. Imaging of the heart and lungs as well as other diagnostic procedures to detect cardiopulmonary disease can then be used to establish a more definitive diagnosis.

Secondary absolute polycythemia caused by increased erythropoietin production (physiologically inappropriate polycythemia)

Physiologically inappropriate polycythemia occurs when erythropoietin production is increased in the absence of generalized tissue hypoxia. Erythropoietin production may be increased in patients with renal lesions (usually tumors that induce localized renal hypoxia). Increased production of erythropoietin or of an erythropoietin-like substance by nonrenal tumors such as hepatoblastomas also may occur, but this is rare. Animals with physiologically inappropriate polycythemia have normal to slightly decreased Pao_2 and oxygen saturation. Mild hypoxemia may be present as a result of poor perfusion, and patients usually have increased serum erythropoietin concentration. Other diagnostic procedures to evaluate the kidneys, such as imaging, renal aspiration cytology or biopsy, and urinalysis, should be performed.

Primary absolute polycythemia

Primary absolute polycythemia (i.e., polycythemia vera) is a well-differentiated myeloproliferative disorder in which erythrocytes proliferate uncontrollably, producing an increased hematocrit. Unlike most other types of hematopoietic neoplasia, the neoplastic erythroid cells appear to be normal and to have a normal maturation sequence. In humans with polycythemia vera, an abnormal proliferation of neutrophils and platelets often accompanies erythrocyte proliferation, resulting in leukocytosis and thrombocytosis. An abnormal proliferation of cells other than red blood cells is rarely observed in domestic animals; thus, in dogs and cats, the disorder probably should be referred to as primary erythrocytosis rather than as primary polycythemia or polycythemia vera.

The presence of an acquired recurrent mutation within the JAK2 gene has been identified in 90% of human patients with polycythemia vera. This mutation (V617F) is located in the pseudokinase domain of JAK2, leading to constitutive activation of the kinase responsible for the polycythemia. Detection of the mutation is a major diagnostic tool in humans for polycythemia vera diagnosis. Identical mutations of the JAK2 gene giving rise to active JAK2 kinase have been shown in dogs with polycythemia vera, suggesting a common mechanism for the human and canine disease.

While the disorder continues to be diagnosed by excluding other causes of polycythemia, it is likely that detection of the mutation will soon be used for diagnosis. Most cases of primary polycythemia in domestic animals have been reported in dogs and cats, but a few have been reported in horses, cattle, and a llama.

Clinical findings

Clinical findings may be secondary to the underlying cause of the polycythemia or may result from the increased number of erythrocytes per se. In animals with relative polycythemia, dehydration or excitement may be clinically evident. In animals with secondary absolute polycythemia caused by hypoxia, clinical signs associated with congenital heart disease (e.g., murmurs, cyanosis) or with pulmonary disease (e.g., cyanosis, dyspnea, abnormal lung sounds) may be observed. In animals with secondary absolute polycythemia caused by inappropriate erythropoietin production, clinical signs associated with renal disease often are not apparent.

Clinical signs associated with erythrocytosis are secondary to increased blood volume and viscosity. They include deep-red mucous membranes, sometimes with slight cyanosis. Increased blood viscosity may result in sluggish blood flow and subsequent decreased tissue perfusion and oxygen transport as well as hemorrhage and thrombosis. Mild to severe central nervous system signs associated with decreased

oxygen transport, such as lethargy, ataxia, blindness, or seizures, also may be observed. Polyuria and polydipsia occasionally are reported and are thought to result from impaired release of vasopressin release. Splenomegaly rarely is observed in domestic animals; however, human patients commonly have splenomegaly, may have generalized pruritis, and eventually may develop marrow fibrosis and lymphoid neoplasia.

Diagnostic approach

When PCV is increased, one should consider if the patient is excited or dehydrated and then perform a second complete blood count to confirm that the finding is repeatable. If the total protein concentration also is increased, the polycythemia likely is relative, secondary to dehydration and decreased plasma volume. Sometimes, however, animals with rapid fluid shifts, such as those with gastrointestinal disease, may not have an increased total protein. Moreover, total protein may be decreased or normal in dehydrated animals that have decreased protein intake, production, or increased loss.

If relative polycythemia is excluded, secondary absolute polycythemia due to hypoxemia from congenital heart disease or pulmonary disease should be considered. Hypoxemia is best diagnosed by performing an arterial blood gas analysis to determine the PaO_2 and oxygen saturation. If the PaO_2 is less than 60 mm Hg, then hypoxemia likely is the cause of polycythemia. Imaging using thoracic radiographic and ultrasonic examination will provide additional information.

If hypoxemia is excluded, secondary absolute polycythemia caused by increased erythropoietin production should be considered. Tumors of the kidney are the most common cause of increased erythropoietin production. In these cases, imaging with renal ultrasonography or intravenous urography is indicated. Serum erythropoietin concentration usually is increased in animals with hypoxemia or inappropriate erythropoietin production and is normal to decreased in animals with primary polycythe mia (Table 9.1). Erythropoietin concentrations appear to be more useful in dogs than in cats. If secondary polycythemia caused by inappropriate erythropoietin production is excluded, then the likely diagnosis is polycythemia vera.

Table 9.1 PaO_2 and erythropoietin in animals with polycythemia.

Polycythemia	PaO_2	Erythropoietin
Relative	Normal	Normal
Secondary		
Caused by hypoxemia	Decreased	Increased
Caused by inappropriate erythropoietin production	Normal	Increased
Primary	Normal	Normal or decreased

Other laboratory findings are not particularly helpful. Affected humans commonly have neutrophilia and thrombocytosis, but these findings are rare in domestic animals. Neutrophilia associated with stress or inflammation is a more likely finding. Other than mild increased cellularity and mild erythroid hyperplasia, bone marrow aspirates usually are normal in appearance. Measuring total red cell mass with a dye technique or radioisotope-labeled erythrocytes, though infrequently performed, can help to establish a more definitive diagnosis.

Therapy

Relative polycythemia is treated by therapy for the underlying disease and correction of dehydration with fluid therapy. The underlying disorder also is treated in animals with secondary polycythemia caused by hypoxemia or inappropriate erythropoietin production. Phlebotomy may be contraindicated in animals with hypoxemia, because the erythrocytosis is physiologic. If the PCV is very high in these patients, however, then tissue perfusion may be impaired, and phlebotomy may be helpful.

Primary polycythemia most commonly is treated—and often with long-term success—by performing repeated phlebotomy to maintain the PCV in the high-normal range. Injectable iron may need to be given to avoid iron-deficiency anemia. Chemotherapy to decrease red cell production also may be used; oral hydroxyurea is the most common such treatment. Dose and frequency are variable, depending on the response. A reported complication in cats is methemoglobinemia and Heinz-body anemia. Alternately, radioactive phosphorus has been used with success in some cases. A veterinary oncologist should be consulted for up-to-date treatment options.

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10 Introduction to Leukocytes and the Leukogram

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Interpretation of leukocyte concentrations in blood provides insight regarding potential processes that may be occurring in the patient. The complete set of numeric data in the leukocyte profile, along with any noted morphologic abnormalities, is known as the leukogram. An abnormal leukogram usually leads to identification of a pathologic process (e.g., inflammation), but not to establishment of a specific diagnosis. Interpretation of leukocyte abnormalities into a process coupled with clinical findings, however, may lead to a diagnosis.

To interpret leukocyte patterns in disease, one must first learn the normal characteristics of the leukogram as a basis for recognizing abnormal patterns. This chapter presents background information regarding the normal leukogram that is necessary for building skills in its interpretation.

Common blood leukocytes: General functions and morphology

This section reviews pertinent characteristics of blood leukocytes, such as general functions and morphologic features, including species variations in morphology.

Neutrophils

Neutrophils participate in inflammatory responses by means of chemoattraction into tissue sites of inflammation and phagocytosis of organisms and other foreign material. After phagocytosis, lysosomal granules fuse with phagosomes to kill organisms and then degrade the material by enzymatic digestion.

Neutrophil morphology is introduced in Figure 10.1. The neutrophilic metamyelocyte is not present in normal blood. It has a bean-shaped nucleus that, as it matures, changes to the horseshoe shape that is characteristic of the band neutrophil. The band nucleus has smooth, parallel sides and no constrictions in the nuclear membrane. The band neutrophil may be present in normal blood in small concentrations. Segmented neutrophils have a horseshoe-shaped nucleus with variable degrees of indentation and constriction along its perimeter (Fig. 10.1). As the nucleus develops constrictions, it may fold into various shapes (Fig. 10.2). Neutrophils have numerous small, very poorly stained granules. These vary among individual animals from colorless, invisible granules to lightly staining granules. Neutrophilic granules of the cow often stain faintly pink, giving the cytoplasm a slightly orange-pink tint overall (Fig. 10.3). Neutrophils observed in cytologic samples may on occasion have altered staining of the neutrophilic granules. The granules may appear more prominent and stain pink. This change is most likely to be observed in neutrophils exudated in airway samples.

Lymphocytes

Blood lymphocytes represent a diverse set of lymphocyte subpopulations, but these subpopulations cannot be distinguished by blood-film examination or by techniques routinely used in clinical veterinary laboratories. The subpopulations include B lymphocytes, which are responsible for humoral immunity, and T lymphocytes, which are responsible for cell-mediated immunity and cytokine responses. T lymphocytes may be further classified as T-inducer (i.e., helper; CD4-bearing) cells and T-cytotoxic/suppressor (CD8-bearing) cells. Null cells are a third population present at small concentrations. Null cells consist of at least several lymphocyte subtypes, including large granular lymphocytes, natural killer cells, and other cells with killer activity. Lymphocyte subtypes may be differentiated by surface immunoglobulin and cluster designation (i.e., CD) markers; however, this technology is not yet part of the routine hemogram. These measurements are currently made in specialized laboratories, usually in cases of leukemia

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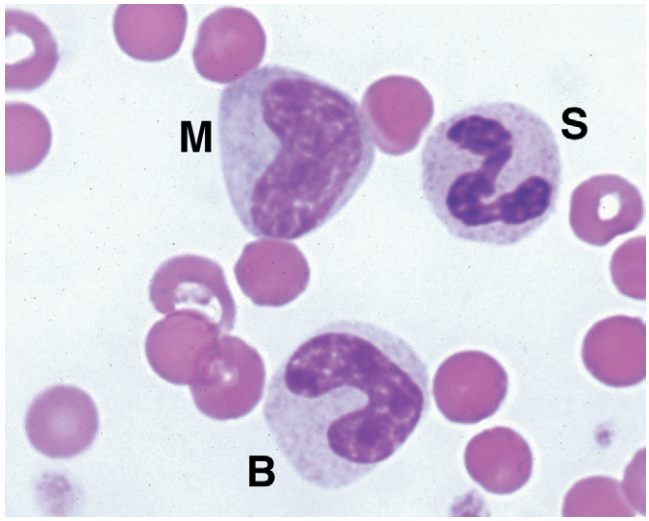

Figure 10.1 Neutrophil maturation sequence commonly seen in blood. The segmented or mature neutrophil (S) has an irregular nuclear membrane, with one or more constrictions. Note the small, faintly staining neutrophilic granules in the cytoplasm. The neutrophilic granules vary in prominence from animal to animal. The band neutrophil (B) has a horseshoe-shaped nucleus with smooth, parallel sides. The metamyelocyte (M) has a bean-shaped nucleus. (Wright's Giemsa stain, high magnification.)

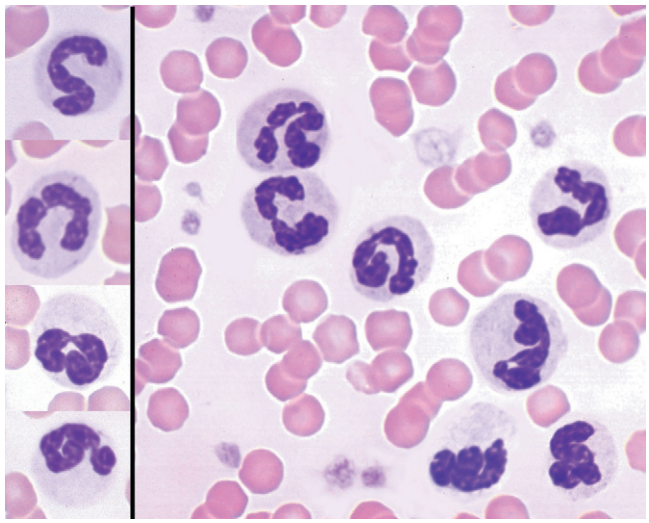

Figure 10.2 Representative segmented neutrophils illustrating variation in nuclear shape. Segmented neutrophils start with the horseshoe-shaped nucleus of the band cell. As the neutrophil nucleus develops more constrictions, it may more easily fold into various shapes. Note the "S"—and horseshoe-shaped nuclei in the upper left. Then, note the various nuclear shapes that result from folding and superimposition of the folded nucleus on itself. Cells are arranged in this figure with greater degrees of folding moving toward the bottom. (Wright's Giemsa stain, high magnification.)

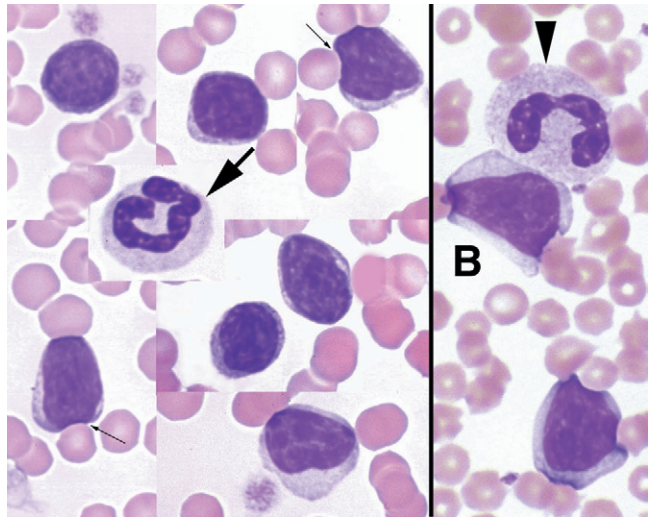

Figure 10.3 Variation in normal lymphocyte morphology in comparison to neutrophils. In the left panel, note that the lymphocyte nucleus may vary from round to oval. The cell shape, including the nucleus, may be indented by adjacent erythrocytes (thin arrows). The amount of cytoplasm varies from virtually none to a modest amount. Lymphocytes in most species have smaller diameter than adjacent neutrophils (thick arrow). An exception is indicated in the right panel: Bovine lymphocytes (B) may be larger in diameter than lymphocytes of other common species and may have the same diameter as that of adjacent neutrophils (arrowhead). Note that the bovine neutrophil has slightly pink neutrophilic granules. (Wright's Giemsa stain, high magnification.)

(see Chapter 13). Such laboratories may provide special procedures for quantitation of certain subpopulations (e.g., B- and T-cell concentrations).

Lymphocytes are recognized by a round to oval nucleus and a minimal amount of clear, almost colorless cytoplasm. The amount of cytoplasm may be variable, as illustrated in Figure 10.3. Normal circulating lymphocytes have smaller diameters than those of neutrophils. In ruminants, lymphocytes may be more irregular in size and have diameters equal to those of neutrophils (Fig. 10.3). Less common forms of lymphocytes include reactive lymphocytes and granular lymphocytes (Fig. 10.4). Reactive forms likely are B cells capable of producing immunoglobulin. They have intensely basophilic cytoplasm, and the nucleus may be more irregularly shaped. In addition, the nucleus may have a cleft or an amoeboid shape. Large reactive lymphocytes are observed normally in juveniles of most species. Granular lymphocytes have a small number of pink-purple granules. These are large granular lymphocytes, some of which are thought to be natural killer or T cells. Large granular lymphocytes are most commonly observed in normal ruminant blood.

Monocytes

Monocytes also participate in inflammatory responses. Monocytes in blood are regarded as intermediate on a continuum

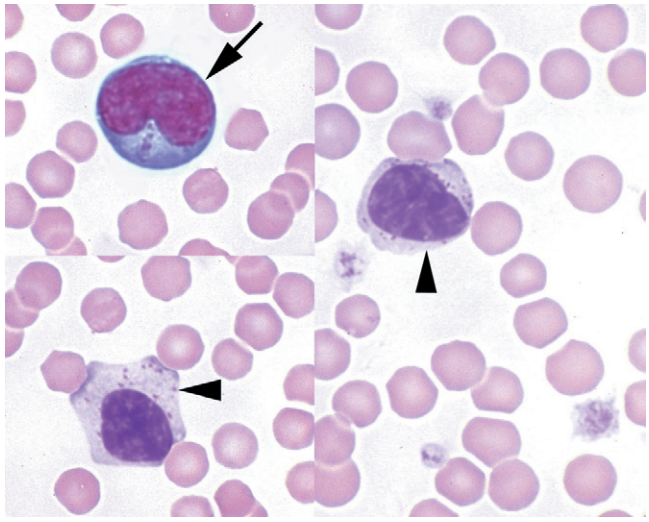

Figure 10.4 Variations in lymphocytes less commonly seen in blood. The reactive lymphocyte (arrow) is characterized by royal-blue cytoplasm. Its nuclear shape may be irregular, often with an indentation or cleft. Large granular lymphocytes (arrowheads) have an increased amount of light-staining cytoplasm, with a sparse sprinkling of azurophilic granules. The granules may vary in size. Large granular lymphocytes are most frequently seen in normal ruminants. (Wright's Giemsa stain, high magnification.)

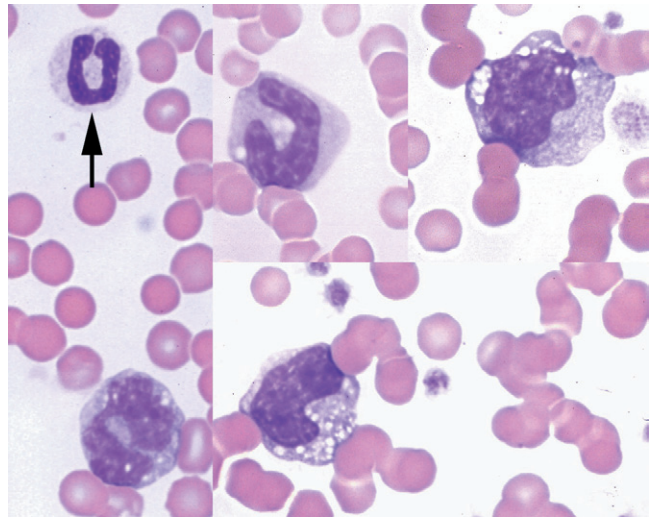

Figure 10.5 Variation in blood monocyte morphology; note the cells not marked by an arrow. Monocytes are typically larger than neutrophils (arrow). Monocytes may have cytoplasmic vacuoles, but this is not consistent. The monocyte nucleus is highly variable in shape: It may be round to bean shaped to amoeboid shaped, or it may be horseshoe shaped and even segmented (like the nuclei of neutrophils). Inexperienced observers frequently confuse monocytes with horseshoe-shaped nuclei for neutrophils. The consistent features of monocytes are larger diameter than an adjacent neutrophil (arrow) and darker blue-gray cytoplasm compared with neutrophils. (Wright's Giemsa stain, high magnification.)

of maturation. Monocytes migrate into tissues, where they continue to develop into macrophages. Mononuclear phagocytes may phagocytize bacteria, larger complex organisms (e.g., yeast and protozoa), injured cells, cellular debris, and foreign particulate debris. These cells play an important immunoregulatory function by presenting processed antigen to T lymphocytes. These cells are also responsible for normal erythrocyte destruction, associated metabolic iron recycling, and most pathologic erythrocyte destruction.

Monocytes are the most misidentified cell on blood films, particularly in the veterinary hospital laboratory. The nucleus may be of almost any shape, including oval, bean, ameboid, or horseshoe shaped (like that of neutrophils). The chromatin pattern may be slightly less condensed than that of neutrophils. The key distinguishing features are a larger diameter and more grayish coloration to the cytoplasm compared with adjacent neutrophils (Fig. 10.5). The cytoplasm may contain extremely fine, light-purple granules. When uncertainty exists regarding monocyte identification, view at low power to make cell-to-cell comparisons (Fig. 10.6). At low power, monocytes will stand out as larger cells. Species differences in monocyte morphology are not remarkable.

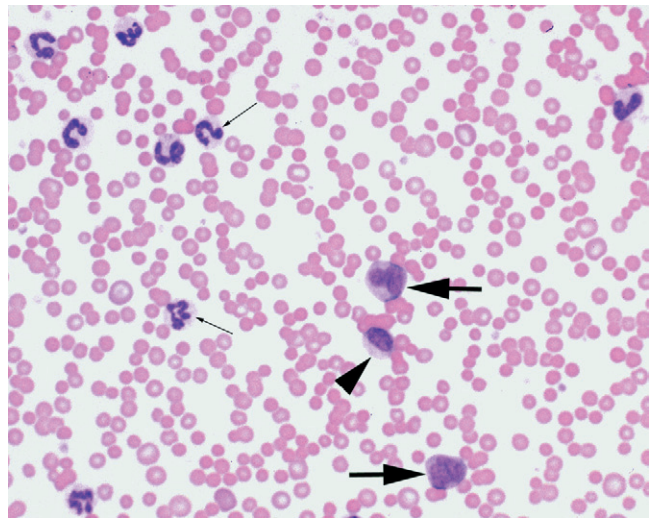

Figure 10.6 Low-magnification comparison of neutrophils and monocytes. When in doubt regarding identification of monocytes, use a lower magnification to make cell-to-cell comparisons that may be difficult at higher magnification. Note that the two monocytes (thick arrows) have larger diameters than the representative neutrophils indicated by thin arrows. A lymphocyte (arrowhead) is smaller than the adjacent neutrophils. (Wright's Giemsa stain, low magnification.)

Eosinophils

The functions of eosinophils are not well understood, even though a considerable number of studies and observations have been reported. Eosinophils contain proteins that bind

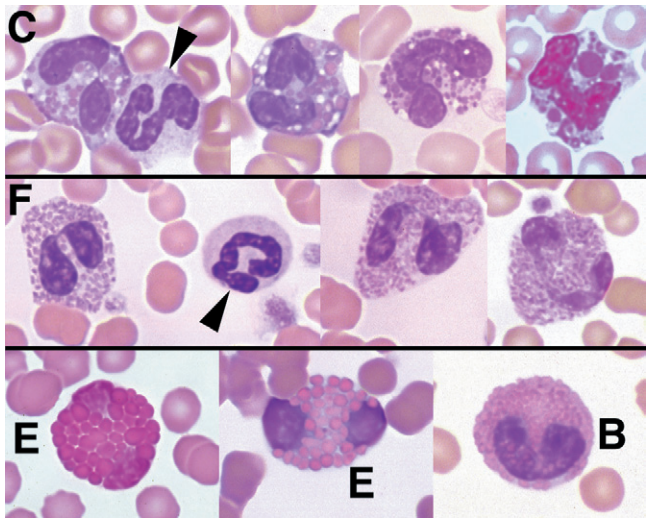

Figure 10.7 Species variation in eosinophil morphology. Representative neutrophils are shown for comparison (arrowheads). Eosinophils are typically larger in diameter than neutrophils. Canine eosinophils are shown in the top band (C). Note the variation in eosinophil granule size in dogs, which also may have eosinophil granules that appear to dissolve during the staining process, leaving a clear space that resembles a cytoplasmic vacuole. Feline eosinophils are shown in the middle band (F). Eosinophil granules of the cat are shaped like barrels or short rods. The density of the granularity may vary as shown. Large animal eosinophils are indicated in the bottom band. Equine eosinophils (E) have large, brightly staining granules that may obscure the nucleus, whereas bovine eosinophils (B) have smaller, brightly staining granules that are densely packed within the cytoplasm. (Wright's Giemsa stain, high magnification.)

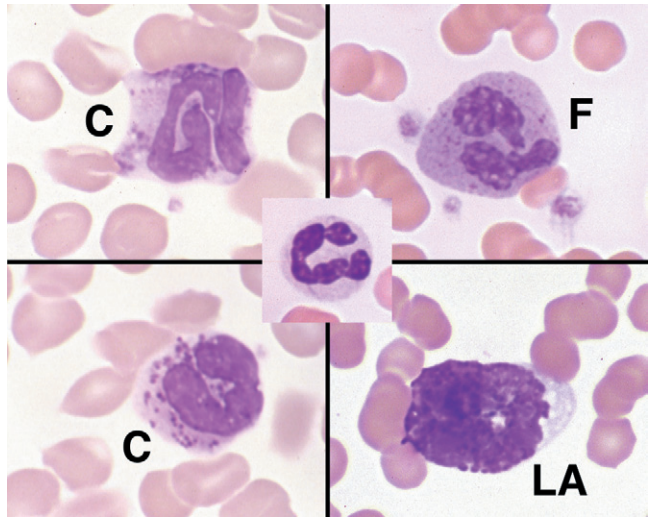

Figure 10.8 Species variation in basophil morphology. A representative neutrophil is shown in the center for comparison. Basophils are larger in diameter than neutrophils. Canine basophils (C) are poorly granulated. Note the sprinkling of basophilic granules in the cytoplasm. Feline basophils (F) have cytoplasm packed with large, poorly staining gray granules that are arranged like pavement stones. Large animal basophils (LA) have numerous dark-staining granules that often obscure the nucleus. (Wright's Giemsa stain, high magnification.)

to and damage parasite membranes, and they are responsible for providing a defense mechanism against larval stages of parasitic infestation. They are also involved in the modulation of allergic inflammation and immune-complex reactions.

Eosinophils vary in morphology among species (Fig. 10.7). The nucleus is segmented (like that of neutrophils). The hallmark feature of eosinophils are prominent, red-orange granules that are tinctorally similar to erythrocytes. Canine eosinophils have highly variable granule size and number per cell. On rare occasions, a few large granules the size of erythrocytes may be present. Eosinophil granules also may wash out during the staining process, leaving what appears to be an empty vacuole; this observation is most pronounced in greyhound dogs. Feline eosinophils are densely packed, with uniform, rod- or barrel-shaped granules. Equine eosinophils have a raspberry appearance because of numerous round, very large granules that usually obscure the nucleus. Ruminant eosinophils have uniform, numerous round granules.

Basophils

The function of basophils is, basically, unknown. Basophils contain histamine and heparin. The cytoplasmic membrane has bound immunoglobulin E, like mast cells; however, their pathophysiologic role in the circulation is unknown. No convincing evidence has been reported that blood basophils migrate into tissues and become tissue mast cells. Concentrations of basophils in the circulation are very low, and they usually are not encountered in the routine differential count.

Basophils are larger in diameter than neutrophils. The nucleus is segmented (like those of other granulocytes). The granule morphology varies among species (Fig. 10.8). Dogs have a small number of dark-violet granules. Cats have large, faint-gray granules that form a pavement-stone arrangement. Large animal basophils are packed with dark-violet granules that are so numerous they often obscure portions of the nucleus.

Reference values: The normal leukogram

The approach to interpretation of the leukogram involves a series of steps to arrive at a conclusion regarding what is normal or abnormal. Interpretive attention should focus only on the absolute values within the differential count (see Chapter 1). When examining the hematology report, one should look first at the total leukocyte concentration. The total leukocyte concentration is only used to calculate absolute differential concentrations; it is not directly interpreted. For interpretation purposes, it only provides some

Table 10.1 Reference intervals for absolute leukocyte concentrations of common domestic animal species.

Leukocyte	Dog	Cat	Horse	Cow	Sheep	Pig
Total WBC (cells/μL)	6000–17,000	5500–19,500	5500–12,500	4000–12,000	4000–12,000	11,000–22,000
Differential WBC:						
Band neutrophils (cells/μL)	0–300	0–300	0–100	0–100	0–100	0–800
Segmented neutrophils (cells/μL)	3000–11,500	2500–12,500	2700–6700	600–4000	700–6000	3200–10,000
Lymphocytes (cells/μL)	1000–5000	1500–7000	1500–5500	2500–7000	2000–9000	4500–13,000
Monocytes (cells/μL)	0–1200	0–800	0–800	0–800	0–800	200–2000
Eosinophils (cells/μL)	100–1200	0–1500	0–900	0–2400	0–1000	100–2000
Basophils (cells/μL)	Rare, 0–100	Rare, 0–100	0–200	0–200	0–300	0–400

WBC, white blood cell.

gross guidance for what to anticipate when interpreting the differential concentrations. If the total count is decreased, examine the absolute concentration of each cell type to determine which are deficient. If the total count is increased, examine the absolute concentration of each cell type to determine which are present in excess. Even if the total concentration is normal, examine the absolute concentration of each cell type to determine if any abnormalities in distribution are present. Identified abnormalities in the absolute concentrations of individual leukocyte types are then interpreted into processes (see Chapter 12).

Reference values are given in Table 10.1. These values are patterned after general guidelines that that have been used for decades (from the original work of Schalm) and are similar to those used by most veterinary laboratories. A more comprehensive, population-based set of reference intervals generated by newer technology for automated cell counting is needed. This has been done in some teaching hospital laboratories for specific automated systems used in the respective settings. Improved precision of automated cell counting as well as improved procedures for statistical analysis may provide more useful interpretive guidelines in the future.

The clinician interprets leukocyte abnormalities by learning to examine the individual differential leukocyte concentrations and then noting any morphologic abnormalities or abnormal cell types currently present that should not be present in normal blood. Differential leukocyte concentrations are reported in cells per microliter for each cell type. Abnormal nucleated cells include blasts, nucleated erythrocytes, mast cells, and immature granulocytes. Morphologic abnormalities include inherited and transiently acquired morphologic changes. Abnormal morphology is presented in Chapter 12.

Suggested reading

Weiss DJ, Wardrop KJ (eds.) (2010) *Schalm's Veterinary Hematology*. 6th ed. Ames, IA: Blackwell Publishing Ltd.

Lee GR, Bithell TC, Foerster J, Athens JW, Lukens JN (eds.) (1993) *Wintrobe's Clinical Hematology*. 9th ed. Philadelphia: Lea & Febiger.

11 Neutrophil Production, Trafficking, and Kinetics

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General trends regarding the trafficking and kinetics of neutrophils in blood have been observed. Although species differences are not well characterized, they appear to be unimportant. An understanding of these behaviors by neutrophils helps to interpret the timing of responses to disease and the sequential changes between hemograms.

Production of granulocytes

Neutrophils are produced almost exclusively in the active bone marrow of healthy, adult domestic animals. Some production may be found in extramedullary sites, most notably the spleen, in juvenile animals. With long-standing increased demand for neutrophils (e.g., in chronic inflammatory disease), extramedullary production may be observed in adult animals. This will be most prominent in the spleen, but it may also be seen in the liver and lymph nodes.

Neutrophils originate from the pluripotential stem cell system, which gives rise to a more differentiated stem cell that has the capacity to create granulocytes and monocytes (GM stem cells). A subpopulation of these GM stem cells enters a pathway of committed differentiation of blood granulocytes, consisting of neutrophils, eosinophils, and basophils. The stem cells are not morphologically distinct, because they are present in small numbers and are probably morphologically indistinguishable from lymphocytes. Once a cell makes this entry commitment, it undergoes both proliferative and maturational events to propagate blood granulocytes. These proliferative and maturational events are associated with morphologically recognized stages of granulocytes. Recognition of the general progression of these stages is important in the evaluation of bone marrow samples and the identification of cells in blood in response to disease. The morphologic stages of granulocytes are indicated in Figure 11.1.

The myeloblast is the first recognizable cell that is committed to granulocyte production. Myeloblasts are difficult to distinguish from primitive blasts of most other lineages. Once committed, the myeloblast produces primary (i.e., azurophilic) granules, the presence of which identifies the progranulocyte stage. At subsequent stages of maturation, the primary granules change their staining character and become indistinguishable in conventional blood stains. In the next stage, the myelocyte begins to produce secondary (i.e., specific) granules that identify whether the cell will be a neutrophil, eosinophil, or basophil. Historically, the naming of the specific granules and the cell type has related to the dye component of the polychrome blood stains taken up by the specific granule. Neutrophil granules have neutral staining affinity; because of poor dye affinity, the granules are very faint or not visible. Eosinophil granules have an affinity for the orange-red dye and stain intensely orange-red. Basophil granules have affinity for basic dyes and stain intensely dark violet. Myeloblasts, progranulocytes, and myelocytes have the ability to undergo cell division as well as to mature from one stage to the next. These stages are relatively rich in ribosomes, giving the cytoplasm a bluish tint. Nuclear features include round to oval shape and relatively fine chromatin pattern.

More mature stages are characterized by the loss of ability to undergo cell division and include metamyelocytes, bands, and segmented neutrophils. Maturation consists mostly of progressive nuclear condensation and change in nuclear shape. The cytoplasm loses most or all of its bluish tint as the ribosome content decreases. The metamyelocyte has a nucleus that has developed an indentation. The band cell nucleus forms a horseshoe shape and has smooth, parallel nuclear membranes. The segmented or mature neutrophil progressively develops indentations or constrictions in the nuclear membrane. See Chapter 10.

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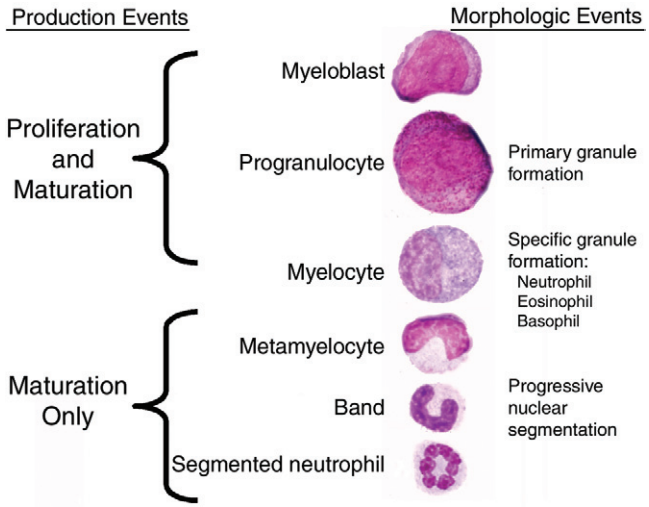

Figure 11.1 Morphologic features of stages of neutrophil maturation. Six morphologic stages are identified on a continuum of maturation, as indicated by the named cells. Cells capable of both cell division and maturation are at the top; cells capable of maturation only are at the bottom. Major changes associated with maturation are indicated on the right. (See text for a more complete description.)

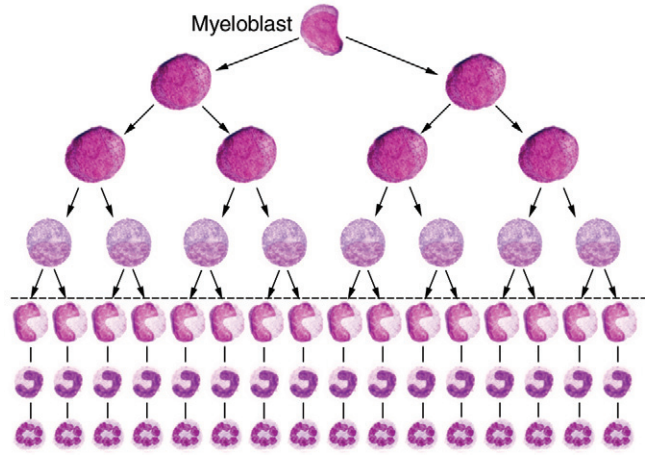

Figure 11.2 Orderly production of neutrophils in bone marrow. Note the progressive increase in relative cell numbers as maturation progresses. The myeloblast may give rise to approximately 16–32 cells before proliferative ability is lost. Cell stages above the dashed line are capable of cell division; cell stages below the dashed line are only capable of maturation. Please refer to Figure 11.1 for reference to cell stages.

Maturation and orderly production

Production normally results in a progressive increase in the relative numbers of more mature stages, as indicated in Figure 11.2. This results from the combined events of proliferating early forms, which amplify both the number of cells and the progress toward more mature stages. In the process, each myeloblast may produce approximately 16 to 32 segmented neutrophils. The pattern of production seen in the marrow is a mixture of a relatively small number of primitive cells, a larger number of intermediate stages, and numerous more mature stages. This progression of a few immature cells to many more mature cells is described as orderly production. Both normal production and accelerated production in response to increased granulocyte demand have this orderly appearance. Cells are also delivered to the blood in this orderly fashion (see the discussion of left shift in Chapter 12). Disorderly production is characterized by a disproportionate relative number of primitive forms and a relative decrease or absence of more mature forms. Disorderly production is one of the features used to identify certain pathologic patterns (e.g., myeloproliferative disorders).

Neutrophil pools and trafficking

To understand neutrophil responses in disease, it is helpful to visualize a set of compartments and pools consisting of bone marrow, blood, and tissues, as depicted in Figure 11.3. The bone marrow compartment may be conceptually divided into a stem cell pool, a proliferative pool, and a maturation and storage pool. The proliferative pool consists of neutrophils at stages during which they still have the ability to

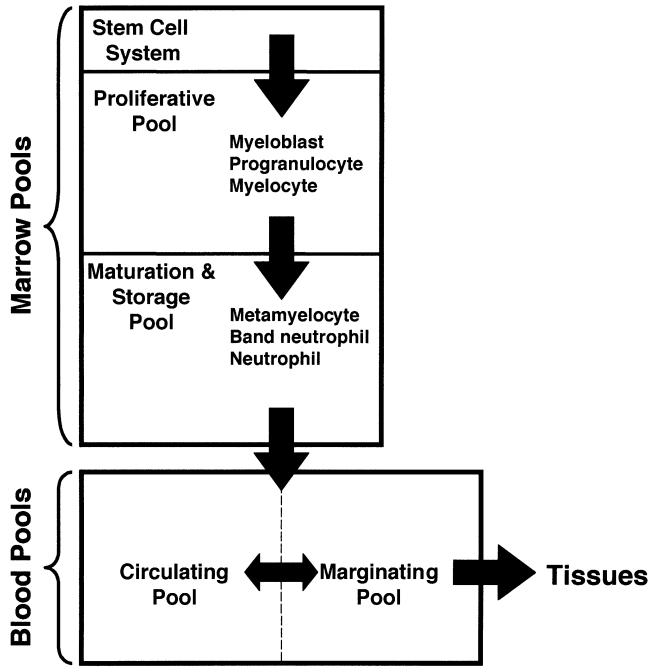

Figure 11.3 Bone marrow and blood neutrophil pools. Single arrows indicate unidirectional movement of cells; double arrow indicates bidirectional movement of cells. (See text for description of various compartments and progress through them.)

undergo cell division and is largely responsible for the amplification of cell numbers. The maturation and storage pool consists of cells having no ability to divide and that are completing morphologic maturation. These cells may accumulate to create a modest storage reserve that is variable in size, which depends on the species. The storage capacity is greatest in dogs, least in ruminants, and intermediate in cats and horses.

Neutrophils make a unidirectional migration to the blood compartment, which is divided into the circulating and margination pools. The circulating pool is located in large vessels in which no interaction normally occurs between neutrophils and the endothelial lining of the vessel. Blood samples taken by venipuncture are from the circulating pool. The margination pool consists of the microcirculation. Cells may move bidirectionally between the circulating and margination pools. Neutrophils interact with the endothelial lining of small vessels and capillaries by their property of stickiness. Neutrophils may then unidirectionally migrate into adjacent tissue spaces (i.e., the tissue compartment). It is in the tissue compartment that neutrophils participate in their host-defense purposes.

All neutrophil responses in disease may be understood as being mechanisms and disturbances occurring in this set of pools. They are discussed in detail in Chapter 12.

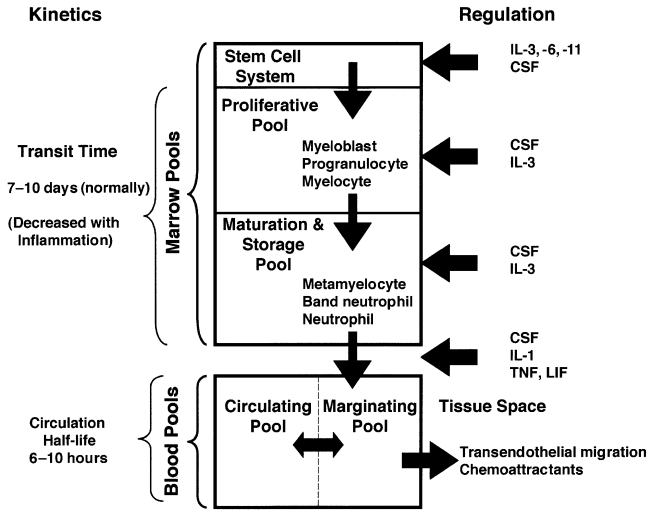

Figure 11.4 Bone marrow and blood neutrophil pools. Kinetic information is given on the left and regulation information on the right. Neutrophil production is regulated by a concert of growth factors and cytokines that act at multiple sites. The transit time is normally 7–10 days but may be shortened with increased demand. The circulation half-life is approximately 6–10 hours.

Growth factors and regulation of production and blood concentration

In health, the concentration of neutrophils in the blood is regulated to stay within a relatively narrow range compared with the range that is possible in disease. Regulation of production is mediated by a complicated set of cytokines and growth factors, a simplified version of which is shown in Figure 11.4. The family of cytokines and growth factors depicted in Figure 11.4 work in concert at various stages to regulate neutrophil production. Colony-stimulating factor (CSF) is a group of characterized molecules; most notable are granulocyte-CSF and GM-CSF. These factors originate from numerous and diverse sites, including mononuclear cells, endothelium, fibroblasts, and other cell types. Mononuclear cells at sites of inflammation are probably the most important source of CSF and may modulate the release of CSFs from the other cell types. Interleukins (ILs) also participate in stimulation of production. The release of neutrophils from the marrow space to blood may be accelerated by IL-1, tumor necrosis factor (TNF), and leukocytosis-inducing factor (LIF). Because of variation in experimental conditions and methods, LIF may be the same as IL-1 and TNF.

In the normal steady state, production is balanced by the transendothelial migration of neutrophils into tissues. This balance yields blood neutrophil concentrations in the normal range. Increased levels of growth factors and cytokines are responsible for marked acceleration of the events to produce neutrophils in response to inflammation. This may result in a dramatic increase in neutrophil production and delivery to blood. Migration into the site of inflammation is accelerated and focused by chemoattractants that are released in the inflammatory lesion. The net result is an increase in the flux of neutrophils from the bone marrow to the inflammatory lesion. After resolution of the inflammatory lesion, blood neutrophil concentrations return to normal. This suggests the presence of some negative-feedback mechanism, but its nature is currently unknown.

Neutrophil kinetics

Some basic information about the kinetics of neutrophils in various pools is helpful in the interpretation of sequential changes in the leukogram. The transit time for production and the circulation time in blood are the two key benchmarks for neutrophil kinetics.

The transit time is the amount of time needed for the myeloblast to complete the maturational events and become a segmented neutrophil in blood (see Fig. 11.4). In the normal steady state, the transit time is approximately 7 days. When the bone marrow is stimulated by the inflammatory response, the transit time may become as short as 2–3 days.

The circulation time is the amount of time between release of the neutrophil to the blood and its subsequent egress into tissues. Neutrophils randomly migrate into tissues, so their

circulation time is variable and not related to cell age. The circulation time is approximately 6–10 hours, encompassing some species variation. This means that the blood neutrophil pools are renewed approximately two to three times per day. The circulation time may be shortened considerably when neutrophils are consumed at a more rapid rate (e.g., at a site of inflammation). Given the rapid rate of blood neutrophil renewal in blood, marked changes in the blood neutrophil concentration may occur very rapidly in response to disease. The magnitude of these changes in the cell concentration that may be observed on hemograms sampled only hours apart is often dramatic and surprising.

Suggested reading

Athens JW (1993) Granulocytes—neutrophils. In: Lee GR, *et al*. *Wintrobe's Clinical Hematology*. 9th ed. Philadelphia: Lea & Febiger, pp. 223–66.

Quesenberry PJ, Colein GA (2001) Hematopoietic stem cells, progenitor cells, and cytokines. In: Beutler E, *et al*. *William's Hematology*. 6th ed. New York: McGraw-Hill, pp. 153–74.

Weiss DJ, Wardrop KJ (eds) (2010) *Schalm's Veterinary Hematology*. 6th ed. Ames, IA: Blackwell Publishing Ltd.

12 Interpretation of Leukocyte Responses in Disease

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To communicate about leukocyte responses, one must first become familiar with the descriptive terminology associated with abnormal patterns of cell concentrations in blood. To identify and interpret leukocyte responses, the rules for interpreting abnormal concentration patterns as indicators of disease processes must be learned. This chapter presents terminology, abnormal morphologic features encountered in the laboratory, and guidelines for interpretation of leukocyte patterns.

Terminology of abnormal leukocyte concentration patterns

Suffixes

Abnormal concentrations are described using a variety of suffixes attached to the name of the cell type(s) involved.

The suffix -penia refers to a decreased concentration of the cell type in blood. A general term, cytopenia, refers to a decrease in cell concentration in a nonspecific manner. Cytopenias that are important for interpretation include neutropenia, lymphopenia, and eosinopenia. Cytopenia does not apply to monocytes, because a decreased concentration of this cell type is not important. It also does not apply to band neutrophils, metamyelocytes, basophils, metarubricytes, and other abnormal cells because the absence of these cells is normal.

The suffixes -philia or -cytosis refer to an increased concentration of the cell type in blood. Examples include:

- neutrophilia or neutrophilic leukocytosis
- eosinophilia
- basophilia
- monocytosis
- lymphocytosis
- metarubricytosis.

Left shift

Left shift refers to an increased concentration of immature neutrophils in blood. This usually indicates band neutrophils, but metamyelocytes and earlier forms may accompany increased bands. (See Fig. 10.1 for neutrophil and left-shift morphology.) A left shift may occur with neutrophilia. A left shift also may occur with neutropenia; this indicates a more severe consumption of neutrophils by a more aggressive inflammatory lesion or an early repopulation of blood following a reversible stem cell injury. An orderly left shift suggests an inflammatory stimulus; in this case, the term orderly means that the concentration of each cell stage decreases with the degree of immaturity of the cell stage.

Leukemia

Leukemia refers to the presence of neoplastic cells in the circulation. The neoplastic cell type that is present designates more specifically the classification of the leukemia present. The classification may be determined by a combination of cell population morphologic differentiation features seen on the blood film, surface marker cytometry panels, and immunocytochemistry reactions (see Chapter 13). Examples include myelomonocytic leukemia and lymphocytic leukemia. The concentration of neoplastic cells may vary from detectable on blood film scanning to extremely high.

Proliferative disorder

Proliferative disorder is a nonspecific term for a hematopoietic cell neoplasm that is distributed in blood, bone marrow, other tissues, or a combination of these and other sites. Proliferative disorders are classified into lymphoproliferative and myeloproliferative categories. The distinction between the lymphoid and bone marrow stem cell systems is somewhat artificial, but these two classes of proliferative disorders have different biologic behavior and case management prognosis. Proliferative disorders are discussed separately in Chapter 14.

Veterinary Hematology and Clinical Chemistry, Second Edition. Edited by Mary Anna Thrall, Glade Weiser, Robin W. Allison, and Terry W. Campbell.

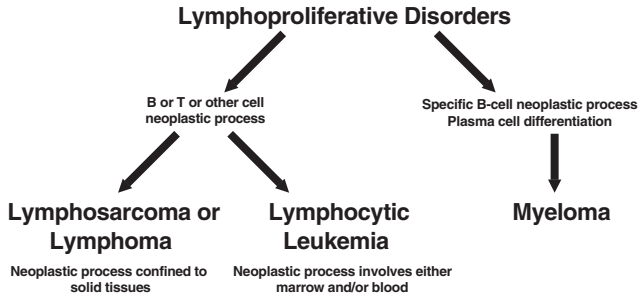

Figure 12.1 Organization and general terminology for lymphoproliferative disorders. See text for discussion.

Lymphoproliferative disorders

Lymphoproliferative disorders, which are characterized in Figure 12.1, are neoplastic processes with lymphoid cell differentiation. If the neoplasm is confined to solid tissues, it is termed lymphosarcoma or lymphoma. If it involves blood and/or bone marrow, it is termed lymphocytic leukemia. A specific form with plasma cell differentiation is termed myeloma, which is usually associated with production of a monoclonal immunoglobulin that may be detected in blood. Immunoglobulin light chains also may be detected in urine. More extensive and detailed classifications of lymphoproliferative disorders based on cellular morphology and immunophenotyping are available (see Chapters 13 and 14 and Suggested Reading).

Myeloproliferative disorders

Myeloproliferative disorders arise from the bone marrow stem cell system. More extensive and detailed classifications of myeloproliferative disorders based on cellular morphology and surface markers are available (see Chapter 13 and 14 and Suggested Reading). The recognized lines of differentiation and associated terminology for specific myeloproliferative disorders are detailed in Figure 12.2. Note that more differentiation pathways are recognized for myeloproliferative disorders than for lymphoproliferative disorders. Granulocytic, monocytic, and erythroid differentiations are the most common myeloproliferative disorders; the others are rare.

In recent years, it has become apparent that confirmation of lymphoproliferative disorders or identification of cell lineage in proliferative disorders is limited with morphology. Occasionally, the distinction between reactive and neoplastic lymphoid proliferation is difficult. Primitive blasts having no specific differentiating morphologic features may be difficult to impossible to classify by morphology alone. These are often called lymphoid on initial examination. It has been learned that lymphoid, monocytic, granulocytic, and megakaryocytic blasts can be morphologically indistinguishable. Immunocytochemistry and flow cytometry are now used to identify cell lineage when it is important for treatment considerations. These procedures use panels of chemistry reactions and/or antibody labeling to identify either cytoplasmic activities or surface markers to aid in classification. Furthermore, these tools may be used to determine lymphocytic subpopulation identification. This is the subject of Chapter 13.

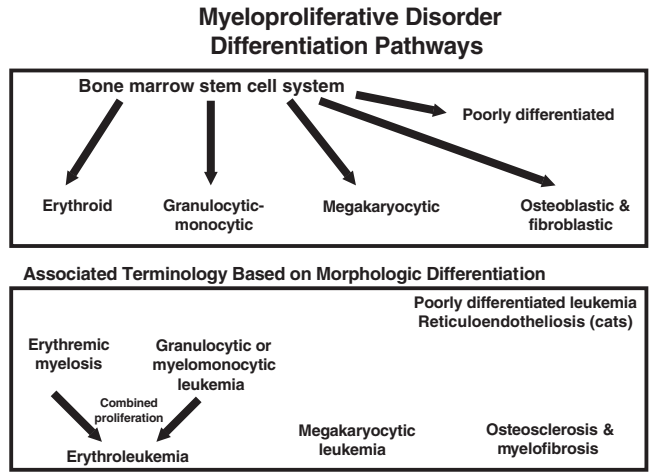

Figure 12.2 Organization and general terminology for myeloproliferative disorders. The top box shows general differentiation pathways based on morphologically recognized cell lineages. The bottom box shows historical and commonly applied terminology for the myeloproliferative disorders based on morphologic identity. See text for discussion.

Acquired changes in leukocyte morphology neutrophil toxic change

Neutrophil toxic change may be observed in association with inflammatory responses. The term toxic change is unfortunate, because it originated from early observations that these alterations in cell morphology were associated with toxemia in human patients. The term implies that the cells are injured or impaired. Today, however, we understand that the morphologic change is attributable to altered bone marrow production and that the cells have normal function. When an inflammatory stimulus is delivered to the bone marrow (see Fig. 11.4), neutrophils are produced at an accelerated rate. As a result, the cells may have increased amounts of certain organelles that are present during early development. The principal manifestation is cytoplasmic basophilia (Fig. 12.3). This is attributable to a larger-than-normal complement of ribosomes. Other, less common manifestations accompanying cytoplasmic basophilia include Döhle bodies and cytoplasmic vacuolation. Döhle bodies are aggregates of endoplasmic reticulum and appear as gray-blue cytoplasmic precipitates. Dohle bodies are seen more commonly in cats (Fig. 12.3).

The interpretation of toxic change is that neutrophils are made under conditions of accelerated production that occurs

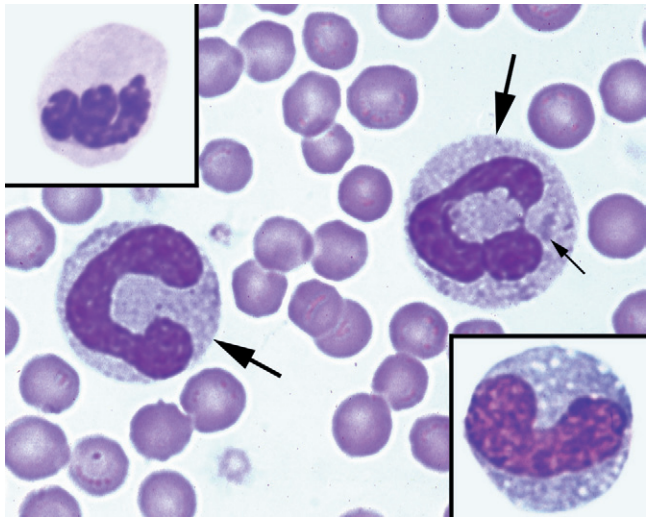

Figure 12.3 Neutrophils with marked toxic change (arrows). Note prominent cytoplasmic basophilia. A Döhle body is indicated by the thin arrow. A toxic neutrophil with fine cytoplasmic vacuolation is shown in the lower right inset. For comparison, a normal neutrophil is shown in the upper left inset. Wright-Giemsa stain, high magnification.

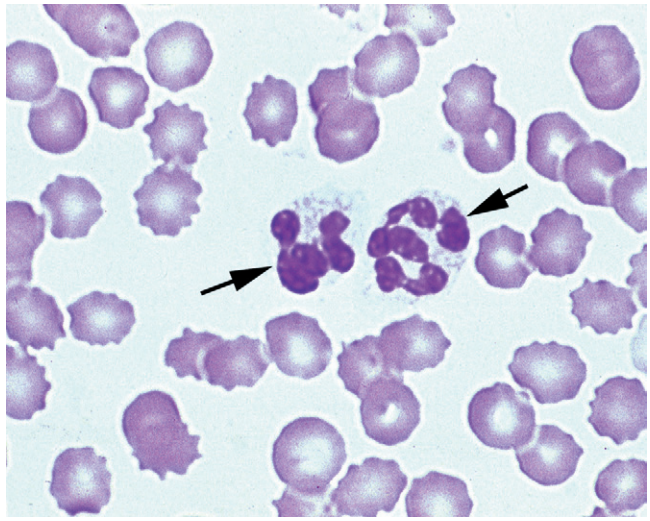

Figure 12.4 Neutrophils with hypersegmentation (arrows). Note the nuclear constrictions to a filament of chromatin that separates approximately 5–7 chromatin lobes. Wright-Giemsa stain, high magnification.

as part of the inflammatory response. As a result, toxic change often accompanies other quantitative changes in the inflammatory leukogram presented later in this chapter.

Neutrophil hypersegmentation

Neutrophil hypersegmentation is the normal progression of nuclear maturation in the neutrophil. The progression from band shape to segmentation to hypersegmentation is a continuum that occurs in a matter of hours. Normally, the process of continued segmentation and, finally, pyknosis occurs in neutrophils after egression to tissues. Hypersegmentation observed on the blood film results from longer than normal retention of neutrophils in the circulation (Fig. 12.4). The interpretation of hypersegmentation is relatively unimportant (it is usually associated with steroid effect on the leukogram presented in this chapter).

Neutrophil degeneration

Neutrophil degeneration is a description ordinarily applied to neutrophils from samples other than blood (e.g., cytopathologic specimens). Neutrophils exposed to an unhealthy environment outside of blood may rapidly degenerate. This is accelerated in cytopathologic specimens, which either have a bacterial component or are from epithelial surfaces such as skin, airways, or the gastrointestinal tract (Fig. 12.5). Features include cytoplasmic vacuolation and nuclear swelling seen as a loss of chromatin pattern and light staining. These changes may progress to cell lysis. It is an artifact in blood seen on the blood film if that film is made from blood that has aged for 12 hours or longer after collection from the animal (Fig. 12.5). In blood, it therefore is interpreted as an artifact of improper sample handling.

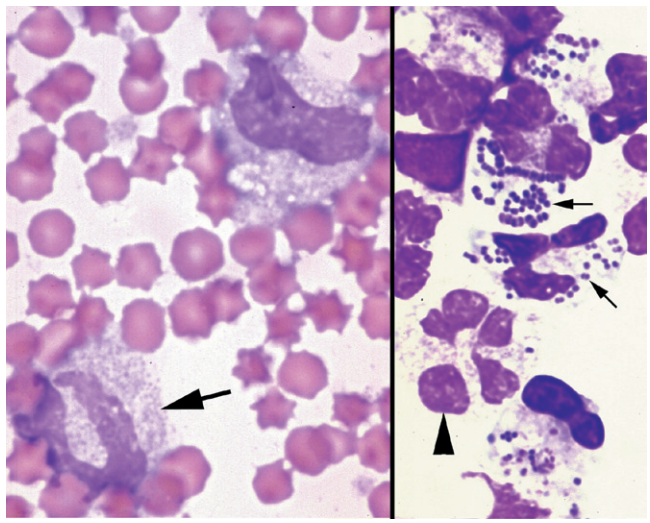

Figure 12.5 Neutrophil degeneration. The left panel shows neutrophil (arrow) degeneration on a blood film that is an artifact of aging in the collection tube before blood-film preparation. Note the swollen chromatin that results in lighter staining and loss of chromatin detail. The right panel shows neutrophils in various stages of degeneration in a cytologic preparation. This results from an unhealthy environment that is created, in part, by numerous bacteria (thin arrows). A neutrophil with chromatin swelling and loss of detail is indicated by the arrowhead. Wright-Giemsa stain, high magnification.

Leukocyte agglutination

Leukocyte agglutination is an immunoglobulin-mediated agglutination of leukocytes in vitro. It may affect either neutrophils or lymphocytes. This phenomenon does not

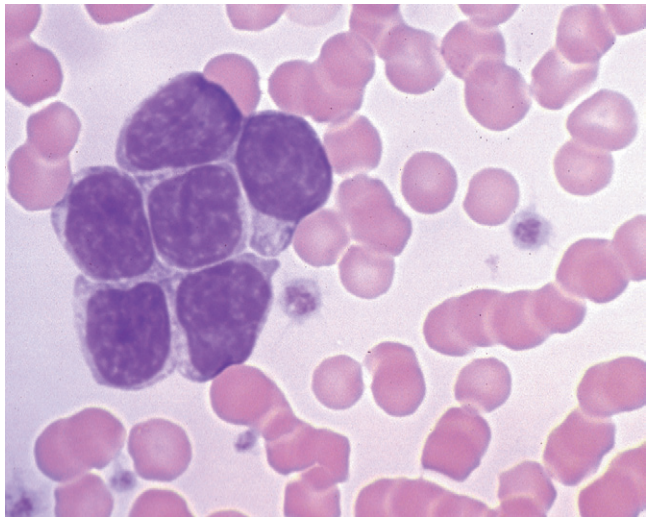

Figure 12.6 Leukoagglutination involving lymphocytes. Note the tight adherence of cells in a cluster. Multiple clusters are observable at low magnification. These cell clusters result in falsely low white-blood-cell counts when present in the counting fluid diluent (see text). Wright-Giemsa stain, high magnification.

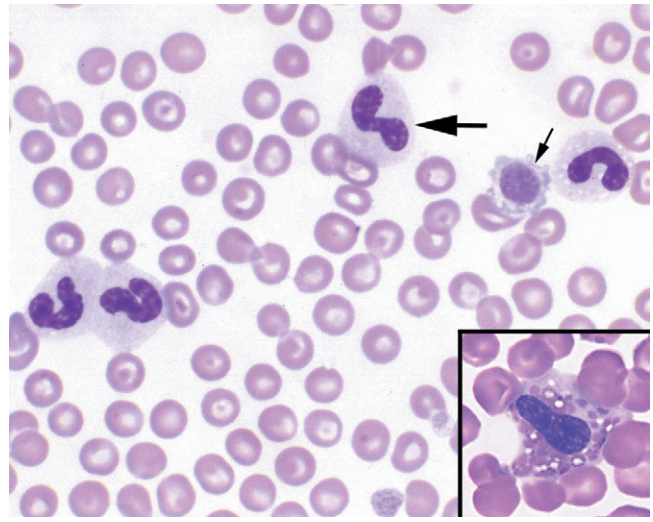

Figure 12.7 Granulocytes from a dog with Pelger-Huët anomaly. Four hyposegmented neutrophils (thick arrow) are present. The lower right inset shows a hyposegmented eosinophil. A macroplatelet, present by coincidence, is indicated by the thin arrow. Wright-Giemsa stain, high magnification.

occur in the animal at body temperature, and it likely has no pathologic consequence in vivo. It is thought to be attributable to a cold-reacting immunoglobulin that acts at temperatures well below body temperature. When the blood cools to room temperature or below, this abnormal immunoglobulin binds to its leukocyte target and bridges cells into agglutinated particles. It therefore occurs in the blood tube after collection from the patient. Its importance is that it may result in a falsely low total white-blood-cell concentration, because agglutinated leukocytes may not be counted by instruments. It is observed on scanning the blood film (Fig. 12.6).

Lymphocyte vacuolation

Lymphocyte vacuolation may be an acquired change associated with ingestion of certain plants containing the toxic substance swainsonine. An example is locoweed ingestion in horses or cattle. The appearance is similar to that of lymphocyte vacuolation associated with inherited storage disorders (discussed later; see Fig. 12.11).

Inherited abnormalities of leukocyte morphology and function

Inherited abnormalities of neutrophil morphology and/or function

Inherited abnormalities of neutrophil morphology include Pelger-Huët anomaly, Birman cat neutrophil granulation anomaly, mucopolysaccharidoses, and Chédiak-Higashi syndrome.

Pelger-Huët anomaly

Mature, hyposegmented neutrophils are seen in heterozygotes for Pelger-Huët anomaly. These cells have an immaturely shaped nucleus (i.e., band or myelocyte form) but a coarse, mature chromatin pattern (Fig. 12.7). Neutrophils function normally, and affected animals are healthy. Typically, no segmented neutrophils are seen in blood films from these animals. Eosinophils are also affected and appear as band forms. The importance of recognizing Pelger-Huët anomaly is to prevent misidentification of a major left shift and misinterpretation as an inflammatory response in an otherwise apparently healthy, affected individual.

Birman cat neutrophil granulation anomaly

Neutrophils from affected cats contain fine eosinophilic to magenta-colored granules (Fig. 12.8). This anomaly is inherited in an autosomal recessive manner. Neutrophil function is normal, and cats are healthy. This granulation must be distinguished from toxic granulation, which is rare, and from that seen in neutrophils from cats with mucopolysaccharidosis, which usually is more coarse.

Mucopolysaccharidoses

Neutrophils from animals with mucopolysaccharidosis (MPS) typically contain numerous distinct, dark-purple or magenta-colored granules (Fig. 12.9). Lymphocytes also usually contain granules and vacuoles.

Mucopolysaccharidosis is a group of heritable, lysosomal storage disorders caused by a deficiency of lysosomal enzymes needed for the stepwise degradation of glycosaminoglycans (i.e., mucopolysaccharides). Common features

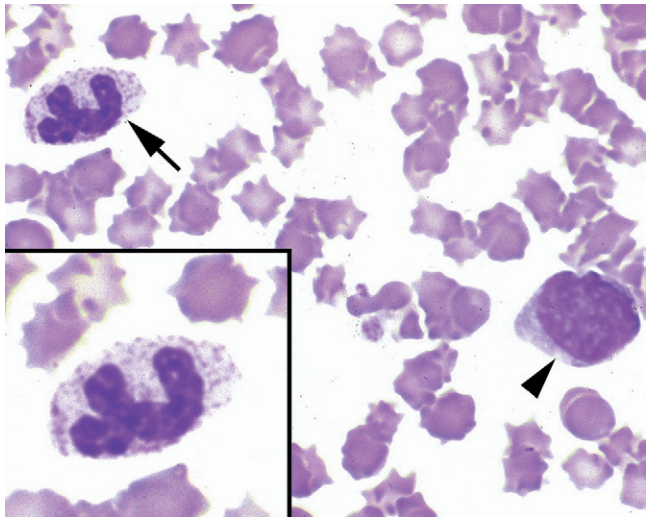

Figure 12.8 Granulated neutrophil from a cat with Birman cat neutrophil granulation anomaly (arrow). The lower left inset shows an enlarged view of the same cell. Note the fine granulation as compared with mucopolysaccharidosis (see Fig. 12.9). Lymphocytes (arrowhead) are not affected. Wright-Giemsa stain, high magnification.

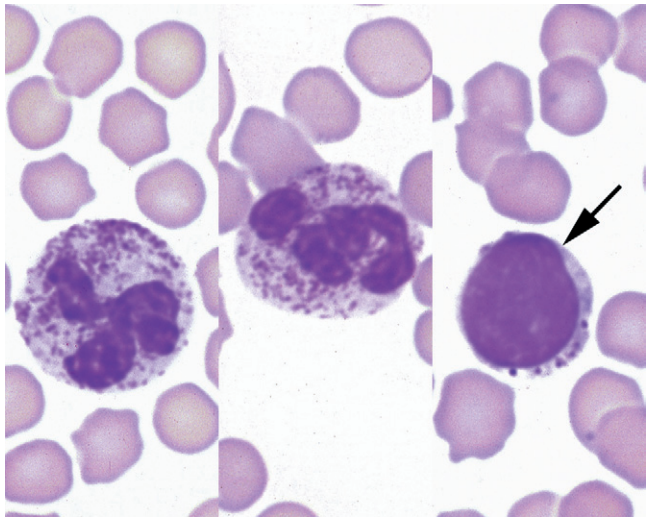

Figure 12.9 Granulated leukocytes from a cat with mucopolysaccharidosis VI. Note the prominently granulated neutrophils at the left and center. A lymphocyte with sparse granulation is typical of mucopolysaccharidosis (arrow). Wright-Giemsa stain, high magnification.

include dwarfism (except feline MPS I), severe bone disease, degenerative joint disease including hip subluxation, facial dysmorphia, hepatomegaly (except feline MPS VI), corneal clouding, enlarged tongue (canine MPS), heart-valve thickening, excess urinary excretion of glycosaminoglycans, and metachromatic granules (i.e., Alder-Reilly bodies) in blood leukocytes. These granules are more distinct in MPS VI and VII than in MPS I. Granules usually are not apparent when

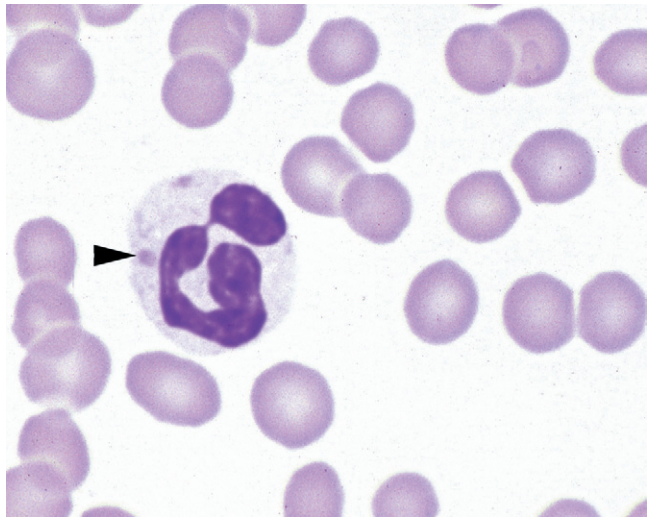

Figure 12.10 Neutrophil from a cat with Chédiak-Higashi syndrome. Note the large eosinophilic granule in the cytoplasm (arrowhead). Wright-Giemsa stain, high magnification.

stained with Diff-Quik. The disease is progressive, with clinical signs becoming apparent at 2–4 months of age. Affected animals may live several years, but locomotor difficulty is progressive.

Chédiak-Higashi syndrome

Neutrophils in cats affected by Chédiak-Higashi syndrome have large, fused, 2.0-mm lysosomes that stain lightly pink or eosinophilic within the cytoplasm (Fig. 12.10). Approximately one in three or four neutrophils contain one to four fused lysosomes. Eosinophilic granules appear slightly plump and large. These cats have a slight tendency to bleed, because platelet function is abnormal. Although neutrophil function is also abnormal, cats are generally healthy. The syndrome has been reported in cats of Persian ancestry and is inherited in an autosomal recessive manner.

Bovine leukocyte adhesion deficiency (BLAD)

Bovine leukocyte adhesion deficiency is a lethal, autosomal recessive disorder identified in Holstein cattle. The defect is a mutation in the CD 18 gene. This results in neutrophils with a deficiency of beta-2 integrin surface molecules that are essential for normal leukocyte adherence and emigration into tissues; hence there is a functional defect. Clinical signs of "poor doing" appear at 1–2 weeks of age. Affected calves may appear stunted and have signs related to respiratory and gastrointestinal tracts. They are predisposed to recurring bacterial infections and typically do not live beyond 2–8 months of age. A hematologic feature is marked, persistent neutrophilia (often >100,000/μL) with no left shift. On examination of tissues, there are few neutrophils, except within vessel lumens, because they persist in the circulation and have impaired entry into the tissues. Testing is available

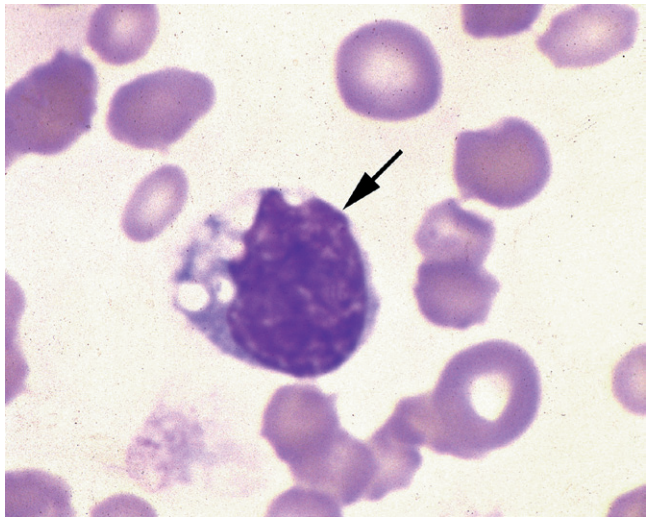

Figure 12.11 Cytoplasmic vacuolation of a lymphocyte (arrow) from a cat with a lysosomal storage disorder (alpha-mannosidosis). Wright-Giemsa stain, high magnification.

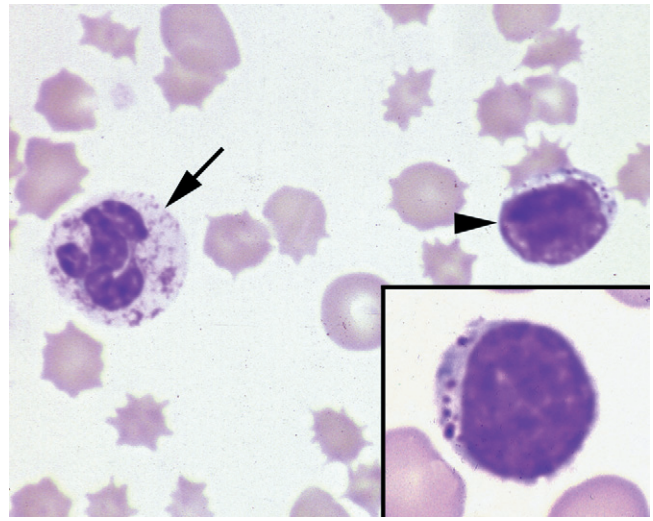

Figure 12.12 Leukocytes from a cat with G_{M2} gangliosidosis. Neutrophils (arrow) may have granulation similar to that seen with mucopolysaccharidosis. Lymphocytes (arrowhead) also have small numbers of granules with some degree of cytoplasmic vacuolation. The lower right inset shows an enlarged lymphocyte. Wright-Giemsa stain, high magnification.

to detect carriers. Incidence of the defect is decreasing due to testing for the carrier state and removal of carriers from breeding stock.

Inherited abnormalities of lymphocyte morphology

Cytoplasmic vacuolization is the most significant inherited abnormality of lymphocytes and usually is associated with lysosomal storage disorders (Fig. 12.11). Those lysosomal storage diseases described in domestic animals that result in vacuoles within the cytoplasm of lymphocytes include the MPS (also have granules in neutrophils); G_{M1} and G_{M2} gangliosidosis (G_{M2} gangliosidosis also has granules in lymphocytes and neutrophils) (Fig. 12.12); alpha-mannosidosis; Niemann-Pick types A, B, and C; acid-lipase deficiency; and fucosidosis. All these disorders, except for MPS and acid-lipase deficiency, result in severe, progressive neurologic disease that is ultimately fatal.

Interpretation of leukocyte responses

Perspective

Most leukocyte response patterns are not interpreted into specific diagnoses, although leukemias may be an exception. Instead, responses are interpreted into basic processes occurring in the animal. These processes must then be coupled with other clinical information to work toward a clinical diagnosis.

Hematologic response to inflammation

Inflammation is the most important—and one of the most common—blood leukocyte responses. The nature of the response is best understood by considering a modified neutrophil trafficking model (Fig. 12.13). It also may be helpful to review the steady-state neutrophil trafficking model in Chapter 11 (see Fig. 11.3). When inflammation is established, an orchestra of chemical mediators modulates many

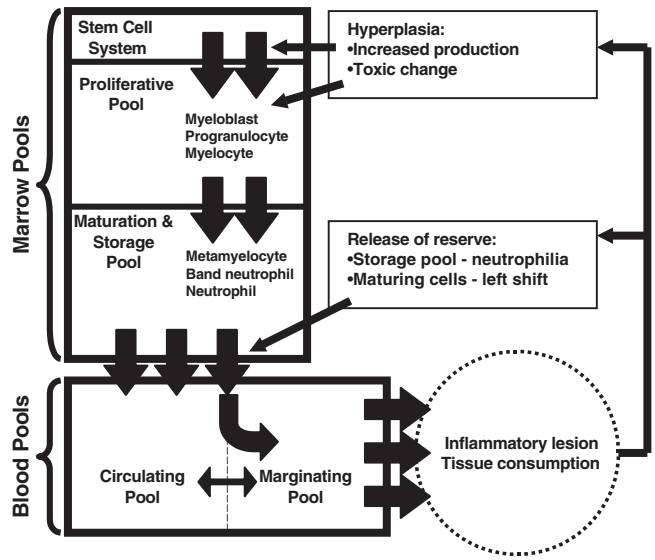

Figure 12.13 Modified neutrophil trafficking model illustrating effects of the inflammatory response on blood and bone marrow. Note the cycle of events leading to increased neutrophil delivery to blood and tissues at the inflammatory site: release of mediators from an inflammatory lesion, increased marrow hyperplasia, increased delivery from marrow to blood, and increased consumption at the site of inflammation.

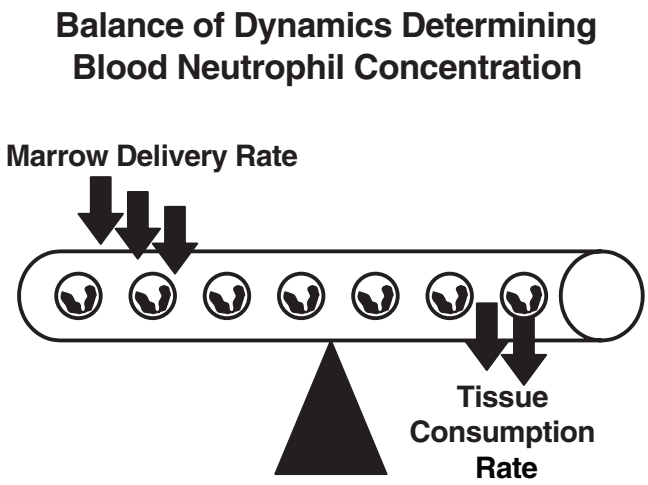

Figure 12.14 Balance between production and consumption. All inflammatory processes may be understood as a balance between marrow delivery and inflammatory-site consumption. When marrow delivery exceeds consumption, blood neutrophilia develops. When tissue consumption exceeds marrow delivery, neutropenia with a left shift develops.

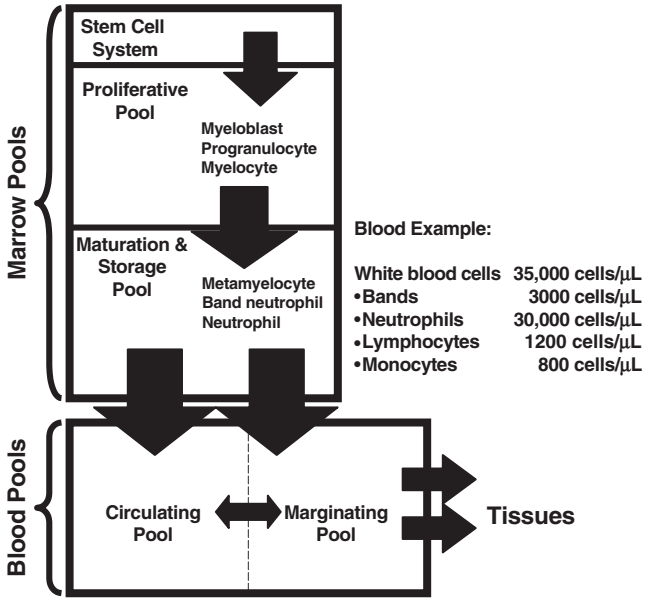

Figure 12.15 Modified neutrophil trafficking model used to illustrate a moderate inflammatory response. Also illustrated is an example of the balance between production and consumption. Note that in this case, marrow delivery exceeds tissue consumption. The example is described as leukocytosis caused by neutrophilia (30,000 cells/µL) and a left shift (3000 bands/µL). The neutrophil pattern is interpreted as inflammation.

events. Vasodilation and chemotactic substances work to increase the egress of neutrophils from the local marginated pool into the inflammatory lesion. Cytokines released from local mononuclear cells (see Fig. 11.4) make their way to the bone marrow, where they increase the rate of release of maturing neutrophils and the rate of production by increasing stem-cell entry, proliferative events, and maturation events. The net result is that the marrow response dramatically increases the delivery rate of neutrophils to blood. In summary, a complete cycle of consumption, production, and release is activated, with the goal of delivering a supply of neutrophils to the inflammatory lesion until it resolves.

The pattern of neutrophil concentrations seen in blood may vary from severely decreased to markedly increased. It is helpful to think of the pattern being dependent on a balance between consumption by the lesion and production and release by the marrow (Fig. 12.14). This balance may explain all neutrophil concentration patterns encountered during inflammation. In small animals, most inflammatory processes result in some degree of neutrophilia, indicating that marrow releases more cells to blood than are consumed at the site of inflammation. This is illustrated using the neutrophil trafficking model in Figure 12.15. Inflammatory patterns manifesting in neutrophilia may be regarded as mild to severe responses that are managing the lesion. The severity of the process may be roughly predicted by the magnitude of the left shift and the presence of toxic change in neutrophils.

Very severe—and typically acute—inflammatory lesions, on the other hand, may consume neutrophils more rapidly than the neutrophils can be delivered to blood. When this occurs, neutropenia develops, as shown in the neutrophil trafficking model in Figure 12.16. In this case, a left shift is

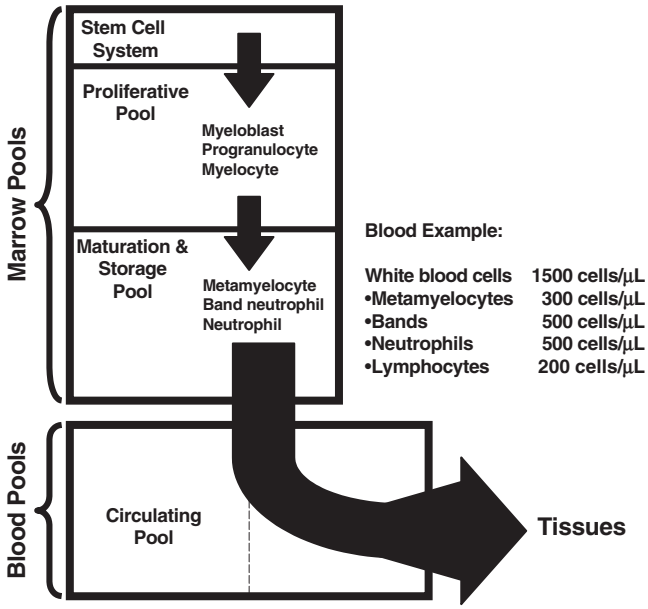

Figure 12.16 Modified neutrophil trafficking model used to illustrate a severe inflammatory response. Also illustrated is an example of the balance between production and consumption. Note that in this case, tissue consumption exceeds marrow delivery. The example is described as leukopenia caused by neutropenia (500 cells/µL) and a left shift (300 metamyelocytes/µL and 500 bands/µL). The neutrophil pattern is interpreted as severe, acute inflammation.

Table 12.1 Comparative bone marrow contribution to neutrophil trafficking and relationship to ranges of neutrophilia seen with the inflammatory response in various species.

Species	Marrow Reserve	Regenerative Capacity
Dog	Relatively high	Rapid
Cat	Intermediate	Intermediate
Horse	Intermediate	Intermediate
Cow	Relatively low	Slow

Species	Range of Possible Neutrophilia (neutrophils/μL)
Dog	20,000–120,000
Cat	20,000–60,000
Horse	15,000–30,000
Cow	10,000–25,000

Species	Interpretation of Neutropenia During Acute Inflammation
Dog	Very severe lesion
Cat	Very severe lesion
Horse	Probable severe lesion
Cow	Usual findings, regardless of severity

expected. At one or more time points, the concentration of bands and other left shift cells may be greater than that of segmented neutrophils.

The balance between neutrophil consumption and delivery by bone marrow is affected by species differences, as outlined in Table 12.1. Species may vary in the amount of neutrophil reserve and in the proliferative capacity of the marrow. Dogs have the largest reserve and the greatest ability to produce neutrophils; cows and other ruminants form the other extreme. Cats and horses are somewhat intermediate in their capacities to deliver cells to blood.

These differences translate into magnitudes of neutrophilia that can occur with inflammatory disease in each species. They also influence how neutrophil concentrations are interpreted with respect to chronicity and severity of the process in various species. For example, in chronic, closed-cavity inflammatory processes, neutrophilia may go as high as 120,000 cells/μL in dogs, but a corresponding process in cows will result in a maximum of approximately 25,000 cells/μL. Cats and horses will be intermediate, as indicated in Table 12.1.

Similarly, bone marrow behavior influences how neutropenia is interpreted during acute inflammation. Because of the canine ability to deliver cells to blood, neutropenia only occurs with inflammatory states involving severe consumption. Neutropenia caused by inflammation may be regarded as a medical emergency in dogs; to some extent, this is also true in cats and horses. Neutropenia in cows is interpreted differently. Because of the minimal neutrophil reserve in this species, the expected response in the acute bovine inflammatory leukogram is neutropenia. Acute inflammatory lesions in cows consume neutrophils from the blood and marrow within a matter of hours. The result may be profound neutropenia that lasts for a few days. After that time, repopulation of blood with neutrophils, with a left shift, occurs as the marrow production increases.

Factors modulating the magnitude of neutrophilia in the inflammatory response

The type of inflammatory lesion may influence the balance between consumption and marrow release. Acute inflammation is a lesion with increased local blood flow and swelling. This results from inflammatory mediators that promote local vascular dilation. Chemotactic factors released within the lesion in conjunction with the vascular events have ample opportunity to promote consumption of neutrophils. An example is cellulitis associated with a bite wound, which results in a balance between consumption and production that is reasonably well matched. The blood inflammatory pattern then consists of mild to moderate neutrophilia with a variable left shift, depending on the severity of the lesion. Acute peritonitis due to gut rupture is an example of a major consumer of neutrophils that may exceed the marrow capacity for production; in this example, it is possible to see neutropenia with a prominent left shift.

Chronic, walled-off inflammatory lesions, on the other hand, may result in very high neutrophil concentrations. Examples include pyometra in dogs or a chronic, walled-off abscess that does not resolve. These are also known as closed-cavity inflammatory lesions (as opposed to diffuse inflammation; discussed above). These lesions continue to stimulate the marrow to achieve maximal production; however, the rate of consumption is curtailed by the nature of the lesion, thus tipping the balance toward production exceeding consumption. In these cases, neutrophil concentrations may approach 70,000–120,000 cells/μL in dogs.

Excitement response: Epinephrine release

The excitement response is an immediate change associated with epinephrine release and is also known as the "fight-or-flight" response. Epinephrine release results in cardiovascular events that, in turn, result in increased blood flow through the microcirculation, particularly in muscle. Strenuous exercise just before bleeding may have the same effect. This results in a shift of leukocytes from the marginated pool to the circulating pool, as depicted in the neutrophil trafficking model (Fig. 12.17). On the leukogram, this manifests as an approximate doubling of leukocytes and is noted in the neutrophils and/or lymphocytes. Within the neutrophil population, no left shift occurs, because mature cells in the

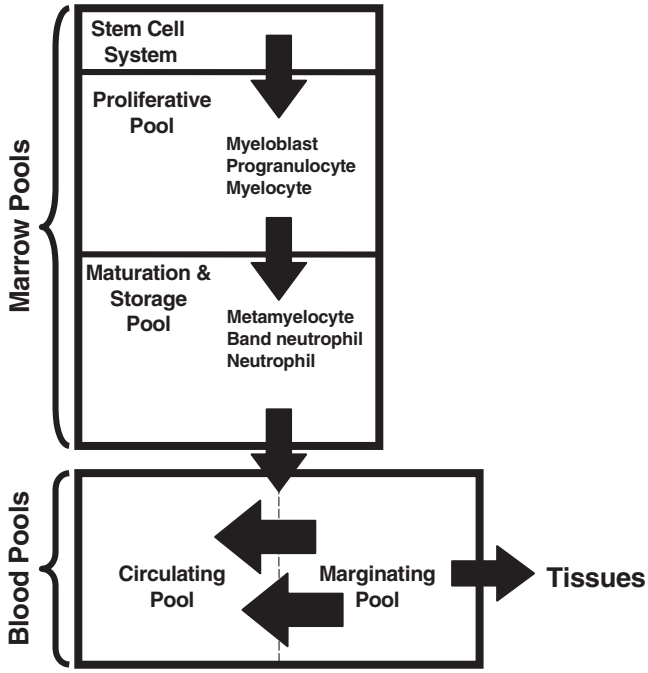

Figure 12.17 Modified neutrophil trafficking model used to illustrate the excitement response. Note that the change involves cell movement from the marginating pool to the circulating pool, resulting in an approximate doubling of resting leukocyte concentrations. Marrow delivery and tissue consumption are unchanged.

microcirculation being flushed to the circulating pool cause the neutrophilia.

The excitement response is recognized most frequently in cats. Lymphocytosis up to a maximum of approximately 20,000 cells/μL is the prominent feature of the feline excitement response. Mature neutrophilia may occur if the resting neutrophil concentration was at the upper end of normal before initiation of the excitement response. In large animals, the excitement response is recognized in association with exercise before bleeding or events that may induce excitement, such as trucking or movement through chutes for blood collection. The excitement response is least common in dogs, because this species is usually accustomed to physical handling related to blood collection.

Stress response: Corticosteroid release or administration

This is likely the most common leukocyte response. Physiologic stress is a body response mediated by release of adrenocorticotropic hormone by the pituitary gland and resultant release of cortisol by the adrenal gland. This occurs in response to major systemic illnesses, metabolic disturbances, and pain. Examples of conditions eliciting the stress response include renal failure, diabetic ketoacidosis, dehydration, inflammatory disease, and pain associated with trauma. The response may be detected in the leukogram by changes in multiple cell types.

The most consistent change is lymphopenia. Steroids may induce lymphocyte apoptosis and may alter patterns of recirculation. The second most consistent change is an approximate doubling of the circulating neutrophils. Steroids cause decreased stickiness and margination, resulting in slightly longer than normal retention in the circulation. As a result, hypersegmentation may be observed. When the resting neutrophil concentration is in the upper 50th percentile of the normal range, neutrophilia is expected. A left shift will not occur unless inflammation is superimposed. Eosinopenia is the next most consistent change. Monocytosis is variable, but occurs most consistently in dogs. The importance of interpreting the steroid leukogram is to look for an underlying physiologic disturbance (if it has not yet been recognized) and to avoid interpreting a simple steroid pattern as inflammation. An inflammatory condition may frequently cause a combined inflammatory and steroid response. The inflammatory component will take priority in determination of the magnitude of neutrophilia and any associated left shift. The steroid component may only recognizable by the concurrent presence of lymphopenia.

Lastly, it is important to note that a steroid response not being present in a very sick animal should prompt the consideration of hypoadrenocorticism (i.e., Addison disease).

Summary: Approach to neutrophilia

In summary, neutrophilia has three causes. Thus, it is useful to develop an orderly approach to looking at the leukogram to rapidly arrive at the proper interpretation of the neutrophilia. The flowchart in Figure 12.18 develops this approach. When neutrophilia is identified, one should next examine the leukogram for the presence of a left shift. If a left shift is present, the interpretation is inflammation. If a left shift is not present, the lymphocyte concentration should be examined. If lymphopenia is found with a neutrophilia and no left shift, the interpretation is steroid response. If the lymphocyte concentration is upper normal or increased within certain limits, the interpretation of excitement response should be considered. Keep in mind that clear neutrophilia with a left-shift inflammatory pattern may have a superimposed steroid response; this is identified by the presence of lymphopenia in conjunction with the neutrophil inflammatory pattern.

Lymphocytosis

Lymphocytosis has two common causes. The first is the excitement response (discussed above), and the second is lymphocytic leukemia. The approach to interpreting lymphocytosis involves analysis of both cell concentration and cell morphology (Fig. 12.19). The lymphocyte morphology should be critically examined when lymphocytosis is present. If the cell concentration is only modestly increased and the

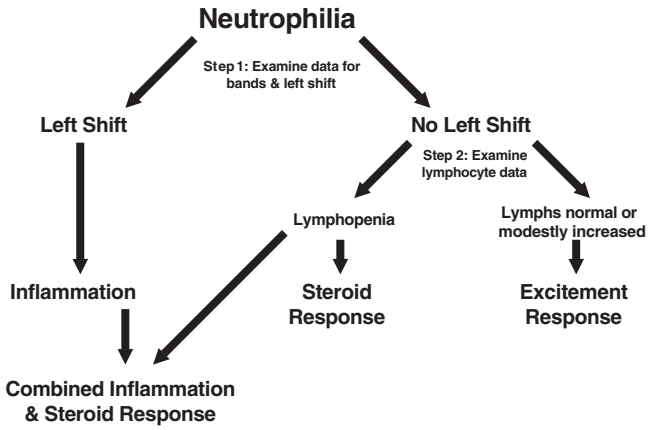

Figure 12.18 Summary flow chart for interpretation of neutrophilias. When neutrophilia is seen, the observer should examine the data for a left shift (Step 1). If a left shift is present, then the interpretation is inflammation. If no left shift is present, then the observer should examine the lymphocyte data (Step 2). Lymphopenia in conjunction with a mature neutrophilia indicates a steroid response. If the lymphocyte concentration is normal to increased, an excitement response should be considered. Also, note that an inflammatory pattern may have a superimposed steroid response that is recognized as lymphopenia occurring in conjunction with the inflammatory pattern.

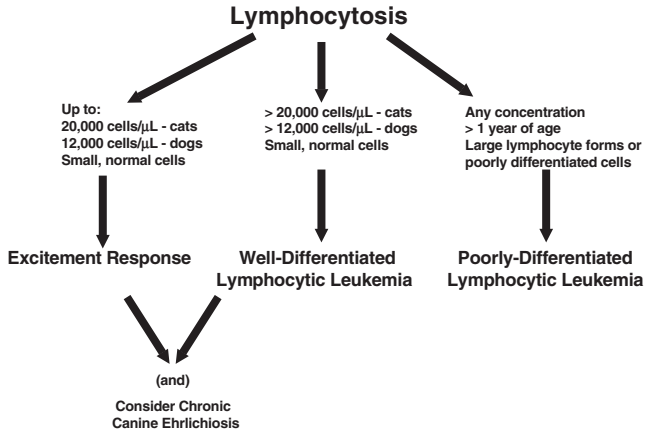

Figure 12.19 Summary approach to interpretation of lymphocytosis. This flow chart may be useful for distinguishing the excitement response from lymphocytic leukemias based on lymphocyte concentration and morphology guidelines. Inflammatory disease is rarely associated with lymphocytosis; however, chronic canine ehrlichiosis is an exception. See text for discussion.

cells are morphologically small, normal-appearing lymphocytes, then an excitement response should be considered. As a guideline, this modest increase is suggested to be a lymphocyte concentration of up to approximately 12,000 and 20,000 cells/µL in dogs and cats, respectively. If the concentrations exceed this guideline or the animal was not excited, then a lymphocytic leukemia should be considered. Repeating the hemogram the next day while making note of the possibility of excitement during blood collection also may be helpful. When lymphocyte concentration is of this magnitude with normal morphology, the confirmation of the diagnosis of leukemia is usually difficult. It involves exclusion and more extensive diagnostics; see Chapter 13. The higher the concentration, the greater the probability that the cause is a lymphoproliferative disorder with leukemia.

A common misconception is that lymphocytosis may occur with chronic inflammatory diseases. This concept likely is extrapolated from the knowledge that inflammatory disease results in an immune system response that includes lymphoid hyperplasia. This process does occur, but the expansion is confined to lymphoid tissues and rarely manifests as lymphocytosis in blood. An exception is the chronic form of canine ehrlichiosis, which has been documented to result in lymphocytosis and also monoclonal gammopathy. The monoclonal gammopathy is expected to be superimposed on an underlying polyclonal gammopathy. When the lymphocytosis is examined, a high proportion of large granular lymphocytes (see Fig. 10.4) may be observed. In dogs, chronic ehrlichiosis should be considered with lymphocyte concentrations up to about 30,000 cells/µL.

Abnormal lymphocyte morphology in conjunction with lymphocytosis makes the diagnosis of leukemia less difficult. Abnormal morphology generally means lymphocyte forms that are normally not found in blood. These cells have one or more features of a cell undergoing proliferation, as opposed to the small, resting lymphocyte that is ordinarily seen in blood (Fig. 12.20). These features may include a diameter larger than that of adjacent neutrophils, a fine chromatin pattern resulting in a lighter-staining nucleus, a visible nucleolus, and increased cytoplasm (Figs. 12.20 and 12.21). If cells with abnormal features for blood, e.g. prolymphocytes and/or lymphoblasts, are present in the circulation, leukemia is a diagnostic consideration even with normal to mildly increased lymphocyte concentrations. Lymphoproliferative disorders and lymphocytic leukemia are presented in more detail in Chapter 14.

Bovine persistent lymphocytosis may occur in cattle infected with bovine leukemia virus (BLV). Persistent lymphocytosis is defined as a lymphocyte concentration of greater than 7500 cells/µL on two or more hemograms. The morphology may be normal. Persistent lymphocytosis is part of a continuum in BLV-infected cows that eventually may progress to a diagnosis of lymphocytic leukemia or lymphosarcoma. Historically, hemograms, with an emphasis on the lymphocyte concentration, have been used as a screening test for BLV infection.

Neutropenia

Neutropenia resulting from acute inflammatory consumption

Neutropenia resulting from overwhelming consumption by an inflammatory lesion was discussed earlier (with the

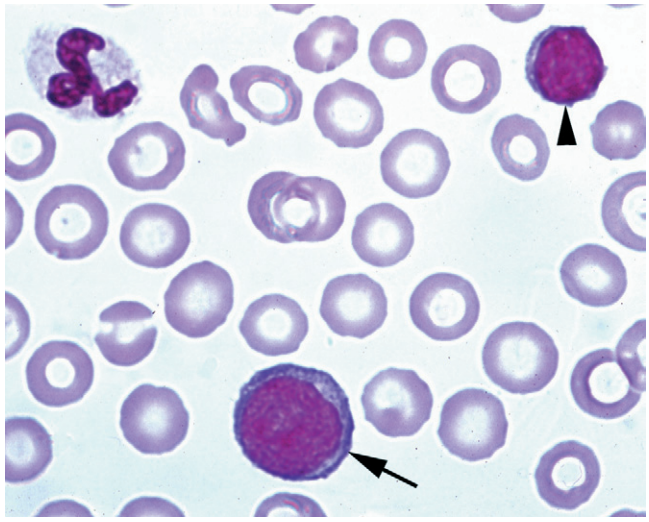

Figure 12.20 Lymphocyte morphology in relationship to evaluation of lymphocytosis. Normal blood lymphocyte morphology consists of small, resting lymphocytes (arrowhead). Note that the diameter is less than that of adjacent neutrophils, the chromatin is condensed, and cytoplasm is scant. An abnormal lymphocyte (arrow) in blood suggests a lymphoproliferative disorder involving blood. Note the increased size, increased cytoplasm, and more fine chromatin pattern. This cell also has a visible nucleolar ring in the nucleus. Wright-Giemsa stain, high magnification.

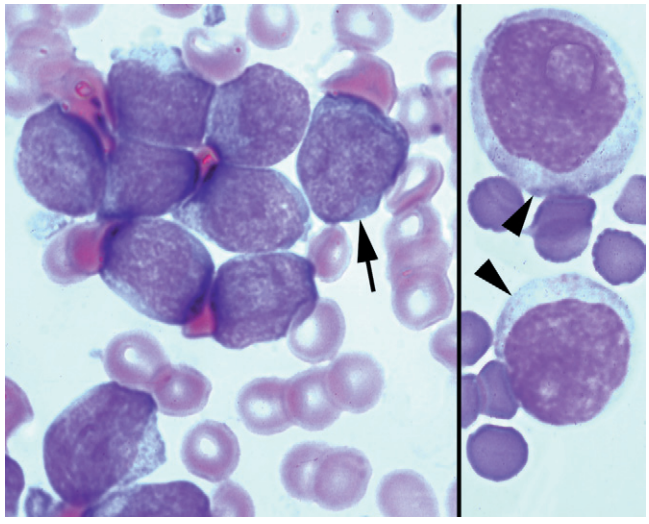

Figure 12.21 The left pane shows large, abnormal lymphocytes (arrow) from a dog with lymphoblastic leukemia (~70,000 lymphocytes/µL). Note the fine, granular chromatin pattern as well as the occasional, faint nucleoli and the large size. The right panel shows two lymphoblasts (arrowheads) from a cat with lymphoblastic leukemia. Note the large cell size, fine chromatin pattern, and prominent nucleolar rings. Wright-Giemsa stain, high magnification.

inflammatory response). Neutropenia resulting from consumption is associated with a left shift. Toxic changes are also expected within a few days of the onset of the process. An alternative form of consumptive neutropenia is immune-mediated neutropenia in which immunoglobulin that recognizes epitope(s) on the neutrophil surface or adsorbed onto the surface results in destruction of both circulating neutrophils and late stages of maturation within the marrow. This may result in profound neutropenia not associated with a demonstrable inflammatory lesion.

Neutropenia resulting from stem cell injuries

The various stem cell injuries may be considered modifications of the neutrophil trafficking model in Figure 12.22. Stem cell injuries have numerous causes, ranging from very acute, transient injury of variable duration to permanent, irreversible injuries. Stem cell injuries are nonspecific in that all cell lines of marrow are involved. Evidence of marrow failure manifested in blood is related to the duration of the injury in relationship to the circulating time or life span of various cell types. Because neutrophils are renewed in blood most rapidly, neutropenia develops first with a stem cell injury. Thrombocytopenia is seen second, because platelets last

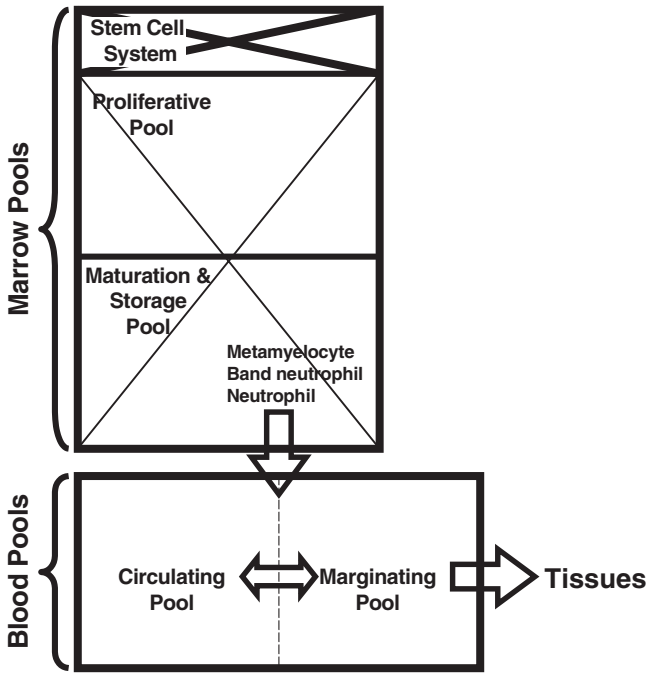

Figure 12.22 Modified neutrophil trafficking model used to illustrate neutropenias caused by stem cell injury. Injury occurs to the stem cell system, which results in a lack of recruited cells to proceed through the proliferative and maturation stages. The end result is interruption in the supply of neutrophils to blood. Because tissue consumption is not interrupted, profound neutropenia in the blood pools may occur within a few days or less.

approximately 7 days in the circulation. Nonregenerative anemia occurs last because of the relatively long erythrocyte life span.

Neutropenia caused by reversible stem cell injuries

Several acute, transient stem cell injuries are caused by the tropism of viruses for rapidly dividing cells. Canine parvovirus and feline panleukopenia are notable examples; these result in injury to intestinal lining, lymphoid cells, and the bone marrow stem cell system. Profound neutropenia is attributable to two mechanisms. First, stem cell injury results in transient failure of production. Second, neutrophil consumption increases at the site of gastrointestinal injury. The stem cell injury involves all marrow cell lines, but is so transient that marrow repopulation occurs before thrombocytopenia and nonregenerative anemia can develop. If anemia is observed, it likely is caused by blood loss into the gastrointestinal tract. Acute neutropenia persists for only 24–48 hours. During the short period of neutropenia, a left shift is not observed. As the marrow repopulates, a left shift with progressively increasing neutrophil concentration is observed. An inflammatory pattern, consisting of neutrophilia and left shift, is usually observed during recovery.

Reversible stem cell injury of varying duration also has numerous causes. These generally are present for days or longer; thus, varying degrees of thrombocytopenia and nonregenerative anemia accompany the neutropenia. One group of causes is chemicals or drugs that injure rapidly dividing cells. Most chemotherapeutic drugs are in this category. Estrogen overdosage and phenylbutazone administration are characterized toxicities in dogs. Very high, repeated doses of estradiol may cause stem cell injury in dogs, but not in cats. Historically, an alternate form of a long-acting, potent estrogen—estradiol cypionate—has been used to prevent unwanted pregnancies in dogs. This drug has been used safely in small doses to treat incontinence. Naturally occurring estrogen toxicity may occur in ferrets if ovulation is not stimulated. Phenylbutazone, a common medication for pain and lameness that is used safely in horses, may cause marked stem cell injury in dogs. An example of an infectious cause is ehrlichiosis in dogs; ehrlichiosis may induce cytopenias, possibly by an immune-mediated mechanism that appears to act on cells in the marrow.

Neutropenia caused by irreversible stem cell injuries

This category of stem cell injury may be regarded as a continuum of proliferative abnormalities of the bone marrow stem cell system. The underlying nature and mechanism of these injuries are poorly understood. Causes include infection with feline leukemia virus, idiopathic hypoproliferative disorders, myelodysplasias, and myeloproliferative disorders. Because these are long-standing disorders, any combination of neutropenia, nonregenerative anemia, and thrombocytopenia may occur. These relatively irreversible stem cell injuries are considered in detail in Chapter 13.

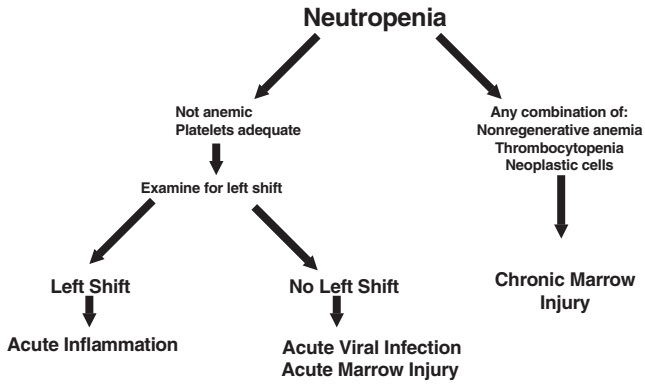

Figure 12.23 Summary approach to interpretation of neutropenia. This flow chart may be useful for distinguishing the various causes of neutropenia. When confronted with neutropenia, the observer should first examine the platelet and erythrocyte data for evidence of production problems. If these cell lines appear to have normal production, then a selective neutropenia is present. The observer should next examine the data for a left shift. If a left shift is found, then the interpretation is severe, acute inflammation (e.g., see Fig. 12.16). If no left shift is found, then an acute failure to produce neutrophils should be considered (as in Fig. 12.22). If the neutropenia is accompanied by evidence of failure to produce other cell lines (e.g., platelets and/or erythrocytes), then a more chronic marrow injury should be considered. The presence of neoplastic cells may indicate an underlying hematopoietic cell neoplasm and is also a possible cause of marrow failure. See text for discussion.

Approach to neutropenia

The approach to interpretation of neutropenia is summarized in Figure 12.23. The observer should first determine if the neutropenia is associated with a left shift. If a prominent left shift is observed with toxic change, then an inflammatory disease is the cause of the neutropenia. If no left shift is seen, then the other cell lines should be assessed. If any combination of thrombocytopenia, nonregenerative anemia, or evidence of hematopoietic cell neoplasia is found, then marrow injury should be considered.

Lymphopenia

Lymphopenia is usually attributable to a steroid response; other causes are uncommon to rare. Lympholytic acute viral infections induce lymphopenia that is accompanied by neutropenia; however, neutropenia is the more important finding. Combined immunodeficiency syndrome of Arabian foals is an inherited disorder with severe deficiency of both T- and B-cell lymphocyte functions. The lymphocyte concentration may be used as a screening test for this disorder in newborn Arabian foals. A lymphocyte concentration of greater than 1000 cells/μL is a finding that rules out the

disease. If lymphopenia is found, more confirmatory tests may be performed.

Monocytosis

Monocytosis is a relatively unimportant change. It may accompany both acute and chronic inflammatory responses. Monocytosis that accompanies an inflammatory response is interpreted as a response to increased demand for mononuclear cells in tissues. Monocytes in blood are regarded as immature cells that become macrophages after migration to tissue sites. Monocytosis also may occur in the steroid response, particularly in dogs.

Eosinophilia

Eosinophilia is interpreted as a nonspecific response that requires consideration of parasitism, hypersensitivity, or an unusual lesion producing eosinophil chemoattractants. Tissue-invading parasitisms are frequently associated with eosinophilia. Notable examples include heartworm disease and hookworm infestation in dogs. Inflammation at epithelial surfaces rich in mast cells (e.g., skin, respiratory tract, gastrointestinal tract) may be associated with eosinophilia, particularly if a component of hypersensitivity is present. Examples include fleabite allergic dermatitis, inhalant allergen disease or asthma-like syndromes, feline hypereosinophilic syndromes, and poorly characterized gastroenteritis that may have an allergic component.

Basophilia

Basophilia is uncommon. In fact, basophils are so rare in normal animals that they usually are not encountered in the 100-cell microscopy differential. The interpretation of basophilia is unknown or not clear. It most frequently accompanies eosinophilia. When this happens, it is described as eosinophilia and basophilia, but it is eosinophilia that is interpreted as indicated earlier.

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13 Molecular Diagnostics of Hematologic Malignancies

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Clonality

The development of cancer is the result of a series of genetic mutations that render a cell resistant to growth controls. The cell divides unchecked, resulting in a tumor mass that is derived from that single original cell, and harboring the unique DNA sequences of the original cell. In most cancers, the unique DNA sequences consist of the genetic mutations that resulted in the development of cancer—oncogenes. In addition to oncogenes, cancers of lymphocytes (lymphoma and leukemia) have another form of unique DNA sequence. During normal lymphocyte development, the genes that encode antigen receptors (immunoglobulin for B cells and T cell receptor for T cells) are assembled at random from a pool of gene segments. Therefore, the antigen receptor gene in each developing B and T cell is unique. When these cells divide, as a result of antigenic stimulation or cancer, the daughter cells inherit the unique antigen receptor genes. The detection of oncogenes and unique lymphocyte genes gives us a very powerful diagnostic tool for detecting malignancy and for making predictions about prognosis and treatment.

Lymphocyte development and generation of antigen receptor genes

In order to understand how the unique DNA sequences in lymphocytes can be used for diagnostic purposes, it is first important to understand how these sequences are generated. Lymphocytes develop in the bone marrow (B cells) and the thymus (T cells) from a lymphocyte precursor that is also found in the bone marrow. The job of these cells is to identify the millions of different foreign antigens carried by potential pathogens. In order to accomplish this, lymphocytes have developed a system to generate enormous diversity in the antigen binding portion of their receptors. The B cell receptor for antigen is an antibody, and the T cell receptor for antigen is called the T cell receptor.[1] The genes encoding these two proteins use the same process for generating diversity, so only antibody genes will be discussed.

The antigen combining regions of an antibody (the variable region) is generated by bringing together three different genes—V (variable) genes, D (diversity) genes, and J (junctional) genes. In the dog, there are 80 variable regions genes found on chromosome 8—each gene is approximately 200–300 bases long, but the genes are separated by thousands of bases of noncoding DNA. Dogs have 6 D genes, ranging from 12 to 30 bases, and 6 J genes of approximately 50 bases.[2] The arrangement of the genes is shown schematically in Figure 13.1.

The arrangement of genes in Figure 13.1 is called the germ line configuration. This is the arrangement of genes found in all cells of the body except fully developed B cells. The way that B cells alter this configuration is described next.

During the development of a B cell, the V, D, and J genes of the antibody heavy chain are brought together so they form one contiguous gene in a process called recombination. Recombination is essentially random, so that any V can be combined with any D and any J. While V regions genes have similar sequences, they are not identical. The same is true for D and J genes—they are similar to one another, but not identical.

This random recombination alone creates a tremendous number of different genes, but in addition, nucleotides can be added between segments during the process, or trimmed from the ends of V, D, and J genes. The process is again random. Thus any given B cell will have not only a unique concatenation of V, D, and J genes, but within this new gene will be further diversity as a result of nucleotides being added and trimmed. The end result is that virtually every newly developed B cell will carry a unique DNA sequence. The length of the new VDJ gene will also be different because

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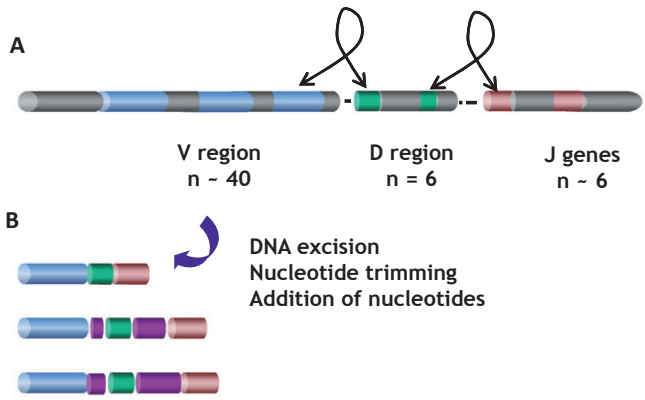

Figure 13.1 A. Arrangement of immunoglobulin gene segments on canine chromosome 8. There are approximately 40 functional variable region gene segments (V, blue), 6 diversity segments (D, green), and approximately 6 joining segments (J, pink). Grey and dotted lines indicate noncoding DNA. This diagram is not to scale. Excerpted from Bao *et al*.[2] B. When V, D, and J regions genes rearrange, the resulting product is a different length in each B cell because variable numbers of nucleotides (purple) can be added during the joining process. Nucleotides could also be trimmed from the ends of the V, D, and J segments during the process.

of the addition and trimming of nucleotides. The same is true for T cells, because the T cell receptor beta gene is also comprised of multiple V, D, and J genes. The antibody light chain genes, and T cell receptor alpha, gamma, and delta genes all undergo a similar process, with the exception that none of these genes contains a D segment.[3]

Lymphocyte division

When mature lymphocytes with their unique antigen receptors encounter an antigen recognized by those receptors, they are stimulated to divide. The progeny cells contain the same antigen receptor gene. Similarly, if a lymphocyte becomes neoplastic at some time during its development and divides unchecked, all of the progeny cells of this cancer will have the same antigen receptor gene.

The response to a pathogen will involve hundreds to thousands of molecularly different B and T cells. This is because even the simplest pathogen is comprised of multiple proteins, which can be recognized by the antigen receptors of many different lymphocytes. Even a single protein has many different antigenic structures, and can stimulate the division of multiple lymphocytes. Cancer, on the other hand, is characterized by the unrestricted division of a single cell, called clonal proliferation. Thus a population of lymphocytes which are all the progeny of a single clone is most likely cancer, and a population of lymphocytes that have multiple different types of cells (called polyclonal) is most likely reactive. The ability to distinguish a clonal from a polyclonal population of lymphocytes has many diagnostic applications. For example, a dog with dental disease presents to his veterinarian because his submandibular lymph nodes are swollen. The enlarged lymph nodes can be due to antigenic stimulation by a heavy burden of oral pathogens. Other clinical factors, however, may raise concern for lymphoma—these can include age, breed, and cytologically suspicious cells. In order to distinguish a purely reactive process from a neoplastic one, it is possible to determine if the lymphocytes are mainly derived from a single clone (neoplastic) or multiple lineages. The assay used to make this distinction is called a clonality assay, and can also be called PARR (PCR for antigen receptor rearrangements).[4]

Principles of the PARR assay

The PARR assay measures the size of all the VDJ genes in a collection of lymphocytes. If all the lymphocytes have the same sized VDJ gene, the lymphocytes are considered clonal. If, however, there are multiple sized VDJ genes, then the population is polyclonal. To accomplish this, DNA is extracted from the lymphocyte in question—for example in the case above, a dog with enlarged submandibular nodes and dental disease, lymphocytes would be collected by aspiration from the submandibular node. PCR primers that will amplify the entire VDJ gene will be added as shown in Figure 13.2.

The resultant DNA products are separated by size using any one of a variety of methods. A dominant single sized product indicates that the population of cells was derived from a single clone. On the other hand, the presence of products of multiple sizes indicates that a heterogenous population of lymphocytes is present, and the process is most likely reactive. Figure 13.2 shows the results of the PARR assay analyzed by capillary gel electrophoresis to demonstrate what each of these two results would look like. In practice, only 1% of the cells in any given sample need to be neoplastic for the result to be interpreted as clonal. This is because the remaining nonneoplastic cells are so heterogeneous that the PCR products from these varied cells are outcompeted by homogeneous product from the neoplastic cell. This idea is not necessarily intuitive, but has been born out experimentally.

Interpretation and uses of PARR

The PARR assay is used when there is suspicion of lymphoma by cytology or histology, but not a definitive diagnosis. Some common reasons for carrying out the assay are: enlarged lymph nodes where the cytology or histology cannot definitively conclude that lymphoma is present, pleural or peritoneal fluid characterized by occasional suspicious looking cells, and the presence of atypical appearing lymphocytes on a peripheral blood smear.

In most cases the assay is performed by using multiple sets of PCR primers—some of which detect antibody VDJ regions, and some of which detect T cell receptor genes. B cell lymphomas will be characterized by single sized PCR products when the antibody primers are used, but multiple products

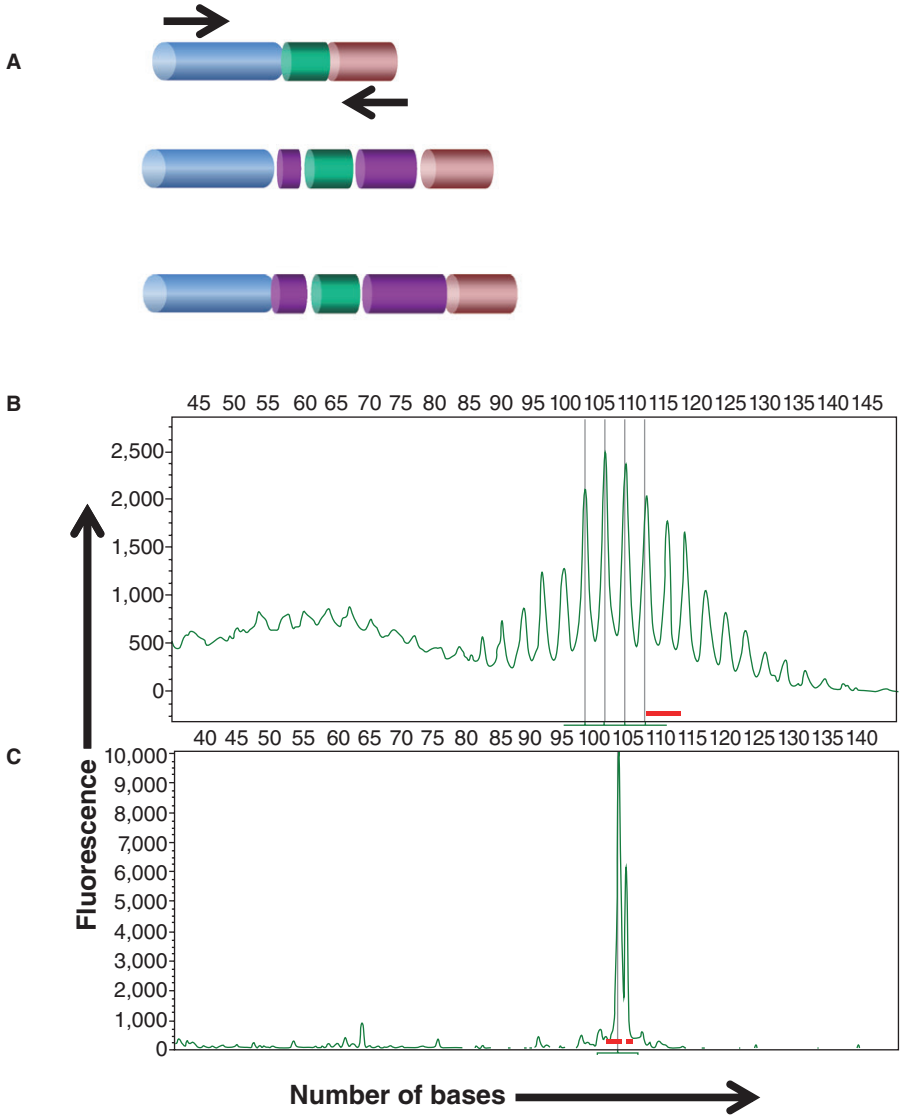

Figure 13.2 Placement of PCR primers, and separation of PCR products by size. A. PCR primers placed as indicated by the arrows will amplify multiple sized PCR products when used on a reactive lymph node (B), and a single sized PCR product when used on a cells derived from a case of B cell lymphoma (C). Samples are separated on the basis of size (x axis). The height of the peak (y axis) indicates the amount of the PCR product.

or no products at all when T cell receptor primers are used. T cell lymphomas, conversely, will be characterized by single sized products when T cell receptor primers are used, and multiple or no products when antibody primers are used. Thus the nature of the clonal PCR product is a clue to the lineage of the neoplasm. As with any assay, there are exceptions to this rule, but for the majority of cases, the clonally rearranged gene reflects the lineage of the tumor. Plasma cell tumors and multiple myeloma are B cell origin tumors and will be positive with this assay.

Not all cases of lymphoma and leukemia can be detected by PARR. This is because there are likely to be V and J regions genes whose sequences differ enough from the PCR primers that the primers will not bind. If the patient's tumor uses one of these V or J genes, then no amplification of tumor DNA will be seen. Therefore, as with many tests, the PARR assay cannot be used to rule out neoplasia, only to support a positive diagnosis.

Detection of oncogenes

Cancer involves a collection of mutations in the DNA of a cell that accumulate as the cell divides. These mutations are generally found in genes that control the susceptibility to cell death, or pathways that stimulate growth. They can be

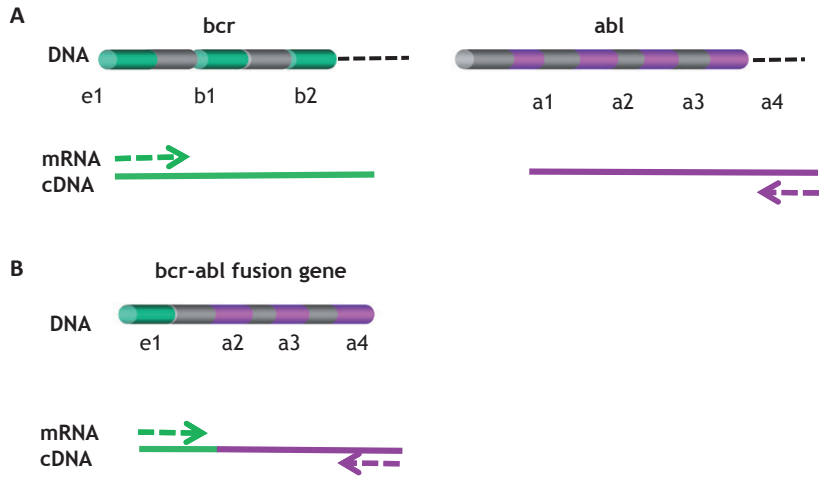

Figure 13.3 Translocation of the abl gene to the bcr gene creates a fusion gene which can be detected by PCR. A. In nonleukemic cells, primers located as indicated (arrows) will not amplify any product, because they anneal to two distinct cDNAs. B. When bcr and abl are brought together by a translocation event, the primers both anneal to the same cDNA, and will amplify this product. E1, b1 etc. refer to exon numbers.

the result of single nucleotide mutations, duplications and deletions within genes, and large scale chromosomal rearrangements. In human medicine, the presence of certain oncogenes indicates a better or poorer prognosis. For example, some cases of acute myeloid leukemia in people are characterized by an internal tandem duplication of a protein called nucleophosmin.[5]

Detection of this mutation indicates a favorable prognosis. Detection of oncogenes can also be used to monitor treatment using sensitive PCR methods. Almost all cases of chronic myelogenous leukemia (CML) in people carry the bcr-abl translocation.[6] This translocation brings abl gene on chromosome 9 next to the bcr gene on chromosome 22, creating a novel fusion gene called bcr-abl. The fusion gene can be detected by PCR using one primer that binds to the bcr gene, and another primer that binds to the abl gene. If a product can be amplified using these two primers, the fusion gene is present (Fig. 13.3). This observation allows for the monitoring of residual tumor cells after chemotherapy, and can detect as few as 1 tumor cell in a background of a million normal cells.

Oncogenes in veterinary medicine

Approximately 20% of canine mast cell tumors harbor a mutation in a gene called c-kit.[7] C-kit is the tyrosine kinase receptor for the growth factor called stem cell factor. The mutations, which fall into three different categories, result in the c-kit gene being permanently phosphorylated, resulting in a constitutive "on" signal. Thus the cells continuously receive a growth signal.

The most common mutations in this gene are called internal tandem duplications, in which a small segment of the gene is duplicated, such that a given sequence is repeated in tandem. One of these mutations in found in exon 8, where a 12 base segment is duplicated, creating a slightly larger version of the gene.[8] The mutation is readily detectable by PCR amplification of exon 8, since it will result in a larger product. Figure 13.4 shows the placement of PCR primers, and what the product will look like. Mast cell tumors will also harbor internal tandem duplications in exon 11 which can be detected the same way (Fig. 13.4).

Mast cell tumors do not usually present a diagnostic challenge, but there are occasions where poorly granulated mast cell tumors might be difficult to distinguish from other round cell neoplasms. In such cases the presence of a c-kit internal tandem duplication would help identify a round cell tumor as mast cell in origin. The more common reason for detecting this mutation, however, is to guide therapy. There are two types of chemotherapy protocols used for mast cells: antimicrotubule drug vinblastine coupled with corticosteroids, or one of different class of drugs, the tyrosine kinase inhibitors. When the c-kit gene is mutated, resulting in permanent phosphorylation, tyrosine kinase inhibitors are more effective than vinblastine. When there is no evidence of a c-kit mutation, vinblastine is more effective. Thus detection of the mutation has now become a routine part of the diagnosis of mast cell tumors for owners who wish to pursue therapy for their dogs.

Although there are currently no other diagnostics which employ the detection of oncogenes in companion animal medicine, the presence of the bcr-abl translocation has been demonstrated in two cases of chronic myelogenous leukemia.[9,13] This finding opens the door for bcr-abl detection to be used in the diagnosis of CML in dogs. Such a test would be useful in establishing that a high, mature neutrophil

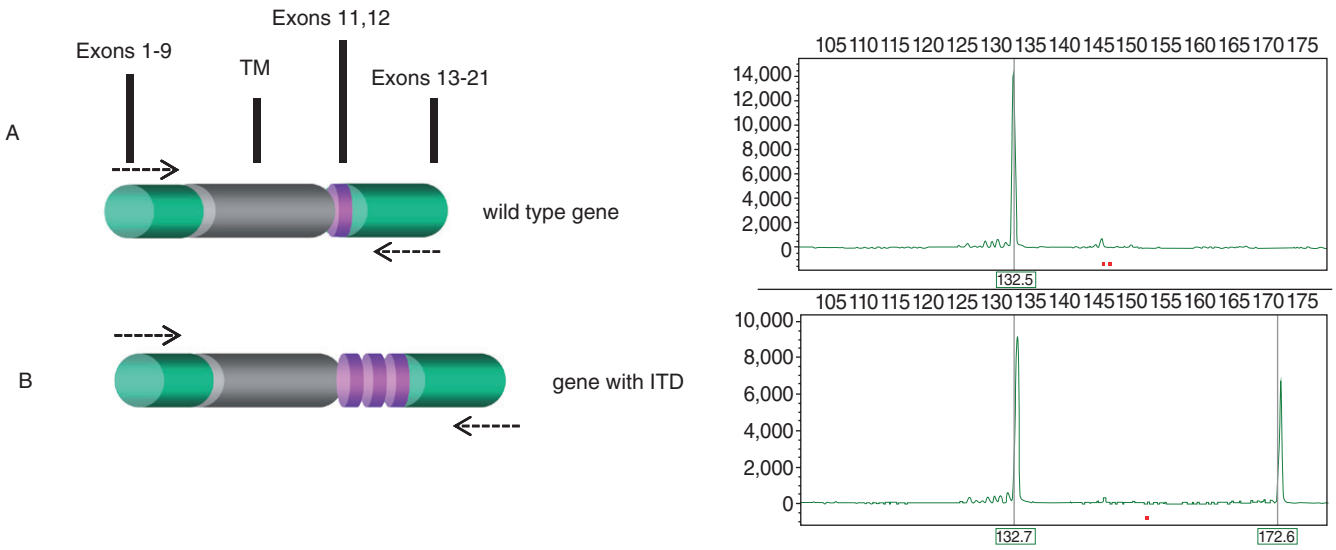

Figure 13.4 PCR amplification of c-kit exon 11. The c-kit gene consists of 21 exons. Internal tandem duplications can be found in exons 11 or 12 (purple), and can be detected by PCR amplification using primers that surround these exons. A: Wild type c-kit gene and the PCR product resulting from amplification of DNA from normal tissue, or a mast cell without the mutation. B: c-kit gene with ITD, and the PCR products resulting from amplification of a mast cell tumor containing a mutated gene. Both wild type and larger products are visible, because nontumor cells are always included in clinical samples, and because there is one wild type copy of c-kit in the tumor cells.

count is the result of CML rather than chronic infection—a distinction that at present can sometimes be hard to make.

Uses of molecular diagnostics in research

The PARR assay and detection of c-kit mutations have been used to answer a number of clinical questions that provide insight into these diseases. These applications provide a model for employing future tests that might become available.

One way in which both the PARR assay and c-kit detection have been used is to compare the relatedness of two tumors arising sequentially in the same patient. The PARR assay was used to investigate a case of multiple myeloma in a dog which had previously been treated for B cell lymphoma. Since both of these tumors are derived from B cells, it was of interest to determine if the original lymphoma had differentiated into the plasma cell tumor, and this kind of progression had not yet been reported in the human or veterinary literature. The PCR product of the VDJ region in both the B cell lymphoma and the myeloma were the same size, suggesting the same clone gave rise to both tumors. Sequencing subsequently confirmed this idea. Thus the PARR assay uniquely identifies the clonal that gave rise to a tumor, and can be used to investigate the relatedness of tumors that appear at different times in the same patient.[10]

The internal tandem duplication in exon 11 of the c-kit gene is slightly different in every tumor. This observation was used to show that three mast cell tumors arising sequentially over a period of three years in one patient were all derived from the same clone. Each time the tumor arose, it was completely excised. The clinical implications of this finding are that the original tumor was never completely eradicated, even though no evidence of the disease could be detected between the three events.[11]

A refinement of the PARR assay has been used to detect minimal residual disease (MRD) in patients with lymphoma. For MRD detection, the VDJ region genes are sequenced, and PCR primers made that will detect only the tumor—they will not bind to any VDJ region genes other than the one carried by the tumor, and are thus able to detect tumor cells with very high sensitivity.[12] MRD detection in people is a prognostic exercise—patients with leukemia and lymphoma whose disease is eradicated (no MRD detected by sensitive methods such as PARR) have a better prognosis. While MRD detection on a routine basis is probably cost prohibitive in veterinary medicine, it may be useful for conducting clinical trials of new chemotherapies. A drug can be assessed by its ability to reduce the tumor burden after treatment as assessed by MRD detection, rather than waiting a year or more to determine remission and survival times.

Flow cytometry

In the context of hematologic malignancy, flow cytometry is used to identify proteins on the surface of lymphocytes, although the technology has a large variety of other uses. As discussed above, lymphoma and leukemia are the result

of unchecked expansion of a single cell. The progeny cells resemble the original cancerous clone. Therefore another way to determine if a population of lymphocytes is neoplastic would be to show they are all the same phenotype—all B cells, CD4 T cells or CD8 T cells. This is not equivalent to showing that the cells are all derived from the same clone, but in practical terms, homogenous expansion of a single lymphocyte subset is usually neoplastic, because reactive processes will result in the expansion of many different lymphocyte subtypes. Thus if the lymphocytes in a submandibular lymph node aspirate consist of 98% B cells, the process in that node is neoplastic. If the lymph node aspirate, however, consists of 30% B cells, 50% CD4 T cells and 20% CD8 T cells this finding is more consistent with some kind of reactive process—response to infection or autoimmune disease, or response to a metastatic tumor of nonlymphoid origin.

Cell surface antigens

Most proteins found on the surface of hematopoietic cells are identified by a number, preceded by the letters "CD" (CD stands for "cluster of differentiation," a term that partly reflects the fact that different proteins are expressed at different points in the life of a cell). CD3, CD4, CD5, and CD8 are all proteins found on the surface of T cells, and were among the earliest identified. CD21 and CD22 are proteins found on B cells, but not on T cells. Monoclonal antibodies specific for virtually all CD antigens are available for both people and mice, and a significant repertoire is also available for dogs, horses, cattle, and sheep. There are fewer antibodies for cats. These antibodies are generally (but not always) species specific—an antibody to canine CD4 will not recognize feline CD4, and vice versa.

In order to determine how many CD4 T cells, CD8 T cells, and B cells there are in any given collection of lymphocytes, commercially available monoclonal antibodies are incubated with the cells in question (for example cells from a lymph node aspirate). The antibodies are conjugated to fluorescent molecules which come in a large array of different colors. Thus if the antibody to CD4 is conjugated to a red molecule, cells that have red fluorescence are CD4+ T cells. If the antibody to CD8 is conjugated to a green molecule, cells that have green fluorescence are CD8+ T cells. A flow cytometer is used to count the number of cells bearing different fluorescent molecules, and the process of enumerating cells of different subtypes is called *immunophenotyping*.

Principles of flow cytometry

Flow cytometry is the analysis of cells and particles in liquid suspension. Flow cytometers are equipped with one or more lasers, which emit light of a single wavelength. The suspension to be analyzed is focused into a narrow stream which passes in front of the laser one particle or cell at a time. When a cell passes through the beam, several aspects of the interaction between the cell and the light are recorded by detectors. First, the cell scatters light in several ways. The *forward light scatter* is an estimate of the size of the cells—large cells produce greater forward light scatter. The complexity of a cell's cytoplasm is indicated by the *side scatter*. Cells such as eosinophils and neutrophils, with granular cytoplasm, have high side scatter, whereas lymphocytes which have little cytoplasm, have low side scatter (Fig. 13.5).

The other important parameter detected by the flow cytometer is the amount and color of the fluorescent molecules bound to the cell. That characteristic is determined by which, if any, monoclonal antibodies specific for CD antigens have bound. The laser light is a single wavelength of light, which excites the fluorescent dye. That dye then emits light of a narrow spectrum—what our eyes would see as green, red, blue, etc. A detector records the amount of fluorescence for each cell that passes in front of the laser, and stores it together with forward and side-scatter information. The amount of fluorescence is proportional to the number of antibody molecules on the cell, which is proportional to the number of CD proteins recognized by that antibody. This principle is illustrated in Figure 13.5.

Clinical applications of flow cytometry

Flow cytometry is most useful when you want to determine if an expanded population of lymphocytes is neoplastic (homogeneous) or reactive (heterogeneous). It can also provide prognostic information in some cases.

Example 13.1. Homogeneous lymphocyte expansion

An 8-year-old female spayed mixed breed dog presents with lethargy and inappetance. Her CBC shows mild anemia (PCV 38), normal neutrophil and monocyte counts, slightly elevated eosinophil counts, and a lymphocyte count of 10,000 cells/μL. Differentials for lymphocytosis in dogs include: lymphocytic leukemia or blood involvement of lymphoma, thymoma, Addison's disease and *Ehrlichia canis*. Other causes of lymphocytosis in dogs are very rare.

Flow cytometry is a good diagnostic test in this case, because it will tell you the phenotype of the lymphocytes. The results showed that 85% of the lymphocytes were CD8 T cells (resulting in a CD8 T cell count of 8500 cells/μL, normal in dogs is 450–1000 cells/μL) (Fig. 13.6). B cells and CD4 T cells were within normal range. This homogeneous expansion of CD8 T cells is most consistent with T cell leukemia. In rare cases, *Ehrlichia canis* can also cause this kind of CD8 T cell expansion, but *E. canis* can easily be ruled out by serology.

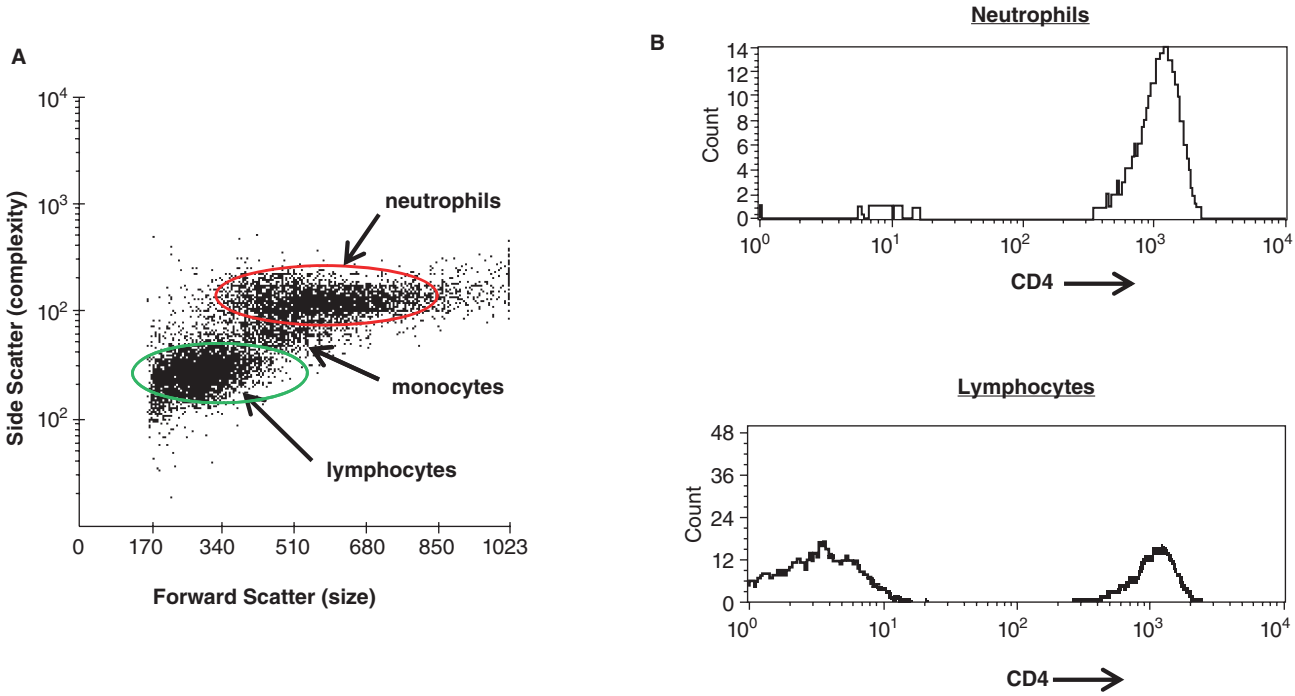

Figure 13.5 A. Light scatter properties of canine peripheral blood. Each dot represents a cell, and each cell is plotted along the X and Y axes based on their forward and side light scatter properties. Light scatter values do not have units. In this example, forward scatter is a linear scale, and side scatter a log scale. Side scatter is also often depicted on a linear scale. B. Expression of CD4 on neutrophils and lymphocytes. Peripheral blood was stained with anti-CD4 conjugated to a green fluorescent molecule. The level of fluorescence is shown on the x axis, and the height of the histogram indicates how many cells fluorescence at that level. The top histogram shows neutrophils (the cells in the red circle in A), almost all of which are CD4 positive. The bottom histogram shows lymphocytes (green circle in A), approximately half of which express CD4.

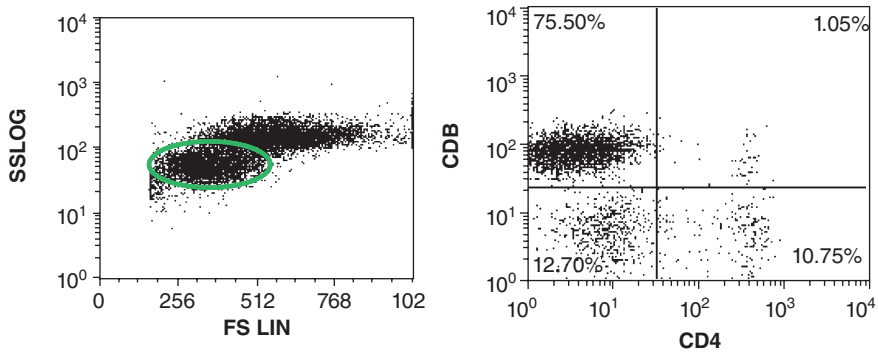

Figure 13.6 Flow cytometry study of a case with CD8 T cell leukemia. The panel on the left indicates the light scatter properties of the peripheral blood, and the panel on the right indicates staining for CD4 (x axis) and CD8 (y axis) on lymphocytes (green circle). The graph shows that the majority of lymphocytes (75.5%) are CD8 T cells. The lymphocyte count in this patient was 11,300 cells/μL, resulting in a CD8 count of 8500 cells/μL (high normal CD8 count in dogs is 1000 cells/μL).

Example 13.2. Heterogeneous lymphocyte expansion

A dog presents as above (same signalment and blood work). In this case, however, the results show that there are 2000 B cells/μL (normal high 300 cells/μL), 4000 CD4 T cells/μL (normal high 1900), and 4000 CD8 T cells/μL (normal high 1000). In this case there is a heterogeneous expansion of all lymphocyte subsets. Although this finding doesn't rule out leukemia, it is more consistent with a reactive or physiologic process. Addison's disease would be a consideration in this case, although there are likely other conditions which can cause reactive lymphocytosis in dogs which have not yet been defined. Thymoma would be less of a consideration in this case, because in thymoma, only CD4 and CD8 T cells are increased.

Example 13.3. Pleural effusion

A 10-year-old male neutered cat presents with a pleural effusion. The effusion was not chylous but contained a very high number of small, mature lymphocytes. Flow cytometry on this fluid revealed that 47% of the lymphocytes were B cells, and 48% were T cells (Fig. 13.7), with an equal mixture of CD4 and CD8 T cells (not shown). This finding supports a reactive process, rather than a neoplastic one, because the population is heterogeneous.

Example 13.4. Cells with an abnormal phenotype

An 11-year-old golden retriever presents with adult onset demodex and a lymphocyte count that is just outside the normal range (5500 lymphocytes/μL). The remainder of the blood work is normal. Flow cytometry shows that the dog has a population of T cells that has an abnormal phenotype: these cells express the T cell antigen CD3, but do not express the pan-leukocyte antigen CD45. This is aberrant phenotype is only associated with neoplasia and not with reactive processes and allows for a definitive diagnosis of T cell leukemia or lymphoma, despite the very mild lymphocyte expansion. This result also illustrates a unique power of flow cytometry—the ability to evaluate more than one antigen at a time on any given cell. This kind of evaluation is particularly useful for establishing the presence of lymphoma or leukemia even when there is no lymphocyte expansion, and for detecting residual neoplastic cells in treated patients (minimum residual disease detection).

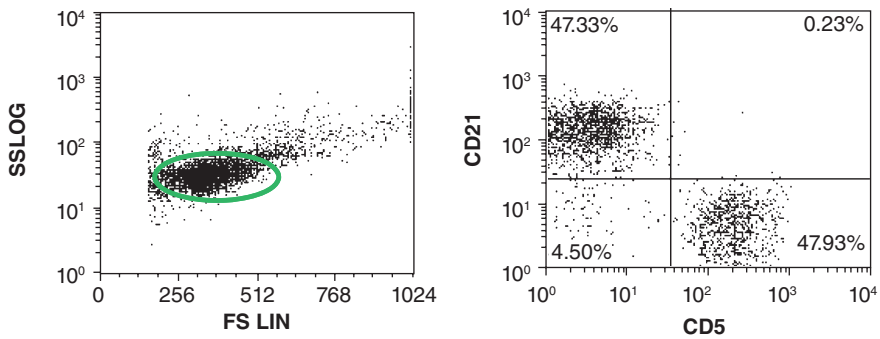

Figure 13.7 Flow cytometry from a cat with pleural effusion. The light scatter properties of the cells (left panel) indicate they are mostly small lymphocytes. The panel on the right shows staining with anti-CD5 (a T cell antigen) on the x axis, and staining with anti-CD21 (a B cell antigen) on the y axis. The findings indicate that there are equal proportions of both B and T cells. This finding is more consistent with a reactive process.

Immunocytochemistry and cytochemistry

As described above, flow cytometry is ideal for determination of cell population marker profiles using marker specific antibodies when cells to be tested are present in a suspension, such as blood or bone marrow. Cytometry also provides the advantage of quantitation of cell subpopulations in the sample. However, on occasion it may be practical to qualitatively evaluate marker profiles by microscopic examination of cells using special techniques of immunocytochemistry and cytochemistry. These techniques can be applied to air-dried slides prepared from blood, bone marrow, and tissue aspirates. They may also be applied to histologic sections where the technique is termed immunohistochemistry. These applications are utilized as an aid in identification of cell lineage in hematopoietic neoplasia, but can also be used to aid characterization of normal cell types in unusual animal species. The techniques can be used to determine cell lineage that is indistinguishable by routine blood cell microscopy; an example is the distinction between T- and B-lymphocytes. The technique is also useful for determination of cell lineage when the population is poorly differentiated such that features typically present by routine microscopy are absent. The determination of cell lineage is important for selecting tailored therapy and rendering prognosis. As experience in diagnosis and treatment of proliferative disorders grows, tailored therapy and prognosis knowledge will be refined.

Microscopic identification of hematopoietic cell neoplasia should start with a routine morphologic evaluation, typically Wright's stained, to determine if any features are present that suggest a specific cell lineage. However, many blast cell populations are poorly differentiated and have no specific identifying features. Identification of lineage then progresses to evaluation of a battery of immunocytochemical markers and cytochemical stains. The laboratory should be consulted for details regarding sample requirements and interpretive guidelines for these techniques.

Immunocytochemistry uses specific antibodies to detect antigen markers that may be present on cells. Cells are incubated with a primary antibody that binds to the antigen of interest. This is followed by addition of a secondary antibody conjugated to an enzyme or fluorochrome that is used to generate a positive readout signal. The secondary antibody binds to the primary antibody on cells positive for the antigen of interest. Examples of common antigenic markers for identification of various cell types are summarized in Table 13.1. Cytochemistry involves procedures using special stains or substrates in microscopy to detect the presence of specific biochemical content or enzyme activity within cell populations. Examples of cytochemical stains and expected reactions are summarized in Table 13.2.

Table 13.1 Examples of cellular antigens useful for cytometry and immunocytochemistry in cell lineage identification. For technical reasons, some antibody reagents are typically limited to use in flow cytometry (indicated by "*") and some are limited to ICC applications (indicated by "#"). Others, with no designation, may be utilized in either procedure. Laboratories should be consulted for specific applications and interpretations.

Cell Type	Antigen Expression
T cells	CD3, CD5*
T cell subsets	CD4*, CD8*
B cells	CD20#, CD21*, CD79a, BLA.36
Monocytes	CD14*, CD18
All leukocytes	CD45*
Antigen-presenting cells	CD1
All cells except neutrophils	MHC II*
Stem/precursor cells	CD34
Dendritic cells	CD1, CD11, CD18
Neutrophils (canine)	CD4*, CD18
Megakaryocytes	CD41, CD61

Table 13.2 Examples of leukocyte cytochemical stains useful in cell lineage identification. Cells are typically positive for stains listed, and may be positive for stains in parentheses.

Cell Type	Expected Positive Stain (Possible Positive Stain)
Neutrophils	PER, SBB, CAE, PAS (ACP)
Eosinophils—canine	PER, SBB, ACP (PAS, LAP)
Eosinophils—feline	ACP, LAP (PAS)
Basophils	CAE (ACP, LAP, PAS)
Monocytes—canine	NSE, ACP (PER, SBB, LAP, PAS)
Monocytes—feline	NSE (ACP, PER, SBB, PAS)
Lymphocytes—canine	NSE (ACP, CAE, LAP, PAS)
Lymphocytes—feline	NSE (ACP)

PER = peroxidase, SBB = Sudan black B, CAE = chloroacetate esterase, ACP = acid phosphatase, LAP = leukocyte alkaline phosphatase, NSE = nonspecific esterase, PAS = periodic acid-Schiff.

Lymphoma is the most common hematopoietic proliferative disorder. Once lymphoma or lymphocytic leukemia is diagnosed, it is useful to determine if the neoplastic cells are T or B cells. The primary antibodies used in ICC for determining if a lymphoma is T or B cell are anti-CD3 or anti-CD79a, respectively. Both of these antibodies recognize cytoplasmic portions of the antigen, but the ICC process results in permeabilization of the cell that allows the anti-

bodies to bind to intracellular antigen. In some cases, cells may label for a particular marker with ICC although they are negative for the same marker by flow cytometry. This occurs if the particular antigen is present only in the cytoplasm and is not being expressed on the cell surface, in which case ICC may provide crucial diagnostic information. Further differentiation of lymphocyte subtype (for example CD4 or CD8 expression) is not possible with this method as it is currently applied. Nonetheless, if a clinician is simply trying to distinguish B cell from T cell lymphoma and does not have access to fresh sample, this method can be useful.

Summary

Molecular diagnostic techniques such as clonality, detection of oncogenes, and flow cytometry can be used to diagnose lymphoma and leukemia. Many of these assays are widely available and now used routinely. They can provide objective confirmation of diagnoses made by cytology and histology, and can help clarify an equivocal diagnosis made by more subjective methods. It is likely that a great many more molecular diagnostic assays will become available within a few years because rapid sequencing technologies allows for much more efficient identification of oncogenes and other genetic alterations in cancer.

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14 Laboratory Evaluation of Bone Marrow

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Cytologic evaluation of a bone marrow aspiration biopsy specimen is helpful in animals with unexplained hematologic abnormalities when a diagnosis cannot be established based on examination of the blood. Examples of such abnormalities include nonregenerative anemia, neutropenia, thrombocytopenia, gammopathy, and suspicion of neoplastic marrow disease (e.g., lymphoma). In horses, bone marrow aspirates are useful to determine if anemias are regenerative, because equine species do not release immature erythrocytes into the peripheral blood. Contraindications to bone marrow aspiration are few, but marrow aspirates from the ribs or sternum of horses with clotting disorders have resulted in death because of hemothorax or cardiac tamponade. Hemorrhage usually can be prevented in thrombocytopenic animals by applying pressure to the aspiration site for several minutes.

Technique

The sites that most commonly are used for bone marrow aspiration in dogs are the proximal end of the femur at the trochanteric fossa, the iliac crest, and the proximal humerus (Fig. 14.1). The trochanteric fossa and humerus are the preferred sites in cats, and the ilium, ribs, or sternum usually are aspirated in horses, cattle, and camelids. If general anesthesia or sedation is not used, a local anesthetic is indicated. Both the subcutis and periosteum should be infiltrated with anesthetic. Bone marrow biopsy needles (16–22 G) are commercially available (Fig. 14.1); conventional hypodermic needles without stylets tend to plug with bone and are not suitable. After surgical preparation of the skin, the needle is introduced. In thick-skinned animals, the skin may be incised to facilitate introduction of the needle. Once the needle is against cortical bone, it should be rotated until firmly seated in the bone and then advanced a few more millimeters, all while keeping pressure on the stylet to prevent any backward movement and subsequent bone plugging (Fig. 14.2). The stylet then is removed, the syringe attached, and negative pressure applied, but only until marrow becomes visible in the syringe barrel. Aspiration of a larger volume results in contamination of marrow with blood. Once the marrow is collected, it should be placed in an EDTA (disodium ethylenediaminetetraacetate) tube, or slides made very quickly, because clotted samples are nondiagnostic. Alternatively, two or three drops of 10% EDTA solution can be placed in the syringe before aspiration. Pull films are prepared by placing a drop of marrow on a glass slide, gently placing a spreader slide directly atop the drop with little or no manual pressure, briefly allowing the drop to spread, and then pulling the two glass slides apart (Fig. 14.3). Slides are air-dried and then stained with a Romanowsky (i.e., Wright-type) stain. Because the preparations usually are quite cellular, staining time should be increased beyond that used for blood films.

If marrow cannot be aspirated even though multiple sites are attempted, a core biopsy is indicated. Core biopsies are collected using a Jamshidi marrow biopsy needle. An infant- or pediatric-sized needle should be used for small animals. After collection, the core of marrow can be gently rolled onto the surface of a glass slide for cytologic evaluation before placing the core in formalin solution for fixation.

Cells encountered in bone marrow films

Erythroid series

Erythroid precursors tend to have round nuclei, coarse chromatin, and moderate to deep blue cytoplasm that becomes more pink in color as hemoglobin is produced by more-differentiated cells. The developmental stages of the erythroid series, from immature to mature, are the rubriblast,

Veterinary Hematology and Clinical Chemistry, Second Edition. Edited by Mary Anna Thrall, Glade Weiser, Robin W. Allison, and Terry W. Campbell.

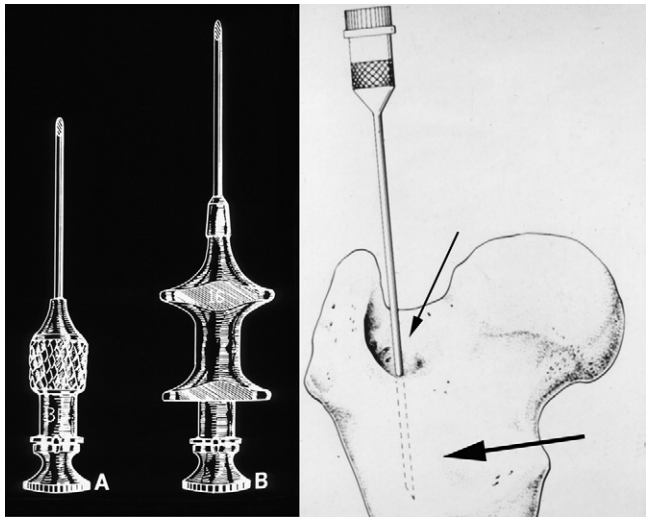

Figure 14.1 Left. Examples of commercially available bone marrow needles with stylets. Right. Correct placement of bone marrow needle in the trochanteric fossa.

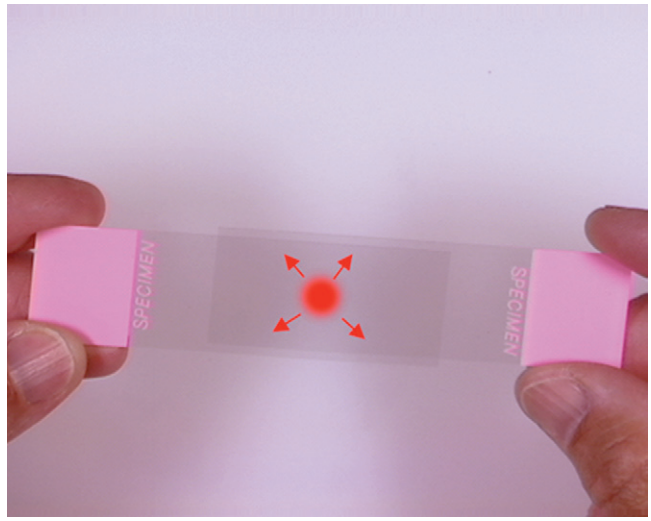

Figure 14.3 Two-slide technique for preparing bone marrow films. The drop of marrow is allowed to spread slightly before pulling the two glass slides apart.

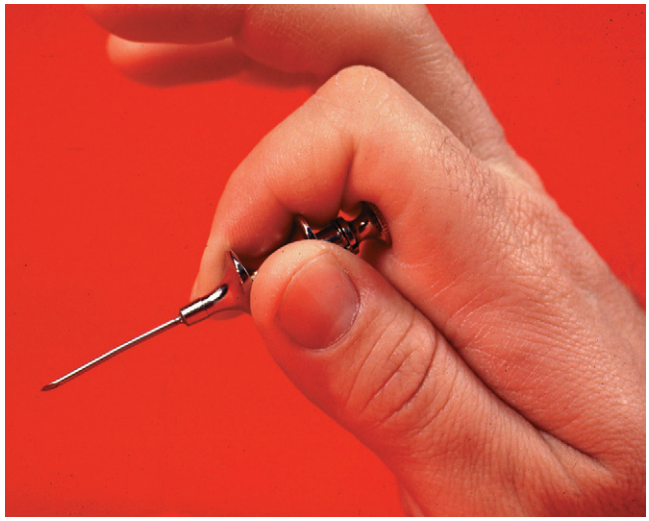

Figure 14.2 The bone marrow needle must be held with pressure against the stylet to keep the stylet in place within the needle, thus preventing bone plugs.

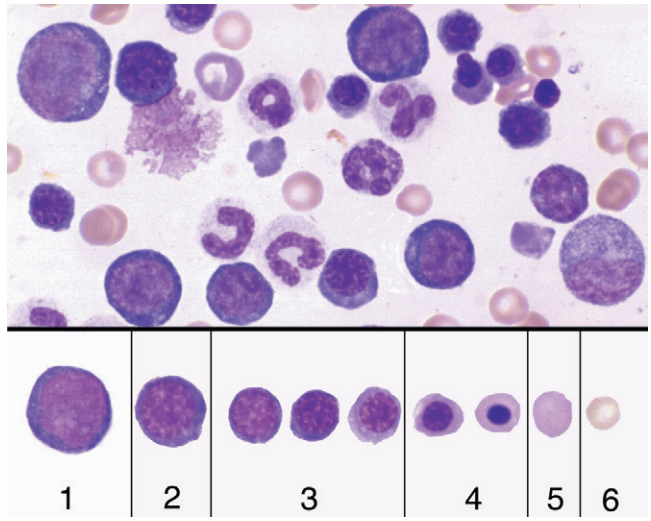

Figure 14.4 Top. Bone marrow aspirate from a dog showing numerous erythroid precursors with round nuclei, coarse chromatin, and blue- to hemoglobin-colored cytoplasm. Bottom. Maturation stages of erythroid precursors, from immature to mature. 1, rubriblast; 2, prorubricyte; 3, rubricytes; 4, metarubricytes; 5, polychromatophilic erythrocyte; 6, mature erythrocyte. Wright stain.

prorubricyte, rubricyte, metarubricyte, polychromatophilic erythrocyte, and mature erythrocyte (Fig. 14.4).

Rubriblasts are the most immature cells that are recognizable in the erythroid series. These cells are relatively large, have round nuclei, slightly coarse chromatin, and nucleoli. The nucleus:cytoplasm ratio is high, with a scant amount of deeply basophilic cytoplasm. A clear Golgi zone may be present as well.

Prorubricytes, which are the next stage in erythrocyte maturation, have a round nucleus, slightly more coarse chromatin, and no visible nucleolus. The cytoplasm is slightly less blue, and it is also more abundant than that of the rubriblast.

Rubricytes are the most mature stage of maturation in which mitosis can still occur. These cells have smaller nuclei, very coarse chromatin, and blue to blue-pink (i.e., polychromatophilic) cytoplasm.

Metarubricytes are the most mature cells of the erythroid series that still contain a nucleus. The nucleus is very small, dark, and dense, and the cytoplasm is either polychromatophilic or the red-orange color of mature erythrocytes. Nuclei are extruded from metaruribricytes, thereby resulting in polychromatophilic erythrocytes.

Polychromatophilic erythrocytes are anucleate, blue-pink in color, and larger than mature erythrocytes. They also may contain nuclear remnants (i.e., Howell-Jolly bodies). When stained with supravital stains (e.g., new methylene or brilliant cresyl blue), their mRNA and organelles clump, thereby resulting in blue-staining dots and fibrils (i.e., reticulum) throughout the cells. When stained in this manner, polychromatophilic erythrocytes are termed reticulocytes.

Mature erythrocytes are red-orange in color. Evaluation of mature erythrocyte morphology in bone marrow preparations usually is not indicated, but it can be diagnostically useful in that abnormalities such as red cell parasites, spherocytes, or hypochromasia occasionally may be observed.

Granulocyte (myeloid) series

Granulocytic precursors tend to have irregularly shaped and, sometimes, eccentric nuclei, with fine to stippled chromatin patterns and abundant, lavender-colored cytoplasm. At certain stages of maturation, they contain azurophilic (i.e., red-purple) to pink granules within the cytoplasm. As the cells mature, the nuclei elongate, from amoeboid or round in shape to kidney-bean or horseshoe shaped to segmented. The developmental stages of the myeloid series, from immature to mature, are the myeloblast, progranulocyte (promyelocyte), myelocyte, metamyelocyte, band granulocyte, and segmented granulocyte (Fig. 14.5). When the maturation process is hastened, whether resulting from inflammation or other causes, the cytoplasm of the myeloid precursors at all stages of maturation is more basophilic and sometimes vacuolated.

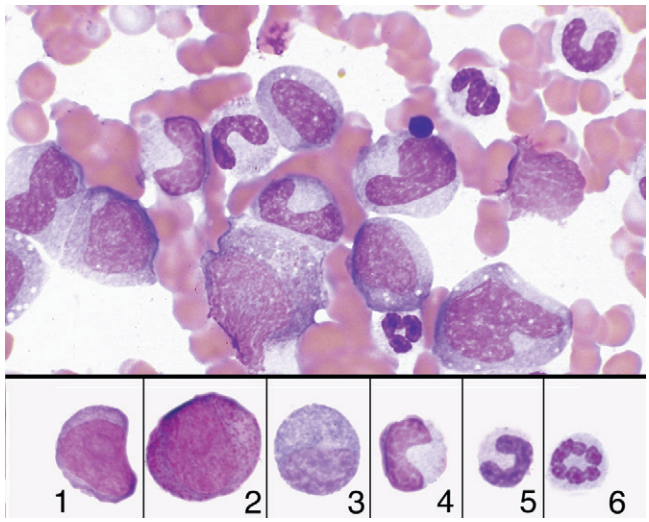

Figure 14.5 Top. Bone marrow aspirate from a dog showing numerous granulocytic (myeloid) precursors. Note the irregularly shaped nuclei, fine chromatin patterns, and lavender-colored cytoplasm. Bottom. Maturation stages of myeloid precursors, from immature to mature. 1, myeloblast; 2, promyelocyte; 3, myelocyte; 4, metamyelocyte; 5, band neutrophil; 6, segmented neutrophil. Wright stain.

Myeloblasts are subclassified into type I and type II. Type I myeloblasts, which are the most immature cells that are still recognizable in the granulocytic series, are large cells with round to oval nuclei, finely stippled or smooth nuclear chromatin, one or more nucleoli, a small amount of moderately blue cytoplasm, and no azurophilic granules. The nucleus usually is centrally located, and the nuclear outline may be slightly irregular. The nucleus:cytoplasm ratio is high (>1.5), and the cell size is approximately 1.5–3.0 times greater than the red cell diameter. The cytoplasm has a "ground-glass" appearance and, rarely, contains small vacuoles. Type II myeloblasts are very similar to type I, except that some small, azurophilic granules (primary granules) are scattered in the cytoplasm and the nucleus may be central or eccentric.

Promyelocytes are cells with smooth or slightly stippled nuclear chromatin, with or without a nucleolus, and many distinct azurophilic granules dispersed in slightly to moderately blue cytoplasm. The nucleus is central or eccentric. Prominent nucleoli may be present, even in cells with a high concentration of granules. A clear Golgi zone may be present as well.

Myelocytes, which are the last maturation stage in which mitosis can occur, are smaller than progranulocytes, have round to oval nuclei, light blue cytoplasm, and no primary granules within the cytoplasm. In these cells, the primary granules have been replaced by secondary (i.e., specific) granules, which are difficult to see in neutrophil precursors but are very distinct in eosinophil and basophil precursors. Eosinophil precursors contain pink (i.e., eosinophilic) granules, and basophil precursors contain azurophilic to dark purple granules (Fig. 14.6).

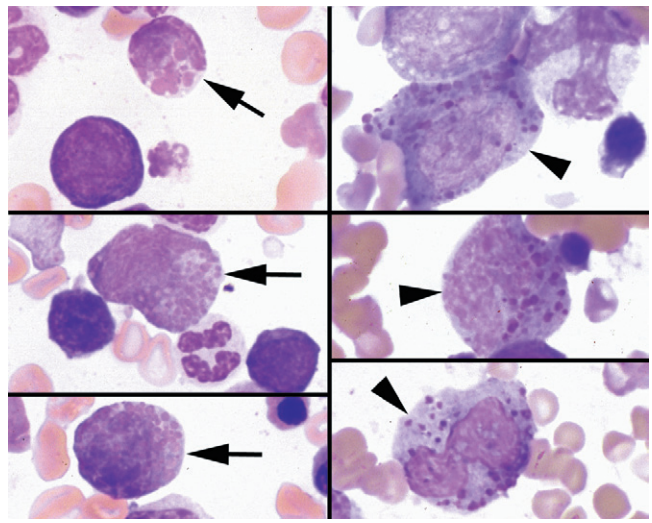

Figure 14.6 Left. Various maturation stages of eosinophil precursors (arrows). Right. Various maturation stages of basophil precursors (arrowheads). Granules may obscure the nucleus, thus making identification of specific maturation stage difficult. Wright stain.

Metamyelocytes have kidney bean–shaped nuclei. The cytoplasm is similar in appearance to that of myelocytes.

Band granulocytes have nuclei that are curved and elongated, with parallel sides. Some chromatin clumping is present, and the cytoplasm is similar to that of myelocytes and metamyelocytes.

Segmented granulocytes have lobulated or markedly constricted nuclei, with large and dense chromatin clumps. The cytoplasmic characteristics are generally similar to those of myelocytes, metamyelocytes, and bands.

Monocyte series

Cells of the monocyte series are relatively few in concentration, and they are very difficult to distinguish from those of the myeloid series in normal marrow. A distinctive feature is their irregular nuclear outlines. Monoblasts appear similar to myeloblasts, and promonocytes appear similar to myelocytes and metamyelocytes. Mature monocytes have the same appearance as monocytes in peripheral blood (Fig. 14.7). Monocyte precursors usually are recognizable only in animals with monocytic leukemia.

Monoblasts are large cells with round, irregular or folded nuclei and finely reticular nuclear chromatin, one or more prominent nucleoli, and a moderate amount of basophilic, agranular cytoplasm. A Golgi zone often is prominent at the site of nuclear indentation. The nucleus:cytoplasm ratio usually is less than that of myeloblasts.

Promonocytes are large cells with cerebriform nuclei and prominent nuclear folds, stippled or lacy chromatin, and no distinct nucleolus. They also have more abundant and less basophilic "ground-glass" cytoplasm than that of monoblasts.

Megakaryocyte series

Megakaryocytes are very large cells, and their cytoplasmic fragments become platelets, which are important in the clotting process. Although these cells undergo mitosis, they do not divide, thus becoming very large and multinucleated, with as many as 16 or more nuclei. The nuclei are not separate entities, however, and they appear as a large, multilobulated structure in the center of the cell. The developmental stages of the megakaryocyte series, from immature to mature, are the megakaryoblast, promegakaryocyte, and megakaryocyte (Fig. 14.8).

Megakaryoblasts are first recognizable when their size exceeds that of other types of precursors. The nuclei usually appear to be more dense than those of other types of blast cells, and the cytoplasm usually is deeply basophilic.

Promegakaryocytes have from two to four nuclei, which usually are connected by thin strands of nuclear material and deep blue agranular cytoplasm. They also usually are several-fold larger than rubriblasts or myeloblasts.

Megakaryocytes are very large (diameter, 50–200 µm), with numerous nuclei that form a lobulated mass of nuclear material. The cytoplasm stains more lightly than that of promegakaryocytes. As megakaryocytes mature, they become larger, gain more nuclei, and contain cytoplasm that becomes granular and, sometimes, light pink in color. Naked nuclei of megakaryocytes commonly are observed in bone marrow films.

Other cells

Small lymphocytes in bone marrow appear as they do in peripheral blood, with a round and usually indented nucleus, a diffuse chromatin pattern without visible nucleoli, and

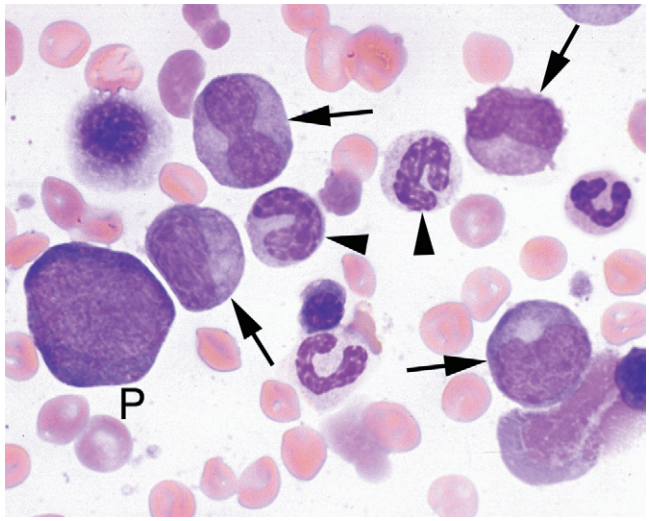

Figure 14.7 Bone marrow aspirate from dog with granulocytic and monocytic hyperplasia. Monocyte precursors (arrows) are difficult to distinguish from granulocytic precursors (arrowheads). Chromatin pattern is more coarse in granulocytic precursors. P, progranulocyte. Wright stain.

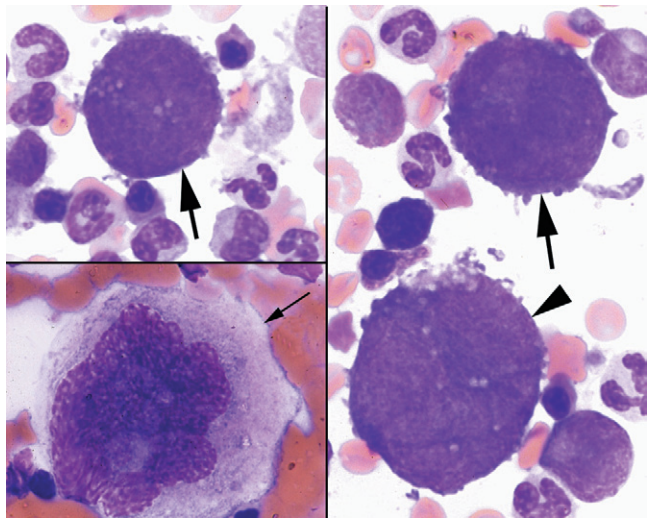

Figure 14.8 Various maturation stages of megakaryocyte series. Large arrows, megakaryoblasts; arrowhead, promegakaryocyte; small arrow, mature megakaryocyte. Wright stain.

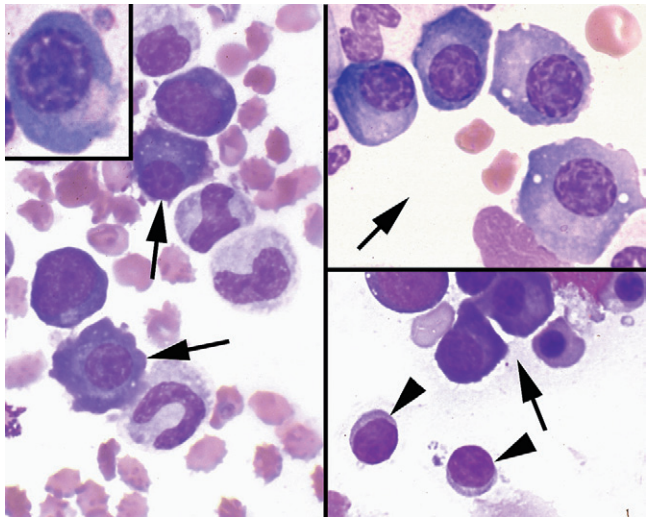

Figure 14.9 Plasma cells (arrows) have a variable appearance, depending on thickness of preparation and degree of flattening of the cells. Flattened plasma cells usually appear to have abundant cytoplasm and obvious, clear Golgi areas. Inset. Higher magnification of a plasma cell. Note the coarse chromatin and clear Golgi area. Lymphocytes (arrowheads) have a small amount of cytoplasm. Wright stain.

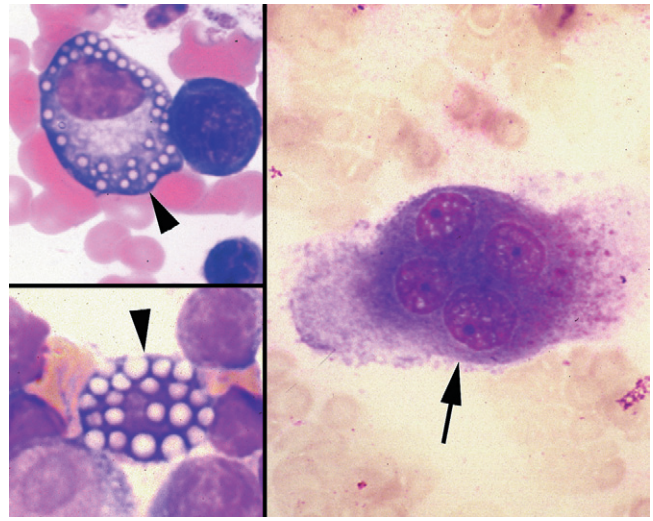

Figure 14.10 Left. Vacuolated plasma cells (Mott cells) containing packets of immunoglobulin (Russell bodies). Right. Osteoclast, which can be differentiated from a megakaryocyte because the osteoclast nuclei are separate rather than lobulated. Wright stain.

scant, light blue cytoplasm. They are slightly smaller than neutrophils (Fig. 14.9). Plasma cells are differentiated lymphocytes that produce immunoglobulin, and they are similar in size to neutrophils. The appearance of plasma cells is very similar to that of rubricytes, except that the cytoplasm of plasma cells is light blue and more abundant, with a clear Golgi zone adjacent to the often eccentric nucleus sometimes being apparent (Fig. 14.9). The nuclei are round, with very coarse and dense chromatin, and nucleoli are inapparent. The cytoplasm of plasma cells occasionally may contain either very eosinophilic material (i.e., "flame cells") or round, clear to light blue structures that represent immunoglobulin (i.e., Russell bodies). Plasma cells that contain Russell bodies are called Mott cells (Fig. 14.10).

Lymphoblasts rarely are seen in the bone marrow aspirates from normal animals, and their presence often is indicative of a lymphoproliferative disorder. Lymphoblasts are small to large cells with a round to oval nucleus, finely stippled to slightly course nuclear chromatin, one or more nucleoli, and a small to moderate amount of pale blue cytoplasm without azurophilic granules. The nuclear outline may appear to be slightly indented or irregular. The nucleus:cytoplasm ratio usually is greater than that of myeloblasts. Lymphoblasts are distinguished from myeloblasts by the slightly more coarse chromatin, less cytoplasm, and the absence of azurophilic granules. Lymphoblasts may appear similar to rubriblasts, but the nuclei of lymphoblasts are less perfectly round.

Macrophages derive from monocytes and are present at a low concentration in normal bone marrow. The appearance of macrophages is highly variable. The nuclei usually are round to slightly kidney bean in shape, and the nucleoli usually are small and inconspicuous. The cytoplasm is gray-blue and usually vacuolated; small, pink granules may be present in the cytoplasm as well. Macrophage nuclei may contain several small nucleoli. Macrophages commonly phagocytize cellular debris, including nuclei that have been extruded from metarubricytes, and they often contain hemosiderin, which is a red cell breakdown product containing iron.

Osteoblasts and osteoclasts may be seen in the bone marrow aspirates from young animals and from those in which bone remodeling is occurring. Osteoclasts are very large, multinucleated cells that may appear similar to megakaryocytes, but their nuclei are individual and not connected to each other (unlike those of megakaryocytes). The cytoplasm is basophilic, and may contain a few pink to azurophilic granules. Osteoclasts are specialized macrophages that derive from monocytes, and they function in the lysis of bone (Fig. 14.10). Osteoblasts are similar in appearance to plasma cells but are larger (Fig. 14.11). They have eccentric, round to oval nuclei that appear to be falling out of one end of the cell; they also have abundant basophilic cytoplasm and a clear Golgi area. Small pink or azurophilic granules may be present in the cytoplasm as well.

Mast cells are easily recognized in the bone marrow, and although rarely observed, they normally are present at very low concentrations. Mast cells are large, round, and discrete cells with abundant small metachromatic granules in the cytoplasm (Fig. 14.11). They usually can be distinguished from basophil myelocytes, because mast cell granules are

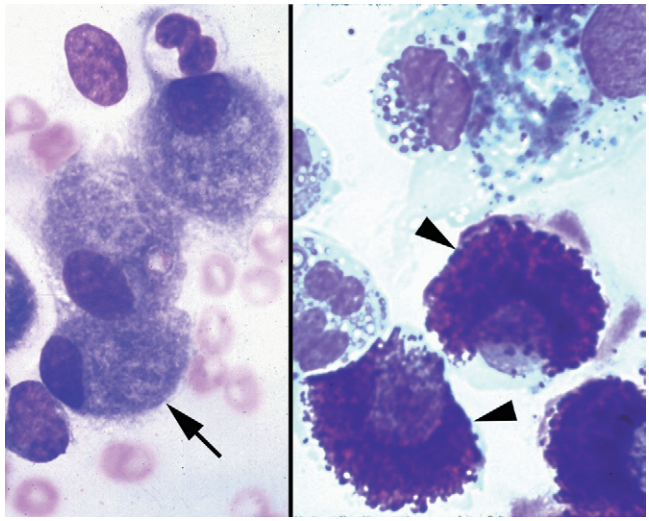

Figure 14.11 Left. Osteoblasts, which have a similar appearance to plasma cells but are larger, with a less condensed chromatin pattern and less distinct cytoplasmic margins (arrow). Right. Mast cells with abundant cytoplasmic granules that tend to obscure the round nucleus (arrowheads). Wright stain.

smaller and more numerous. Mast cells are more apparent and, possibly, increased in concentration in bone marrow that is hypocellular, such as that which may be seen with ehrlichiosis. When mast cells are abundant, infiltration by mast cell neoplasia is likely.

Fibrocytes and fibroblasts are seen only infrequently, even in aspirates from animals with myelofibrosis, because they do not exfoliate easily. The nuclei are round to oval, and the cytoplasm is lightly basophilic and spindle-shaped.

Cytochemistry and immunophenotyping

Cytochemical reactions sometimes are useful in the process of cell identification. These stain reactions are based on various cell types having different amounts, distribution, and types of enzyme activities. The stains most commonly used include peroxidase, Sudan black B, chloroacetate esterase, α-naphthyl acetate esterase, α-naphthyl butyrate esterase, and alkaline phosphatase (ALP). Peroxidase, Sudan black B, and chloroacetate esterase are myeloid (i.e., granulocytic) markers. The nonspecific esterases α-naphthyl acetate esterase and α-naphthyl butyrate esterase, which can be inhibited by sodium fluoride, and are monocyte markers, but their staining patterns vary. Monocytes may have a few small, round granules that stain positive for Sudan black B. Reactivity for ALP is somewhat confusing, however, because ALP positivity is rare in the immature neutrophils of normal animals but ALP-positive myeloid cells are common in animals with acute myelogenous leukemia. Moreover, ALP activity is present in some types of lymphoid cells as well as in cells with monocytic differentiation in animals with acute myelomonocytic leukemia. Cytochemical staining of blood and bone marrow films can facilitate the classification of neoplastic cells, but in many cases, negative staining occurs, perhaps because of abnormalities in hematopoietic differentiation that are associated with the neoplastic process.

Immunophenotypic analysis is based on using monoclonal antibodies that are directed against antigens on the surface of hematopoietic cells to determine the phenotypic profile of those cells, thus identifying the cell type. Very little sample quantity usually is necessary, and flow cytometric analysis using the antibodies makes the technique relatively simple to perform. Briefly, monoclonal anti bodies directed against cell surface proteins are conjugated to fluorescent molecules and then mixed with the cells, after which the cells are analyzed by flow cytometry. Flow cytometry provides information regarding the size of the cells, expression of any particular surface protein, and concentration of the surface protein. Phenotypes of both normal and neoplastic cells are continuously being classified as more monoclonal antibodies become available. Immunophenotyping likely will eventually replace cytochemistry for use in the classification of hematopoietic cells. See Chapter 13 for more discussion.

Evaluation and interpretation of bone marrow films

Bone marrow films must be evaluated and interpreted in conjunction with the analysis of concurrent complete blood count (CBC) data. For example, if an animal has a decreased platelet concentration (i.e., thrombocytopenia), the megakaryocyte concentration is particularly important to evaluate.

Cellularity

The low-power (×10) objective should be used to scan the slide at ×100 magnification to assess the degree of cellularity and amount of fat that is present (Fig. 14.12). Hemodiluted marrow samples are difficult to evaluate for cellularity. Normal marrow cellularity varies, but in general, approximately 50% of the marrow consists of fat and 50% of cells. Cellularity is increased when production in either the myeloid or the erythroid cell line is increased in response to cell loss, destruction, or consumption. Abnormal causes of increased cellularity include lymphoproliferative and myeloproliferative disorders as well as other neoplastic disorders. Overall cellularity may be decreased with disorders such as myelofibrosis, certain infectious agents (including *Ehrlichia* sp. in dogs and feline leukemia virus [FeLV]), estrogen toxicity (in dogs and ferrets), drug toxicities (including some

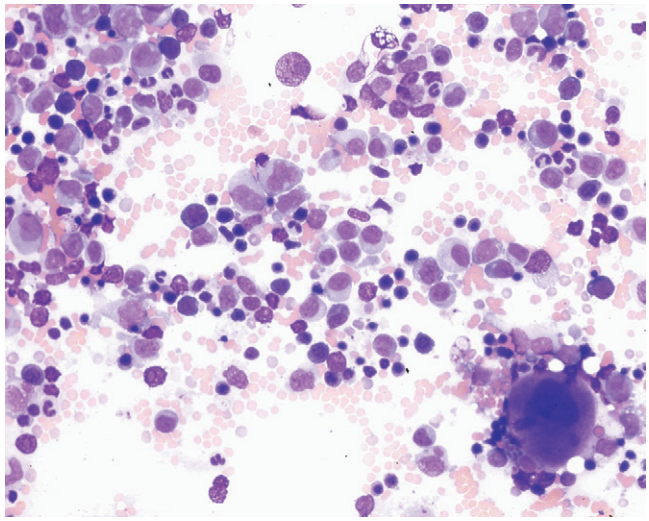

Figure 14.12 Bone marrow aspirate from a dog, low magnification. The degree of cellularity is adequate to increased. Cellularity is judged by the density of sheets of cells, as exemplified in this figure, or by estimating the ratio of fat to cells in particles. Wright stain, low power.

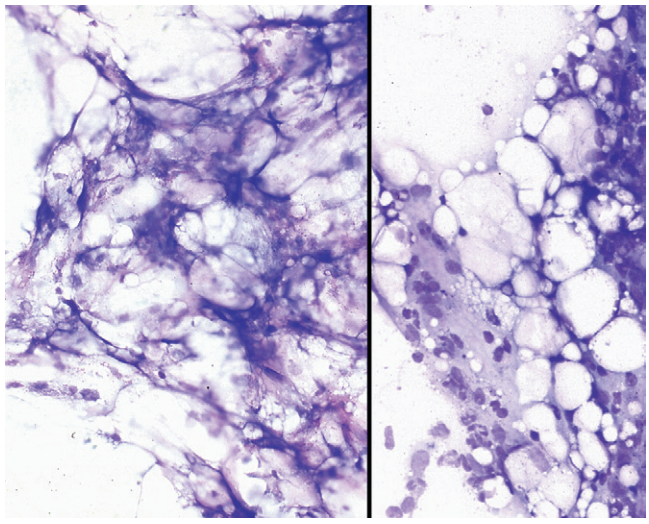

Figure 14.13 Bone marrow aspirate from a cat with generalized marrow hypoplasia, low magnification. Right. Numerous adipocytes are present, with very little hematopoietic cellularity. Left. Broken adipocytes and stroma are present, with few hematopoietic cells. Wright stain, low power.

commonly used chemotherapeutic agents), chemicals that are toxic to the marrow, radiation, and immune-mediated disorders in which stem cells are destroyed (Fig. 14.13). A decrease in cellularity is termed hypoplasia, and a complete absence of cells is termed aplasia. Hypoplasia of only one cell line is relatively common, whereas aplasia usually involves all cell lines. Erythroid or myeloid aplasia is rare. Histopathologic evaluation of a core biopsy specimen is indicated when the cellularity cannot be determined by examination of the marrow aspirate.

Megakaryocytes

Using the low-power (10×) objective, the megakaryocyte concentration should be estimated as either increased (i.e., hyperplasia), decreased (i.e., hypoplasia), or adequate. Interpretation of this estimate depends on the platelet concentration in the peripheral blood. Areas with high cellularity normally contain at least a few megakaryocytes, and unless the sample is markedly hemodiluted, at least 5–10 megakaryocytes should be present on the slide. In animals with increased platelet consumption (e.g., animals with disseminated intravascular coagulopathy) or destruction (e.g., animals with immune-mediated thrombocytopenia), the megakaryocyte concentration in the marrow should be increased. Animals with megakaryocytic hyperplasia may have as many as 50 or more megakaryocytes in cellular areas of the slide. Increased concentrations of megakaryoblasts, promegakaryocytes, and smaller, more immature megakaryocytes typically are seen with megakaryocytic hyperplasia. In thrombocytopenic patients with megakaryocytic hyperplasia, the platelet size usually is increased because of the early release of platelets; this increase in size is analogous to the increased size of immature erythrocytes. Animals that are thrombocytopenic because of the lack of platelet production have very few—or even no—megakaryocytes in the marrow film. Megakaryocytic hypoplasia without erythroid and myeloid hypoplasia is rare and may be caused by immune-mediated destruction of megakaryocytes.

Myeloid:erythroid ratio

Using the ×10 objective, appropriate areas that are not too thick and in which cells are not broken can be chosen for further examination of the bone marrow using the ×50 or ×100 oil objectives (to magnify 500- and 1000-fold, respectively). At these higher magnifications, erythroid and myeloid precursors can be identified, and the myeloid:erythroid (M:E) ratio can be estimated (Fig. 14.14). Usually, estimation of this ratio is just as informative as actual quantification. To quantify the M:E ratio, 300–500 nucleated cells are classified as being either myeloid or erythroid. This classification should be performed while examining several different areas, because some fields may be predominantly granulocytic and other areas predominantly erythroid.

Normal M:E ratios differ with the species, but in general, they range from 0.5:1 to 3:1. Decreased or increased production of either cell line shifts the M:E ratio, and such shifts must be interpreted in light of the CBC results, particularly the packed cell volume and the neutrophil concentration. For example, if the M:E ratio is increased, the animal is anemic, and the blood neutrophil concentration is normal, then the ratio is increased because of a decrease in red cell production rather than an increase in neutrophil produc-

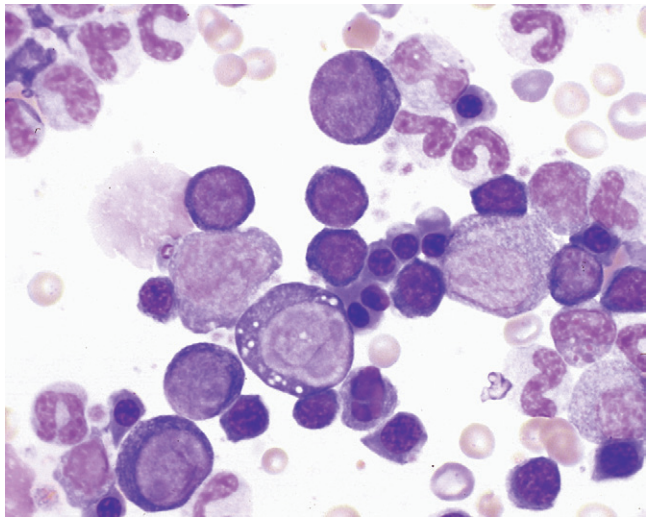

Figure 14.14 Bone marrow aspirate from a dog. Both myeloid and erythroid precursors are present, with a normal myeloid:erythroid ratio of approximately 1. Wright stain.

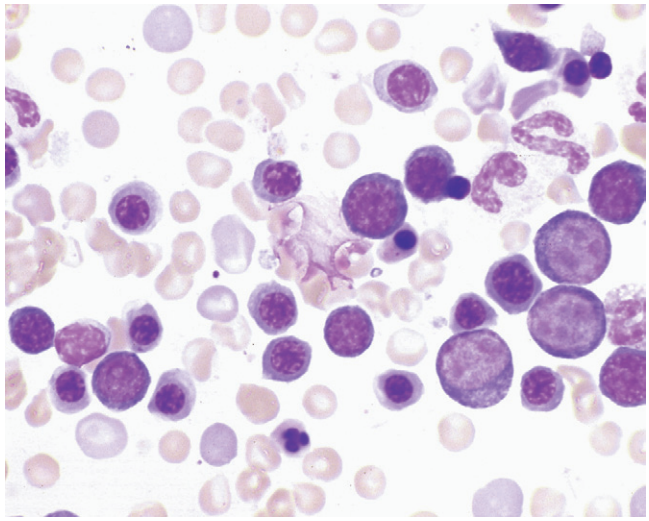

Figure 14.15 Bone marrow aspirate from a dog with regenerative anemia. The myeloid:erythroid ratio is decreased because of increased red cell production (erythroid hyperplasia). Wright stain.

tion. Conversely, if the animal is not anemic and the neutrophil concentration is increased, then the increased M:E ratio results from an increased neutrophil production rather than a decreased erythrocyte production.

Decreased M:E ratio

A decreased M:E ratio may be indicative of increased red cell production, such as that seen with a regenerative anemia (i.e., erythroid hyperplasia); a decreased neutrophil production (i.e., myeloid hypoplasia); or a combination of the two (Fig. 14.15). Myeloid hypoplasia without erythroid hypoplasia is rare but, when present, usually is associated with myelodypsplasia or myeloproliferative disorder.

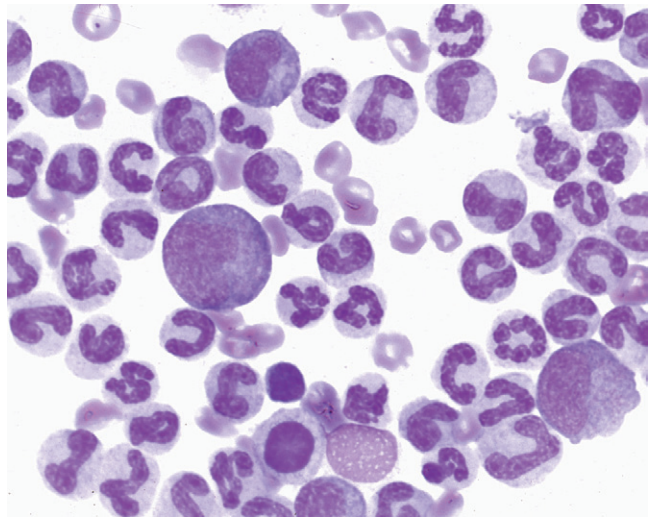

Figure 14.16 Bone marrow aspirate from a dog. The myeloid:erythroid ratio is markedly increased because of increased granulocyte production (myeloid hyperplasia). Wright stain.

Increased M:E ratio

An increased M:E ratio may be indicative of increased granulocyte production (i.e., myeloid hyperplasia), decreased in red cell production (i.e., erythroid hypoplasia), or both (Fig. 14.16). Granulocytic hyperplasia usually results from inflammation, but it also may be seen in animals with immune-mediated destruction of neutrophils and in those recovering from viral-induced marrow damage, such as parvovirus infections in dogs (i.e., parvoviral enteritis) and cats (i.e., panleukopenia). Causes of erythroid hypoplasia are discussed in Chapter 7 and include renal failure, endocrinopathies, and anemia of inflammatory disease. Anemia of inflammatory disease (i.e., anemia of chronic disease) is one of the more common causes of mild erythroid hypoplasia in domestic animals. Granulocytic hyperplasia and increased iron stores (i.e., hemosiderin) also usually are seen in the marrow from these patients. Pure red cell aplasia is rare but, when present, usually is caused by immune-mediated destruction of very early erythroid precursors.

Orderliness of maturation

The orderliness and completion of maturation in erythroid and myeloid cells should be determined. Blast cells divide to ultimately produce 16–32 mature cells. Thus, approximately 80–90% of the cells should be more mature forms (i.e., metamyelocytes, bands, and neutrophils in the myeloid series, and rubricytes and metarubricytes in the erythroid series), and polychromatophilic erythrocytes should be present. Orderly progression of maturation usually is referred to as a "pyramid," with the few immature forms comprising

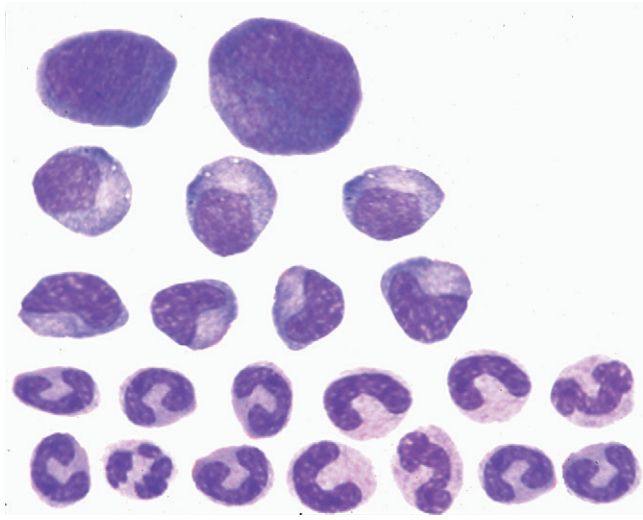

Figure 14.17 A normal "pyramid," illustrating an orderly maturation of myeloid precursors. A few very immature cells form the top of the pyramid, with numerous more mature cells forming the bottom.

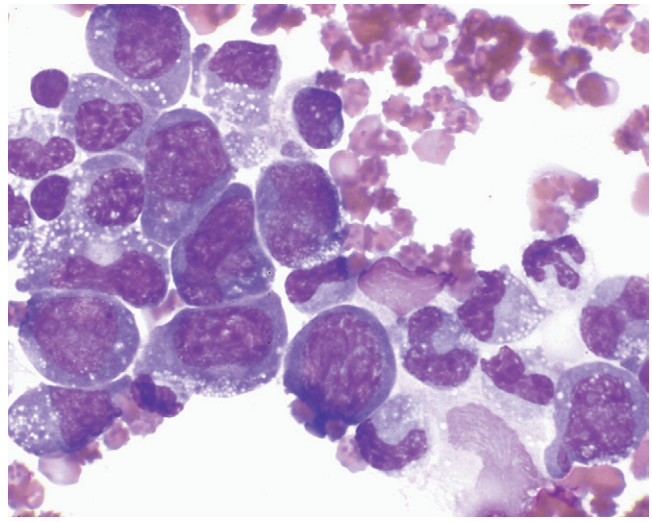

Figure 14.18 Bone marrow aspirate from a dog with immune-mediated neutropenia. Marked myeloid hyperplasia is evident, with an increased proportion of more immature granulocyte precursors and few mature granulocytes because of the immune-mediated destruction of more mature cells. Note that the cytoplasm is basophilic and vacuolated, likely because of the increased rate of cell production. Wright stain.

the top and the numerous more mature forms comprising the broad bottom (Fig. 14.17).

Disorderly maturation of erythroid and myeloid precursors commonly is seen in animals with leukemia and myelodysplasia, but it also may be seen in animals with nonneoplastic conditions. An apparent arrest in maturation of the erythroid series, often at the rubricyte stage of maturity, may be seen in animals with immune-mediated destruction of immature erythroid cells. These animals do not have a typical regenerative response, such as that usually seen in animals with immune-mediated hemolytic anemia. Metarubricytes and polychromatophilic erythrocytes often are decreased to absent in the marrow from such patients.

A similar apparent arrest of maturation in the granulocytic series, which often occurs in conjunction with marked myeloid hyperplasia, commonly is seen in marrow aspirates from animals with immune-mediated neutropenia (Fig. 14.18). This "arrest" may appear at any stage of granulocytic maturity, but it often occurs at the metamyelocyte stage. Marrow from animals with immune-mediated destruction can appear similar to that from patients with granulocytic leukemia, but the concentration of myeloblasts usually is lower in those with immune-mediated disease. Other conditions that cause disorderly maturation of granulocytes include marked inflammatory disease (with consumption of more mature forms) and recovery from viral-induced neutropenia.

Macrophages and iron stores

Macrophages (i.e., histiocytes) normally are present in small concentrations (<1% of nucleated cells), and phagocytosis of red cells and nuclear debris by macrophages occasionally may be seen in normal animals. The concentration of macrophages may be increased in animals with immune-mediated disorders, and macrophages that have phagocytized nucleated red cells, platelets, and neutrophils occasionally are observed (Fig. 14.19). Other causes of increased cell destruction, such as marrow necrosis secondary to drugs, toxins, or radiation, may result in increased macrophage concentration. In these cases, other morphologic evidence

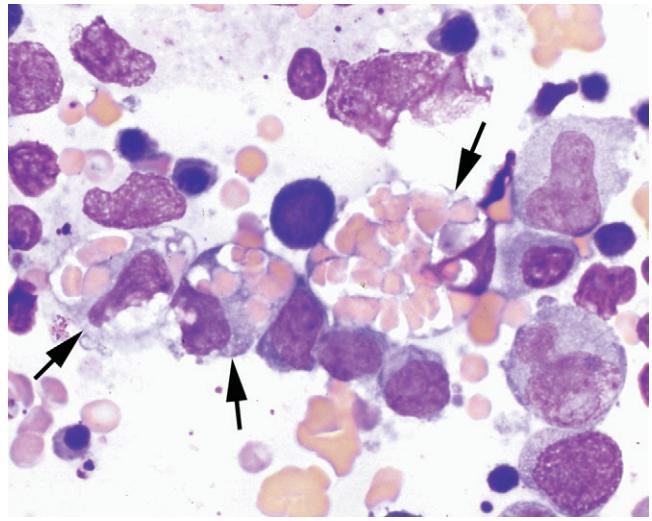

Figure 14.19 Bone marrow aspirate from a cat. Macrophages (arrows) are increased and have phagocytized many erythrocytes. This degree of phagocytic activity is abnormal and suggestive of either immune-mediated destruction of red cells or hemophagocytic syndrome. Wright stain.

of necrosis, such as pyknosis and increased cytoplasmic vacuolation, usually is observed.

A marked increase in the concentration of macrophages may be seen in animals with hemophagocytic syndrome, which also is called hemophagocytic histiocytosis and is a condition characterized by a benign, histiocytic proliferation secondary to infectious, neoplastic, or metabolic diseases. One retrospective study[1] found hemophagocytic syndrome in 3.9% of dogs that had bone marrow aspiration. This syndrome is associated with cytopenia of at least two cell lines and greater than 2% hemophagocytic macrophages in the marrow. It must be distinguished, on the basis of red cell morphology (i.e., lack of spherocytes and agglutination) and a negative Coombs test, from the much more commonly occurring immune-mediated diseases. Macrophages are a prominent cellular component in the marrow from animals with hemophagocytic syndrome, and they appear to be normal and well differentiated, with amoeboid nuclei and abundant, light blue cytoplasm. Many macrophages are observed with phagocytized hematopoietic cells within their cytoplasm (Fig. 14.20). Dogs with hemophagocytic syndrome often exhibit fever, icterus, splenomegaly, hepatomegaly, and diarrhea, and those with infection-associated hemophagocytic syndrome are thought to have a better survival rate than dogs with other causes of hemophagocytic syndrome. An increased concentration of macrophages also is seen in animals with malignant histiocytosis, which is a neoplastic proliferation of histiocytes (see below).

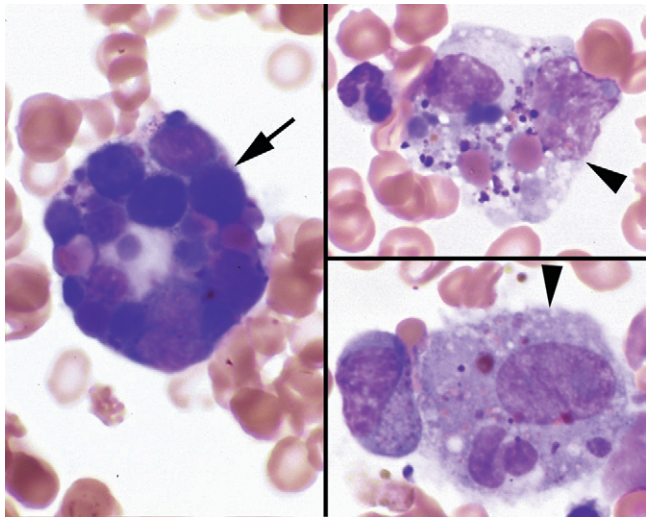

Figure 14.20 Bone marrow aspirate from a dog. Left. A macrophage (arrow) that has phagocytized erythrocytes as well as nucleated erythrocytes. Upper right. A macrophage (arrowhead) that has phagocytized erythrocytes, a large nucleated cell, platelets, and cellular debris. Lower right. A macrophage (arrowhead) that has phagocytized a neutrophil and contains hemosiderin. Phagocytosis of platelets and immature cells may be seen with immune-mediated disease and with hemophagocytic syndrome. Wright stain.

The presence or absence of hemosiderin (i.e., iron stores) in macrophages should be noted (Fig. 14.21). Special stains for iron, such as a Prussian blue stain (Fig. 14.21), usually are not necessary, because hemosiderin can be readily visualized with use of Romanowsky stains. Hemosiderin rarely is seen in the marrow aspirates from normal cats, but it usually is abundant in that from normal dogs and horses. Animals with iron deficiency anemia lack iron stores in the marrow, and animals with anemia of inflammatory disease may have increased iron stores.

Other cells

The presence and percentage of other types of cells, such as lymphocytes and plasma cells, should be noted as well. In animals that have been antigenically stimulated, the plasma cell concentration may be markedly increased, and the plasma cells may be present in small groups. Normally, approximately 2% or less of the marrow cells are plasma cells. Approximately 15% or less of the cells observed in a bone marrow film from healthy dogs may be lymphocytes, whereas as much as 20% of the cells may be lymphocytes in normal cats. The concentrations of plasma cells and lymphocytes in the bone marrow film usually vary from one area of the film to the next.

Microorganisms

Microorganisms occasionally may be found in bone marrow aspirates. Bacteria very rarely are seen, but *Histoplasma capsulatum* (Fig. 14.22), *Toxoplasma gondii* (Fig. 14.23), *Leishmania donovani* (Fig. 14.24), *Cytauxzoon felis*, and rarely, *Ehrlichia* sp. can be observed. Red cell parasites such as

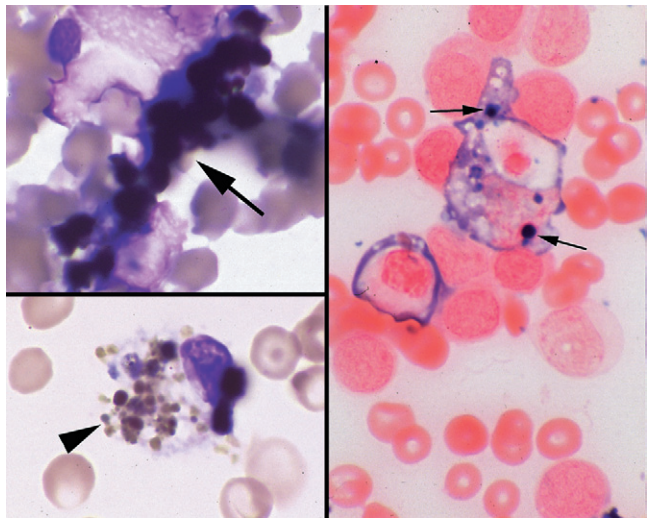

Figure 14.21 Bone marrow aspirate from a dog. Upper left. A clump of hemosiderin (storage iron; arrow) from a broken macrophage. Wright stain. Lower left. A macrophage (arrowhead) containing hemosiderin. Wright stain. Right. Prussian blue iron stain, showing the presence of blue-staining iron (small arrows).

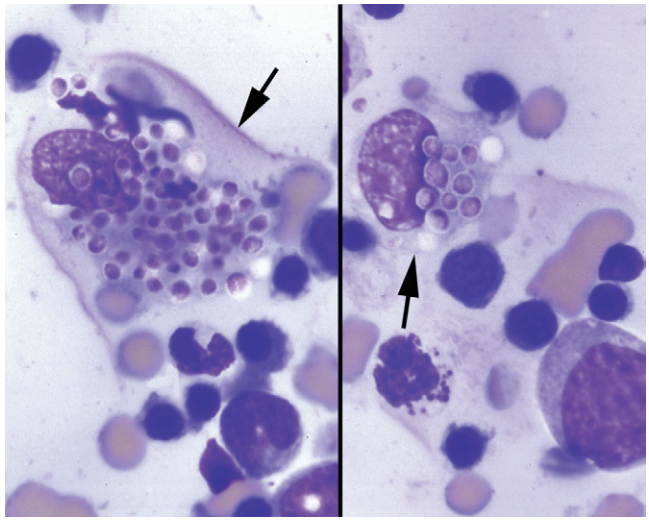

Figure 14.22 Bone marrow aspirate from a cat. Macrophages (arrows) contain numerous *Histoplasma capsulatum* organisms, which are round yeast cells with a well-defined, thin capsule. Wright stain. (Specimen courtesy of Antech Diagnostics.)

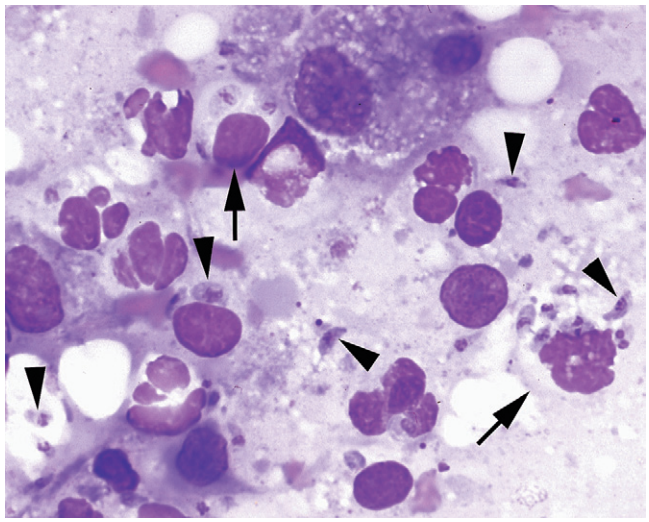

Figure 14.23 Bone marrow aspirate from a cat. Macrophages (large arrows) contain trophozoites of *Toxoplasma gondii*. Individual trophozoites (arrowheads) have a characteristic crescent shape and a central nucleus. Wright stain.

Mycoplasma sp. or *Babesia* sp. also may be observed in marrow aspirates.

Stem cell disorders of marrow

Reversible stem cell injuries

Reversible injury is transient in nature and, therefore, usually manifests as neutropenia because of the short half-life of neutrophils in blood (see Chapter 11). Causes include

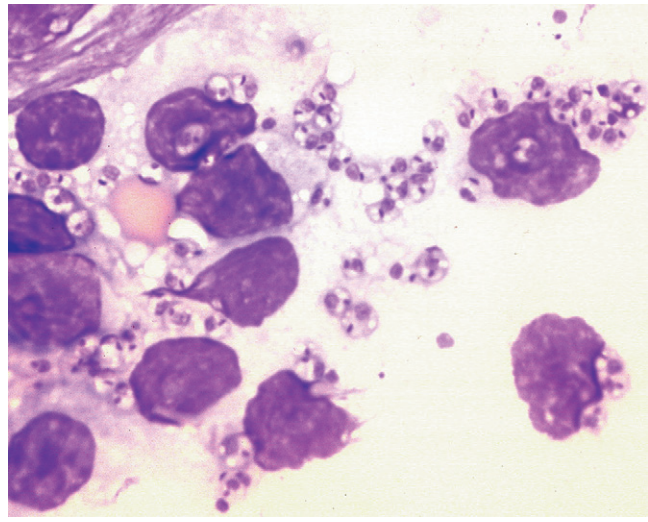

Figure 14.24 Bone marrow aspirate from a dog. Note the broken mononuclear cells with numerous *Leishmania donovani* organisms. These organisms are oval, with a typical, dark-staining, rod-shaped structure (kinetoplast). Wright stain.

viral injury, drugs or chemicals, and chemotherapeutic drugs, such as doxorubricin, that injure rapidly dividing cells. Although neutropenia is recognized initially, thrombocytopenia and nonregenerative anemia may occur if the injury lasts for more than 1–2 weeks. In general, if the animal does not have complications associated with the cytopenias, the stem cell system can be expected to recover and repopulate the blood with normal concentrations of cells.

Some drugs and chemicals apparently are directly cytotoxic to stem cells. Drugs that have been associated with stem cell injury in animals include estrogen (in dogs and ferrets), phenylbutazone (in dogs), and albendazole, which is a broadspectrum anthelmintic (in dogs and cats). Estrogen toxicosis may occur in bitches given exogenous estrogen for mismating, termination of pseudopregnancy, or urinary incontinence. Myelosuppression may occur either from administration of excessive amounts of estrogen or from an idiosyncratic sensitivity to estrogen. Endogenous estrogen, either because of Sertoli cell tumors in male dogs or cystic ovaries in female dogs, also may result in bone marrow suppression. Because ferrets are induced ovulators, marrow suppression from endogenous estrogen is a common and potentially fatal disorder in this species. The mechanism of estrogen toxicosis is unclear but is thought to result from secretion, by thymic stromal cells, of an estrogen-induced substance that inhibits stem cells. Marrow suppression is preceded by an initial thrombocytosis and neutrophilia.

Other drugs may induce cell destruction by immune-mediated mechanisms. In dogs, trimethoprim-sulfadiazine, cephalosporin, and phenobarbital have been associated with pancytopenia that may be immune-mediated. Methimazole,

which is used for treating cats with hyperthyroidism, is associated with neutropenia and thrombocytopenia in approximately 20% of the cats given this drug. Stem cell injury that is drug related and immune-mediated usually responds to discontinuation of the drug. Idiopathic immune-mediated stem cell injury usually responds to immunosuppressive therapy; however, it may take several weeks to respond and, often, requires long-term treatment for resolution.

Myelofibrosis may develop in response to various types of marrow injury. Any agent that is directly toxic to hematopoietic cells presumably may damage the microvasculature of the marrow, thereby leading to necrosis and subsequent fibrosis. Myelofibrosis also has been associated with myeloproliferative and lymphoproliferative disorders, other types of neoplasia, chronic hemolytic anemia secondary to pyruvate kinase deficiency, radiation, and other unidentified causes.

Irreversible stem cell injuries

In contrast to reversible stem cell injuries, irreversible injuries result from an intrinsic defect in proliferative behavior or regulation of stem cell entry into differentiated hematopoiesis. These types of injuries generally are regarded as being irreversible, because they do not spontaneously correct themselves and therapeutic intervention almost never corrects the proliferative abnormality (with the exception of bone marrow transplantation, in which defective stem cells are replaced by normal donor stem cells). The causes of this form of stem cell injury are not well understood. The best-characterized causative association in domestic animals, however, is infection with FeLV in cats. In other domestic animals, the cause almost always is unknown. Chronic exposure to benzene-related chemical compounds is an employment hazard in humans and, rarely, may cause similar injury in animals. Radiation also may induce such an injury in a number of species. Manifestations of stem cell injury are highly variable (Fig. 14.25). These manifestations

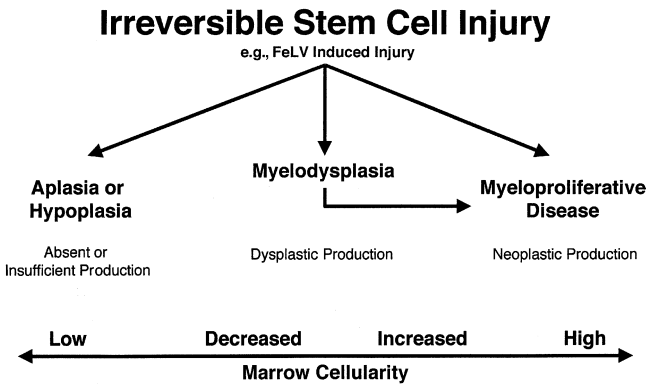

Figure 14.25 Organizational diagram of irreversible stem cell disorders. Myelodysplasia may progress to neoplasia over time. Expected cellularity of proliferative abnormalities are indicated at the bottom.

are best regarded as being a continuum, from lack of cell production on one extreme to uncontrolled, neoplastic proliferation at the other. In the middle of this continuum is dysplastic cell production, which usually is associated with one or more cytopenias and with subtle morphologic abnormalities of the blood cells. Many cases likely begin as dysplasia and then, with time, progress to either hypoplasia or neoplasia. The stage observed at the initial examination is variable, depending on when during the course of the disease the animal is presented to the veterinarian. (More detailed descriptions of the points on this continuum are presented in Chapter 15.)

Aplasias or hypoplasia

Marrow aplasia is a relatively rare disorder in dogs and cats. Causes include chronic ehrlichiosis, Parvovirus, and FeLV infections; drug and toxin exposure; and idiopathic causes. Diagnosis is based on cytopenias in the blood and hypoplastic to aplastic bone marrow, with the marrow space replaced by adipose tissue. Treatment is dependent on determining the underlying cause of the bone marrow failure, and the outcome is variable. The hematologic result may be either somewhat selective, severe, and nonregenerative anemia (i.e., pure red cell aplasia or hypoplasia) or pancytopenia in which neutropenia and thrombocytopenia accompany the anemia (i.e., aplastic anemia). Establishing the morphologic diagnosis depends on the examination of marrow particles or histopathology to distinguish the hypocellularity from a hemodiluted marrow sample. Plasmacytosis of the marrow, in conjunction with the lack of hematopoietic cells, is often present with chronic ehrlichiosis, and is sometimes so marked that it must be distinguished from multiple myeloma (see Chapter 14). Most cases of pure red cell aplasia in dogs, as well as those that are not associated with FeLV in cats, likely are immune-mediated, and many respond to immunosuppresive therapy.

Dysmyelopoiesis

Dysmyelopoiesis is defined as a hematologic disorder characterized by the presence of cytopenias in the blood and dysplastic cells in one or more hematologic cell lines in the blood or bone marrow. The causes of dysmyelopoiesis include acquired mutations in hematopoietic stem cells (myelodysplastic syndromes) congenital defects in hematopoiesis, and secondary dysmyelopoietic conditions associated with various diseases, drugs, or toxins. Causes of secondary dysmyelopoiesis include immune-mediated hematologic diseases, lymphoid malignancies, and exposure to chemotherapeutic drugs. Secondary dysmyelopoiesis is also referred to as nonneoplastic syndromes of ineffective hematopoiesis in which dysmorphic maturation of cells occurs. Without methods to confirm clonality by cytogenetic analysis, the diagnosis of neoplastic myelodysplasia in dogs is based on light microscopic examination of bone marrow

films (see Chapter 15). The characteristic morphologic and cytochemical features of neoplastic myelodysplasia and nonneoplastic ineffective hematopoiesis in dogs are discussed elsewhere in detail.[2]

Neoplastic disorders involving bone marrow other than lymphoproliferative or myelopoliferative disorders

Mast cell leukemia may be seen in dogs and cats with systemic mastocytosis secondary to mast cell tumors (Fig. 14.26). Although examinations of bone marrow aspirate commonly are performed to stage mast cell tumors, involvement of the marrow by mast cell tumors very rarely is observed. Buffy coat examination for mast cells also rarely is useful, because circulating mast cells occasionally may be seen in animals without mast cell tumors. The current recommendation is that bone marrow aspiration not be performed for routine staging but may be indicated for those dogs having either an abnormal CBC or presenting for tumor regrowth, progression or new occurrence.

Malignant histiocytosis is a rapidly progressive—and ultimately fatal—proliferative disorder of the mononuclear phagocyte system that has been described in adult dogs, including Bernese mountain dogs and other breeds. An increased incidence of the disorder has been suggested to occur in the golden retriever and flat-coated retriever breeds. The disorder often is characterized by the systemic proliferation of large, pleomorphic, single and multinucleated histiocytes with marked cellular atypia and phagocytosis of erythrocytes and leukocytes. The bone marrow as well as lung, lymph nodes, liver, spleen, and central nervous system commonly are involved. Positive reactivity of neoplastic cells to histiocytic markers (e.g., lysozyme and α_1-antitrypsin) can be demonstrated by immunohistochemistry (IHC). This immunohistochemical reactivity aids in the differentiation of neoplastic histiocytic cells from lymphoid and epithelial neoplasms, and it is important for establishing a definitive diagnosis of the neoplasm. The cellularity of bone marrow aspirates containing neoplastic histiocytes is consistently very high. These histiocytes are pleomorphic, large, discrete, and markedly atypical mononuclear cells, and the nuclei are round to oval or reniform. Features of malignancy include marked anisocytosis and anisokaryosis, prominent nucleoli, bizarre mitotic figures, marked phagocytosis of erythrocytes, leukocytes, other tumor cells, and moderate amounts of lightly basophilic, vacuolated cytoplasm (Fig. 14.27). The presence of multinucleated giant cells also is supportive of the diagnosis. Other findings vary and may include erythroid hypoplasia, with prominent cytophagia of marrow elements, or generalized marrow hypoplasia, with neoplastic infiltration of atypical histiocytes and marked phagocytosis. Hematologic abnormalities such as anemia and mild to marked thrombocytopenia also may be present, correlating with marrow changes.

Epithelial and mesenchymal tumors rarely metastasize to the bone marrow. Epithelial tumors (i.e., carcinomas) tend to form groups of cohesive cells that are easy to distinguish from normal hematopoietic cells (Figs. 14.28 and 14.29). Metastatic sarcomas are more difficult to diagnose, however, and are characterized by large, discrete, spindle-shaped cells

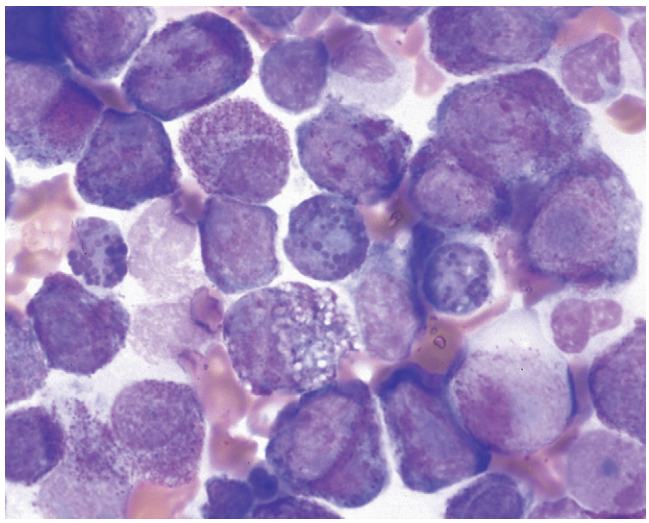

Figure 14.26 Bone marrow aspirate from a dog with poorly differentiated mast cell leukemia. Almost all the cells present are mast cells with metachromatic cytoplasmic granules. This dog also had mast cells on the blood film. Wright stain.

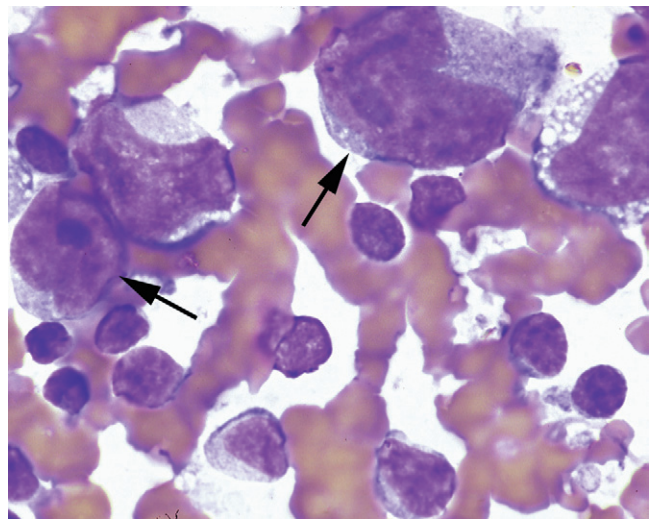

Figure 14.27 Bone marrow aspirate from a dog with malignant histiocytosis. Note the large, neoplastic histiocytic cells with prominent, irregularly shaped nucleoli (arrows). Most of the other nucleated cells in the field are small lymphocytes. Wright stain.

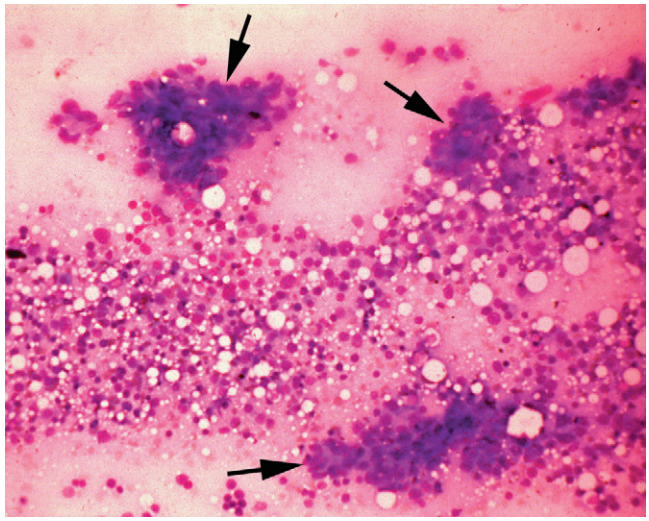

Figure 14.28 Bone marrow aspirate from a dog with metastatic mammary carcinoma, low magnification. The islands of cells (arrows) are neoplastic epithelial cells and can be differentiated from the normal hematopoietic cells by their tendency to adhere to each other. Wright stain. Low power.

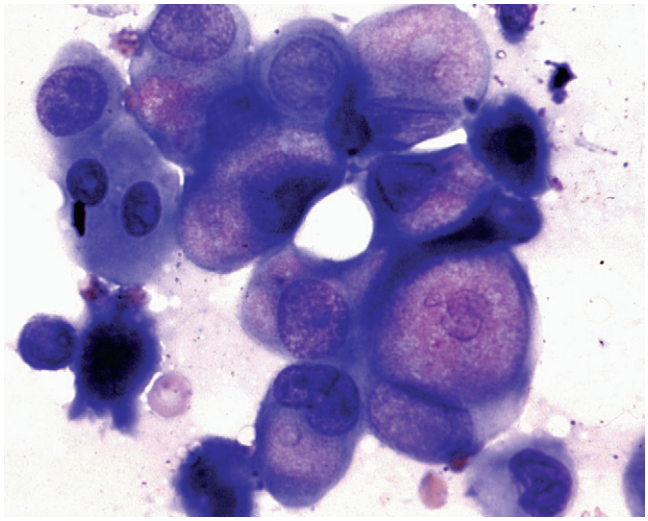

Figure 14.29 Bone marrow aspirate shown in Figure 14.28, high magnification. Note the large epithelial cells that exhibit numerous criteria of malignancy, including nuclear molding, binucelate cells, and prominent nucleoli. Wright stain.

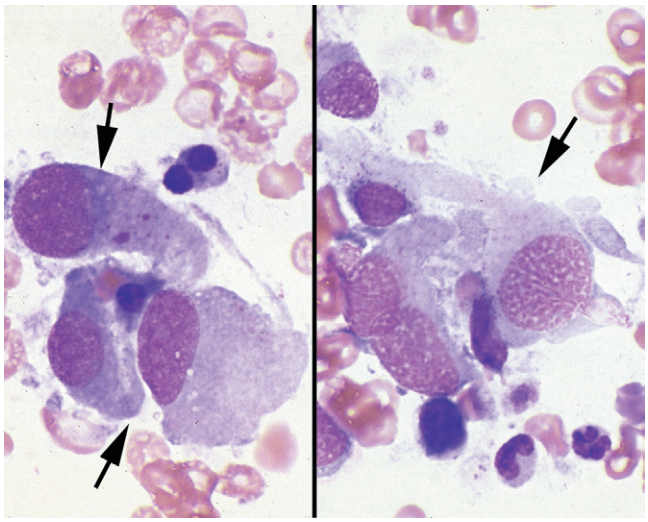

Figure 14.30 Bone marrow aspirate from a dog with metastatic hemangiosarcoma. Spindle-shaped neoplastic cells (arrows) exhibit numerous criteria of malignancy, including variability in nuclear size, variability in cell size, ropy chromatin, and prominent nucleoli. Note that some cells have fine, azurophilic granules in their cytoplasm. Wright stain. (Specimen courtesy of Dr. Kyra Somers, Idexx.)

that meet multiple criteria for malignancy (Fig. 14.30). These cells must be distinguished from fibroblasts that may be observed in myelofibrosis.

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15 Lymphoproliferative Disorders and Myeloid Neoplasms

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Overview of myeloproliferative and lymphoproliferative disorders (leukemia)

Leukemia, which is a neoplastic proliferation of hematopoietic cells within the bone marrow, is defined by the presence of neoplastic blood cells in the peripheral blood or bone marrow, and is classified broadly into myeloid neoplasms and lymphoproliferative disorders. The diagnosis of these disorders is established based on finding characteristic cells in the blood or bone marrow and associated hematologic abnormalities. Specific cell types are identified by their morphologic appearance in Wright-stained blood and bone marrow films, cytochemical staining properties, electron microscopic appearance, and monoclonal antibody binding to surface antigens. In some cases, cells may appear so morphologically undifferentiated that classifying the disorder into either the myeloproliferative or the lymphoproliferative category may be difficult (Fig. 15.1). Myeloid neoplasms include neoplastic proliferation of erythrocytes, granulocytes, monocytes, and megakaryocytes. Multiple cell lines may be neoplastic if the affected stem cell is multipotential; an example is myelomonocytic leukemia, in which both neutrophils and monocytes have been neoplastically transformed. Lymphoproliferative disorders include acute lymphoblastic leukemia, chronic lymphocytic leukemia, and multiple myeloma.

Leukemias are also classified according to the concentration of neoplastic cells that are circulating in the blood. With leukemic leukemias, many neoplastic cells are circulating, thereby resulting in a markedly increased nucleated cell count. In patients with subleukemic leukemias, however, the nucleated cell count is near normal, with only a few neoplastic cells circulating. No circulating cells are observed on blood films from patients with aleukemic leukemia. Establishing the diagnosis of leukemia when few or no cells are circulating usually is based on examination of the marrow aspirate.

Leukemias may also be further classified as either acute or chronic based primarily on the maturity or degree of neoplastic cell differentiation as well as by the clinical course. The neoplastic cells in acute leukemias are immature (i.e., blasts), and the patient survival time usually is quite short. By definition, 20% or more blast cells in the marrow is diagnostic of acute myeloid leukemia. The percentage of blast cells in the blood, however, is quite variable in these patients. Chronic leukemias are characterized by the predominance of mature, more well-differentiated cells in the blood and marrow, and the patient survival time usually is longer. Neoplastic cells commonly can be found in organs other than the bone marrow in patients with leukemia. The spleen commonly is involved, and the liver and lymph nodes also may contain neoplastic cells (Fig. 15.2). Genetic mutations similar to those seen in people have been described in dogs with lymphoid and myeloid leukemia. In one study of 210 dogs with leukemia,[1] 51 had acute lymphoblastic leukemia, 33 had acute myeloid leukemia, 61 had chronic lymphocytic leukemia, and 65 had Grade V lymphoma with involvement of bone marrow. Anemia, neutropenia, and thrombocytopenia were more common and severe in dogs with acute leukemias than in dogs with Stage V lymphoma or chronic leukemias. Similar results were observed in a series of 64 dogs;[2] 25 dogs had acute lymphoblastic leukemia, 22 had acute myeloid leukemia, and 17 had chronic lymphocytic leukemia. Golden retriever dogs in the study population were overrepresented in comparison with a control population of dogs.

Myeloid neoplasms

Myeloid neoplasms are cancers of hematopoietic cells, and are distinct from cancers of lymphoid cells. They manifest as either a lack of normal blood cells, or an increase in neoplastic cells in blood. While lymphoid leukemia may pre-

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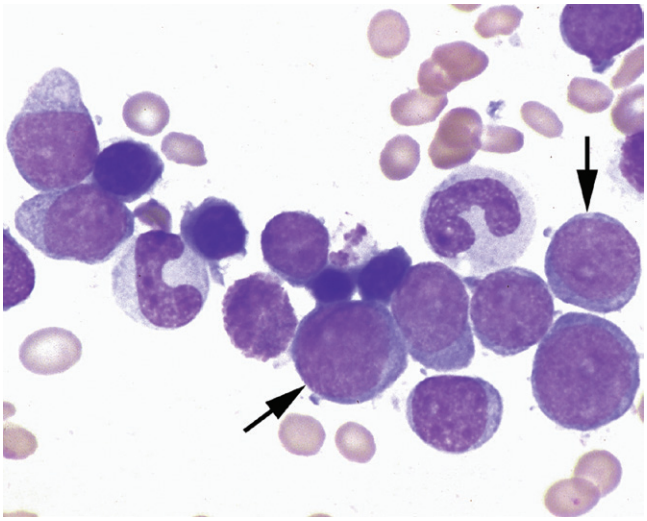

Figure 15.1 Bone marrow aspirate from a cat. Large undifferentiated cells (arrows) are difficult to classify based on their morphologic appearance. Cells may be lymphoblasts or type 1 myeloblasts. Wright stain.

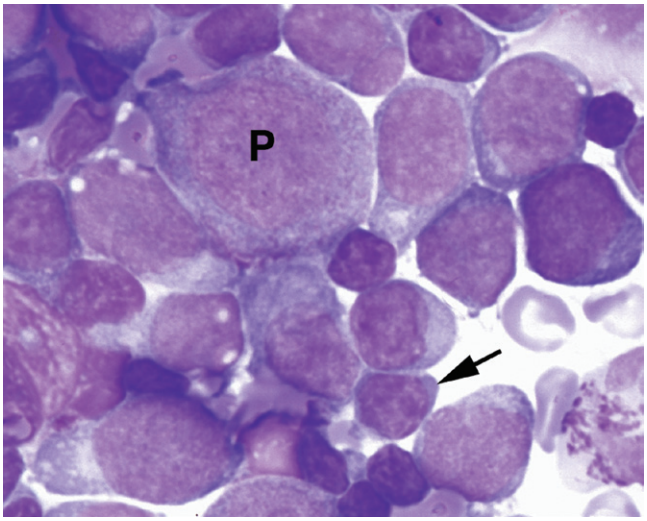

Figure 15.2 Lymph node aspirate from a dog with granulocytic leukemia (M2). Most of the large blast cells cannot be differentiated from lymphoblasts based on their morphology, but some are differentiating toward promyelocytes (P). Note the small lymphocyte (arrow). Wright stain.

dominantly affect bone marrow, it is not referred to as a myeloid neoplasm. Myeloid neoplasms include cancers associated with both rapid and gradual disease progression. Percentage of blast cells in marrow is used to distinguish rapid (acute) from gradual (chronic). The rapidly progressing myeloid cancers are referred to as acute myeloid leukemias, and the myeloid cancers with more gradual progression are classified as either myelodysplastic syndromes or myeloproliferative neoplasms (formerly termed "chronic leukemias").

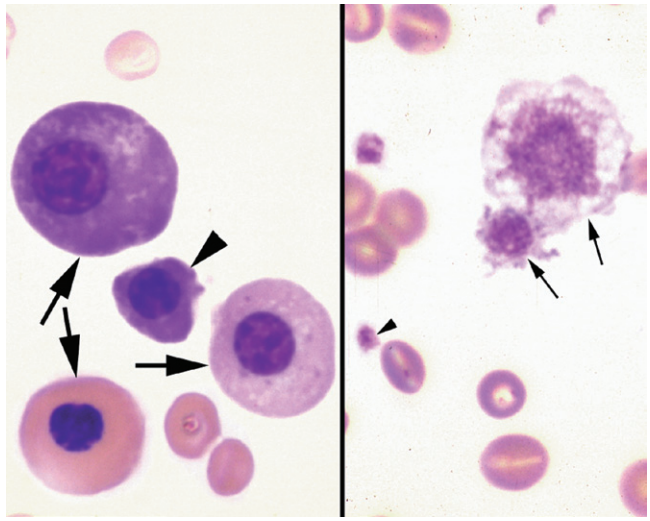

Figure 15.3 Left. Bone marrow aspirate from a cat with myelodysplasia. Note the three rubricytes with dysynchrony of nuclear and cytoplasmic maturation (arrows) and the more normal-appearing metarubricyte (arrowhead). Right. Blood film from a cat with myelodysplasia. Note the giant atypical platelets (small arrows) and the normal-appearing platelet (small arrowhead). Wright stain.

Myelodysplastic syndromes

Myelodysplastic syndrome (MDS) is a variable manifestation with some subtle, morphologic changes in blood cells. The hematologic manifestations almost always involve some form of cytopenia, and this may include any single abnormality or combination of nonregenerative anemia, thrombocytopenia, and neutropenia. Cellularity of marrow is variable. The marrow may be hypocellular, of normal cellularity, or hypercellular, thereby making it difficult to distinguish this condition from a myeloproliferative disorder. Characteristic morphologic abnormalities include large, highly variable erythroid precursor size and dysynchrony of nuclear and cytoplasmic maturation events (Fig. 15.3). The disturbed erythroid production in cats commonly leads to establishment of marked macrocytosis and increased erythrocyte volume heterogeneity (i.e., anisocytosis) seen as a widening of the erythrocyte histogram. Cats that are positive for FeLV infection may have mean red cell volumes of 70 fL or greater (reference interval, 40–55 fL). Macrocytosis also has been reported in dogs with myelodysplasia. Other features in blood may include extreme platelet macrocytosis (Fig. 15.3). Megakaryocyte differentiation may be altered as well, with both hypo- and hyperlobulation of nuclei (Fig. 15.4). Neutrophils of unusually large diameter may be observed, with nuclear changes that may include both hyper- and hyposegmentation (Fig. 15.5). Very early precursors are not found in blood.

Several prognostic scoring systems based on number of bone marrow blast cells, cytogenetic findings, number of

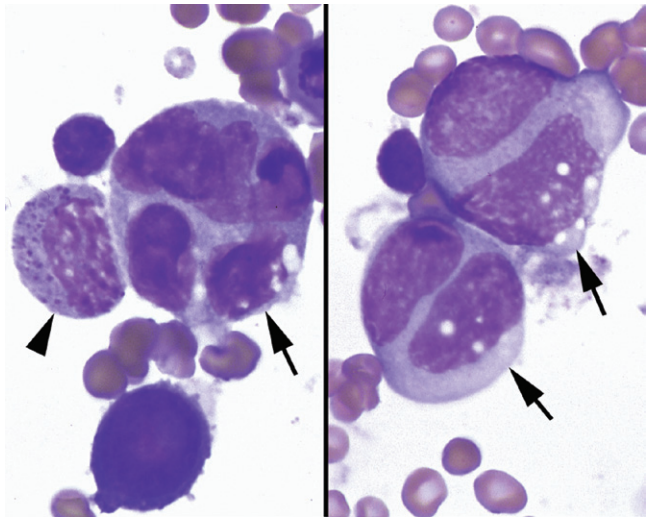

Figure 15.4 Left. Bone marrow aspirate from a cat with myelodysplasia. Note the dysplastic megakaryocyte (arrow) and the granulocytic precursor with retained primary granules (arrowhead). Right. Dysplastic megakaryocytes with hypolobulation of the nuclei (arrows). Wright stain.

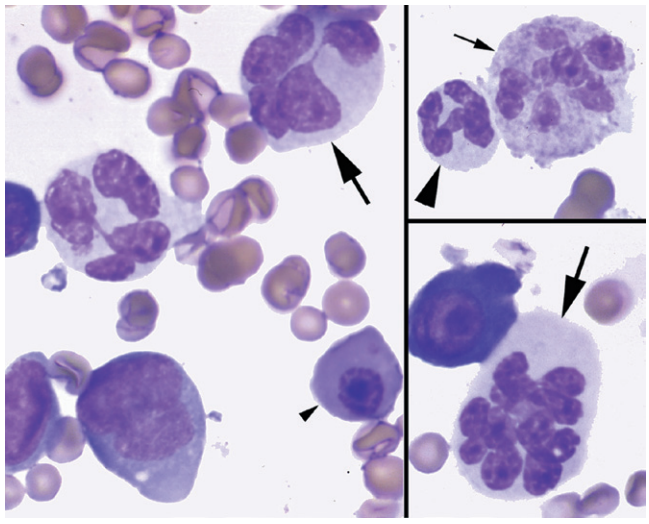

Figure 15.5 Bone marrow aspirate from a cat with myelodysplasia. Left and lower right. Note the giant hypersegmented neutrophils (arrows) and megaloblastic erythrocyte precursor (small arrow head). Upper right. Note the giant hypersegmented neutrophil (small arrow) and the neutrophil of normal size (large arrowhead). Wright stain.

hematopoietic lines affected by cytopenia, and transfusion dependence have been developed for use in humans with myelodysplastic syndrome. Classification schemes used for animals are quite simple in comparison. The Animal Leukemia Study Group in 1991 recommended two MDS categories: MDS (M:E>1.0) and MDS-Erythroid (M:E<1.0). Since that time, recommendations for three subtypes have been made. MDS with excess blasts (MDS-EB) has blast cell percentages in marrow that are equal to or greater than 5% but less than 20%, MDS with refractory cytopenia (MDS-RC) which has blast cell counts less than 5%, can have an indolent course, and MDS-ER (M:E ratio <1.0) which has a poor prognosis and short survival time. In general, high blast percentages (>5%) multiple cytopenias, and marked morphologic atypia are usually considered negative prognostic markers.

Other differential diagnoses for hypercellular marrow and cytopenias include the recovery stage of marrow damage, such as might be seen with parvovirus infection; immune-mediated disease, with destruction of more mature cells; and consumption of neutrophils due to an overwhelming inflammatory process.

Myelodysplastic syndromes have been reported in cats, dogs and one horse. Cats with myelodysplasia almost always are positive for FeLV. Clinical signs usually include lethargy, anorexia, and weight loss. Animals may die within weeks of diagnosis, without progression to leukemia, but overt leukemia is a common sequela.

Overview of acute myeloid leukemias and myeloproliferative neoplasms

Acute myeloid leukemia (AML) is a neoplasm of hematopoietic cells resulting in rapid progression of disease. Myeloproliferative neoplasms (MPN) comprise various types of clonal neoplastic conditions of hematopoietic tissue characterized by gradual disease progression. In general, these neoplasms are characterized by bone marrow hypercellularity, loss of orderliness in maturation, and a tendency for neoplastic cells to be released into the blood. Myeloid neoplasms are more common in cats than in other domestic animals and, as mentioned, usually are associated with FeLV infection. Hematopoietic precursors are infected by FeLV, and viral proteins are thought to interact with host cell products that are important in cell proliferation, resulting in recombination or rearrangement events involving host gene sequences that encode products involved in the normal regulation of cell growth. Feline immunodeficiency virus (FIV) also appears to be associated with stem cell disorders of cats, although FIV does not directly infect myeloid or erythroid precursors. The mechanism probably relates to infection of other cells in the bone marrow microenvironment or to the virus or viral antigen affecting hematopoiesis in some way.

Clinical signs usually relate to the crowding out of normal hematopoietic cells in the bone marrow, but they may also result from the infiltration of different organs by neoplastic cells. Lethargy, weakness, pallor, bleeding, shifting leg lameness, and bone pain frequently are seen, as are hepatomegaly and splenomegaly. Typical CBC findings include an increased nucleated cell count, neoplastic cells in the periph-

eral blood, nonregenerative anemia, and thrombocytopenia, although thrombocytosis may be present, particularly in cats. Other abnormal laboratory findings are variable, depending on the type and degree of organ dysfunction.

The response of these disorders in dogs and cats to therapy usually is disappointing, and the prognosis is poor, particularly in animals with acute myeloid leukemia. Chemotherapeutic drugs may produce remissions of very short duration (e.g., usually a few weeks). Types of recommended chemotherapy differ with the type of leukemia and the species. A veterinary oncologist should be consulted for advice on new protocols for therapy. Bone marrow transplantation offers the potential for a complete cure, but is expensive and requires intensive care. Cats that are negative for FeLV and FIV and have a sibling that can serve as a marrow donor are reasonably good candidates for bone marrow transplantation. Animals with myeloproliferative neoplasms have a longer survival time after the diagnosis is established, but they almost always eventually develop a terminal blast crisis and die.

Classification of acute myeloid leukemias

Acute myeloid leukemia is morphologically and biologically variable. Most cases of AML in humans are associated with genetic abnormalities that affect myeloid cellular proliferation and maturation. Thus, cytogenetic analysis is a routine component of the diagnosis in people, and plays a significant role in treatment modality and prognosis. The classification scheme in humans consists of the following groupings: AML with myelodysplasia-related changes; therapy-related myeloid neoplasms, and AML not otherwise categorized. Subgroups of "AML not otherwise categorized" are subgrouped according to the type of cell involved. AML that develops in cats with FeLV is most similar to the human "AML with myelodysplasia-related changes," but most of the cases in domestic animals are comparable to "AML not otherwise categorized."

Traditionally, acute myeloid leukemias in domestic animals have been characterized as being granulocytic (i.e., myeloid, neutrophilic), myelomonocytic (i.e., neutrophils and monocytes), monocytic, eosinophilic, basophilic, megakaryocytic, erythremic myelosis (i.e., erythrocytes), or erythroleukemia (i.e., erythrocytes and granulocytes). Diagnostic criteria have varied considerably, however, and agreement on nomenclature and classification of hematopoietic neoplasms has been lacking. Because of potential differences in response to various treatment protocols and prognosis, in 1991 an animal leukemia study group standardized the definitions for acute myeloid leukemias by using a human classification scheme based primarily on the number and morphology of blast cells in Wright-stained blood and bone marrow films. To classify a myeloproliferative or myelodysplastic disorder, 200 cells are differentiated to calculate an M:E ratio and to determine the percentages of blast and other cell types. Blast cell percentages in the bone marrow are calculated in relation to all nucleated cells as well as to nonerythroid cells. Lymphocytes, macrophages, mast cells, and plasma cells are excluded for all-nucleated-cell counts, and erythrocyte precursors are excluded for nonerythroid cell counts. At the time of this study, 30% blasts in the marrow was considered the lowest percentage that could be present and still diagnose AML. Our physician counterparts, in collaboration with oncologists, have since constructed a new World Health Organization (WHO) standard, which lowered the blast threshold from 30% to 20% for diagnosing acute myeloid leukemia (AML). The AML alphanumeric designations (M1, M2, etc.) described below have been discontinued in humans as numbers of subtypes have increased. The system established in 1991 has been revised below to lower the blast threshold to 20%. The alphanumeric designations and blast threshold should be formally reassessed by veterinary clinical pathologists and oncologists as to their accuracy and usefulness.

Cytochemical stains and immunophenotyping, as discussed earlier, may be useful adjuncts in the classification of leukemia (Figs. 15.6 and 15.7). The clinical relevance of cytomorphologic, cytochemical, and immunophenotypic characterization of acute myeloproliferative diseases remains to be determined, although given the importance of karyotyping in human AML, prognostically significant chromosomal abnormalities are likely present in animals with AML. Moreover, the classification of leukemia in a patient may

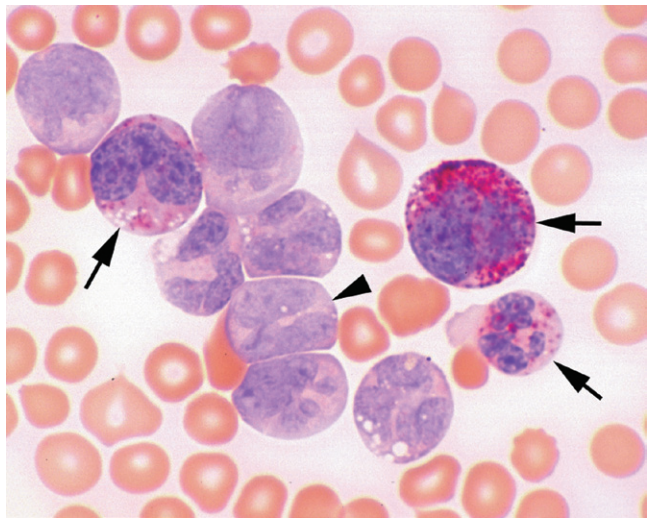

Figure 15.6 Blood film from a dog with myelomonocytic leukemia (M4) stained with chloracetate esterase (CAE), a granulocyte marker. Note the metamyelocyte and neutrophil with red-staining granules in the cytoplasm (arrows). Several monocytes (arrowhead) are present that do not stain positive with CAE. (Specimen courtesy of Dr. Wendy Sprague, Colorado State University.)

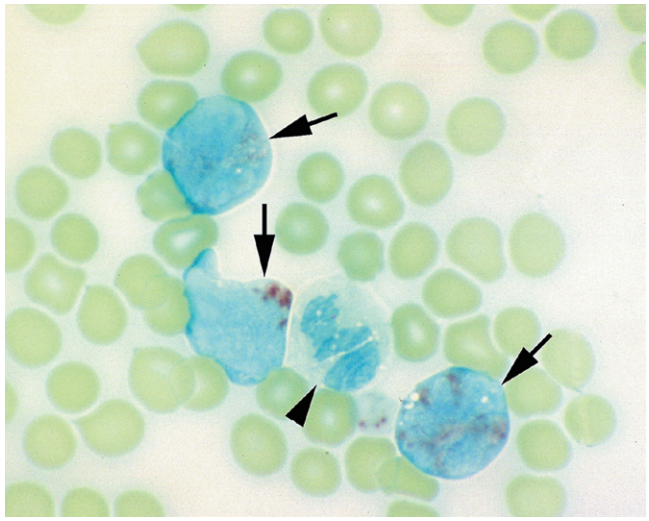

Figure 15.7 Blood film from dog with myelomonocytic leukemia (M4) stained with α-naphthyl butyrate esterase (ANBE), a monocyte marker. Note the brown-staining granules in the monocytes (arrows) and the neutrophil (arrowhead) that does not stain with ANBE. (Specimen courtesy of Dr. Wendy Sprague, Colorado State University.)

change as the disease progresses; for example, erythremic myelosis may convert to erythroleukemia or acute myelogenous leukemia. A classification scheme showing historically used terminology, current terminology, and a summary of bone marrow findings is presented in Table 15.1.

Undifferentiated leukemia

The diagnosis of undifferentiated leukemia is established when approximately 100% of the cells in the bone marrow are blast cells that cannot be properly classified according to the usual morphologic and cytochemical criteria. The diagnosis can be based on electron microscopy, ultrastructural cytochemistry, or immunophenotyping. Included in this category are cases of what previously were termed reticuloendotheliosis in cats, in which a predominance of blast cells have pseudopodia, eccentric nuclei, and sometimes, features of both erythroblasts and myeloblasts (Figs. 15.8 and 15.9). Some cells may contain azurophilic granules. If the neoplastic cells do not appear to be maturing toward erythroid or myeloid cells, they are categorized as being undifferentiated.

Table 15.1 Classification of leukemias.

Historical Terminology	FAB Subtype	Description
Acute leukemias (≥20% blasts in marrow)		
Reticuloendotheliosis	AUL	Acute undifferentiated leukemia, myeloid and erythroid features
Granulocytic leukemia	M1	Myeloblastic leukemia with differentiation
Granulocytic leukemia	M2	Myeloblastic leukemia with neutrophilic differentiation
Myelomonocytic leukemia	M4	Combination of myeloblasts and monoblasts
Monocytic leukemia	M5a	Monocytic leukemia without differentiation
Monocytic leukemia	M5b	Monocytic leukemia with differentiation
Erythroleukemia	M6	Combination of myeloblasts and rubriblasts
Erythremic myelosis	M6Er	Erythroid leukemia
Megakaryoblastic leukemia	M7	Increased megakaryoblasts in blood and marrow
Chronic myeloid leukemias (<20% blasts in marrow)		
Chronic granulocytic leukemia		Mature neutrophilia, left shift, similar to granulocytic hyperplasia
Chronic myelomonocytic leukemia		Combination of mature neutrophilia, left shift, and monocytosis
Chronic monocytic leukemia		Mature monocytosis in blood and bone marrow
Chronic eosinophilic leukemia		Eosinophilia with left shift, basophilic predominance in marrow
Chronic basophilic leukemia		Basophilia with left shift, basophilic predominance in marrow
Essential thrombocythemia		Marked increase in platelets, megakaryocytic hyperplasia in marrow
Polycythemia vera (erythrocytosis)		Mature erythroid proliferative disorder, erythroid hyperplasia
Lymphoid leukemia		
Acute lymphoblastic leukemia		Lymphoblasts in blood or bone marrow
Chronic lymphocytic leukemia		Lymphocytosis, >30% lymphocytes in marrow

FAB, French-American-British.

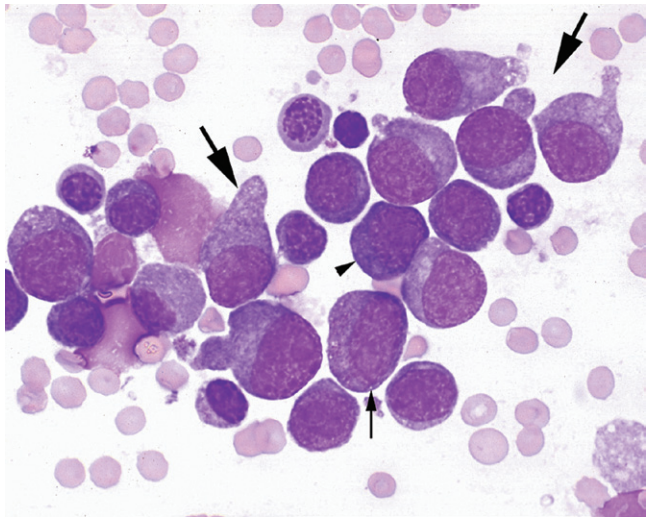

Figure 15.8 Bone marrow aspirate from a cat with undifferentiated leukemia. The cells have features of both erythroid and myeloid precursors. Cytoplasmic pseudopodia (large arrows) typically are present. Note the cell with obvious erythroid characteristics (arrowhead) and the cell with myeloid features and primary granules (small arrow). Wright stain.

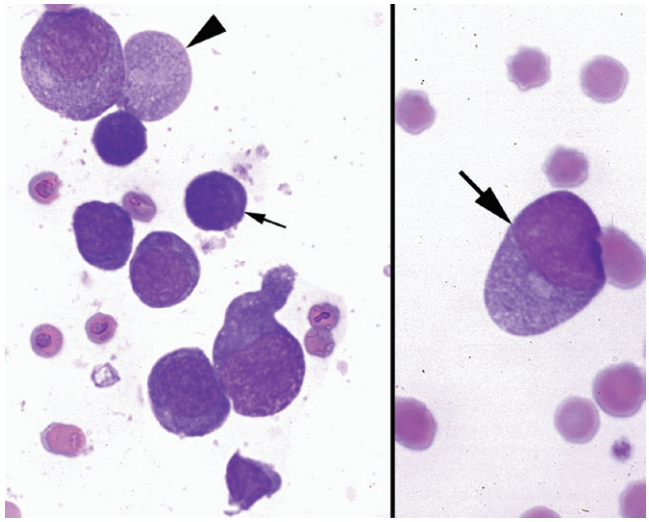

Figure 15.9 Left. Bone marrow aspirate from a cat with undifferentiated leukemia. Note the cytoplasmic pseudopodia that has detached from the cell (arrowhead). When present in blood, these cytoplasmic fragments may be mistaken for platelets. Note the rubricyte (small arrow) as well. Right. Blood film from a cat with undifferentiated leukemia. Note the typical undifferentiated cell with primary granules and an eccentric nucleus (large arrow). Wright stain.

Myeloblastic leukemia (M1)

The predominant cell in the bone marrow in animals with myeloblastic leukemia is the type I myeloblast; type II myeloblasts are only seen infrequently (Fig. 15.10). Both types of blasts comprise more than 90% of all nucleated cells. Differentiated granulocytes (promyelocytes to neutrophils and eosinophils) comprise less than 10% of the nonerythroid cells.

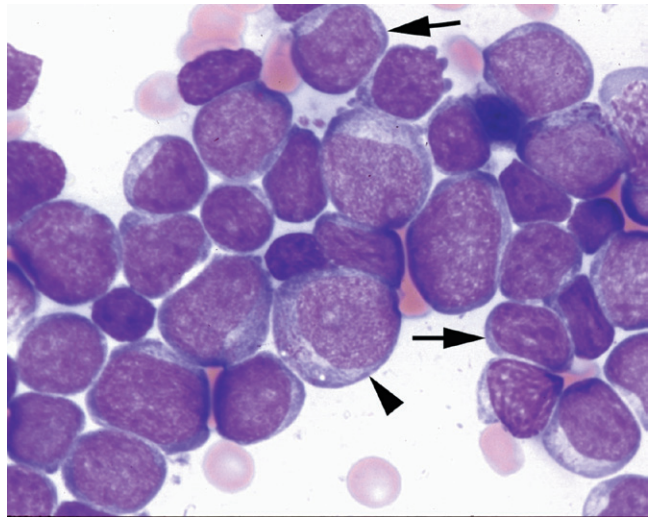

Figure 15.10 Bone marrow aspirate from a dog with granulocytic (myeloblastic) leukemia (M1). Almost all the cells present are type 1 myeloblasts (arrows). A type II myeloblast with cytoplasmic primary granules (arrowhead) is present as well. Type I myeloblasts are morphologically similar to lymphoblasts, and without the presence of more differentiated cells, immunophenotyping may be necessary to correctly classify the leukemia. Wright stain.

Myeloblastic leukemia with maturation (M2)

Myeloblasts constitute from more than 20% to less than 90% of all nucleated cells, with a variable number of type II myeloblasts being present (Figs. 15.11 and 15.12). Differentiated granulocytes comprise more than 10% of the nonerythroid cells, usually with a predominance of promyelocytes.

Myeloblastic leukemia with maturation and atypical granulation of promyelocytes (M3)

Although myeloblastic leukemia with maturation and atypical granulation of promyelocytes is one of the classifications for human leukemia, no such cases have been reported in domestic animals. This type of myeloblastic leukemia is characterized by either hypergranular, hypogranular, or microgranular promyelocytes with folded, reniform, or bilobed nuclei.

Myelomonocytic leukemia (M4)

Myeloblasts and monoblasts together constitute more than 20% of all nucleated cells, and differentiated granulocytes and monocytes comprise more than 20% nonerythroid cells (Figs. 15.13 and 15.14).

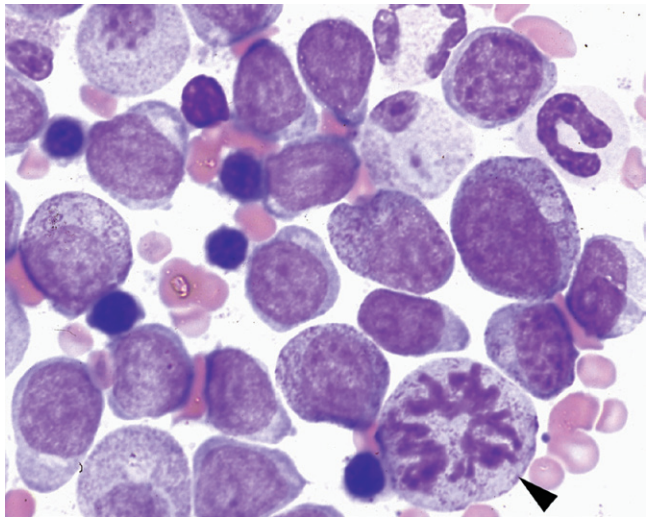

Figure 15.11 Bone marrow aspirate from a cat with granulocytic (myeloblastic) leukemia (M2). Numerous type II myeloblasts with cytoplasmic granules are present, as is a cell in mitosis (arrowhead). Note that cells are more differentiated than those seen in marrow aspirates of patients with M1. Wright stain.

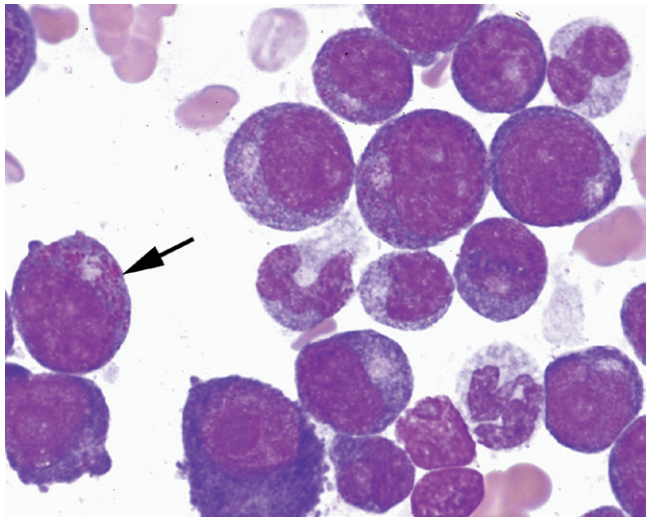

Figure 15.12 Bone marrow aspirate from a cat with granulocytic (myeloblastic) leukemia (M2). Note that most of the cells present are type II myeloblasts or progranulocytes (arrow). Most of these cells have clear Golgi areas. A few more differentiated myeloid precursors are present as well. Wright stain.

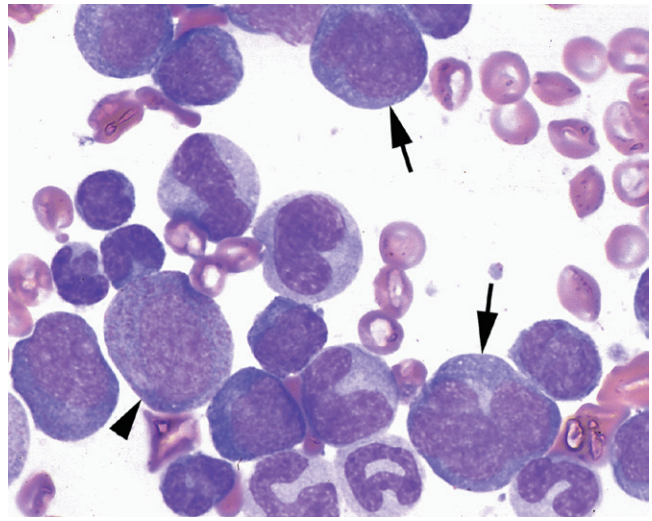

Figure 15.13 Bone marrow aspirate from a dog with myelomonocytic leukemia (M4). Both monocyte precursors (arrows) and myeloid precursors (arrowhead) are present. Wright stain.

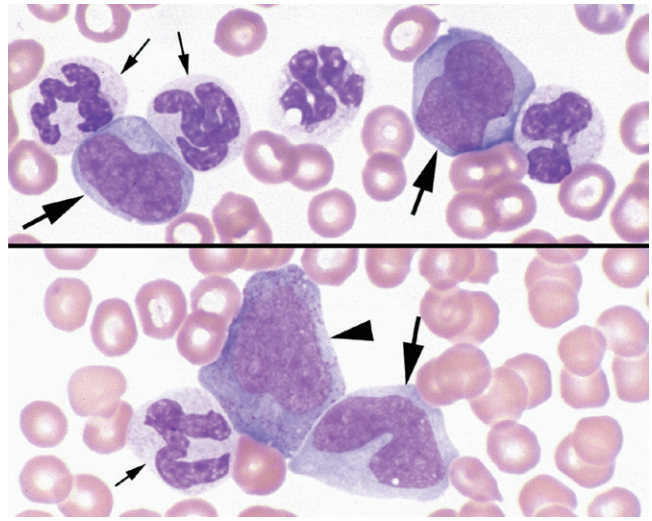

Figure 15.14 Blood film from a dog with myelomonocytic leukemia (M4). Top. Note the monoblasts (large arrows) and normal-appearing, segmented neutrophils (small arrows). Bottom. Note the segmented neutrophil (small arrow), monocyte (large arrow), and type II myeloblast (arrowhead). Wright stain.

Monocytic leukemia (M5)

The predominant population is monocytic, as determined by the characteristic nuclear morphology and confirmed by cytochemical staining for nonspecific esterase. Monoblasts and promonocytes constitute more than 80% of nonerythroid cells in M5a (Figs. 15.15 and 15.16), while M5b has more than 20% to less than 80% monoblasts and promonocytes with prominent differentiation to monocytes (Figs. 15.17 and 15.18). The granulocytic component is less than 20%.

Erythroleukemia (M6)

The erythroid compartment in M6 is more than 50%, and the myeloblasts and monoblasts combined are less than 20%

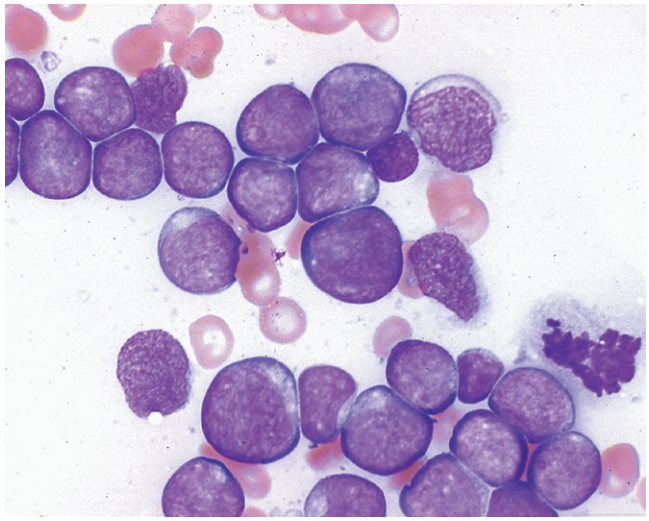

Figure 15.15 Bone marrow aspirate from a dog with monocytic leukemia (M5a). Almost all the cells present are undifferentiated monoblasts. These cells appear to be morphologically similar to lymphoblasts and type I myeloblasts, but immunophenotyping and cytochemistry determined this was a very undifferentiated type of monocytic leukemia. Wright stain.

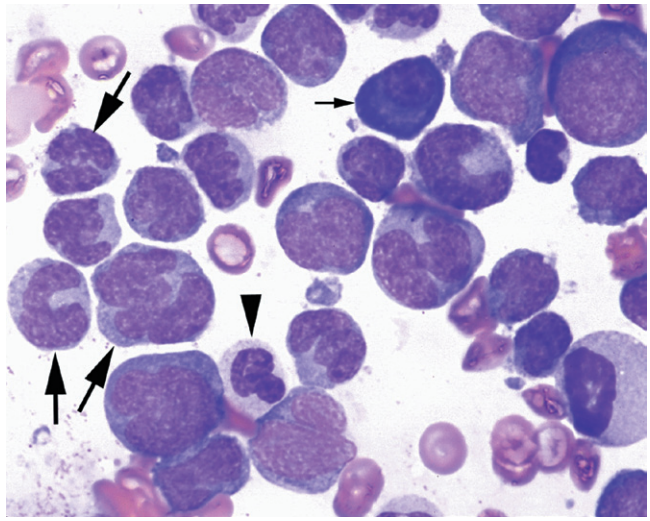

Figure 15.17 Bone marrow aspirate from a dog with monocytic leukemia (M5b). Note the numerous monocytes in various stages of maturation (large arrows), the segmented neutrophil (arrowhead), and the plasma cell (small arrow). Wright stain.

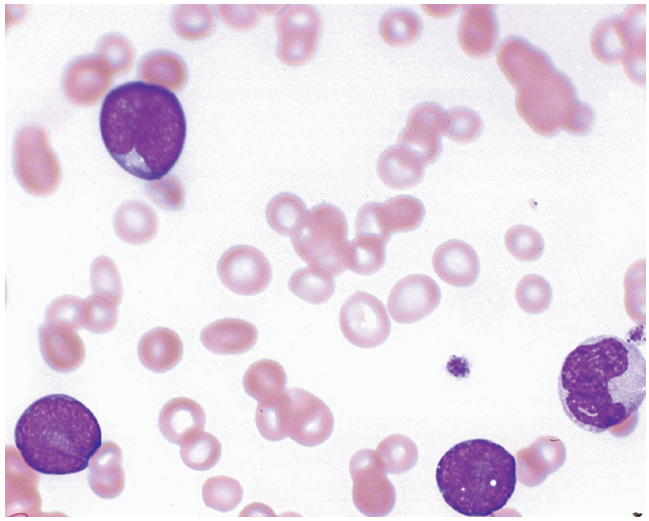

Figure 15.16 Blood film from a dog with monocytic leukemia (M5a). Cells were classified as monoblasts based on the presence of other cells that appeared to be differentiating to monocytes as well as on the results of cytochemical analysis and immunophenotyping. Wright stain.

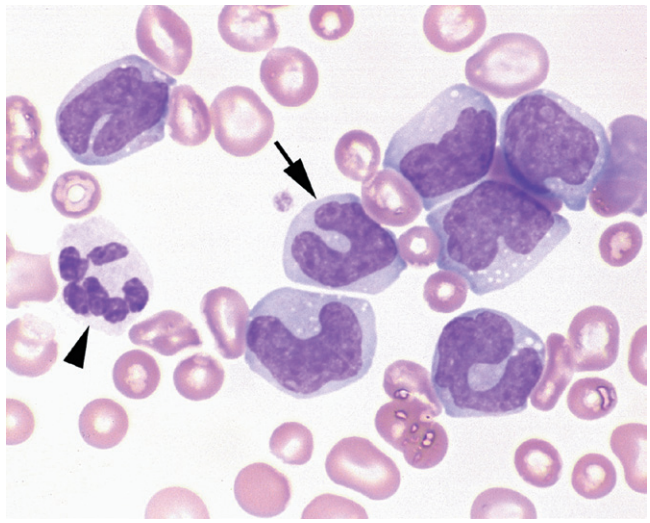

Figure 15.18 Blood film from a dog with monocytic leukemia (M5b). Note the numerous monocytes (large arrow), and compare the blue color of the cytoplasm and density of the nuclear chromatin with that of a segmented neutrophil, which has more dense nuclear chromatin and pink cytoplasm (arrowhead). Wright stain.

of all nucleated cells. The M6 classification is recognized when either of the following criteria are met: myeloblasts and monoblasts constitute more than 20% of nonerythroid cells, or blast cells (including rubriblasts) constitute more than 20% of all nucleated cells. An M6Er designation is used to define the latter situation when there is a predominance of rubriblasts in the erythroid component. Erythremic myelosis, which is a myeloproliferative disorder of erythroid precursors, may fall under the designation of M6Er or MDS-Er, because the erythroid component constitutes more than 50% of all nucleated cells and the blast cell concentration, (including rubriblasts) may constitute more than 20% (i.e., M6ER) or less than 20% (i.e., MDS-Er) (Figs. 15.19 and 15.20).

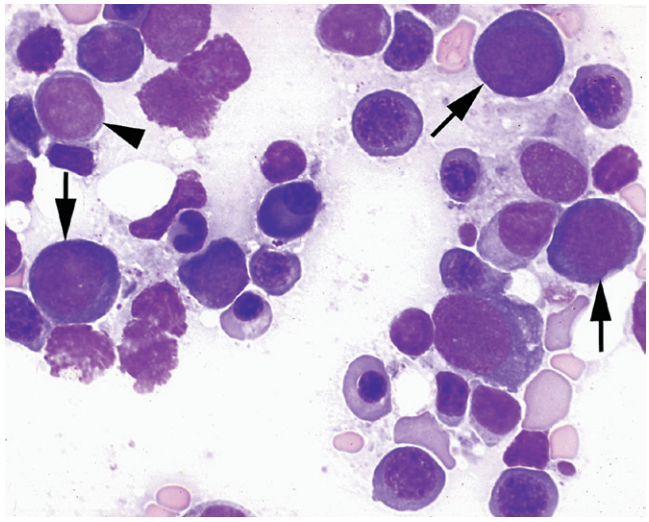

Figure 15.19 Bone marrow aspirate from a cat with erythremic myelosis (M6Er). Almost all the cells present are erythroid precursors. Note the rubriblasts (large arrows) and the nonerythroid blast (arrowhead), which probably is a myeloblast. Wright stain.

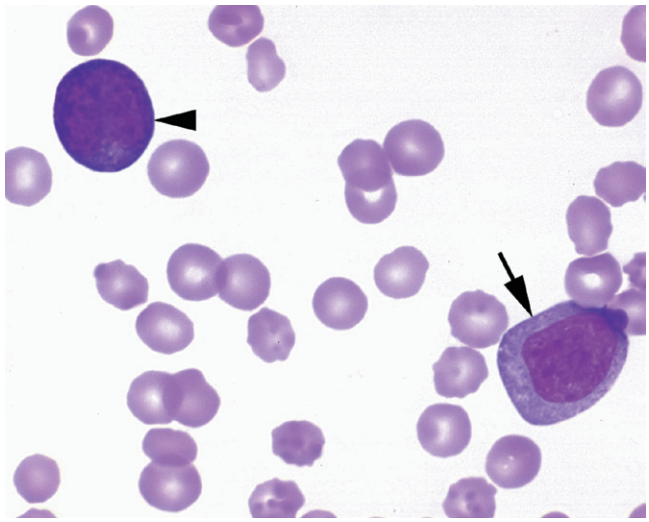

Figure 15.20 Blood film from a dog with erythroleukemia (M6). Note the rubriblast (arrowhead) and myeloblast (arrow). Also note the typical lack of polychromasia, because the erythroid precursors do not mature normally. Wright stain.

Megakaryoblastic leukemia (M7)

More than 20% of all nucleated cells or nonerythroid cells is comprised of megakaryoblasts in the M7 stage. An increased concentration of megakaryocytes may be present as well, and megakaryoblasts usually are detected in the blood (Fig. 15.21). Animals often are thrombocytopenic, although thrombocytosis has been reported. Immunohistochemical techniques to detect reactivity for factor VIII–related antigen and platelet glycoprotein IIIa sometimes are necessary to definitively identify megakaryoblasts. Primitive

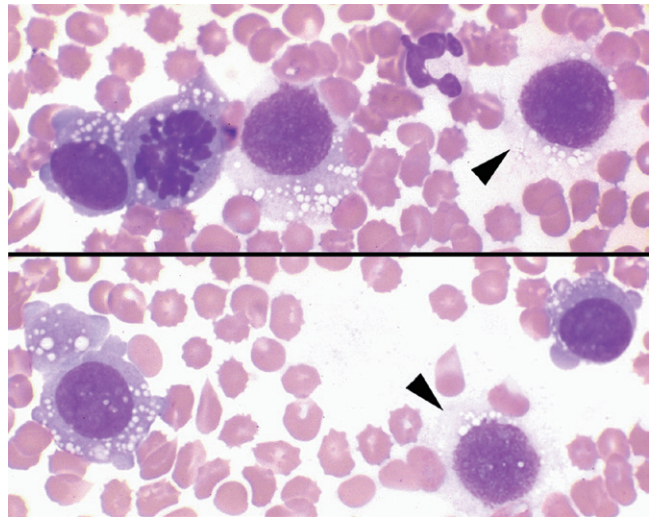

Figure 15.21 Blood film from a dog with megakaryoblastic leukemia (M7). Top. Note the numerous megakaryoblasts (arrowhead), one of which is in mitosis. Also note the abundant vacuolated cytoplasm with ruffled borders. Bottom. Note the broken megakaryoblast (arrowhead). Wright stain.

megakaryoblasts may also stain positive for acetylcholine esterase, a specific cytochemical marker for this cell line. This leukemia is rare in animals. While most cases of AML are rapidly fatal in domestic animals, one dog survived 2 years while being treated with chemotherapy for acute megakaryoblastic leukemia.

Chronic myeloproliferative neoplasms

Myelopoliferative neoplasms in people are classified as chronic myelogenous leukemia (CML), chronic neutrophilic leukemia (CNL), polycythemia vera (PV), essential thrombocythemia (ET), primary myelofibrosis (PMF), chronic eosinophilic leukemia (CEL) and CEL not otherwise specified (CEL, NOS), mastocytosis, and myeloproliferative neoplasm unclassifiable (MPN,U). The phenotypic diversity among these neoplasms is attributable to various genetic mutations. Best characterized among these mutations is a gene mutation located in the Philadelphia chromosome that is associated with CML. Other mutations have been discovered for most of the other types of myeloproliferative neoplasms. CML in people is a clonal stem cell disorder with proliferations involving several or all of the hematopoietic cell lineages, and is characterized by neutrophiliia, basophilia, and eosinophilia. CNL in people is rare, and characterized by a marked leukocytosis composed of segmented neutrophils and band forms. Polycythemia and essential thrombocythemia have a relatively indolent course that results in a slight decrease in lifespan. Polycythemia in people may transform to AML. Primary myelofibrosis is

characterized by proliferation of megakaryocytes and granulocytic precursors, with progressive myelofibrosis. CEL and CEL, NOS result in persistent blood, bone marrow and tissue eosinophilia and must be distinguished from hypereosinophilia. Mastocytosis in people results from clonal expansion of mast cells, and is divided into localized or diffuse cutaneous mastocytosis, and systemic mastocytosis with variable involvement of bone marrow. Systemic mastocytosis has three possible manifestations: accumulations of mast cells in lymph nodes, spleen, liver, and GI tract with an indolent course; mast cell leukemia with a rapid course, and mast cell sarcoma with development of mast cell leukemia. Most of the unclassifiable myeloproliferative neoplasms are early stages of other types of myeloproliferative neoplasms that have not developed diagnostic features. This section presents a brief discussion of these relatively rare disorders in animals. Polycythemia vera, which is a chronic myeloproliferative disorder of erythrocytes, is discussed in Chapter 9.

In animals, the diagnosis is usually based on clinical and morphologic features. Cells are usually relatively normal to mildly dysplastic. The difficulty of making these diagnoses is that they are difficult to distinguish from hyperplasia, and thus other causes for increased leukocytes, platelets, or erythrocytes must be eliminated.

Chronic granulocytic (myelogenous) leukemia

The disorder that has been historically described in dogs and cats as CML more closely resembles human CNL, as neutrophilia predominates, and there is an absence of eosinophilia and basophilia. The morphologic equivalent of human CML has not been described in detail in animals. These chronic leukemias are rare in domestic animals and are characterized by marked neutrophilia, a left shift that often is disorderly, and anemia. A monocytosis may be present as well. Chronic myelogenous leukemia has been reported more frequently in dogs than in cats. Dysgranulopoiesis may be present and include hypersegmented nuclei and giant metamyelocytes and bands (Fig. 15.22). These leukemias, however, can be differentiated from myelodysplastic syndrome by the marked leukocytosis in the blood. Inflammatory responses can mimic myeloproliferative neoplasms, and such "leukemoid reactions" often are misdiagnosed as leukemias. Marrow examination may not be helpful in distinguishing the two, because marked inflammatory leukograms can be associated with marked granulocytic hyperplasia and a pronounced increase in the M:E ratio and the orderliness of maturation may appear disrupted. Histopathologic evaluation of the spleen and liver is not always helpful, because these organs may exhibit marked granulopoiesis with some types of inflammatory disease. Animals with myeloproliferative neoplasms usually eventually develop a disorderly left shift, and have a "blast crisis," during which myeloblasts appear in the blood (Fig. 15.23). Animals with myeloproliferative neoplasms also usually develop much more severe anemia than animals with inflammatory disease.

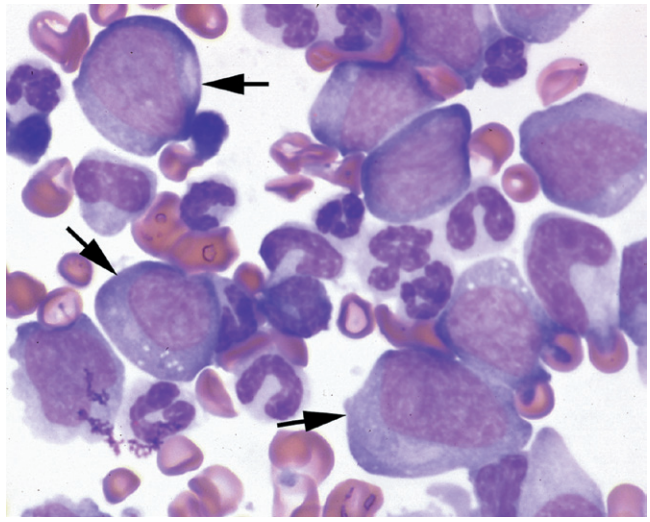

Figure 15.22 Bone marrow aspirate from a dog with chronic myelogenous leukemia. Note the increased concentration of myeloblasts (arrows). Although some degree of maturation to segmented neutrophils is occurring, the maturation appears to be disorderly. Very few erythroid precursors were present in the marrow, and none is present in this field. Wright stain.

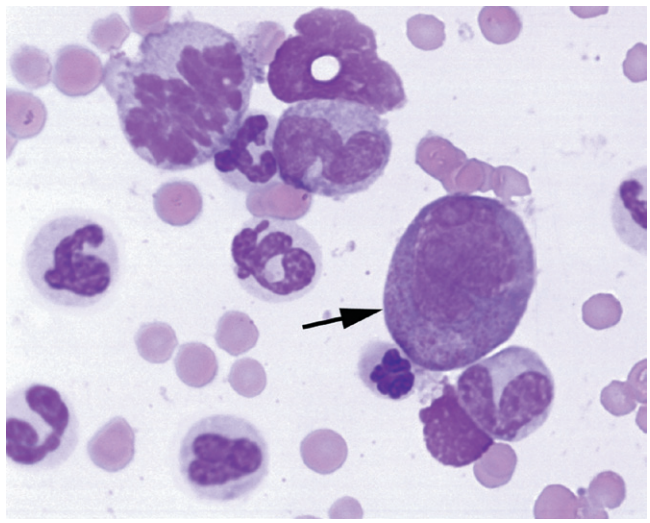

Figure 15.23 Blood film from a dog with chronic myelogenous leukemia in blast crisis. Note the cell in mitosis (upper left corner) and the myeloblast (arrow). The nucleated cell concentration in this dog was 150,000 cells/μL. Wright stain.

Eosinophilic leukemia

Eosinophilic leukemia is rare but has been reported primarily in FeLV-negative cats. It is characterized by eosinophilia, immature eosinophils in the blood, eosinophil predominance in the marrow (Fig. 15.24), and infiltration of various

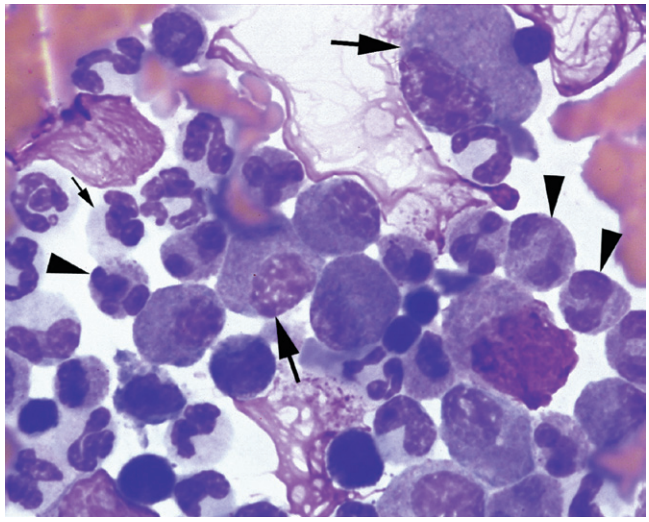

Figure 15.24 Bone marrow aspirate from a cat with eosinophilic leukemia or hypereosinophilic syndrome. Note the eosinophil precursors (large arrows) and the numerous, mature eosinophils (arrowheads). For comparison, note the neutrophil (small arrow). Wright stain. (Specimen courtesy of Antech Diagnostics.)

organs with eosinophils. This disorder is difficult to differentiate from feline hypereosinophilic syndrome, in which the same characteristics can be seen, although the eosinophilic left shift may be more orderly with hypereosinophilic syndrome. Intestinal involvement is typical as well. Recent reports are suggestive that the separation between the two disorders may be artificial, and that they both may represent a neoplastic proliferation of eosinophils. Clinical signs are similar to those seen in animals with other myeloproliferative disorders. Typically, however, they also include thickened bowel loops, diarrhea, and vomiting, because the intestine usually is infiltrated. Most cats die within 6 months of the diagnosis being established, but hydroxyurea in combination with prednisone may prolong survival.

Chronic basophilic leukemia

Chronic basophilic leukemia is very rare, but has been reported in dogs and cats. Abnormal blood findings include marked basophilia with an orderly left shift of the basophilic series, anemia, and occasionally thrombocytosis. Multiple organs usually are infiltrated. Chronic basophilic leukemia must be differentiated from mast cell leukemia. Basophils have segmented nuclei, whereas mast cells have round nuclei. Basophilic myelocytes, however, may be difficult to differentiate from mast cells, and animals with systemic mast cell neoplasia may have a mild basophilia.

Essential thrombocythemia

Essential thrombocythemia is a very rare chronic myeloproliferative disorder that is characterized by a marked increase in the platelet concentration (>1,000,000). Platelets may appear atypical, with hypo- or hypergranularity, and giant forms may be present. The concentrations of megakaryocytes and megakaryoblasts usually are increased in the bone marrow as well. The platelet concentration may be increased secondary to many other disorders, such as iron deficiency anemia, inflammation, antineoplastic drug therapy, corticosteroids, and neoplasia (particularly lymphoma).

Lymphoproliferative disorders

Although the term lymphoproliferative disorder can be used to describe any abnormal proliferation of lymphoid cells, it more commonly is used to describe neoplastic proliferations. Tumors that derive from lymphocytes or plasma cells are classified as lymphoproliferative, or lymphoid neoplasms. Lymphoproliferative disorders are more common than myeloproliferative disorders in domestic animals, and they are more common in cats than in any of the other domestic species. As with myeloproliferative disorders, cats with certain types of lymphoproliferative disorders usually test positive for FeLV, FIV, or both. Lymphoproliferative disorders generally are categorized as primary lymphoid leukemia, lymphoma, or plasma cell tumors, including multiple myeloma and solitary plasma cell tumors. In turn, the leukemias can be classified as either acute or chronic, as discussed earlier, and are termed acute lymphoblastic leukemia or chronic lymphocytic leukemia. Use of polymerase chain reaction to detect antigen-receptor rearrangements can identify a clonal, neoplastic population of cells and usually can differentiate nonneoplastic lymphoproliferative disorders from those that are neoplastic.

Lymphoid leukemia differs from malignant lymphoma primarily in the anatomic distribution. Solid neoplastic masses are present in lymphoma, but are less common in patients with primary lymphoid leukemia. At least 10–25% of dogs and cats with lymphoma develop leukemia, however, and some investigators report that approximately 65% of dogs with multicentric lymphoma are leukemic at the time of presentation (if the determination of leukemia is based on the evaluation of blood, bone marrow aspirates, and marrow core biopsy specimens). Lymphoproliferative disease that arises in the bone marrow rather than in the lymph nodes or spleen has a different biologic behavior, response to therapy, and prognosis.

Acute lymphoblastic leukemia (ALL)

Acute lymphoblastic leukemia is characterized by the presence of lymphoblasts in the blood and bone marrow (Figs. 15.25 through 15.28). In both acute lymphoblastic leukemia and the leukemic phase of multicentric lymphoma (Stage V), however, lymphoblasts can be found in the blood and bone marrow, thereby making these two disorders difficult to differentiate. A general rule is that if lymphadenopathy is not present, the disorder most likely is acute lymphoblastic

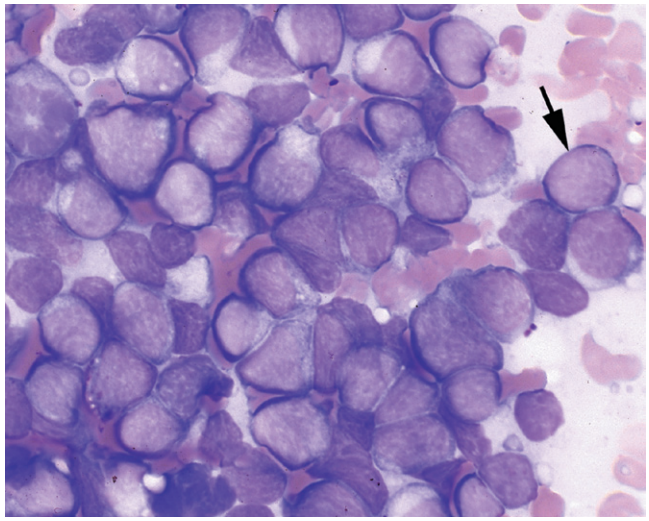

Figure 15.25 Bone marrow aspirate from a dog with acute lymphoblastic leukemia. Note that normal hematopoietic cells are absent, having been replaced by lymphoblasts (arrow). Wright stain.

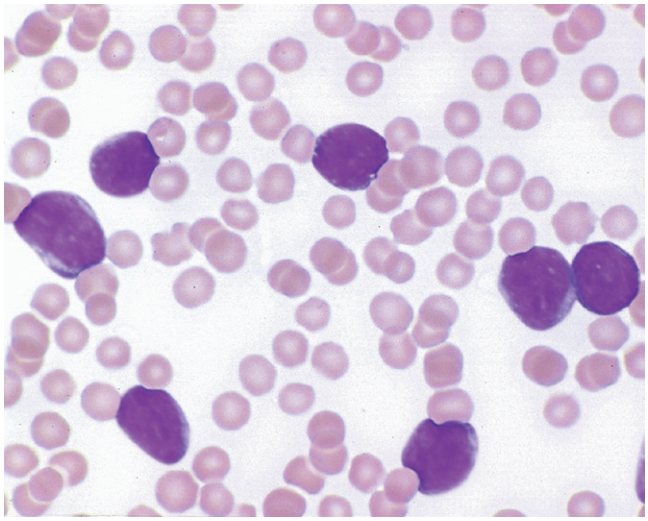

Figure 15.27 Blood film from a dog with acute lymphoblastic leukemia. Note the numerous large lymphoblasts. Wright stain.

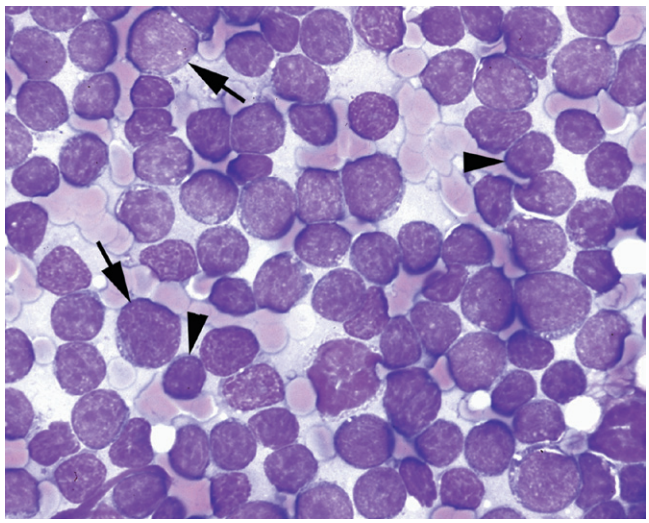

Figure 15.26 Bone marrow aspirate from a dog with acute lymphoblastic leukemia. Numerous intermediate-sized lymphoid cells are present and have completely replaced the normal marrow elements. Note the lymphoblasts (arrows) and lymphocytes (arrowheads). Wright stain.

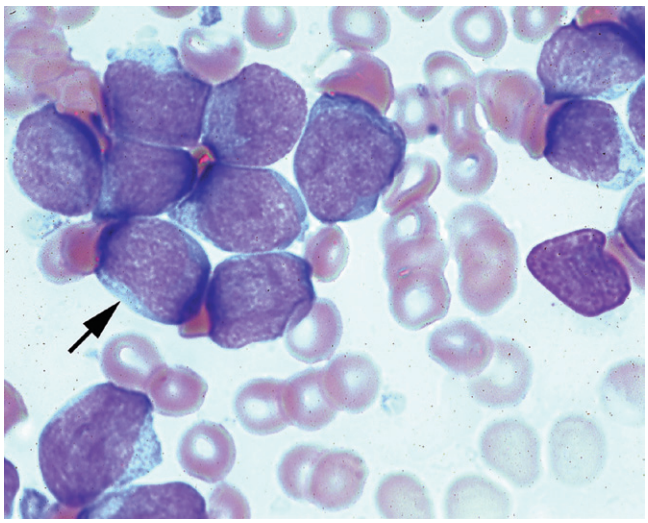

Figure 15.28 Blood film from a dog with acute lymphoblastic leukemia and a nucleated cell count of 300,000 cells/μL. All the cells present are lymphoblasts (arrow). Note the large size, high nucleus:cytoplasm ratio, and nucleoli with the nuclei. Wright stain.

leukemia rather than lymphoma. Approximately half of the dogs with acute lymphoblastic leukemia, however, also have lymphadenopathy. As with the myeloproliferative disorders, clinical signs relate either to a lack of normal hematopoietic cells or to the infiltration of organs by neoplastic cells. Common findings include pale mucous membranes, splenomegaly, and hepatomegaly, lethargy, and weight loss. Common CBC abnormalities include anemia, thrombocytopenia, lymphocytosis, and lymphoblasts in the blood.

Lymphoblasts usually can be differentiated from other types of immature cells based on their characteristic morphology, as described earlier. Occasionally, however, certain types of lymphoblasts (e.g., large granular lymphoblasts) may contain a few fine to coarse azurophilic granules (Fig. 15.29). These cells may be difficult to distinguish from myeloblasts, in which case immunophenotyping (using monoclonal antibodies directed against proteins on the surface of leukocytes) may be very helpful. Cytochemical reactions also may be helpful, because lymphoblasts typically are negative for most of the cytochemical stains except nonspecific esterase. Chemotherapy, usually involving a combination of vincristine, cyclophosphamide, and prednisone, may result in remission,

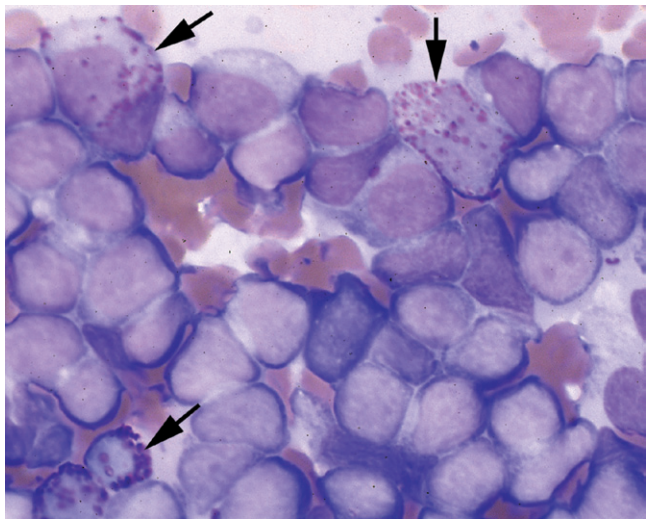

Figure 15.29 Bone marrow aspirate from a dog with lymphoblastic leukemia. Note the presence of a few cells with azurophilic granules within the cytoplasm (arrows), which are referred to as large granular lymphoblasts. These granules make this type of leukemia difficult to distinguish from M1 based on cell morphology alone. Wright stain.

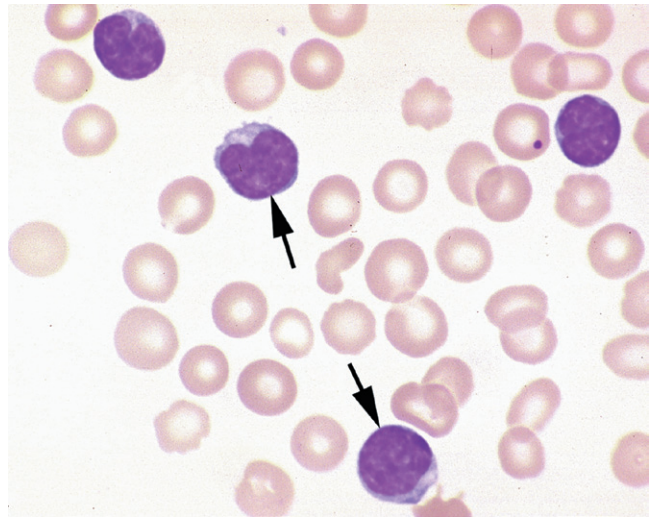

Figure 15.30 Blood film from a dog with chronic lymphocytic leukemia. Note the relatively small, normal-appearing lymphocytes (arrows). The diagnosis of leukemia was based on the high concentration of small lymphocytes in the blood (40,000 cells/μL) and by polymerase chain reaction results. Wright stain.

though usually of short duration. The clinical course is usually rapid, progressive and poorly responsive to therapy. Middle-aged to older dogs are usually affected. Cats are usually younger and FeLV positive. The majority of acute lymphoblastic leukemias and leukemias associated with Stage V lymphoma in dogs have been thought to be of B-cell origin, although one study[2] found that the prevalence of B and T immunophenotypes in acute lymphoblastic leukemia and chronic lymphocytic leukemia was not statistically different.

Chronic lymphocyte leukemia (CLL)

In animals with chronic lymphocytic leukemia, the lymphocytes are small and appear well-differentiated (Fig. 15.30). Chronic lymphocytic leukemia is more common in dogs than in other domestic animals. This type of leukemia, however, must be differentiated from physiologic lymphocytosis in excited cats (usually kittens), in which the absolute lymphocyte count may reach 20,000 cells/μL. Other differential diagnoses include lymphocytosis induced by chronic antigenic stimulation, such as that seen in dogs with chronic ehrlichiosis. Lymphocytosis is rare and usually mild (<10,000 lymphocytes/μL) with other types of antigenic stimulation, however. Lymphocytosis predominated by large granular lymphocytes (LGL) may be seen in animals with ehrlichiosis or chronic lymphocytic leukemia. Mild to moderate lymphocytosis has been reported as an infrequent finding in cats infected with *Bartonella henselae*. The list of major differentials for persistent nonneoplastic lymphocyte expansion in dogs and cats is short and most of these conditions are relatively uncommon. Persistent lymphocytosis of small, mature, or reactive lymphocytes is most commonly the result of chronic lymphocytic leukemia or lymphoma. The first step in distinguishing nonneoplastic from neoplastic lymphocytosis is immunophenotyping by flow cytometry to determine the phenotypic diversity of the circulating cells. Clonality testing using the polymerase chain reaction for antigen receptor rearrangements assay is a useful second step in cases in which the phenotype data are equivocal. Once the diagnosis of malignancy has been established, the immunophenotype also provides prognostic information in dogs (see Chapter 13).

Clinical signs and abnormalities found in ill animals are similar to those seen in animals with other types of leukemia, including lethargy, anorexia, pale mucous membranes, lymphadenopathy, splenomegaly, and hepatomegaly. However, some animals are asymptomatic, and the lymphocytosis is discovered during a wellness exams or presurgical screening. The most striking CBC abnormality is the lymphocytosis, which may range from increased slightly above the reference interval to greater than 300,000/μL. Anemia and thrombocytopenia may be present, but the anemia usually is not as severe as that seen in animals with acute lymphoblastic leukemia. The concentration of small lymphocytes in the marrow is greater than normal, being reported to range from 25% to 93% of cells. Monoclonal gammopathies occasionally are seen in animals with chronic lymphocytic leukemia.

Four main phenotypic classifications have been described in dogs with CLL: CD8+ T-cell, CD21+ B-cell, CD4-8-5+ (aberrant T-cell phenotype), and CD34+ (undifferentiated progenitor). T-cell CLL is more common in dogs and cats than is B-cell CLL, many of the dogs with T-cell leukemia

have LGL leukemia, and T-cells tend to proliferate in the spleen. Immunophenotyping provides an objective method for determining prognosis in dogs with CLL. Expression of CD34 predicts poor outcome with much shorter survival compared with other phenotypes. Within the CD8+ phenotype, dogs presenting with a >30,000 lymphocytes/μL have significantly shorter median survival than those presenting with <30,000 lymphocytes/μL. Within the T-cell leukemias, dogs with CD4-8-5+ leukemia and dogs with the CD8+ T-cell phenotype have a similar survival time. A CD21+ B-cell lymphocytosis composed of large cells was associated with shorter survival time than those with smaller circulating cells. In another study, old dogs with B-CLL survived longer than young dogs, and anemic dogs with T-CLL survived a shorter time than dogs without anemia.

Therapeutic intervention is controversial, because untreated animals may live for months to years. Recommendations for chemotherapy in dogs and cats include a combination of chlorambucil and prednisone; long remissions and survival can be achieved. The median survival time for dogs is more than 1 year. Survival time has been reported to be significantly different in untreated dogs with chronic lymphocytic leukemia (~450 days), as compared to that of dogs with acute lymphoblastic leukemia (~65 days). Chronic lymphocytic leukemia in cats is rarely associated with FeLV infection.

Plasma cell myeloma (multiple myeloma)

Plasma cells derive from B-lymphocytes and typically secrete immunoglobulins. Plasma cell myeloma is a relatively rare lymphoproliferative neoplasm, in which plasma cells or their precursors proliferate abnormally (Figs. 15.31 and 15.32). As implied by the term multiple myeloma, plasma cells proliferate in the bone marrow at multiple sites. The incidence of multiple myeloma in cats is thought to be even less than that in dogs and usually is not associated with FeLV or FIV infections. These plasma cell proliferations may be detected on bone marrow films, but plasma cells only rarely are seen on blood films. When plasma cell leukemia is present, the survival time usually is less. Markedly increased plasma cell concentration in the bone marrow (>20% of all nucleated cells) often results from plasma cell neoplasia, but plasma cell proliferation also may occur secondary to chronic antigenic stimulation. Neoplastic plasma cells often are seen in large aggregates and sometimes appear slightly abnormal or immature, with occasional multinucleated plasma cells being present. Neoplastic cells may appear to be very well-differentiated, however, in which case they are difficult to distinguish from normal plasma cells. Plasma cells occasionally may have a ruffled eosinophilic cytoplasmic margin that appears similar to a flame; these are termed flaming plasma cells or flame cells (Fig. 15.32).

An important diagnostic and clinical manifestation of plasma cell myeloma is a monoclonal or biclonal gammopathy, usually immunoglobulin G or A but, occasionally, immunoglobulin M (Fig. 15.33). The immunoglobulins synthesized by malignant plasma cells also are known as paraproteins. Other diagnostic features include Bence-Jones protein (i.e., light chains of immunoglobulins) in the urine and radiographic evidence of osteolysis (Fig. 15.34). Two or three of these four features traditionally are considered to

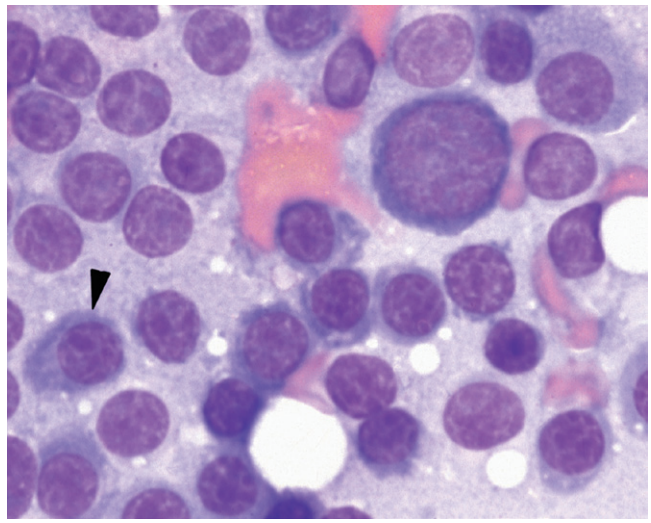

Figure 15.31 Bone marrow aspirate from a dog with plasma cell myeloma. Almost all the cells present are plasma cells. Note the more typical plasma cell with an eccentric nucleus and abundant cytoplasm (arrowhead). Wright stain.

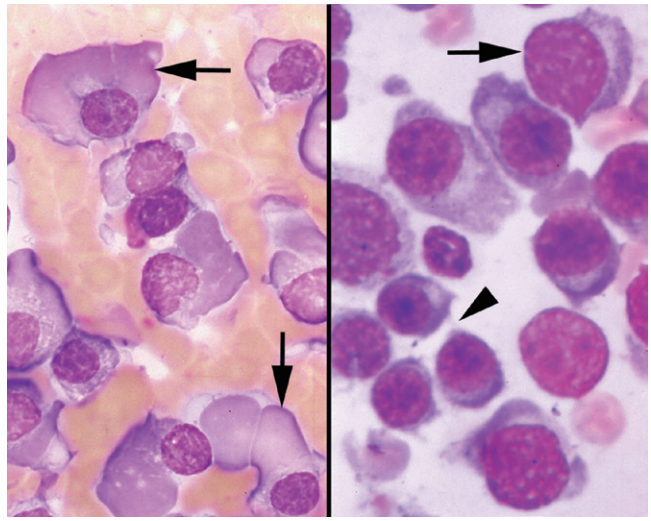

Figure 15.32 Left. Bone marrow aspirate from a dog with plasma cell myeloma. These plasma cells have eosinophilic-colored cytoplasm that is ruffled, and they sometimes are referred to as flame cells. The cytoplasm is filled with immunoglobulin. Right. Bone marrow aspirate from a dog with plasma cell myeloma. Note the variation in cell size, ranging from the large, immature plasma cell with loose chromatin (arrow) to the small cells with more condensed chromatin (arrowhead). Wright Stain.

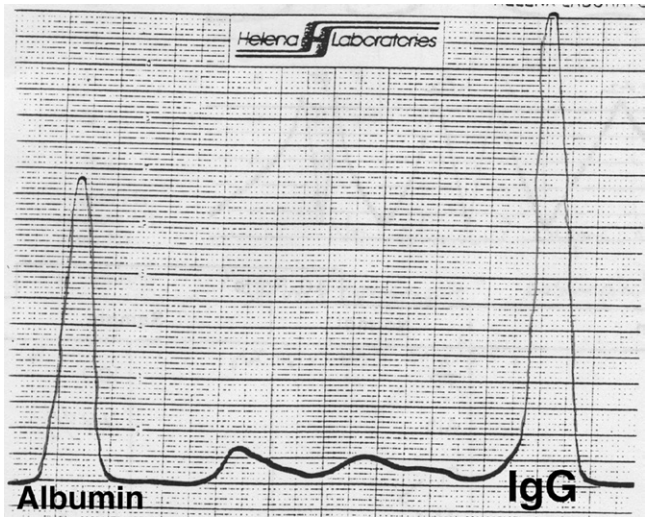

Figure 15.33 Protein electrophoretogram from a dog with plasma cell myeloma and monoclonal gammopathy. Note the monoclonal immunoglobulin (IgG) spike at the right. Albumin is represented by the smaller spike to the left. Wright stain.

be essential for the diagnosis of plasma cell myeloma to be established. Dogs with chronic ehrlichiosis, however, may very rarely have a monoclonal gammopathy, usually within a polyclonal gammopathy, and a markedly increased concentration of plasma cells in the bone marrow. Other disorders in which monoclonal gammopathies have been rarely reported include chronic lymphocytic leukemia, lymphoma, feline infectious peritonitis, and extramedullary plasmacytoma.

Clinical signs associated with multiple myeloma are usually associated with plasma cell infiltration of the bone marrow and other organs or with increased concentration of circulating immunoglobulins, which may result in increased viscosity of the blood (i.e., hyperviscosity syndrome). Lethargy, anorexia, lameness, bleeding from the nares, paresis, polyuria, and polydipsia are relatively common. Fundoscopic changes such as retinal hemorrhages and engorged retinal blood vessels commonly are observed as well. Renal disease is relatively common and usually associated with the abnormal proteins interfering with

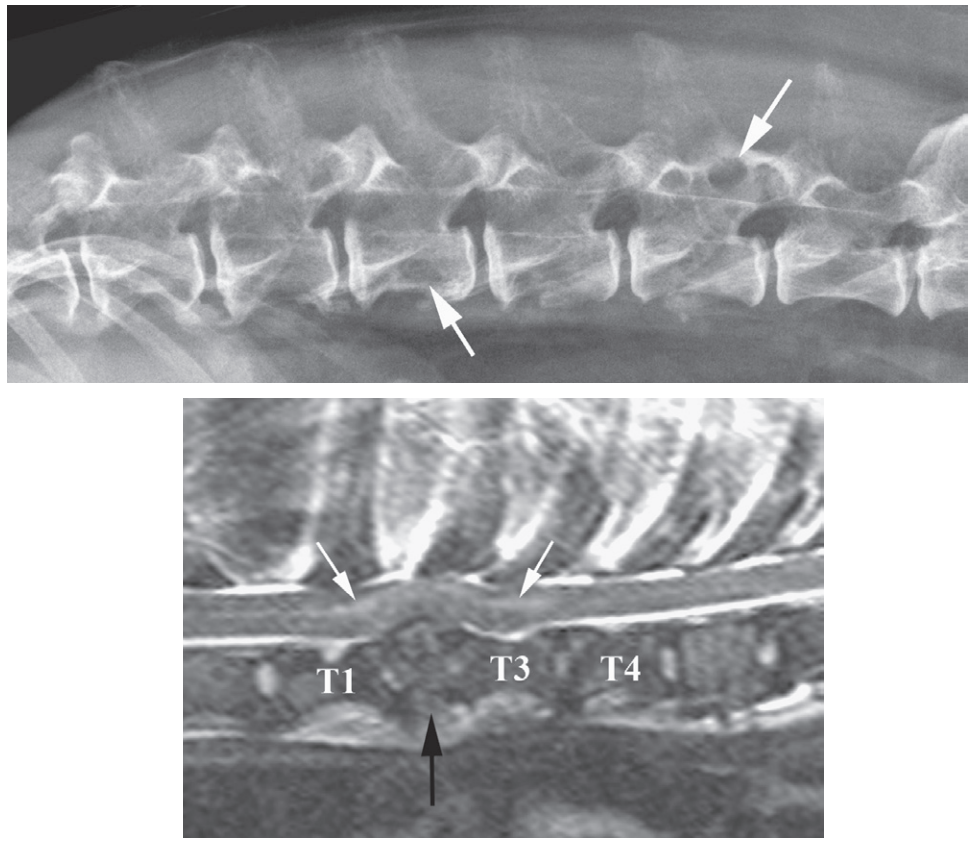

Figure 15.34 (A) Lateral lumbar radiograph of a dog with multiple myeloma. There are numerous small lucencies in the spinous processes and larger lucent lesions in the body of L3 and the lamina of L5 (white arrows) due to bone effacement from neoplastic cells. (B) Sagittal T2-weighted magnetic resonance image of the thoracic spine of a dog with multiple myeloma. Effacement of the 2nd thoracic vertebral body by neoplastic cells has led to a pathologic fracture. Note the foreshortened irregular shape (black arrow) compared to the adjacent normal 1st, 3rd, and 4th thoracic vertebrae (T1, T3, and T4, respectively). A portion of the fractured vertebra is protruding into the vertebral canal and causing spinal cord compression. The increased signal (whiteness) of the spinal cord (white arrows) is due to edema and inflammation. Vertebral fracture causing paralysis or paresis is a relatively common complication of multiple myeloma. (Courtesy Dr. Donald Thrall, North Carolina State University.)

tubular and glomerular function, but it sometimes occurs secondary to hypercalcemia with subsequent calcification of renal tissue. Central nervous system impairment may result from serum hyperviscosity and subsequent sludging of blood in small vessels. Bleeding diatheses, which are seen in approximately one-third of dogs with multiple myeloma, may result from thrombocytopenia, but it also can result from the abnormal immunoglobulins interfering with platelet function. Common findings in feline multiple myeloma include atypical plasma cell morphology, hypocholesterolemia, anemia, bone lesions, and multi-organ involvement. In one retrospective study, all of the affected cats examined had noncutaneous, extramedullary tumors of the spleen, liver, or lymph nodes.

Dogs with multiple myeloma that are treated with alkylating agents (e.g., melphalan or cyclophosphamide) often have survival times of from 1 to 2 years. Reported survival times in treated cats usually are less. Animals with multiple myeloma that are azotemic or have severe anemia, neutropenia, or thrombocytopenia usually have a poorer prognosis. Hypercalcemia, Bence-Jones proteinuria, plasma cell leukemia, and extensive bony lesions also are associated with a shorter survival time. In humans, stem cell transplantation offers significantly improved prognosis and survival rates.

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16 Diagnosis of Disorders of Hemostasis

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The cardiovascular system delivers blood to tissues throughout the body and is susceptible to injury. When mechanical, inflammatory, or other injury occurs to the vascular system on a daily basis, normal animals have a finely controlled system that prevents blood loss, maintains blood flow, and allows healing and repair of damaged vessels.

Overview of hemostasis

The term hemostasis is defined as the arrest of bleeding, and defects of hemostasis vary from excessive hemostasis, with resulting intravascular thrombosis, to excessive bleeding and blood loss. Both extremes may be life-threatening, but intravascular thrombosis is more difficult to detect and manage in animals. This chapter discusses a systematic approach for establishing an effective diagnosis of the commonly encountered disorders of hemostasis, and emphasizes disorders that result in bleeding since they are most common in animals. Effective hemostasis after vascular injury reflects integrated responses by three major components: the soluble circulating coagulation factors (i.e., proteins) that culminate in stable insoluble fibrin, circulating platelets, and the vessel comprised of endothelial cells, smooth muscle cells, and fibroblasts.

Coagulation factors

The term coagulopathy usually refers to excessive bleeding resulting from the abnormal function or absence of one or more circulating coagulation factors. Coagulation factors are present in plasma at very low concentrations (μg/mL), and most are proteases (Table 16.1). Coagulation factors are activated predominantly by exposure to tissue thromboplastin expressed on the surface of circulating microparticles, stimulated endothelial cells or extravascular fibroblasts. After initial activation with production of small amounts of thrombin, coagulation factors are further activated through feedback amplification loops to enhance the initial stimulus. The culminating event of coagulation factor activation is the conversion of fibrinogen to fibrin and the formation of a stable fibrin clot in association with platelets to occlude blood flow from a damaged vessel. Defective coagulation factor activity or absence of coagulation factors will delay the formation of fibrin.

The activation and amplification scheme of hemostasis traditionally has been divided into the intrinsic, extrinsic, and common pathways (Fig. 16.1). This scheme implies two pathways of activation: by exposure to tissue thromboplastin, or by contact activation of basement membrane and collagen (or other negatively charged surfaces). The results of recent kinetic analyses of individual factors, however, suggest a scheme in which the initial activation by tissue thromboplastin forms small amounts of thrombin, which is then amplified by subsequent loop activation of the intrinsic, extrinsic, and common pathways. Important in this loop is the activation of factors VII, XI, and the accelerators (factors V and VIII) by thrombin (factor IIa). (Fig. 16.2). This implies that contact activation is not a significant contributor to coagulation factor activation, and that the intrinsic system primarily functions as an amplification loop that becomes activated after the initial thrombin generation by tissue thromboplastin.[1,2] Evidence supporting this view exists in human patients who do not bleed when they are deficient in any of the contact activator proteins (factor XII, prekallikrein, or high-molecular-weight kininogen (HMWK)). This may not be true in some domestic animals as dogs and horses with prekallikrein deficiency have mild clinical bleeding tendencies.

Some coagulation factors require vitamin K, which serves as a cofactor for the post-translational carboxylation of coagulation factors II, VII, IX, and X as well as anticoagulant proteins C and S, and recently protein Z.[3] Vitamin K

Veterinary Hematology and Clinical Chemistry, Second Edition. Edited by Mary Anna Thrall, Glade Weiser, Robin W. Allison, and Terry W. Campbell.

Table 16.1 Procoagulation factors.

Factor	Trivial Name	Location of Synthesis	Molecular Weight	Plasma Concentration	Plasma Half-Life	Vitamin K Dep.	Species Affected	Inheritance/Disease Name	Clinical Disease
I	Fibrinogen	Liver	340,000	0.1–2.5 g/dL	1.5–6.3 days	–	Man, goats, dogs	Autosomal	Severe
II	Prothrombin	Liver, macrophage	72,000	—	2.1–4.4 days	+	Man, dog	Auto. rec.	Mild
III	Tissue thromboplastin	Lipoprotein, is a constituitent of fibroblasts and smooth muscle cell plasma membrane; lipoprotein can be induced in endothelium, monocytes, and macrophages.			15–24 hours	–	Man	auto. rec., paraphemophilia	Variable, mostly mild
IV	Calcium				1–6 hours	+	Man, dogs	Auto. dom. (auto rec. in man)	Mild
V	Proaccelerin	Liver, macrophages	350,000	—	2.9 days	–	Man, dogs, cats, horses	X-linked rec., hemophilia A	Variable
VI	No factor				24 hours	+	Man, dogs, cats	X-linked rec., hemophilia B	Often severe
VII	Proconvertin	Liver, macrophages	53,000	—	32–48 hours	+	Man, dogs	Auto. dom.	Severe[a]
VIII:C	Antihemophiliac factor	Liver	Ambiguous	—	30 hours	–	Man, cattle, dogs	Auto. rec., hemophilia C	Mild[b]
IX	Christmas factor (plasma thrombo-plastin component)	Liver	56,000	—	48–52 hours	–	Man, cats[c]	Auto. rec., Hageman trait	None
X	Stuart factor	Liver, macrophages	56,000	—	4.5–7.0 days	–	Man		
XI	Plasma thromboplastin antecedent	Liver (probably)	124,000	6 μg/mL	35 hours	–	Man, dogs, horses	Auto. rec.	None to mild
XII	Hageman factor	Liver (probably)	80,000	30 μg/mL	6.5 days	–	Man		
XIII	Fibrin-stabilizing factor	Liver (probably)	320,000	—					
Prekallikrein	Fletcher factor	Liver (probably)	85,000	50 μg/mL					
High-molecular-weight kininogen	Fitzgerald factor	Liver (probably)	110,000	70–90 μg/mL					

auto. dom., autosomal dominant; *auto. rec.*, autosomal recessive; *X-linked rec.*, X-linked recessive.

[a] Severe in neonates, mild in adults.

[b] Severe bleeding may occur after major surgical procedures or trauma.

[c] Normally not present in marine mammals, most reptiles, and fowl.

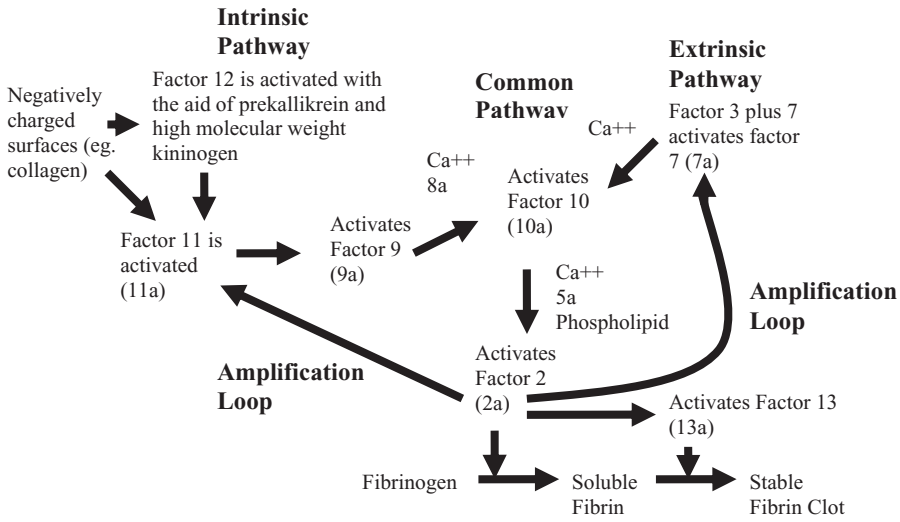

Figure 16.1 The traditional activation cascade of the intrinsic system by contact activation of negatively charged surfaces. Activation of factor XI by thrombin (IIa), however, may be more important. Factor IX is activated by factor XIa (and activated factor VII), and it can activate factor X in the presence of calcium and platelet factor III. Factor VIII:C is not required for activation, but when factor VIII:Ca is present, the rate of factor Xa formation is increased. The designation "a" denotes an activated factor. The extrinsic system is thought to be the predominant method of coagulation activation, and this occurs when factor III (tissue thromboplastin) is in contact with factor VII, which then can activate factor X. Factor X is the first factor of common pathway and is activated by either factor IXa or VIIa. The common pathway culminates in the formation of a stable covalently linked fibrin clot. Activation of prothrombin to thrombin (IIa) does not require factor V, but it proceeds much faster with activated factor V.

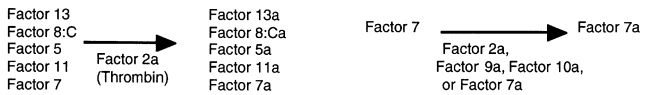

Figure 16.2 Thrombin activates many other factors of the coagulation cascade and amplifies the initial activation of prothrombin to form much more thrombin. Once factor VII has been activated, additional amplification loops generate more factor VIIa to enhance the overall activity of the coagulation cascade. Factor VIIa also activates factor IX to IXa to enhance the formation of factor Xa from X. The purpose of these activation loops is to fully activate the cascade and to generate much more thrombin to, ultimately, shorten the time needed to convert a critical mass of fibrinogen to fibrin for the formation of a stable fibrin clot.

normally is oxidized during carboxylation of coagulation proteins and then reduced back to the active hydroquinone form in a two-step process by the enzyme epoxide reductase.[4,5] In the presence of inhibitors of vitamin K reduction or the absence of dietary vitamin K, the procoagulant and anticoagulant proteins are formed but lack activity. These nonfunctional proteins are designated PIVKA (proteins in vitamin K absence or antagonism), and they can be detected by immunologic methods.

Platelets

Platelets are cytoplasmic fragments of megakaryocytes with numerous cytosolic organelles (Table 16.2), and are in the shape of a flat disk (Fig. 16.3). Platelets are crucial to hemostasis and provide a surface[6] for the formation of the tenase and prothrombinase complex to form thrombin and are responsible for the initial, temporary cessation of blood flow after injury to the microvascular bed.[7] The importance of platelet surfaces for activation of coagulation and hemostasis is evident in some German shepherd dogs that lack normal phosphatidylserine surface exposure and clinically bleed.[8] Platelets respond to vascular injury that exposes collagen, laminin, and fibronectin.[9] Thrombin (factor IIa) formed during activation of coagulation is a strong agonist for platelet activation through PAR-1_1 and PAR-4_4 receptor signaling on platelets. Platelets can adhere to collagen in the basement membrane and extravascular stroma through a surface receptor complex (glycoprotein (GP) 1b-V-IX) that binds the plasma glycoprotein von Willebrand factor (vWF), which in turn binds collagen. Platelets also adhere to collagen directly in shear conditions (as in arteries) primarily through surface GP VI and α2B1. Lack of the GP1b-V-IX receptor (Bernard-Soulier syndrome) or vWF (von Willebrand disease) results in clinical bleeding.[10,11] After adhesion, platelets undergo what is termed "inside-out" signaling through integrin receptors to increase receptor affinity and "outside-in" or second wave signaling to further stabilize the developing platelet/fibrin thrombus.[12] As platelets adhere to one another by glycoprotein IIb/IIIa ($\alpha_{IIB}\beta_3$) binding fibrinogen, they swell, centralize their organelles, and then form pseudopodia (Fig. 16.4). Glanzmann's thrombasthenia is a lack of IIb/IIIa glycoprotein and results in bleeding.[13] Aggregation of

Table 16.2 Ultrastructural and functional anatomy of platelets.

Anatomic-Structure	Constituents	Functions
Exterior coat	Fibrinogen	Platelet aggregation
	Glycoprotein	Platelet adhesion
Unit membrane	Arachidonic acid	Prostaglandin synthesis
	Platelet factor III (phosphatidylserine)	Enhances coagulation
Microtubules	Tubulin	Provides cytoskeleton and contractile system
Microfilaments	Thrombosthenin	Shape change, clot retraction, platelet release
α-Granules	β-Thromboglobulin	Impedes prostacyclin production by endothelium
	von Willebrand factor	Platelet adhesion to subendothelial collagen
	Factor V (Platelet factor I)	
	Fibrinogen	
	Fibronectin	
	Growth factor(s)	Mitosis of fibroblasts, endothelium, smooth muscle
	High-molecular-weight kininogen	
	Platelet factor IV	Antiheparin activity
Dense bodies	Adenine nucleotides	Platelet metabolism and hemostasis
	Histamine	Increases vascular premeability
	Serotonin	Vasoconstriction and enhancement of aggregation
	Calcium	Necessary for platelet stimulation
Lysosomal granules	Acid hydrolases	proteolysis
Dense tubular system	Calcium	Necessary for platelet stimulation
	Enzymes for prostaglandin synthesis	Thromboxane A_2 is important in recruiting more platelets and mobilizing calcium
Open canalicular system	Extensive surface area	Route for exocytosis, endocytosis, phagocytosis

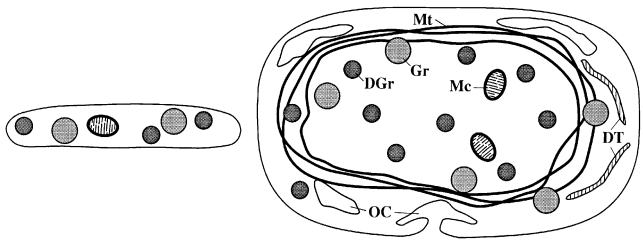

Figure 16.3 The disc shape of a platelet on end (left) and from above (right). DGr, dense granules; DT, dense tubular system; Gr, granules; Mc, mitochondria; Mt, microtubules around the margin; OC, open canilicular system.

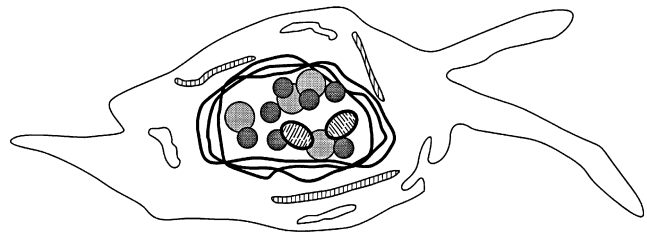

Figure 16.4 The centralization of platelet contents during activation, with pseudopodia formation and fusion of granules to the open canalicular system to release their contents into the extracellular milieu without lysis of the platelet membrane.

platelets and recruitment of additional platelets are promoted by the release reaction that empties products of the platelet granules into plasma. Coagulation factors stored within granules ensure fibrin formation, which is necessary for platelet plug stabilization.[8] Platelet granules empty their contents into the open canalicular system (OCS) that communicates with the exterior, or onto the surface of platelets if they lack an OCS (as in cattle[14]). Platelets do not lyse during aggregation. Platelets also respond to agonists released from platelet granules in a positive feedback fashion through surface receptors. ADP (that stimulates surface receptors P2Y12, the important target for platelet inhibition by the drug clopidogrel), serotonin, histamine are examples of agonists released by platelets.[15] Thromboxane A2 is produced and released to bind the surface receptor (TP) to further activate platelets in a positive feedback fashion. Aspirin and other NSAIDs inhibit platelet function by blocking thromboxane A2 synthesis.

Vessels

Vessels contribute to the cessation of blood loss by reflex vasoconstriction of smooth muscle cells to reduce the vessel

lumen diameter as well as by secretion or expression of thrombogenic substances from endothelial cells to promote clot formation such as platelet activating factor or tissue thromboplastin. Damage or loss of endothelium also reduces the local secretion of mediators such as prostacyclin (PGI_2) that downregulate the reactivity of platelets, thus enhancing platelet responsiveness at that site of injury. Decreased perivascular collagen support in disease conditions such as Marfan syndrome, Ehlers-Danlos syndrome, scurvy, and steroid excess has been associated with increased vascular fragility and bleeding; there is also some evidence for reduced platelet response to abnormal collagen. Vascular abnormalities, when compared with platelet and coagulation factor abnormalities, are the least frequent cause of excessive bleeding but the most difficult to evaluate.

Effective maintenance of vascular integrity and tissue perfusion requires the precise control of clot formation and resolution of the clot for reestablishing blood flow in tissues. Even as the clot forms, lysis of the fibrin clot occurs by plasmin, which is formed by the activation of plasminogen.[16] In vivo activation of plasminogen to plasmin is primarily by active factor XII and by tissue-plasminogen activator from endothelial cells. Factor XII activates plasminogen as well as factor XI, complement, and kinins by the scheme depicted in Figure 16.5. Patients who are deficient in factor XII do not bleed excessively, but they do have a tendency for thrombosis resulting from the insufficient resolution of fibrin clots by plasmin. Other activators of plasminogen are known (epithelial plasminogen activator, urokinase, bilokinase, streptokinase, staphylokinase), but these are less or not important in the normal regulation of intravascular clot resolution. Plasmin degrades fibrin and fibrinogen to small fragments and peptides (Fig. 16.6).[17] Detection of fibrin and fibrinogen (fibrin(ogen)) degradation by plasmin is primarily by the immunologic identification of fragment E in most species.[18] Fibrin(ogen) degradation products (FDPs) normally are removed from the circulation by hepatocytes, and decreased removal results in increased circulating concentration of FDPs. D-dimer is a product of plasmin digestion of crosslinked fibrin and has the same diagnostic interpretation as FDPs; however, D-dimer is more specific for thrombus formation as it only occurs when soluble fibrin has been crosslinked by factor XIII and plasmin has cleaved stable fibrin to form unique neoantigens as opposed to FDPs occurring with plasmin degradation of fibrinogen or fibrin (Fig. 16.6).[17] Evaluation of D-dimer concentration in citrate-anticoagulated blood is by immunologic detection (either by ELISA or Latex agglutination) of the neoantigen, D-dimer. In the absence of liver disease, the usual clinical implication of an increased FDP or D-dimer concentration is increased intravascular coagulation with subsequent clot resolution.[19,20]

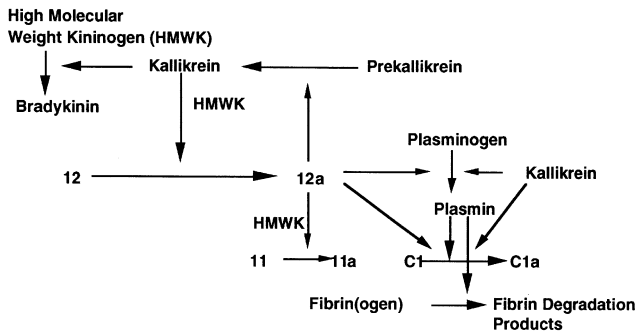

Figure 16.5 Factor XIIa can activate four protein cascades, but the only significant functional loss associated with decreased plasma factor XII activity is thrombosis caused by reduced generation of plasmin from plasminogen.

Anticoagulant proteins

Anticoagulant proteins downregulate the coagulation cascade by inhibiting the procoagulant proteins of the intrinsic, extrinsic, and common pathways. This ensures that activation of coagulation does not exceed the immediate need for hemostasis at the site of vascular injury. These anticoagulant proteins are in balance with the procoagulant proteins, and deficient anticoagulant protein activity relative to the procoagulant protein activity results in thrombosis. This balance can be shifted in favor of thrombosis by the loss of anticoagulant proteins or by selective, increased concentration of procoagulant proteins. Important anticoagulant proteins are listed in Table 16.3.

The most important circulating anticoagulant protein is antithrombin (AT), previously called antithrombin III, which accounts for approximately 70% of the total anticoagulant activity in plasma. Antithrombin requires heparin for activity at the endothelial surface. Heparin allows AT to bind thrombin in a 1:1 ratio and inactivates thrombin. The AT: thrombin complex subsequently is removed by hepatocytes. In addition to the inactivation of thrombin (IIa), AT also inactivates other serine proteases (IXa, Xa). Antithrombin has a very low molecular weight (Table 16.3) and is lost in the urine of patients with severe glomerular nephritis or amyloidosis. Low AT activity in plasma, either through loss or hereditary deficiency, is often associated with clinical thrombosis. Deficiency of other anticoagulant proteins, such as proteins C and S, which inhibit factors Va and VIII:Ca, also may be associated with thrombosis. α_2-Macroglobulin inhibits thrombin, plasmin, and kallikrein, and it accounts for another 20% of anticoagulant activity in plasma. Extrinsic pathway inhibitor is a lipoprotein produced by the liver and endothelial cells that is thought to be the major regulator of extrinsic pathway activation. Extrinsic pathway inhibitor inactivates thromboplastin and factor VIIa, and it requires factor Xa for activation.

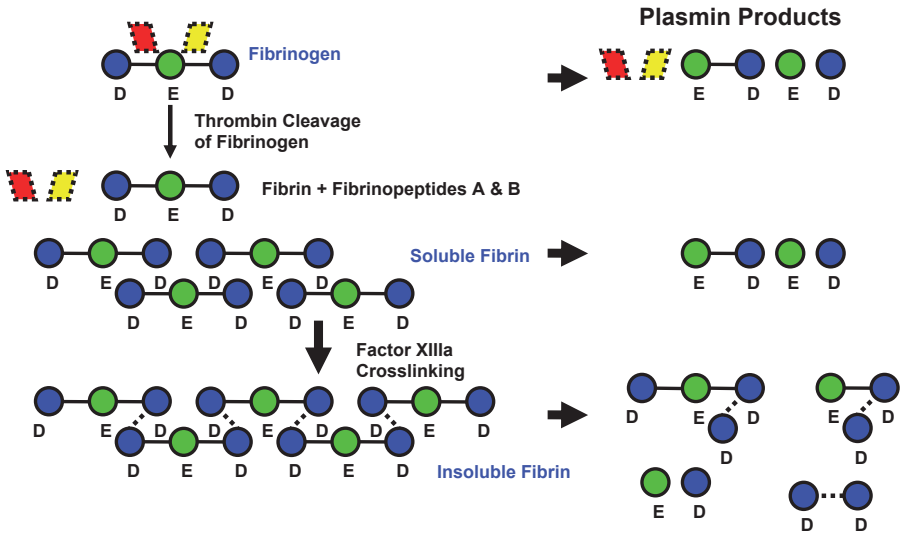

Figure 16.6 The degradation of fibrin and fibrinogen to smaller peptide fragments that can be used in anabolism by hepatocytes. Degradation of fibrin results in neoantigen, d-dimer.

Evaluation of the bleeding patient

When an animal is suspected of having a hemostatic defect, the following case-analysis method may be used to accurately determine if a defect exists and, if so, to determine the nature of a defect.

Patient history is a very important component in the analysis of an animal suspected of having a hemostatic defect. Specific questions should be asked, such as:

1. Have there been large hemorrhages into the subcutaneous tissue in the past?
2. Have there been previous petechial or ecchymotic hemorrhages on the skin?
3. What has been the color of urine?
4. Does the animal have periodic lameness, suggesting intra-articular hemorrhage?
5. What are the color and character of the feces?
6. Did the animal bleed excessively during previous surgery?
7. Has the animal been administered any drug and, if so, when?
8. What is the animal's environment?
9. When did hemorrhage occur relative to other symptoms?

Questions such as these should be asked to determine if the hemorrhage is appropriate for the degree of injury and if it resolves in an appropriate length of time. Persistent, recurrent hemorrhage at one site suggests a local vascular problem rather than a generalized homeostatic defect. Drugs and toxins, including rodenticides, may result in hemostatic defects, and possible access to such chemicals also is important information.

A careful physical examination to determine the nature and severity of the hemostatic defect provides useful information as well. For example, if evidence is found for underlying disease processes such as icterus, mass lesions, or fever, then disorders such as liver disease, hemangiosarcoma, or Rocky Mountain spotted fever, respectively, may be causing secondary bleeding disorders. Also, petechial and ecchymotic hemorrhages are characteristic of platelet abnormalities (Fig. 16.7) and generalized vascular abnormalities, whereas large hematomas, hemarthrosis, and deep muscle hemorrhages are more characteristic of soluble coagulation factor defects (Figs. 16.8 and 16.9).

Appropriate laboratory evaluation is another important component in evaluation of hemostasis and should initially include a complete blood count (CBC), activated partial thromboplastin time (aPTT) or activated coagulation time (ACT), and prothrombin time (PT). The CBC provides information such as platelet concentration and packed cell volume. The aPTT or ACT and PT help to determine if the levels of coagulation factors (except factor XIII) are deficient. A bleeding time may be necessary if the platelet concentration is normal but the animal is still suspected of having a platelet-related bleeding problem (defective platelet function). In addition, clinical chemistry, urinalysis, radiology, ultrasound, or isotope scanning may be helpful in evaluating other system functions in a patient with a hemostatic defect.

Other laboratory tests, available from specialized laboratories, may be required to fully characterize the underlying defect in hemostasis. Light transmittance platelet aggregometry may be necessary to examine platelet responsiveness and thromboelastography may be helpful in assessing overall coagulation of whole blood, especially if hypercoagulability is suspected (see Appendix 16.1).

Table 16.3 Anticoagulant factors.

Factor	Trivial Name	Lacation of Synthesis	Molecular Weight	Plasma Concentration	Plasma Half-Life	Vitamin K Dep.	Species Affected	Inheritance	Clinical Disease
Antithrombin III	Heparin cofactor	Liver	62,000				Man	Autosomal	Thrombosis
CI esterase inhibitor									
Extrinsic pathway inhibitor		Liver, endothelium (lipoprotein)							
α1-Antitrypsin									
α2-Antiplasmin									
Protein C			62,000		6–9 hours	+	Man		Thrombosis (purpura)
Thrombomodulin	Protein C cofactor	Endothelium							
Protein S	Cofactor for activated protein C		75,000	30 μm/mL		+	Man	Autosomal	Thrombosis (purpura)

[a] Normally not present in marine mammals, most reptiles, and fowl.
[b] Severe in neonates, mild in adults.
[c] Severe bleeding may occur after major surgical procedures or trauma.

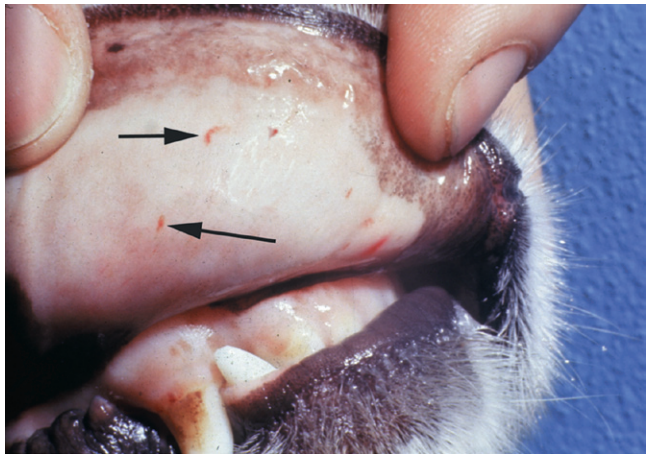

Figure 16.7 Petechial hemorrhages on the gingival surface of the lips are suggestive of thrombocytopenia or a platelet function defect. (*Courtesy of Dr. Ellen Miller.*)

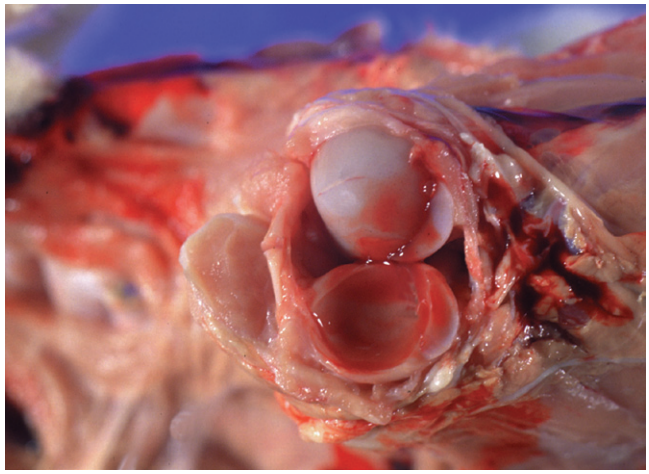

Figure 16.8 Hemorrhage into joints (as in this lamb with a carboxylation defect of coagulation factors) or body cavities suggests deficiency of a coagulation factor (or factors).

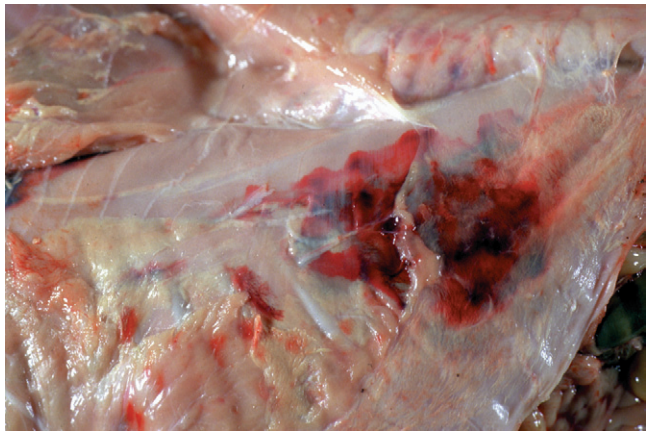

Figure 16.9 Subcutaneous hemorrhage suggestive of a coagulation factor deficiency.

Evaluation of the components of hemostasis

Coagulation factors

All circulating coagulation factors are produced by the liver, and hepatic insufficiency often is associated with clinical bleeding because of the decreased synthesis of one or more coagulation factors. The factors and some of their characteristics are listed in Table 16.1. Of the coagulation factors, only factors VIII:C and V are not proteases; these two factors increase the activity of factor IXa and Xa respectively. Hereditary deficiencies of coagulation factors are uncommon in domestic animals, but hereditary deficiencies of most known factors have been described. The most frequent hereditary coagulation factor deficiency described in veterinary medicine involves factor VIII:C and is termed classic hemophilia or hemophilia A. Deficiencies of factors VIII:C and IX (hemophilia B) are sex-linked traits and bleeding occur more frequently in male patients; the remaining factors are encoded on somatic chromosomes.

Evaluation of coagulation factor activities is usually not performed in the clinic setting but instead at local or regional laboratories because the tests require infrequently used and often expensive equipment. The only exception to this generalization is the ACT test, but even this test can require dedicated hemostasis instruments depending on the system utilized. The ACT does, however, evaluate all coagulation factor activities (except factors VII and XIII). This test uses sterile, diatomaceous earth or other contact activator substances and has an endpoint of loose blood clot formation. An ACT also requires a minimum number of functional platelets ($10^4/\mu L$) (see Appendix 16.1 for specific details of the ACT test). If the ACT and platelet count are normal, then coagulation factor deficiencies are an unlikely cause of the hemostatic defect. Factor XIII deficiency has never been reported in animals, and factor VII deficiency is associated with mild clinical bleeding.

Thromboelastography has been advocated as a method of evaluating overall coagulation, including platelets, and though it has been available for over 60 years, improved technology has been associated with a resurgence of interest[21] and validation for use in domestic animals.[22,23] Citrate anticoagulated blood is utilized in this assay, but it requires dedicated instrumentation to measure the viscous resistance of clotting blood.[22,23] Thromboelastography has been shown to useful in detecting hypercoagulable conditions.[24]

Selective evaluation of the coagulation cascade may be done by collecting blood in 3.2% sodium citrate in a volume of 1:9 anticoagulant:blood. Atraumatic venipuncture and collection of blood, first into a tube that is thrown away before collection into a sample vial to be sent to the laboratory, are important steps in proper blood collection. Blood is then sent to a laboratory for testing, optimally within 4 hours of collection. The aPTT uses one of several contact activators, and the PT uses tissue thromboplastin to activate coagulation. Phospholipid is added as a substitute for plate-

lets in both tests. A prolonged aPTT results if there is less than 25% of normal activity or concentration of any of factors II, V, VIII:C, IX, X, XI, XII, prekallikrein, HMWK, or fibrinogen. A prolonged PT results if there is less than 25% of normal activity or concentration of any of factors II, V, VII, X, or fibrinogen. A thrombin time may also be done, which essentially reflects fibrinogen concentration and may be modified to quantitate the actual fibrinogen concentration. Coagulation factor concentrations as well as activation kinetics to convert fibrinogen to fibrin vary between species, but these details are clinically unimportant. Submission of plasma for an aPTT or PT should be coordinated with the laboratory performing the evaluation in order to optimize reliability of the results and to avoid any delay in testing. A similarly collected blood sample from a normal animal may be required as an additional control for the patient's sample (to control for handling, collection, transportation, etc.), especially if the laboratory is distant from the site of collection. If individual coagulation factor analysis is desired for a complete evaluation, it usually is referred to a specialized laboratory for determination. The clinician should consult with laboratory personnel before collection and delivery of the sample to enhance reliability of the results.

Platelets

Inadequate platelet concentration and, less commonly, abnormal platelet function can be responsible for excessive bleeding. Both platelet concentration and function can be assessed by several methods. Platelet concentration can be determined by counting platelets electronically or manually. The concentration can be estimated on a blood film; at least 5–10 platelets/oil immersion field (magnification, ×1000) should be present. Normal platelet concentrations vary among animal species, but the range for all species is 100,000–800,000 platelets/μL with horses having the lowest and cattle the highest concentrations. Animals do not exhibit bleeding because of low platelet concentration until the concentration is ≤10,000–50,000 platelets/μL. Normal platelets should be smaller than erythrocytes. Pseudothrombocytopenia caused by undercounting larger than normal platelets can occur in the Cavalier King Charles spaniel or in cats that have platelets which overlap erythrocytes in size. Platelet concentration can actually be decreased sufficiently to cause hemorrhage because of decreased production by the bone marrow, increased removal from the blood (i.e., destruction), or activation (i.e., consumption). When the bone marrow production of platelets is decreased, the megakaryocyte concentration within the marrow is decreased; conversely, the bone marrow megakaryocytes concentration is increased when platelets are being destroyed or consumed (Fig. 16.10). Platelets may appear to be larger than normal if production and release are accelerated (Fig. 16.11).

If the platelet concentration is adequate, platelet function can be evaluated by performing a bleeding time or by measuring platelet response to specific agonists in vitro. In general, the bleeding time is the time that it takes blood to cease flowing from a shallow wound that causes injury to capillaries under a hairless skin surface. A bleeding time is performed by creating a wound on the lip, gum, or nasal planum of an animal (see Appendix 16.1 for details). The time that it takes for blood to stop flowing initially is the bleeding time, and it reflects platelet plug formation stopping the capillary blood leakage. If coagulation factors are insufficient, the platelet plug still forms and blood flow is stopped, but the wound begins bleeding again (i.e., rebleed phenomena) because fibrin has not been formed rapidly enough to stabilize the formed platelet plug. If the platelet concentration is decreased, the bleeding time is prolonged. If the platelet concentration is within the reference interval but the bleeding time is prolonged, then platelets are not

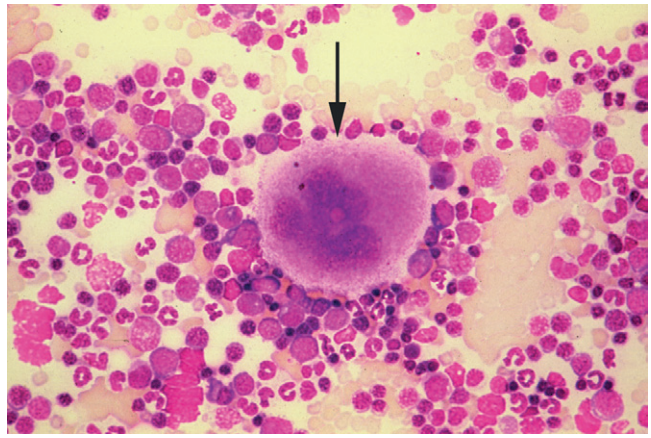

Figure 16.10 Bone marrow film showing a mature megakaryocyte. The cytoplasm is eosinophilic and granular, with a large, syncytial nucleus. (Wright stain, ×500 magnification)

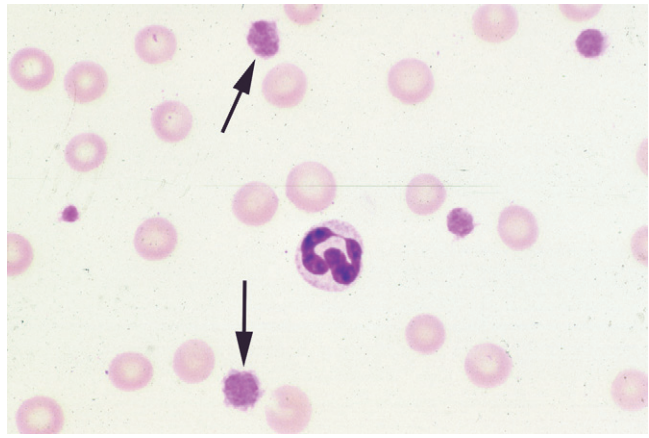

Figure 16.11 Large platelets (macroplatelets) in a thrombocytopenic dog infected with *Ehrlichia canis*. Large platelets suggest accelerated thrombopoiesis and early release of immature forms.

responding appropriately. Once an animal is thought to have a platelet defect, more detailed platelet function analysis can be performed by platelet aggregometry using a wide variety of chemical stimuli for platelets. The pattern of response to known platelet agonists suggests the type of platelet defect. Von Willebrand's disease is the most common platelet function defect in veterinary medicine and is due to abnormal von Willebrand factor synthesis by endothelium and megakaryocytes. Platelets will not respond to collagen appropriately during collagen-induced platelet aggregometry, and measuring plasma von Willebrand factor (vWF) will allow clinical diagnosis. (Von Willebrand's disease is discussed in more detail later.) Thrombocytosis (i.e., a platelet concentration greater than the reference interval) is a nonspecific disorder that usually is not associated with clinical signs in animals but has the potential to predispose to thrombosis and hypercoagulable states. Thrombocytosis is often associated with iron deficiency anemia, inflammatory conditions, epinephrine release, and some forms of myeloproliferative disorders.

Vascular abnormalities

Disorders of hemostasis resulting from defects or abnormalities in the vessel are uncommon. These patients may have a prolonged bleeding time with normal platelet function or a localized vascular injury resulting in bleeding. Methods to evaluate possible vascular causes of bleeding include tissue-incisional biopsy for histologic evaluation of tissue and vessel structure or for biochemical evaluation of collagen. Depending on the species affected, clinical evaluation for scurvy or Cushing syndrome may be appropriate for increased fragility of the skin and collagen in a bleeding patient.

Commonly encountered disorders of hemostasis

Disseminated intravascular coagulation

Disseminated intravascular coagulation (DIC) is associated with many clinical diseases and is caused by excessive activation of coagulation, either regionally or throughout the entire body. Potential causes for the initiation of coagulation can vary from widespread tissue damage, as in heat stroke, to elaboration of procoagulant proteins by neoplastic cell populations, as in some leukemias.[25,26] Diffuse thrombosis, especially in the microcirculation, may occur with resulting exhaustion of coagulation factors and decreased platelet concentrations. In turn, this consumption of coagulation factors and platelets leads to bleeding. Disseminated intravascular coagulation often is life-threatening, and it is difficult to control. Effective control is not possible without determining what induced the DIC and then treating the underlying cause. During DIC, excessive activation of coagulation occurs, and although coagulation times initially may be shorter than normal, most animals present with clinical bleeding and prolonged coagulation times. There are no clear diagnostic parameters to identify DIC, but many of the available tests have abnormal results in most patients with DIC. The aPTT commonly is prolonged, and other frequent abnormalities include a prolonged PT, decreased fibrinogen concentration, decreased platelet concentration, presence of fragmented erythrocytes (i.e., schistocytes), and increased levels of FDPs or d-dimer. Table 16.4 lists the frequency of coagulation abnormalities detected during DIC. No test directly evaluates increased coagulation, but an increased serum FDP or d-dimer concentration provides indirect evidence of increased intravascular coagulation. This is because plasmin simultaneously degrades fibrin clots as they are being formed. Occasionally, FDP and possibly d-dimer concentrations can be increased in patients with resolution of extensive hemorrhage into subcutaneous tissue or pleural or peritoneal spaces, as might occur in patients with warfarin toxicosis.

Table 16.4 Frequency of abnormal coagulation parameters during disseminated intravascular coagulation caused by a variety of diseases.

Coagulation Parameter	Abnormality and % of Time Abnormal
Activated partial thromboplastin time	Prolonged, 87%
Antithrombin III activity	Decreased, 85%
Prothrombin time	Prolonged, 80%
Platelet numbers	Decreased, 80%
Fragmented red blood cells (schizocytes)	Increased, 71%
Fibrin (ogen) degradation products	Increased, 61%
Fibrinogen	Decreased, 61%
Plasminogen activity	Decreased, 49%
Factor 5V activity	Decreased, 46%
Factor VIII:C activity	Decreased, 29%

From Feldman BF, Madewell BR, O'Neill S. Disseminated intravascular coagulation: antithrombin, plasminogen, and coagulation abnormalities in 41 dogs. *J Am Vet Med Assoc* 1981;179;151–154.

Disseminated intravascular coagulation has been associated with many disease conditions, including a wide variety of neoplasms. Dogs with hemangiosarcoma often have associated DIC and thrombosis within the tumor mass. Some leukemias secrete procoagulant proteins that initiate DIC, and many other tumors are associated with DIC as well. Immune-mediated hemolytic anemia, incompatible blood transfusions, and endotoxin-related endothelial injury often initiate DIC.[26] Physical and infectious causes of DIC include heat stroke, snake bite, pancreatitis, and infections with *Rickettsia rickettsii* and *Dirofilaria imitis*.

Hereditary coagulopathies

Inherited coagulopathies and the domestic species in which they have been reported are summarized in Table 16.1. The most common hereditary coagulopathy in domestic animals is hemophilia A, which is a deficiency of coagulation factor VIII:C. Hemophilia A has been described in sheep, dogs, cats, and horses. This disorder is inherited in a recessive manner, and the gene is located on the X chromosome. Hemophilia A often is confused with von Willebrand disease because historically, both these conditions have been termed defects of factor VIII. Factor VIII:C and vWF circulate in plasma in close physical association with one another. Von Willebrand disease, which is the most common hereditary hemostatic defect in domestic animals, is strictly a defect of platelet function. Expected hematologic parameters in patients with hemophilia A and in patients with von Willebrand disease are given in Table 16.5. Whereas hemophilia A is a sex-linked trait and occurs more frequently in male patients, von Willebrand disease is an autosomally transmitted defect with an equal frequency of occurrence in male and female patients.[27]

Table 16.5 Expected coagulation parameter results in hemophilia a or von Willebrand disease.

Test	Hemophilia A	von Willebrand Disease
Activated partial thromboplastin time	Prolonged	Normal to prolonged[a]
Prothrombin time	Normal	Normal
Activated coagulation time	Prolonged	Normal to prolonged
Bleeding time	Normal	Prolonged
Fibrinogen	Normal	Normal
Fibrin(ogen) degradation products	<10 µg/mL	<10 µg/mL
Platelet number	Normal	Normal
von Willebrand factor concentration	Normal	Decreased
Factor VIII:C activity	Decreased	Normal to decreased

[a]Some variable results (normal to decreased or normal to prolonged) reflect the physical relationship that factor VIII and von Willebrand factor have while cirulating in plasma together. Loss of von Willebrand factor may prolong the activated partial thromboplastin time, because factor VIII:C is not appropriately oriented physically but is present.

Other hereditary deficiencies of circulating coagulation factors are infrequent, but examples of all but factor XIII deficiency have been described at least once. Prekallikrein deficiency has been described in several dogs and horses with only mild bleeding tendencies. Deficiency of HMWK was suspected in one horse but was not proved. This horse did not have a bleeding tendency but did have a prolonged aPTT, a normal PT, and normal plasma activity of other intrinsic coagulation factors. Factor XII deficiency has been described in cats and in dogs associated with either prekallikrein deficiency or von Willebrand disease but not purely as a factor XII deficiency. Factor XII deficiency in cats and humans is not associated with bleeding, but in humans, it is associated with a tendency for thrombosis. Interestingly, factor XII is not present in marine mammals, reptiles, or avian species. Factor XI deficiency has been described in cattle and dogs, with severe bleeding in Holstein cattle and milder bleeding tendencies in dogs. Factor IX deficiency (i.e., hemophilia B) has been demonstrated in dogs and cats and, like factor VIII:C, has a sex-linked inheritance pattern. Factor IX deficiency is usually associated with severe bleeding tendencies. Deficiencies of coagulation factors XII, XI, IX, VIII:C, HMWK, or prekallikrein all produce a prolonged aPTT with a normal PT, regardless if these patients bleed clinically or not. Factor X deficiency has been described in dogs and cats with variable bleeding tendencies. Factor VII deficiency has been described in beagle dogs and is usually associated with mild clinical bleeding. These dogs have a prolonged PT and a normal aPTT or ACT. Factor II (i.e., thrombin) deficiency has been described in dogs. Fibrinogen deficiency has been described in dogs and goats and is associated with severe bleeding clinically. Animals with deficiencies of factor X, V, II, or fibrinogen have a prolonged PT, ACT, and aPTT. A deficiency of coagulation factor XIII does not cause a prolonged aPTT or PT because a stable fibrin clot is not necessary for these tests. Hereditary deficiencies of several coagulation factors simultaneously, because of several genetic defects, have been sporadically reported. In addition, hereditary deficiency of all vitamin K–dependent coagulation factors because of abnormal carboxylation has been reported in Devon Rex cats and Rambouillet sheep. Figure 16.12 shows a simple algorithm to aid in establishing the diagnosis of common hereditary deficiencies of coagulation factors.

Acquired coagulopathies

A relatively common cause of acquired coagulopathy in both large and small animals is exposure to vitamin K antagonists that inhibit reduction of oxidized vitamin K back to the active hydroquinone form. Antagonists of vitamin K reduction include coumarin from moldy sweet clover, sulfaquinoxaline (a coccidiastat) that is added to water, and some rodenticides. Rodenticides containing indanedione-type active ingredients have a half-life of 15 to 20 days in the

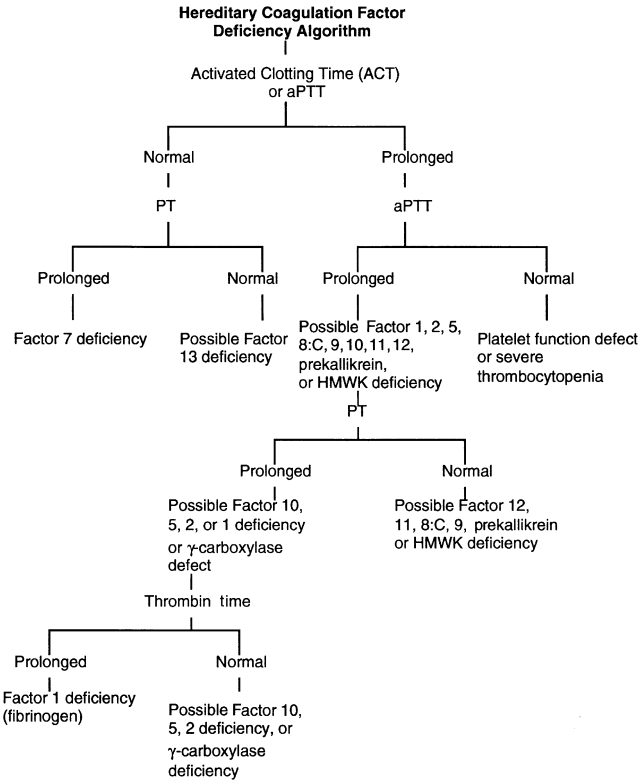

Figure 16.12 Specific coagulation factor deficiencies can be determined by mixing patient plasma with specific factor deficient plasma and then repeating the activated partial thromboplastin time (aPTT) or prothrombin time (PT). The factor-deficient plasma will correct the prolonged coagulation time for all deficiencies except the deficiency that is common to the patient's plasma and the specific factor-deficient plasma. The only exception is factor XIII deficiency, in which a clot urea solubility evaluation is done to determine if factor XIII is active.

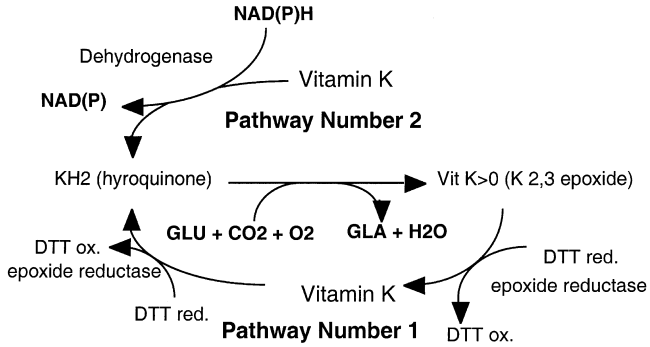

Figure 16.13 This figure depicts the two pathways that mediate the formation of reduced vitamin K_1H_2 (hydroquinone). Pathway number 1 is the physiologic pathway that is normally operative and cycles oxidized vitamin K_1 to the reduced form, but epoxide reductase can be inhibited by warfarin or newly manufactured long-acting antagonists. Pathway 2 is insensitive to warfarin, but requires supraphysiologic concentrations of vitamin K_1 for activity. Therapeutic administration of vitamin K_1 to a warfarin-poisoned patient uses this pathway, but administered vitamin K_1 is exhausted as it is reduced then subsequently oxidixed with accumulation of vitamin K_1 2,3 epoxide in the plasma and liver. Repeated administration of vitamin K1 is required as oxidized vitamin K_1 (2,3 epoxide) is not reduced by pathway 2. DTT = Dithiothreitol, a proposed electron donor, but other molecular species may be the actual electron donor. GLU = glutamic acid in the peptide sequence of the coagulation factor or anticoagulant protein, GLA = gamma-carboxyglutamic acid that has been transformed in the peptide sequence.

body, whereas warfarin-type rodenticides have a half-life of only 40 hours. Administration of vitamin K allows vitamin K to be reduced to the active hydroquinone form through a second pathway, which operates at a much higher concentration of vitamin K than is usually present (Fig. 16.13). Low availability of vitamin K is also associated with obstructive hepatopathy (i.e., lack of bile for absorption of vitamin K), malabsorption syndromes with an inability to absorb lipids, and low dietary levels of vitamin K. In small animals, administration of antibiotics that inhibit production of vitamin K by gut flora may result in deficiency. Severe liver disease commonly results in an acquired coagulopathy. Most coagulation factors are produced by hepatocytes; moreover, the liver is responsible for the removal of activated coagulation factors and FDPs from plasma.

Uncommon causes of acquired coagulopathy include amyloidosis, which is associated with selective factor X deficiency because this factor is incorporated in the amyloid matrix, thereby depleting plasma factor X concentrations. Autoimmune disease, with an autoantibody directed against a coagulation factor, also has been described in domestic animals but occurs only rarely. Figure 16.14 provides an algorithm to aid in establishing the diagnosis of acquired abnormalities and other defects of hemostasis that result in clinical bleeding.

Thrombocytopenia

Numerous conditions that affect the bone marrow may cause decreased platelet production (see Chapter 14). Whole-body irradiation, drugs, toxins, infectious agents, neoplastic processes, or immune-mediated disorders may result in decreased production of platelets. Estrogen toxicosis is a common cause of decreased production in dogs and ferrets.

Increased platelet consumption is associated with DIC (discussed earlier), hemangiosarcoma in dogs, vasculitis, and other types of vascular injury. Blood loss does not result in significant thrombocytopenia; the platelet concentration rarely is less than 100,000/μL secondary to hemorrhage.

Immune-mediated thrombocytopenia is one of the most common causes of thrombocytopenia in dogs. Antibodies may be directed specifically against platelet epitopes or may be antigen (e.g., drugs or infectious agents)–antibody complexes on or adsorbed to platelets. Administration of intravenous heparin may induce mild thrombocytopenia in some

horses, and heparin overdose may result in severe thrombocytopenia in cats. Heparin-induced thrombocytopenia is thought to be immune-mediated in humans secondary to antibodies directed against a heparin–platelet component complex. Recently, better methods to determine if antibodies are present on the surface of platelets have been developed. These methods are either indirect (examining plasma) or direct (examining patient platelets). Direct methods are more sensitive but have less flexibility regarding both time and handling.

The most common infectious cause of thrombocytopenia in dogs is ehrlichiosis. Infection with *Ehrlichia canis* and, less commonly, *Anaplasma platys*, *E. ewingii*, and *E. chaffeensis* cause thrombocytopenia (Figs. 16.15 through 16.17, Table 16.6). *Ehrlichia canis* is thought to initially cause platelet destruction by immune-mediated mechanisms. Then, late in the disease, the agent causes bone marrow aplasia, with a subsequent decrease in platelet production.

Defects of platelet function

Acquired

Platelet function can be inhibited by most nonsteroidal anti-inflammatory drugs, abnormal plasma proteins (myeloma paraproteins), proteins such as FDPs, autoantibodies, phenothiazine tranquilizers, or plasma waste products that accumulate in patients with uremia.

Hereditary

Platelets may not function because of hereditary defects such as von Willebrand disease or intrinsic defects of platelet receptors or signaling that have been described in domestic as well as laboratory animals.[28] Table 16.7 provides several specific examples of hereditary platelet defects that have been described. The most common hereditary platelet defect in domestic animals is von Willebrand disease.[27]

Von Willebrand disease is associated with the lack of vWF, a glycoprotein that is synthesized and secreted by endothelial cells and megakaryocytes. Von Willebrand factor circulates in close physical association with factor VIII:C, and is a heterogeneous glycoprotein composed of various-sized multimers of an identical 270-kDa polypeptide subunit linked to each other by disulfide bonds. Plasma concentrations of vWF can be measured by immunologic methods (immunoelectrophoresis and, more recently, enzyme-linked immunosorbent assay). Results are usually reported as a percentage of normal pooled plasma. Circulating multimers can be separated by sodium dodecyl sulfate–agarose electrophoresis and visualized with labeled antibody after electroblotting (Fig. 16.18). These multimers range in size from 500 to 10^4kDa. The higher-molecular-weight multimers are most effective in mediating platelet adherence and aggregation. Von Willebrand factor binds to surface glycoprotein 1b of platelets and also to IIb/IIIa, which usually also binds fibrinogen. If the level of vWF is deficient or the vWF itself is defective, platelets are not activated, because they do not recognize and respond to collagen in the basement membranes or extravascular tissue. Release of stored vWF in endothelium can be stimulated by 1-desamino-8-D-arginine vasopressin (DDAVP) administration.

Von Willebrand disease is classified into three types depending on the underlying molecular cause. Type I has a decreased level of vWF:Ag (antigen), with levels of all plasma multimers decreased as well. This group includes the Welsh corgi (percentage of breed population affected or carriers, 43%), Doberman pinscher (73%), German shepherd (35%), golden retriever (30%), and poodle (30%). Type I appears to have autosomal dominant inheritance with incomplete penetrance. Type II animals have decreased levels of vWF:Ag, with a disproportionate decrease in the level of high-molecular-weight multimers. The German shorthair pointer dog is a notable member in this category. The inheritance pattern is unknown. Type III animals have undetectable levels of vWF:Ag. The Scottish terrier (30%), Chesapeake Bay retrievers, and Shetland sheepdog (23%) are of this type. The inheritance pattern appears to be homozygous recessive in Scottish terriers and Chesapeake Bay retrievers and autosomal dominant with incomplete penetrance in Shetland sheepdogs. Von Willebrand disease has also been identified in rabbits, cats, horses, and swine. Acquired causes of the disease in dogs are in dispute, but such causes do occur in humans.

Vascular causes of bleeding

Acquired

Injury to the endothelium, as occurs with Rocky Mountain spotted fever or heat stroke, may cause widespread hemorrhage with subsequent activation of coagulation. Acquired collagen disorders such as scurvy may be associated with bleeding because of the increased fragility of vessel walls. Localized inflammatory disease may be associated with hemorrhage. If recurrent localized hemorrhage is detected but a defect in either platelets or coagulation factors is not apparent, then a biopsy may be helpful in determining the cause of hemorrhage.

Hereditary

Hereditary collagen disorders, such as Ehlers-Danlos syndrome and Marfan syndrome, as well as acquired collagen disorders, such as scurvy and Cushing syndrome, have been associated with clinical bleeding. In part, bleeding results from increased vascular fragility caused by defective or decreased collagen, and in some cases may also be caused by decreased platelet responsiveness to abnormal collagen.

Thrombosis

Thrombosis can be life-threatening, and it is a common ultimate cause of death in a wide variety of clinical diseases involving altered blood flow, endothelial injury, or

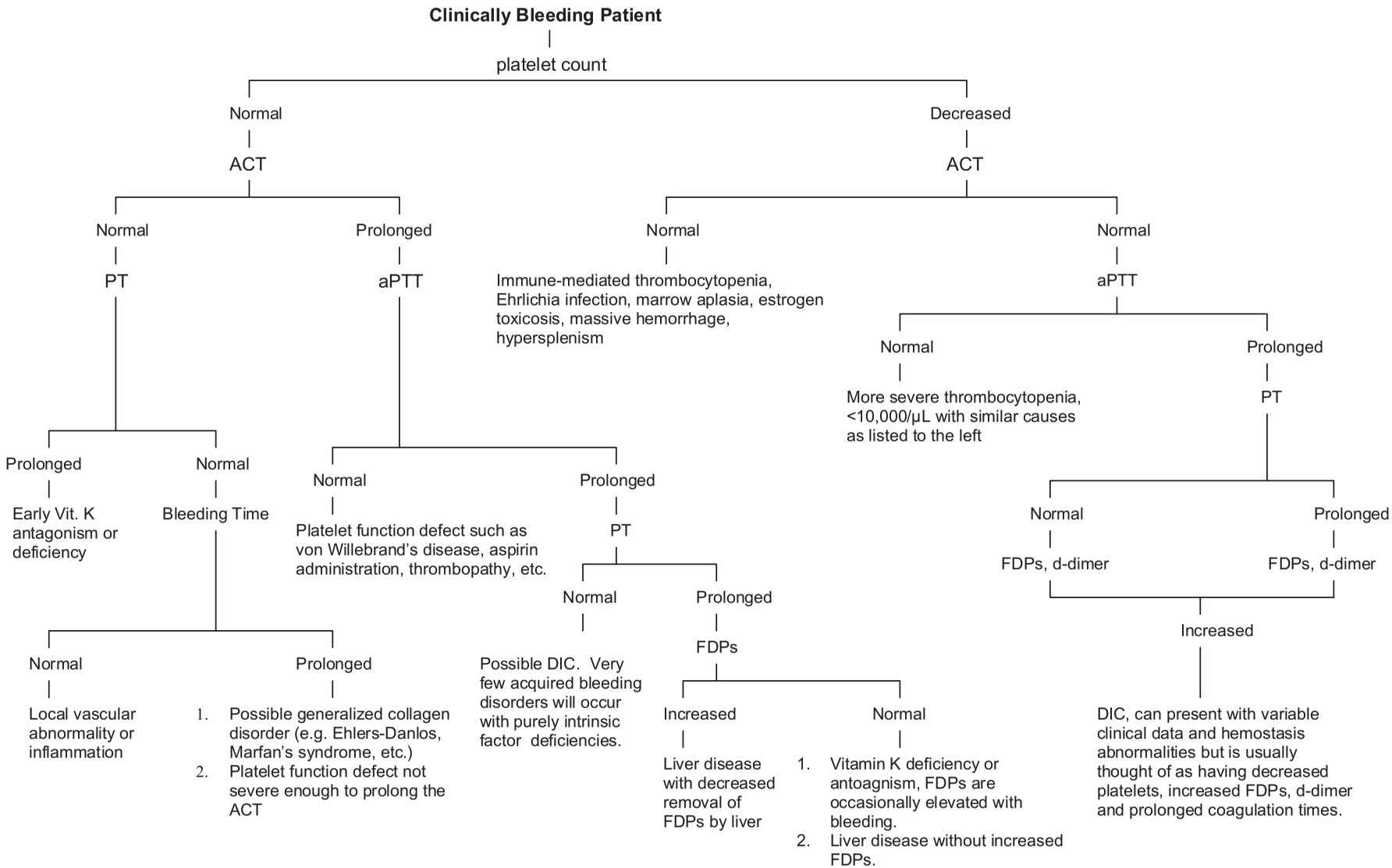
Clinically Bleeding Patient
platelet count
Normal
Decreased
ACT
ACT
Normal
Prolonged
Normal
Normal
PT
aPTT
Immune-mediated thrombocytopenia, Ehrlichia infection, marrow aplasia, estrogen toxicosis, massive hemorrhage, hypersplenism
aPTT
Normal
Prolonged
More severe thrombocytopenia, <10,000/µL with similar causes as listed to the left
PT
Prolonged
Normal
Normal
Prolonged
Early Vit. K antagonism or deficiency
Bleeding Time
Platelet function defect such as von Willebrand's disease, aspirin administration, thrombopathy, etc.
PT
Normal
Prolonged
Normal
Prolonged
FDPs, d-dimer
FDPs, d-dimer
Normal
Prolonged
Increased
Local vascular abnormality or inflammation
1. Possible generalized collagen disorder (e.g. Ehlers-Danlos, Marfan's syndrome, etc.)
2. Platelet function defect not severe enough to prolong the ACT
Possible DIC. Very few acquired bleeding disorders will occur with purely intrinsic factor deficiencies.
FDPs
DIC, can present with variable clinical data and hemostasis abnormalities but is usually thought of as having decreased platelets, increased FDPs, d-dimer and prolonged coagulation times.
Increased
Normal
Liver disease with decreased removal of FDPs by liver
1. Vitamin K deficiency or antoagnism, FDPs are occasionally elevated with bleeding.
2. Liver disease without increased FDPs.

Figure 16.14 This algorithm provides a general guide for a diagnostic approach in clinical practice when presented with a bleeding patient, progressing from tests that can be done in most clinical settings to those that are more difficult to perform. The final diagnosis for each of these disease conditions relies on additional serum biochemical, hematologic, physical, serologic, and tissue evaluations or other specialized tests. This list is not all-inclusive, but it does include the most common clinical diseases in practice. aPTT, activated partial thromboplastin time; FDP, fibrinogen degradation products; PT, prothrombin time.

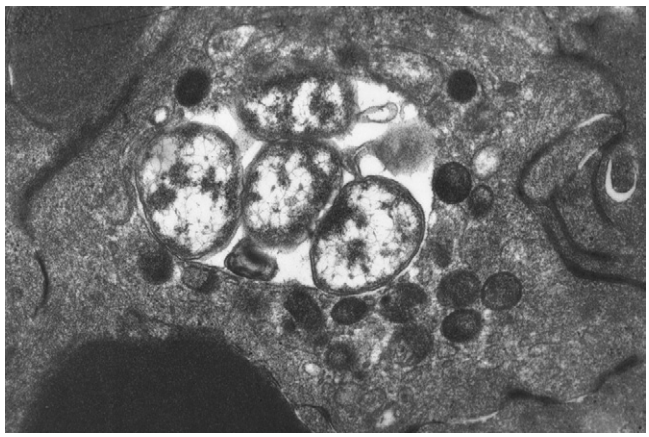

Figure 16.15 Transmission-electron micrograph of a platelet containing a morula of *Anaplasma platys*. The elementary bodies are surrounded and held together by a second membrane.

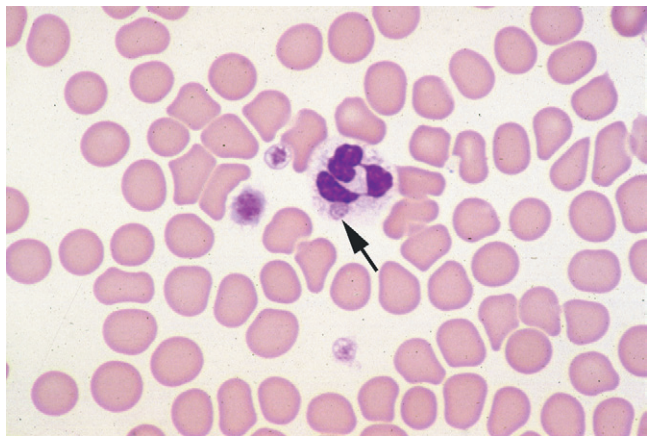

Figure 16.17 Peripheral blood film from a dog containing an *Ehrlichia ewingii* morula (arrow) in a neutrophil. (Wright stain, X1000 magnification)

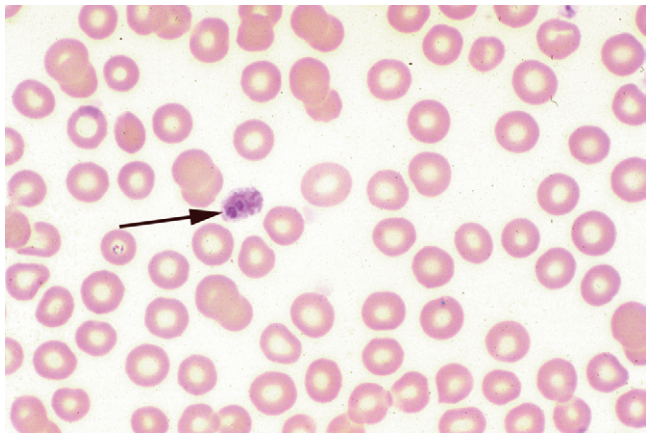

Figure 16.16 Wright-stained blood film from a dog with two *Anaplasma platys* morulae in a platelet (arrow). (X1000 magnification)

hypercoagulable conditions. Clinical thrombosis is especially common in some specific clinical diseases.

Decreased circulating anticoagulant proteins, notably AT, occurs in animals with nephrotic syndrome, because AT is a small-molecular-weight protein that is lost in the glomerular ultrafiltrate. Decreased levels of AT also occur in response to some drugs, such as asparaginase, a chemotherapeutic drug used in patients with lymphosarcoma. Increased concentrations of coagulation factors make blood hypercoagulable, and thrombosis may occur during late pregnancy after the administration of growth hormone implants or of some drugs, such as asparaginase. Cushing disease is associated with thrombosis, but the pathophysiology in dogs is unknown. Localized thrombosis can result from turbulent or misdirected blood flow, and generalized thrombosis can result from widespread vascular injury caused by chemicals, endotoxin, immunologic mechanisms, or infectious agents, such as infectious canine hepatitis virus or *Rickettsia rickettsii*.

Conclusion

Effective hemostasis is the product of coagulation factors, platelets, and vessels working in concert to cease the flow of blood from an injured vessel and allow healing and repair to occur, with subsequent reestablishment of blood flow to the tissues. Defects in any component of this delicate balance may result in excessive hemostasis (i.e., thrombosis) or in adequate hemostasis (i.e., bleeding). The challenge in each clinical case is to logically consider the possible causes and to fully evaluate them. Each clinical case should have at least an aPTT or ACT, a PT, and a platelet count performed to evaluate commonly encountered disturbances of hemostasis. Additional clinical evaluations are determined by the results of these tests and their relationship to the results of a thorough physical examination.

Table 16.6 Species of *Ehrlichia* and related *Anaplasma* spp. important in animals and humans with the disease names, cells infected, vectors, and distribution.

Naturally Occurring *Ehrlichia* and related *Anaplasma* spp.					
Species	**Disease Name**	**Natural Hosts**	**Cells Infected**	**Primary Vectors**	**Distribution**
Ehrlichia chaffeensis	Human monocytic (monocytotropic) ehrlichiosis (HME)	Human, dogs, deer	Primarily mononuclear cells	*Amblyomma americanum* (Lone Star tick)	USA, Europe, Africa, South and Central America
Anaplasma phagocytophilum (previous names: HGE agent, *E. equi*, *E. phagocytophilum*)	Human granulocytic (granulocytotropic) ehrlichiosis (HGE), equine ehrlichiosis, tick-borne fever; pasture fever	Human, deer, wild rodents, elk, horses, llamas, sheep, cattle, bison	Granulocytes	*Ixodes scapularis* and *I. pacificus* in the USA; *I. ricinus* in Europe	USA, Great Britain, Europe
Ehrlichia canis	Canine ehrlichiosis	Dogs, wolves, jackals	Primarily mononuclear cells	*Rhipicephalus sanguineus* (brown dog tick)	Worldwide
Ehrlichia ewingii	Canine and human granulocytic ehrlichiosis	Human and dogs	Granulocytes	*Amblyomma americanum* (Lone Star tick)	USA
Anaplasma platys (previous name: *E. platys*)	Canine cyclic thrombocytopenia	Dogs	Platelets	*Rhipicephalus sanguineus* (brown dog tick)	USA, Japan, Venezuela, Israel, Thailand, Europe, Taiwan, Greece

Table 16.7 Hereditary platelet function defects in domestic and laboratory animals.

Species/Breed Affected	**Type of Defect/Gene Affected**
Otterhounds, Spitz, Great Pyrenees, Thoroughbred cross, Quarter horse, Oldenburg horse (thrombasthenia)	All are mutations involving α_{IIB} of the $\alpha_{IIB}\beta_3$surface integrin. Altered fibrinogen binding.
Rats, mice	Various additional storage pool defects of α or δ granules (dense bodies) in platelets.
Chédiak-Higashi syndrome (cattle, cats, killer whales, beige mice, fox, mink)	Storage pool granule defect (LYST gene defect in Japanese Black Cattle)
Simmental cattle, basset hound, Spitz, Landseer ECT dogs (thrombopathia)	Cytoskeletal assembly (calcium-diacylglycerol guanine nucleotide exchange factor 1)
Fawn-hooded rats	Serotonin release.
Pale ear mouse, gray collies (cyclic hematopoiesis), cocker spaniels	Dense body (δ granule) defects: Mouse counterpart to Hermansky-Pudlak syndrome. Collie has defect in gene. Cocker spaniels have ADP deficiency.
Dogs (Johnson,1999)	Arachidonate insensitivity.
German shepherds	Phosphatidylserine defect, lack procoagulant activity of platelets.
Cavalier King Charles spaniels	Beta-1 tubulin defect suspected; macroplatelets. Dogs don't bleed.

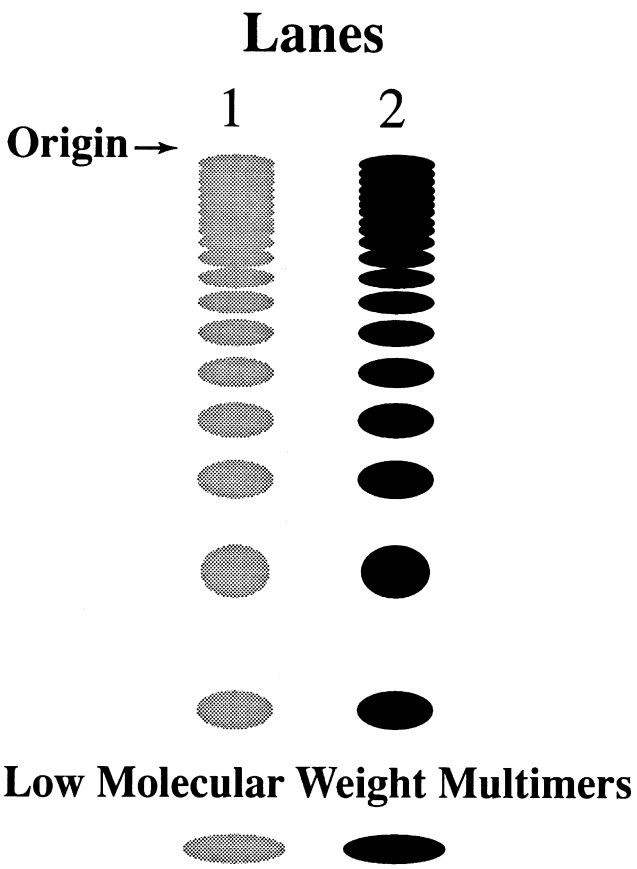

Figure 16.18 Plasma subjected to low resolution, sodium dodecyl sulfate–gel electrophoresis. Large-molecular-weight vWF multimers are closest to the origin, with low-molecular-weight multimers being furthest from the origin. Lane 1 depicts a patient with type 1 von Willebrand disease and a decrease in all types of vWF multimers compared with normal values, as shown in lane 2. Selective loss of large-molecular-weight multimers, or absence of all multimers, characterizes patients with other types of von Willebrand disease.

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Appendix 16.1

Minimum laboratory information base to evaluate a patient with a hemostatic defect

1. Activated clotting time or activated partial thromboplastin time.
2. Prothrombin time.
3. Platelet count.
4. Bleeding time (possibly).

Other tests of hemostasis are may be indicated by the results of these preliminary examinations.

Laboratory methods in clinical practice

Bleeding time

Method. Cause a reproducible wound (#11 BP blade, fixed to penetrate 1/8–1/4 inch, or use a commercial product that creates a small wound 1 mm in depth) on a smooth, non-haired portion of the skin (e.g., gingiva, nasal planum, umbilicus, upturned lips held in place by a gauze tied around the muzzle). Next, gently remove beads of blood from wound margins, not touching the wound itself, with filter paper at 30-second intervals, and then record the time taken to stop bleeding.

Principle. Initial hemostasis reflects platelet function and numbers. May have rebleed phenomenon if fibrin clot does not adequately form after the initial cessation of bleeding, and this may be the result of coagulation factor deficiency.

Reference interval. 1–5 minutes

Note. The results of this test can be influenced by certain nonsteroidal anti-inflammatory drugs and by some sedatives or analgesics.

Activated clotting time (ACT)

Utilizing only 2 mL draw tubes and nonspecialized equipment

Method. Draw 2 mL of venous blood into a prewarmed (37°C) tube (available at http://www.vetlab.com/ACT%20System.htm or http://www.haemtech.com/ACT.htm?gclid=CLKbzKmn9JwCFQtN5QodvSSur) containing sterile, diatomaceous earth. Begin timing with a stopwatch when blood first enters the tube. Invert the tube five times to mix, and then place it in 37°C heating block, removing it every 5–10 seconds after 1 minute of incubation to check for the first soft clot formation.

Principle. Contact activation proteins are fully activated when blood enters the tube. The time to clot formation depends on adequate mixing and activation, temperature, coagulation factor concentrations, and platelet numbers as well as function. Prolonged times suggest intrinsic or common pathway factor deficiencies. Platelet numbers must be very low to prolonged the ACT (<10,000 cells/μL). The ACT does not evaluate factor VII, and it generally does not evaluate platelets.

Reference interval

Dogs	79 ± 7.1 seconds
Horses	163 ± 18 seconds
Cows	145 ± 18 seconds

Note. Salicylates, anticoagulants, some antibiotics, and barbiturates can inhibit clot formation.

Utilizing specialized equipment

Method. Draw fresh whole blood into a tube containing negatively charged particles and time for the formation of a clot in a commercially available endpoint detection machine.

The type of negatively charged particle affects the "normal" length of ACT:

Celite = Diatomaceous Earth

- Used with high circulating levels of heparin—cardiopulmonary bypass
- Black top glass test tube

Kaolin

- Used with high circulating levels of heparin—cardiopulmonary bypass
- Gold top glass test tube

Glass particles

- Used with medium circulating levels of heparin—haemodialysis
- Clear top plastic test tube

Type of endpoint detection machine affects normal and therapeutic values:

Hemochron® (International Technidyne Co.,Edison, NJ)

- A warmed test tube is rotated inside the instrument. As the blood clots, it displaces the magnet within the test tube. The clotting time is determined when the magnet has displaced enough to activate a proximity switch

Medtronic HemoTec® (Medtronic HemoTec Inc., Englewood, CO, USA)

- A mechanical plunger is dipped in and out of a kaolin activated blood sample. The instrument optically senses the

time it takes the plunger to move through the specimen. Clotting is defined by the "drop time" threshold for the plunger.

Principle. Contact activation proteins are fully activated when blood enters the tube. The time to clot formation depends on adequate mixing and activation, temperature, coagulation factor concentrations, and platelet numbers as well as function. Prolonged times suggest intrinsic or common pathway factor deficiencies. Platelet numbers must be very low to prolong the ACT (≤10,000 cells/μL). The ACT does not evaluate factor VII, and it generally does not evaluate platelets.

Note. Salicylates, anticoagulants, some antibiotics, and barbiturates can inhibit clot formation.

Activated partial thromboplastin time (aPTT)

Method. Blood is collected from a vein nontraumatically into 3.2% sodium citrate anticoagulant in a blood:anticoagulant ratio of 9:1. Plasma is harvested by centrifugation and mixed with a phospholipid platelet substitute (ether extract of brain); an activator such as kaolin, diatomaceous silica, or ellagic acid; and calcium. A pooled plasma sample is run as a control, samples are done in duplicate, and an average time is reported. Electrical impedance or optical endpoint systems are used to detect fibrin clot formation. Factor XIII activity is not evaluated with this test, because only a loose, noncovalently linked clot is necessary for initiating an endpoint by optical, impedance, or manual methods.

Principle. Contact activator proteins are fully activated, and platelets are replaced by phospholipid. Normal time reflects adequate levels of intrinsic and common pathway factors.

General reference intervals

Dogs	9–11 seconds
Cats	10–15 seconds
Horse	25–45 seconds

Note. Several types of contact activators are used commercially for the aPTT, and each activator has an associated normal reference interval for a given species. The laboratory performing the test should provide a reference interval for different species. Circulating anticoagulants (heparin, fibrinogen degradation products, autoantibodies) inhibit the reaction. Mix equal volumes of normal plasma and patient plasma, and repeat the test. If the time corrects, there was a deficiency, not a circulating anticoagulant, that caused the prolonged time. Nontraumatic venipuncture is very important, because release of tissue thromboplastin shortens most coagulation times. One or more factors must be decreased by 70 to 80% before significant prolongation occurs in the coagulation times (aPTT, ACT or PT) (Fig. 16.19).

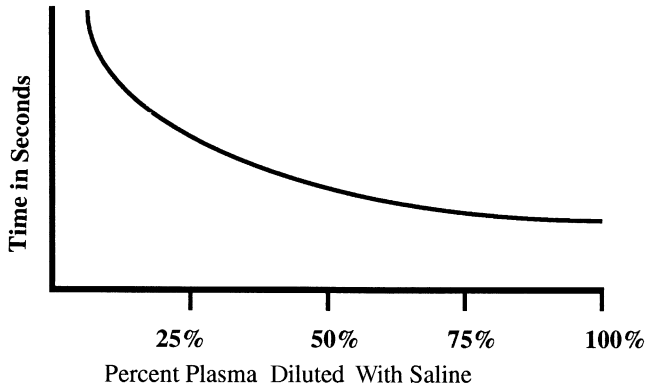

Figure 16.19 (In Appendix 16.1) The approximate change in activated partial thromboplastin time (apt) or prothrombin time (PT) with serial dilutions of normal plasma using saline. A more than approximately 70% loss of plasma coagulation factor activity is associated with a marked change in coagulation time.

Prothrombin time (PT)

Method. Blood is collected using the same method as that described for an aPTT, and plasma is harvested. Plasma is mixed with tissue thromboplastin (rabbit brain tissue thromboplastin) and calcium. The time to clot formation depends on the presence of factor VII and common pathway factors. Electrical impedance or optical endpoint systems are used to detect fibrin clot formation. Factor XIII activity is not evaluated with this test, because only a loose, noncovalently linked clot is necessary for initiating an endpoint by optical, impedance, or manual methods. It should be noted that an assay called PIVKA test (Thrombotest®, Axis Shield, UK) has been advocated for detecting PIVKAs that form with vitamin K antagonists or deficiency, and although claims have been that this test is sensitive to both an increase in PIVKAs (which are supposed to inhibit the reaction) as well as a decrease in the functional coagulation factors II, VII, and X (especially factors VII and X), there is no proof that PIVKAs actually inhibit the reaction. This PIVKA test is merely a modified Prothrombin time.

Principle. Factor III is supplied with a platelet substitute, and normal time reflects adequate levels of factor VII and common pathway factors.

General reference intervals

Dogs	6.4–7.4 seconds
Cats	7–11.5 seconds
Horses	9.5–11.5 seconds

Note. The aPTT and PT can be done in a local hospital, but plasma is best transported on ice. There should not be a long delay between collection and testing.

Fibrinogen concentration

Method. Methods include heat precipitation (see Chapter 29 for details), modified thrombin time, and immunologic methods. Heat precipitation is the least accurate when fibrinogen concentrations are low but the most accurate when fibrinogen is high. Modified thrombin time is most accurate if no dysfibrinogenemia is present. Immunologic methods do not require normal function of fibrinogen, which is the most abundant coagulation factor in plasma.

General reference intervals

Cats	0.05–0.3 g/dL
Cow	0.2–0.7 g/dL
Horses and goats	0.1–0.4 g/dL
Dogs, sheep, and pigs	0.1–0.5 g/dL

Note. Concentrations may be increased during inflammation or decreased when consumed during coagulation (e.g., disseminated intravascular coagulation).

Platelet enumeration

Method. Methods include use of a hemocytometer and automated platelet enumeration by particle counters. Blood must be collected in EDTA using nontraumatic venipuncture, because trauma activates platelets and causes clumping. Feline platelets clump readily. If clumping is noted on the blood film, then the platelet count is factitiously decreased. Platelets can be estimated when looking at a blood film. Less than three platelets per oil immersion field suggests the platelet count is 50,000 platelets/μL or less if platelet clumping is not present.

Reference intervals

Dogs	200,000–500,000 platelets/μL
Cats	200,000–500,000 platelets/μL
Horses	100,000–600,000 platelets/μL
Cows	200,000–800,000 platelets/μL

Note. This test should include examination of blood film platelet morphology when evaluating circulating platelet numbers, and may require examination of bone marrow to evaluate platelet production. Younger platelets are larger when viewed on peripheral blood films.

Fibrin(ogen) degradation products (FDPs)

Method. The most common method is to use the Thrombo-Wellco® Kit from Remel Inc. (http://www.remelinc.com/). Immunoglobulin-coated latex beads that detect fragments D and E of fibrin or fibrinogen are mixed with diluted serum that has had an inhibitor of plasmin added. The beads agglutinate if sufficient D and E fragments are present. Results are sensitive to 2 μg/mL. Other commercial kits are available and perform well.

Principle. Activation of the contact activation system also activates plasmin. Fibrinogen and fibrin forming in a clot are broken down by plasmin, and the fragments circulate in the peripheral blood. Microvascular injury and repair are constantly occurring against a background of FDPs being produced (usually <10 μg/mL). Concentrations of FDPs increase with increased coagulation (as in disseminated intravascular coagulation).

Note. The FDPs inhibit thrombin and platelet function by competing with fibrinogen for binding sites, but they lack the structure to form crosslinks or a fibrin meshwork.

Reference interval. Most animals have FDP concentrations of less than 10 μg/mL.

D-dimer determination

Method. Blood is collected in 3.2% sodium citrate anticoagulant in a ratio of 1 part anticoagulant to 9 parts blood, then mixed. Several kits are available commercially and some have been validated for dogs, cats, and horses. There is no interference from fibrinogen in the assay.

Principle. A monoclonal antibody in most commercial kits detect the neoantigen, D-dimer, found in plasma or fluids in which there has been plasmin degradation of crosslinked fibrin. The interpretation is more specific than FDP quantitation, which also detects degradation of fibrinogen.

Thromboelastography

Method. Blood is collected in 3.2% sodium citrate anticoagulant in a ratio of 1 part anticoagulant to 9 parts blood, then mixed. Blood is then added to a plastic cup placed on an angle with a sensing rod that oscillates and tissue thromboplastin is added to initiate the coagulation cascade. Other versions of instruments rotate the cup relative to the sensing rod. The change in resistance detected by the probe is plotted on a paper strip. The shape of the paper tracing is related to how fast a clot forms and subsequently lyses giving, a nonspecific profile of coagulation strength and clot lysis capability of blood. Disadvantages of TEG include a relatively high coefficient of variation, poorly standardized methodologies, and limitations on specimen stability of native whole blood samples.

Four values are determined by this test in the classic assay: the R value (reaction time), the K value, the angle, and MA (maximum amplitude). The MA represents the clot strength. A mathematical formula determined by the specific manufacturer can be used to determine a Coagulation Index (CI) which is an overall assessment of coagulability.[29]

17 Principles of Blood Transfusion and Crossmatching

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Blood groups (blood types)

Blood groups or types are classifications made on the basis of species-specific antigens on the surface of erythrocytes. Antigens associated with platelets, leukocytes, and plasma proteins may also be important in inducing immune-mediated reactions in host animals during transfusion therapies. Naturally occurring alloantibodies against another blood type can be present in an animal's plasma despite lack of prior exposure to those erythrocyte antigens. More commonly, antibodies against erythrocyte antigens are induced in response to exposure, either via blood transfusion, transplacental exposure or, in the case of neonatal isoerythrolysis, through colostrum. Blood groups in the common domestic and pet exotic species are described here.

Dog

The dog erythrocyte antigen or blood type system is known as the DEA system, and includes DEA 1 (1 neg, 1.1, 1.2, 1.3) and DEAs 3–8. Naturally occurring alloantibodies are of limited clinical significance in dogs, unlike the situation in cats.[1,2] The most important canine blood types are DEAs 1.1 and 1.2, which comprise approximately 60% of blood types in the canine population.[2] These antigens will induce severe transfusion reactions in previously sensitized dogs. DEA 1.3 has been described in German shepherd dogs in Australia.[3] DEA 4 is a high frequency antigen that can result in hemolytic transfusion reactions in DEA 4-negative dogs previously sensitized by DEA 4-positive blood transfusions.[4] In addition, DEA 7 may elicit an antibody response in dogs that lack it, and DEAs 3 and 5 are low incidence antigens in which naturally occurring alloantibodies can occur; anti-DEA 3, 5, and 7 can result in delayed transfusion reactions.[5,6] The high frequency *Dal* erythrocyte antigen has been demonstrated as lacking in some Dalmatian dogs thus resulting in the potential for clinically important transfusion reactions in that breed.[7]

Cat

Three blood types are routinely recognized in the feline AB blood group system. Type A is the most common and is estimated to occur in more than 95% of domestic shorthair (DSH) and domestic longhair (DLH) cats in the United States.[1,8] Type B occurs with varied frequency (<5–25%) in the Abyssinian, Birman, Himalayan, Scottish fold, Somali, Sphinx, Maine coon, Norwegian forest, and Persian cat breeds, whereas the highest frequency (25–50%) has been reported in the British shorthair, Cornish Rex, Devon Rex, and Turkish angora/van breeds.[1,8] A higher percentage of DSH/DLH cats in the West Coast region of the United States, Europe, Japan, India, Turkey, and Australia are reportedly type B;[1,9,10] a recent report found up to 30% of DSH/DLH cats were type B in the United Kingdom.[11] To date, type B has not been found in the Siamese, Burmese, Oriental shorthair, Tonkinese, American shorthair, or Russian blue breeds. Type AB is extremely rare but has been reported in DSH/DLH cats and in certain families of breeds in which type B blood also occurs, including the Abyssinian, Birman, British shorthair, Norwegian forest, Somali, Scottish fold, and Persian.[12] A novel erythrocyte antigen, *Mik*, has been described in DSH cats.[13] It is important to consider that geographical variation of feline blood types is significant, even in mixed breed cats, and the risk of administering a potentially fatal type A or AB blood transfusion to a type B cat, at least in some populations, may be as high as one in five.[11,14]

Cats have naturally occurring alloantibodies: anti-A, anti-B, and anti-*Mik*.[13,15] All type B cats have high serum concentrations of alloantibodies that are strong hemagglutinins and hemolysins against type A erythrocytes. Type A cats generally have weak hemagglutinins and hemolysins against

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type B erythrocytes. Newborn kittens have no alloantibodies because of their endotheliochorial placenta, but colostral transfer of immunoglobulin (primarily IgG) occurs. Neonatal isoerythrolysis (NI) occurs in type A or AB suckling kittens born to type B queens with transfer of the anti-A alloantibodies via the colostrum.[8,15,16] Because DSH/DLH cats have a low frequency of type B blood, less than 2% of random matings produce litters at risk for NI, whereas Birman and Devon Rex matings carry a risk of 15% and 25%, respectively, for producing NI.[16] The *Mik* antigen is clinically relevant in that AB-matched blood transfusions with *Mik*-positive blood to cats lacking this antigen can result in acute post-transfusion hemolysis.[13]

Horse and donkey

The seven internationally recognized blood groups in the horse, A, C, D, K, P, Q, U, include more than 30 antigens.[17,18] Because of various antigenic combinations, no universal donor exists. To minimize transfusion reactions, blood typing of the donor and recipient is ideal but often impractical; at the very least crossmatching prior to transfusion must be performed.[19] Aa and Qa alloantigens are extremely immunogenic; both are hemolysins, and most cases of NI are associated with anti-Aa or -Qa antibodies. In addition, anti-Aa and -Ca are agglutinating antibodies; therefore it is important to note that Qa will be negative on an agglutination test. Blood types vary among horse breeds with Thoroughbreds and Arabians having a high prevalence of antigens Aa or Qa compared to other breeds, and Standardbreds lacking the Qa antigen.[17,20] A unique donkey and mule erythrocyte antigen (donkey factor), not found in the horse, puts all mule pregnancies at risk for NI.[21,22] Although erythrocyte antigens Aa or Qa have been associated with approximately 90% of equine NI cases,[23] other antigens including Ab, Dc, Db, De, Dg, Pa, Qc, and Ua have rarely been associated with NI in foals.[17,20,23,24] Reportedly, the anti-Ca antibody does not cause NI and, in fact, may actually prevent NI by removal of potentially sensitizing cells from the circulation.[25]

Cattle

The eleven recognized blood groups in cattle are A, B, C, F, J, L, M, R, S, T, and Z with groups B and J being the most clinically relevant. The B group itself has more than 60 antigens, thereby making closely matched blood transfusions difficult. The J antigen is a lipid found in plasma that is not a true erythrocyte antigen; it is usually acquired to varying degrees early in life. Cattle with anti-J antibodies, despite having erythrocytes with a small amount of adsorbed J antigen that apparently type negative, can develop transfusion reactions when receiving J-positive blood.[17,26] Vaccinations of blood origin (some anaplasmosis and babesiosis vaccines) may sensitize cattle to erythrocyte antigens which could result in NI in subsequent calves.[17]

Sheep and goat

Seven blood groups have been identified in sheep (A, B, C, D, M, R, X). The B system has over 52 factors.[17] The R system is similar to the J system in cattle (i.e., antigens are soluble and passively adsorbed to erythrocytes). The M-L blood group in sheep is related to active potassium transport in reticulocytes.[27] The blood groups of the goat (A, B, C, M, J) are very similar to those of sheep with the B system equally complex. Many of the reagents used for blood typing of sheep also have been used to type goats.

Exotic pets

No blood groups have been identified to date in ferrets.[28] Blood transfusions can be administered safely without the need for crossmatching even when multiple transfusions are indicated such as for severe estrus-induced aplastic anemia.[29]

Little to no information is available on blood groups in rabbits and exotic pet species of birds or reptiles. In these cases crossmatching prior to transfusion is recommended and homologous transfusions using species-specific blood are advised. A low-volume simplified crossmatch procedure (see Appendix 17.1) can be utilized in these species particularly for cases in which a prior transfusion has been administered.[30,31]

Transfusion therapy

General principles and indications

In general, blood transfusions should not be administered to animals without blood typing and crossmatching the recipient and donor to decrease the likelihood of a transfusion reaction. In addition to potential adverse reactions, the shortened survival of mismatched transfused cells results in ineffective therapy. Crossmatching and blood typing are particularly important in breeding females to avoid primary sensitization and risk of future offspring developing hemolytic disease. General guidelines and information related to transfusion medicine are summarized in Table 17.1; the reader is referred to the text for background and explanation. Blood typing for canine DEA 1.1 and for feline types A and B is feasible in the general veterinary practice.[32,33] More extensive typing is available through universities and/or reference laboratories. Crossmatching and blood typing products are described in this chapter and select procedures are provided (Appendix 17.1).

Blood transfusions are not without risk; therefore, they should be performed only when warranted. Clients should be questioned regarding any history of previous transfusion therapy, which necessitates crossmatching. Whole blood or blood component therapy may be administered depending on availability and the reason for transfusion. Blood component therapy in domestic animals has been reviewed elsewhere,[6,34,35,36] but the primary indication for blood transfusion

Table 17.1 Antigens and pertinent factors in veterinary transfusion medicine.

Species	Major Immunogenic Antigens	Naturally Occurring Alloantibodies	Recommended Donor Type	First Transfusion Risks and Recommendations	Matched Transfused RBC Half-Life
Dog	DEA 1.1, 1.2	Rare; DEA 3, 5, 7; cold reacting	DEA 1.1 type-matched or 1.1 negative for first transfusion. Universal donor for repeat transfusions. **No** prior transfusion	Low Use of universal donor minimizes sensitization. Must crossmatch if prior transfusion.	21 days
Cat	A most common B rare except select breeds. AB very rare – in breeds which also have B. *Mik*	Common Anti-B, usually mild in type A cats Common Anti-A, strong in type B cats Anti-*Mik* reported in DSH	A and B needed A in US for most DSH/DLH and Siamese, Burmese, Oriental shorthair, Tonkinese, or Russian blue breeds A or AB preferred for AB cats. Refer to text for breed-related incidence including breeds with higher percent type B	High for non-type-matched transfusions. Usually low in type-matched transfusions. Type and crossmatch with A/B matched blood. High neonatal isoerythrolysis risk when A or AB kittens nurse B queen. Risk exists with anti-*Mik* in *Mik*-negative cats.	30–38 days
Horse	Complex system of 30+ antigens in seven blood groups Donkey RBC antigen	Occur Anti-Aa, -Qa most important Probably none	None Aa/Qa negative or same breed class is best starting choice.	Considerable; use least incompatible. High neonatal isoerythrolysis risk for mule foals.	2–4 days
Cattle	Eleven blood groups: B and J most important B very complex in ruminants	Occasionally Anti-J	J-negative	Low for first transfusion. Close match difficult. Hemolytic crossmatch recommended.	2–4 days
Sheep Goat	Seven blood groups in sheep: sheep R similar to cattle J; sheep B similar to cattle and goat B. Five blood groups in goat: similar to sheep.	Weak Goat Anti-R	Not defined	Low for first transfusion. Hemolytic crossmatch recommended. Hemolytic crisis rare in sheep.	Unknown
Ferret	None identified	None identified	Not applicable	No risk identified	Unknown

is the treatment of severe anemia caused by hemorrhage, hemolysis, ineffective erythropoiesis, immune-mediated hemolytic anemia, chronic inflammatory or infectious disease, or neoplasia. Animals must be clinically evaluated on an individual basis. A rule of thumb for the treatment of anemia is to transfuse when the packed cell volume (PCV) is less than 10% to 15%.[35,37–39] Animals with acute-onset anemia, however, usually require transfusion before their PCV decreases to 15%, which contrasts with the situation in animals with chronic anemia. For cases of thrombocytopenia, the generally accepted trigger for platelet transfusion is a platelet count of 10,000/μL.[40] Additional indications for transfusion include hypovolemia, primary or secondary clotting factor deficiency, and hypoproteinemia. Detailed labeling of collected blood and recordkeeping are crucial in all cases of blood collection and administration.

Donor selection

Blood typing should be performed to select permanent blood donors. All donors should be healthy young adults that have never received a blood transfusion. In addition, donors must undergo routine physical examinations as well as hematology and clinical chemistry evaluations, receive vaccinations, and be tested free of blood parasites and other infectious diseases. Donors should have normal baseline PCV and total protein concentrations prior to any donation. Blood should be collected aseptically usually via jugular venipuncture. To avoid interference with platelet function,

donors should not be sedated with acepromazine. Blood collection methods for different species have been reviewed in detail elsewhere.[6,30,31,35,36,41]

Dogs

Dogs can donate approximately 15 mL of blood per kilogram (kg) of body weight every 6 weeks.[6] Previously transfused dogs should not be blood donors.[1] For first-time transfusion recipients, donors negative for DEA 1.1 can be considered universal donors and, for this situation, routine typing for other blood types is not clinically warranted.[1] A dog is considered a universal donor when negative for DEA 1.1, 1.2, 3, 5, 7, and positive for DEA 4.[2,6] To minimize potential sensitization of the recipient and improve the odds of identifying compatible donors, the use of universal donors is recommended when periodic transfusions are anticipated. Since about 42% of dogs are DEA 1.1 positive and testing for DEA 1.1 is a practical procedure, having DEA 1.1 positive donor blood available for DEA 1.1 positive recipients is prudent.[1,2] A practical summary is that DEA 1.1 negative dogs are ideal for first time transfusions regardless of recipient blood type, and DEA 1.1 positive donors should be limited to DEA 1.1 positive recipients. An algorithm including details for specific cases is presented in Figure 17.1.

Dog donors should be greater than 25–30 kg, bled less than once per month to prevent iron deficiency, and well-nourished including supplemented with oral iron if collected frequently. To ensure general good health donors should

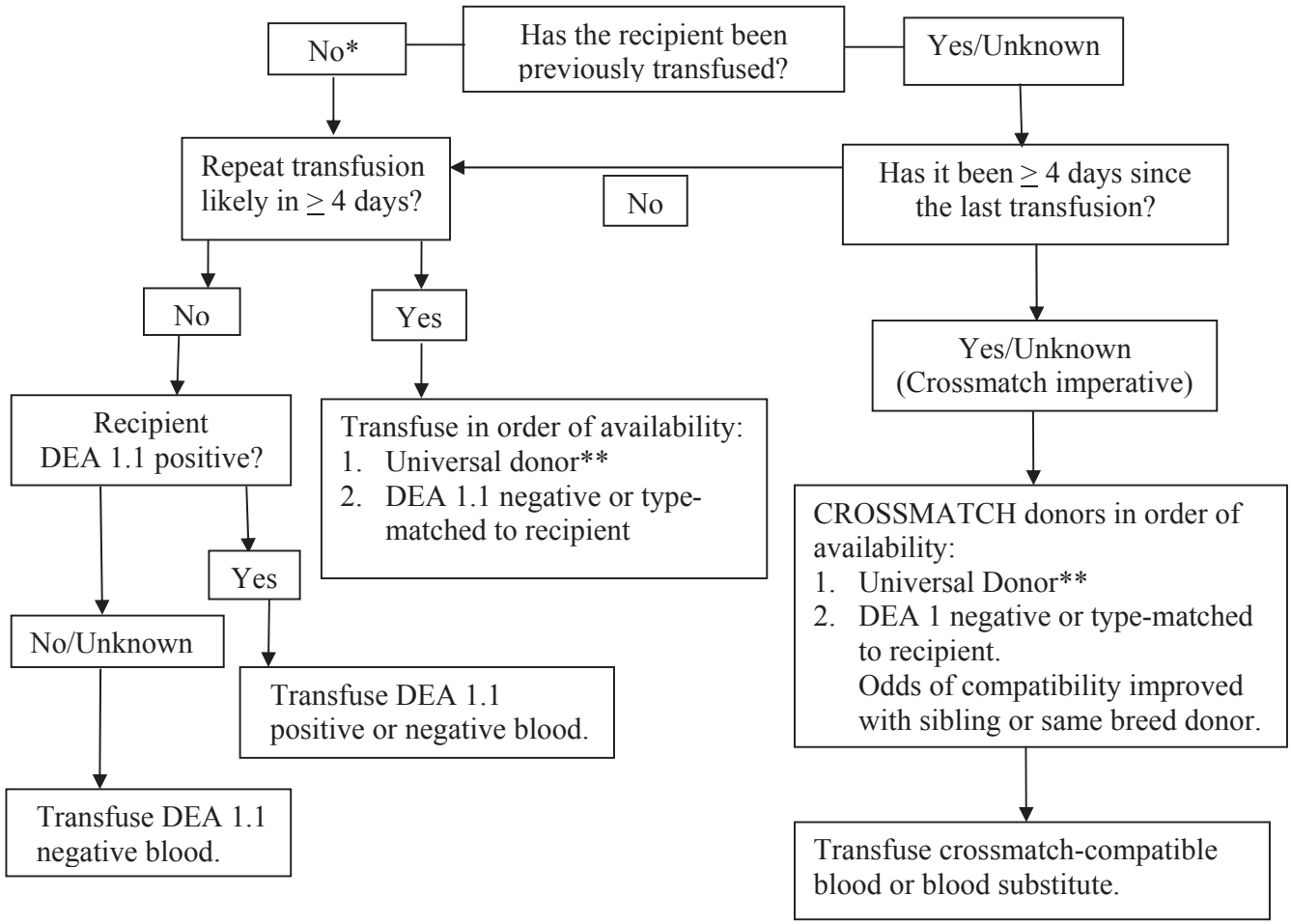

Figure 17.1 Dog type and crossmatch algorithm.

Complete typing and crossmatching will not prevent or predict all sensitizations and transfusion reactions.

* Crossmatching is always advised;[74] alloantibodies that can result in a delayed transfusion reaction may be detected.

** Universal donor: DEA 1.1, 1.2, 3, 5, 7 negative; DEA 4 positive.

have negative fecal and heartworm disease examinations. According to the American College of Veterinary Internal Medicine's (ACVIM) Consensus Statement, donors should test negative for transmissible infectious diseases including: babesiosis, leishmaniasis (especially foxhounds),[42] brucellosis, ehrlichiosis, anaplasmosis, and neorickettsiosis. The Consensus Statement should be consulted for specific recommendations on diseases relevant to particular geographical regions such as trypanosomiasis, bartonellosis, and hemoplasmosis.[43]

Cats

Donor cats can donate between 10 and 12 mL of blood/kg body weight. Healthy adult cats can donate 45–60 mL every 6 weeks and usually require sedation for blood collection.[36,44] Like dogs, donors should have negative fecal and heartworm disease examinations as part of a general health screening. Since the majority of cats in the United States are type A, donors should also be type A, but depending on geography and breed prevalence, type B and AB donors may also be required. Because of the cat's naturally occurring alloantibodies, there is no universal cat donor. Donor cats should be negative for feline leukemia virus (FeLV), feline immunodeficiency virus (FIV), cytauxzoonosis, and hemoplasmosis. As for donor dogs, the Consensus Statement should be consulted for specific recommendations on diseases relevant to particular geographical regions such as anaplasmosis, bartonellosis, ehrlichiosis, and neorickettsiosis.[43]

Horses

Adult horses can safely donate approximately 6–8 L of blood. Whole blood can be collected every 2–4 weeks and plasma collected every week if the erythrocytes are returned to the donor.[45] Donors should be in good health, negative for equine infectious anemia, and have normal PCV and plasma protein concentrations; male donors may be preferred as they are less likely to have been previously sensitized.[41] Mares that have been pregnant or foaled and horses that have received blood or erythrocyte-contaminated plasma transfusions should be excluded as potential donors. A totally compatible blood transfusion is unlikely to be achieved in the horse. Crossmatching to identify the least incompatible donor is recommended to minimize adverse transfusion reactions but will not identify all donor/recipient incompatibilities.[19] Because Aa and Qa erythrocyte antigens are extremely immunogenic, Aa- and Qa-negative donors are the best choice as donors to recipients of unknown blood type. In cases of NI, the dam's washed erythrocytes may be used for transfusion to severely anemic foals whereas a transfusion from the sire to foal would be contraindicated.[17,41] While blood transfusion can be life-saving to foals with NI, number and volume of transfusions must be limited: one study demonstrated that each administration of a blood product to a foal with NI increased its likelihood of *nonsurvival* by greater than eightfold, and administration of four or more liters (total volume) of blood products significantly increased the risk for liver failure in foals.[46] Mule foals with NI could receive a transfusion from a horse not previously sensitized by pregnancy against donkey factor, since horses are known to be free of naturally-occurring antibodies against donkey factor, the implicated antigen in cases of NI in mules.

Cattle and sheep

Cattle can donate 8–14 mL/kg of body weight. Closely matched transfusions are very difficult in cattle; first transfusions are generally low risk, but ideally a donor would be negative for the J antigen.[35] Prion diseases have been shown to be transmitted by blood transfusion in sheep[47] and should, therefore, be a consideration for disease screening prior to blood transfusion in ruminants.

Exotic pets

Large, vaccinated adult male ferrets are the best choice as blood donors, and should have a normal PCV and total protein, be negative for Aleutian mink disease virus, and be screened for heartworm microfilaria.[30,48] These ferrets can donate a total of 6–10 mL of blood depending on body weight.[48]

For pet birds and reptiles, since blood typing is not generally available, using a healthy donor of the same species as the recipient may minimize the likelihood or severity of transfusion reaction and will reportedly result in longer survival of transfused erythrocytes.[31,38] Ideally a bird donor would be negative for chlamydiosis, psittacine beak and feather disease, and polyoma virus.[30]

Anticoagulants

Citrate-phosphate-dextrose-adenine (CPDA-1) is the superior anticoagulant because it maintains higher levels of 2,3-disphosphoglycerate (2,3-DPG) and adenosine triphosphate (ATP) in collected blood. Blood can be stored for approximately 35 days in CPDA-1. Acid-citrate-dextrose (ACD) allows storage of blood for 21 days.[6,36,49,50] When using CPDA-1 or ACD, use 1 mL of anticoagulant for every 7 mL of blood. Blood should be refrigerated in plastic blood collection bags. Heparin activates platelets and is not recommended for blood collection, but if heparin is used as the anticoagulant (5 U per mL of blood),[6] blood must be used immediately. Blood collected for transfusion in pet birds must be administered immediately because use of available storage media will result in a blood product that contains potassium levels that are dangerously high.[38]

Survival and functional usefulness of erythrocytes decrease with increased storage temperature and time because of glucose consumption and depletion of ATP and 2,3-DPG.

Erythrocyte nutrient additives are available for closed-collection blood systems that will prolong the lifespan of stored canine packed red blood cells (pRBCs); a review of storage shelf life of blood component products is available.[36] To preserve platelets, blood should be collected into latex-free plastic bags or plastic syringes (and then sterilely transferred to plastic collection bags for storage). Glass-bottle collection systems also are available for use; however, limitations include loss of platelets because of activation, increased hemolysis and inability to prepare components.[51]

Administration

Blood for transfusion must be collected aseptically. As recommended by the ACVIM Consensus Statement on infectious disease screening, an aliquot from every donated unit of blood should be stored for later testing in cases where disease transmission following transfusion is suspected.[43]

Blood should be filtered either prior to or during administration using 150–170 µm pore, nonlatex filters. An inline filter should also be utilized when administering blood via plastic syringe immediately following collection as for reptiles, birds, or small mammals.[30,31] To prevent hypothermia, blood should be warmed to not greater than 37°C before administration; higher temperatures cause lysis of erythrocytes and inactivation of clotting factors. Blood is administered intravenously through commercially available administration sets with filters. If concurrent crystalloid fluid therapy is indicated or for reconstituting blood components such as packed erythrocytes, use only fluid containing 0.9% saline. Lactated Ringer's solution, 5% dextrose in water, and hypotonic saline are contraindicated for the following reasons: lactated Ringer's solution causes calcium chelation with citrate-containing anticoagulants and subsequent clot formation, dextrose-containing fluids cause swelling and lysis of erythrocytes, and hypotonic fluids will lyse erythrocytes.

Circulatory overload and heart failure can result from excessive and rapid injection of blood or plasma. Generally, blood should be given intravenously at a rate not to exceed 10 mL/kg per hour (always begin more slowly then gradually increase flow rate); however, each patient must be assessed individually to establish an appropriate infusion rate. For example, hypovolemic patients may require an infusion rate of 20 mL/kg per hour, whereas patients with cardiac, renal, or hepatic disease or recumbent calves may require an infusion rate of only 1 mL/kg per hour.[6,35] Exotic species may require a slower initial infusion rate (0.5 mL/kg).[30] If blood is administered too quickly, salivating, vomiting, and muscle fasciculations may occur. Transfusions should be completed within 4 hours to avoid contamination of warmed blood. The amount to be transfused is determined according to the recipient's body weight, estimated blood volume, PCV of the recipient and of the donor, and the goal of therapy. A simple guideline for small animals is that 10–15 mL/kg of packed erythrocytes or 20 mL/kg of whole blood increases the PCV by 10% if the donor has a PCV of approximately 40%.[6,30] One report in horses demonstrated that 15 mL/kg of whole blood and 8–10 mL/kg of packed erythrocytes resulted in a 4% increase in PCV when the donor PCV was 35–40%.[19] More specific calculations for cattle are reported depending on the indication for transfusion; for hemorrhagic shock a general volume rule is 7L of whole blood/600 kg cow.[15] For cases of thrombocytopenia in dogs and cats for which fresh whole blood is used for treatment, the general rule is to administer 10 mL/kg to increase the recipient's platelet count by a maximum of about 10,000/µL.[40] The reader is directed to references on component therapy for dosages and flow rates for specific blood components.[6,35,36,39,41]

In dogs, the matched transfused erythrocyte half-life is approximately 21 days. In cats, the matched transfused erythrocyte half-life is approximately 30–38 days.[52] The survival time of compatible transfused erythrocytes is only 2–4 days in horses and cattle.[53,54] Homologous transfusions in birds result in a longer transfused erythrocyte half-life (approximately 8.5 days) compared to heterologous transfusions (4.5 days).[2,30]

Preparations used for transfusions

Fresh whole blood is indicated for use in acute hemorrhage, anemia, coagulation disorders, and thrombocytopenia. Stored whole blood is indicated for use in anemia but will not provide platelets or coagulation factors. Packed erythrocytes are recommended for anemic animals, particularly those at high risk for volume overload. Preparation methods for blood components are reviewed elsewhere.[35,36,39,41]

Uses for fresh-frozen or stored-frozen plasma include congenital or acquired deficiencies of coagulation factors and hypoproteinemia. Fresh-frozen plasma is indicated for use in failure of passive transfer (hypogammaglobulinemia) in calves, foals, puppies, and kittens.[34,35,50,55] Cryoprecipitate prepared from fresh-frozen plasma is indicated for replacement of coagulation factors, especially factor VIII:C, vWf, and fibrinogen, but not for protein. Cryosupernatant (cryoprecipitate-poor plasma) provides more of coagulation factors II, VII, IX, and X and can be stored for 5 years.[6] Platelet-rich plasma or platelet concentrate is indicated for severe thrombocytopenia or thrombocytopathia. Hyperimmune equine plasma or equine plasma rich in anti-endotoxin antibodies has been used for critically ill foals reportedly improving survival in those that are septic.[56] Hyperimmune serum products are also available for use in cattle with infectious disease.[35]

Blood substitutes are available and have been used for treatment of anemia in different species of animals including dogs, cats, horses, birds, and ferrets. The most widely used product is the hemoglobin-based oxygen-carrying product Oxyglobin® (Biopure Corporation, Cambridge, MA), which has been approved for use in dogs only. Oxyglobin is no longer available. Blood substitutes have several advantages

in that blood typing and crossmatching are not required, risk of infectious disease transmission is minimized and the shelf life is long (3 years for Oxyglobin®). However, the product is expensive and must be discarded if not used within 24 hours; once administered it has a half-life of 18–40 hours (depending on the dosage). Because Oxyglobin is a colloid, patients of all species must be monitored very closely for volume overload, particularly those with compromised cardiac, respiratory, and/or renal function, and very small animals; monitoring for other adverse reactions during administration is also indicated.[6,30,46,57,58,59,60] One should be aware of the potential for abuse of artificial oxygen-carriers in canine and equine athletes.[59] Lastly, these products can interfere with patient monitoring using colorimetric laboratory tests; effect of transfusion in recipients should be monitored using hemoglobin concentration, not PCV.[6,34]

Transfusion reactions and sequelae

Potential transfusion reactions may be acute or delayed. Acute intravascular hemolysis with hemoglobinemia and hemoglobinuria may be seen in animals receiving incompatible transfusions. Release of thromboplastic substances may lead to disseminated intravascular coagulopathy. Hypotension and shock secondary to the release of vasoactive substances, acute renal failure, and death also may occur. Delayed hemolysis is evidenced by a decrease in PCV between 2 and 14 days after transfusion, and it occurs most commonly in previously transfused animals with an antibody titer too low to detect by crossmatching. Usually, hemoglobinemia and hemoglobinuria do not occur; however, hyperbilirubinemia and bilirubinuria may result from extravascular hemolysis.

A first transfusion usually can be safely given to dogs without regard for donor blood type (if the recipient has not been previously transfused), because alloantibodies against the common canine erythrocyte antigens 1.1 and 1.2 do not exist, and sensitization does not occur during pregnancy in dogs.[1] Administering a mismatched first transfusion may sensitize the recipient to immunogenic antigens such as 1.1, 1.2, 7, and others, however, and result in shortened survival times of the transfused cells on first transfusion, and subsequent predisposition to severe transfusion reaction. The strongest antigen in dogs, DEA 1.1, elicits the most severe transfusion reaction.[2]

In cats, AB-mismatched transfusions, whether initial or subsequent, may cause acute hemolytic incompatibility reactions. Because of alloantibodies, erythrocytes are destroyed immediately in cats; this contrasts with the situation in dogs, in which delayed transfusion reactions are more likely to occur. Transfusion of type B blood to a type A cat typically results in mild clinical signs but also shortened erythrocyte survival, thus resulting in ineffective therapy. Transfusion of type A blood to a type B cat results in an acute hemolytic transfusion reaction with massive intravascular hemolysis and serious clinical signs and possibly death — even if it is the first transfusion.[8,16] Type AB cats can safely receive type AB or A blood. Administering *Mik*-positive blood to *Mik*-negative recipients can also result in acute hemolytic transfusion reactions.[13] Because of identification of novel blood groups in cats, even AB-matched transfusions can result in reactions, and therefore crossmatching may be necessary for any feline blood or plasma transfusion.[1]

First transfusions can generally be administered with low risk in cattle; however, transfusing J-positive erythrocytes into J-negative cattle can result in transfusion reactions, and hemolytic reactions to a second transfusion within four days of a first transfusion have been reported.[35]

Neonatal isoerythrolysis is the destruction of erythrocytes in the circulation of offspring by alloantibodies of maternal origin that are absorbed from colostrum. Kittens at risk include those that are type A or AB that suckle colostrum from type B queens in the first 16 hours of life.[1] Nearly all cases in foals are caused by factor Aa in the A system and factor Qa in the Q system (acquired, not naturally occurring, alloantibodies).[17] Signs usually develop 24–36 hours after suckling, with anemia and resultant liver failure and kernicterus (bilirubin encephalopathy) being the primary causes of death in foals.[46] Neonatal isoerythrolysis is also important in mule foals, and calves, and has been rarely reported in puppies.[17,22,50]

Complications that may be unrelated to erythrocyte antigen–antibody reactions include fever, allergic reactions, circulatory overload, citrate toxicosis, ammonia toxicosis, and infection.[6,61,62,63] Fever is a common reaction to a blood transfusion, and it may occur in response to leukocyte or platelet antigens or to sepsis from bacterial contamination of blood. Allergic reactions after transfusions can be seen in dogs, cattle, and horses;[6,19,35] sensitivity to plasma proteins or leukocyte and platelet antigens is generally responsible. Circulatory overload is a potential sequela to whole-blood transfusion, particularly in patients with cardiovascular compromise. Citrate toxicity can result in acute decreases in the level of serum ionized calcium, which can be especially significant in cattle already hypocalcemic during acute illness. Simultaneous administration of calcium in a separate administration line from the transfused blood may be indicated in these cattle.[35] Ammonia toxicity can occur with prolonged storage of blood, because the ammonia concentration increases over time. Patients with liver disease should be monitored closely for these latter two reactions. Infection is a potential risk of blood transfusion as a result of bloodborne parasites and viruses, contamination of stored blood or prolonged administration. Proper health maintenance and screening of donors as well as proper handling and storage of blood and visual inspection of the unit prior to transfusion help to minimize these risks.[43] Storing a properly labeled segment of the donor unit tubing is a convenient way to retain an aliquot for later testing if needed.

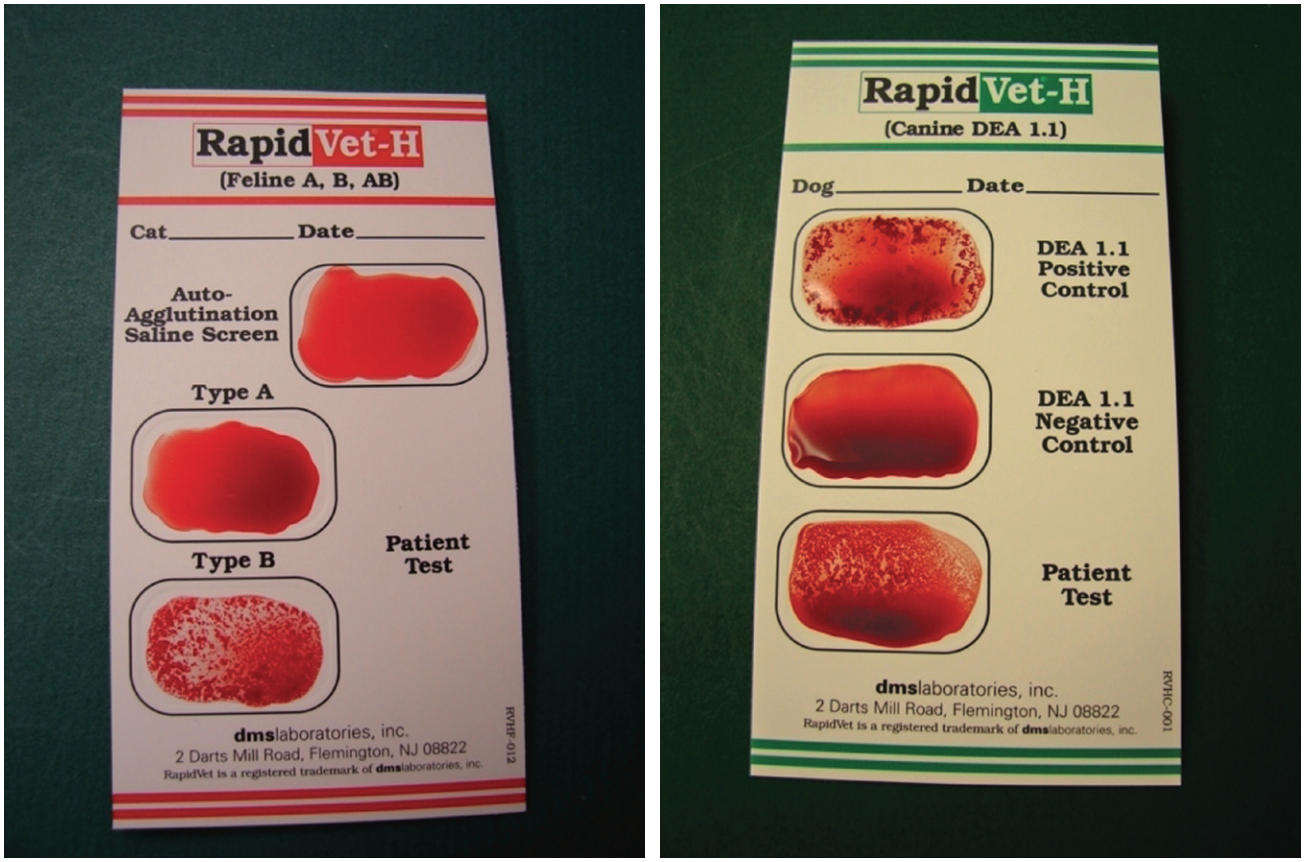

Figure 17.2 RapidVet®-H typing cards demonstrating type B in a cat (left) and type DEA 1.1 in a dog (right). (Courtesy of DMS Laboratories, Inc.)

Rare, but fatal, cases of serum hepatitis have been reported in horses receiving transfusion of commercially available plasma, and, as previously mentioned, liver failure and kernicterus are associated with large volume (greater than or equal to 4 L) transfusions in foals.[46,64]

Common sense and quality medical care mandate that transfusions associated with hemolysis or hemoglobinuria, which is evidence of an acute hemolytic reaction, be immediately stopped and investigated. Visible hemolysis of the donor unit suggests inappropriate storage or bacterial contamination, in which case culture is warranted. Pre- and posttransfusion recipient blood samples as well as donor unit samples should be obtained to allow confirmation of blood types, compatibility by crossmatching, and Coombs testing status.[63] Similar testing may provide insight to an unexplained failure to maintain maximal PCV when delayed transfusion reactions occur.

Typing blood

General principles

Blood typing can be performed in the clinic to screen potential cat and dog blood donors and to type the recipient for appropriate donor selection prior to crossmatch and transfusion. Commercially available blood typing kits include a card-based agglutination assay (DMS Laboratories, Inc., Flemington, NJ), an immunochromatographic cartridge (Alvedia, France), and a gel column diffusion assay (DiaMed, Switzerland);[a] the kits type for A, B, or AB in cats, and DEA 1.1 only in dogs. Brief overviews of the procedures are described; however, current package insert directions should be carefully followed at the time of testing.

Blood typing cards contain lyophilized antisera in designated reaction wells. The dog cards include positive and negative control wells and the cat cards include an autocontrol well. A drop of diluent and a drop of whole blood are mixed onto each reaction well on the card, rocked, and then observed for macroscopic agglutination (Fig. 17.2). The procedure is simple, and results are obtained in less than 2 minutes with no special equipment required. The autocontrol well included on the cat typing card and on separate cards in dogs allows assessment for autoagglutination. Auto-

[a]At the time of press, the distributor (BioRad) indicates the DiaMed (Switzerland) gel column diffusion assay has been discontinued (personal communication, R. Orynich, 11/22/2010).

agglutination appears similar to a positive reaction and may preclude accurate typing. A prozone phenomenon may occur in the presence of inappropriate antigen:antibody ratios that can generate false negative results. Consequently, an additional drop of diluent should be added to dog samples with weak or grainy reactions followed by rocking and reading the card again, and samples from very anemic animals (PCV < 10%) should be concentrated prior to typing (DMS Laboratories package insert).[65]

The immunochromatographic kits utilize a plastic cartridge device and testing requires about 2 minutes. Testing requires simple preparation of a cell suspension and manipulation of the device to properly place the reaction strip into the suspension. This allows erythrocytes to move up the membrane by capillary action. Erythrocytes positive for the antigen in question are trapped by the antibody impregnated in the strip, which then forms a visible line (Fig. 17.3). Erythrocytes negative for the antigen pass by the antibody and do not form a line. The strip is also impregnated with control material that must read positive to confirm the test was performed properly. The manufacturer claims autoagglutination and marked anemia do not interfere. These tests are easy to interpret and archive.[66]

The gel tube typing kits require a simple preparation of a cell suspension, a 10-minute incubation period, and a 10-minute centrifugation in a centrifuge specifically designed to hold the gel tube cartridges (Fig. 17.4). The reaction is grossly visible as a compact to modestly dispersed layer of agglutinated cells at or near the top of a gel column (Fig. 17.5). Nonreacting cells accumulate at the bottom of the column. This method reportedly provides easier result interpretation and higher accuracy for dog and cat typing.[1,32,33]

The cat typing kits are generally reliable, but the card may show weak A reactions in type AB cats resulting in potential misclassification of type AB cats as type B.[33] The kit insert instructs users to add a second drop of diluent to the "A" well; this reduces the chance of a prozone reaction. The gel and card/cartridge methods may give inconclusive or erroneous results, respectively, in FeLV positive cats. All three methods are reportedly accurate for detecting B types, while the gel method is accurate for type AB.[66] Given the potential for error and the inability to routinely type for the *Mik* antigen, crossmatching with even apparently AB type-matched units is strongly recommended to avoid potentially harmful mismatched blood transfusions.[1]

Rare false positive and negative results occur with the dog agglutination test kits and rare false negative results occur with the cartridge kit.[32,66,67] Only 2+ or stronger agglutination reactions should be interpreted as 1.1 positive; some weaker reactions may be related to the DEA 1.2 type. This issue has been corrected according to DMS Laboratories.[68] If performing in-house confirmatory tube-typing, note that only 2+ or stronger reactions with 1.2 antisera should be considered positive for DEA 1.2 (package insert, Animal Blood Resources International); confirmatory testing by an outside laboratory is recommended when weak reactions are obtained. Since the canine kits indicate the presence or absence of DEA 1.1 only, and do not detect DEA 1.2 or 7, they should only be considered as a screen for typing potential donors. It is recommended that potential donors typed as DEA 1.1 negative be further tested with more specific antisera (Animal Blood Resources International, Stockbridge, MI) to at least rule out the presence of DEA 1.2. The kits are adequate for recipient testing provided that all donors are known to be negative for DEA 1.1 and 1.2. Failure to follow this recommendation may sensitize the recipient to the DEA 1.2 antigen. If DEA 1.1/1.2 negative blood is in short supply, DEA 1.1 positive blood should be

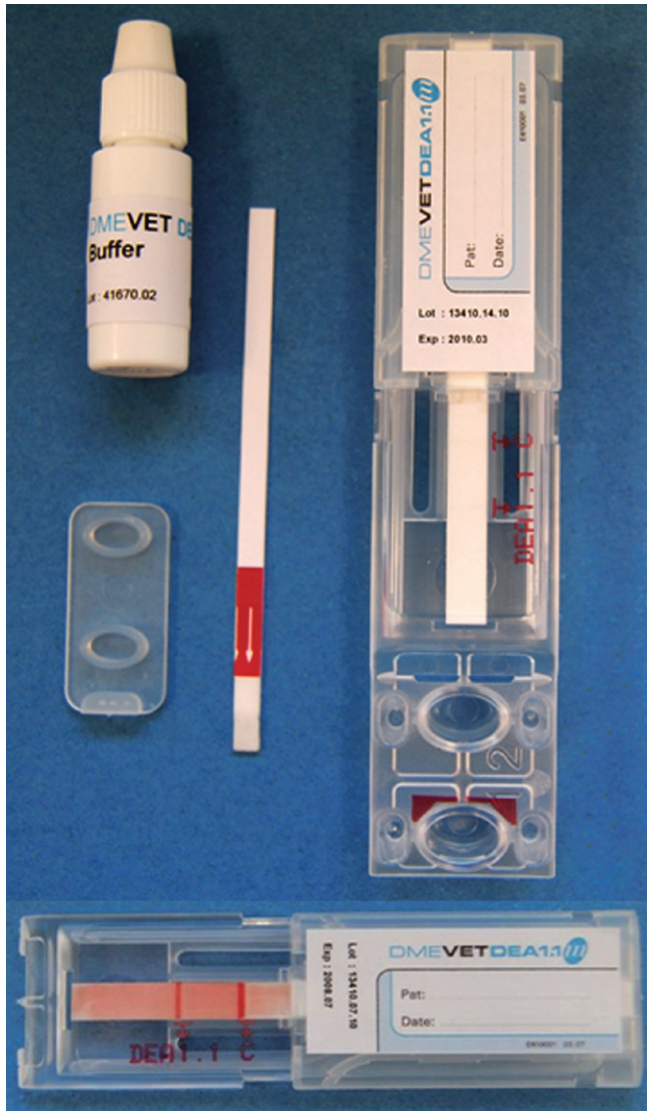

Figure 17.3 Immunochromatographic typing kit supplies (top) and column indicating type DEA 1.1 in a dog (bottom). (Courtesy of Alvedia.)

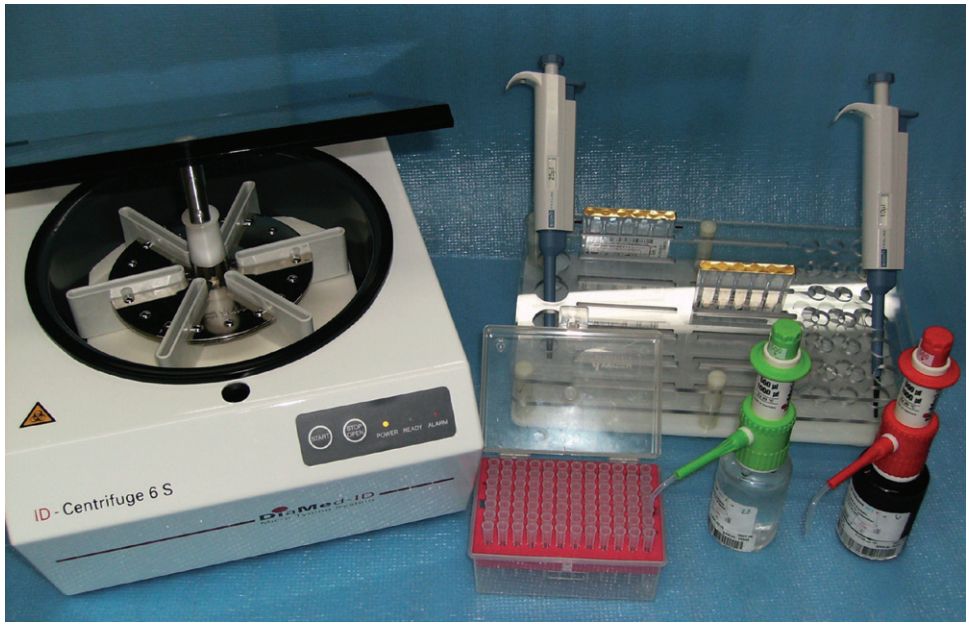

Figure 17.4 Gel typing centrifuge and materials. (Courtesy of Bio-Rad.)

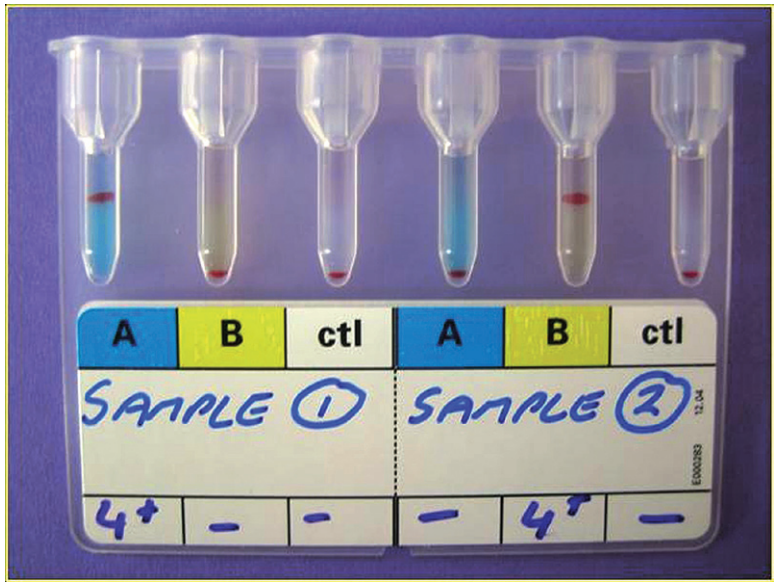

Figure 17.5 Gel typing test card indicating type A cat (left, sample 1) and type B cat (right, sample 2) with negative controls. (Courtesy of Bio-Rad.)

reserved only for those patients typing strongly positive for DEA 1.1.[6]

Tube typing is not available in kit form, but utilizes commercially available lyophilized antisera (Animal Blood Resources International, Stockbridge, MI), borosilicate glass tubes and a blood banking centrifuge capable of 3400 rpm (1000 relative centrifugal force—rcf) in order to obtain appropriate cell packing. An agglutination viewer assists reading macroscopic reactions. In addition to DEA 1.1 and 1.X (includes 1.1, 1.2, and 1.3), DEA 3, 4, 5, and 7 antisera are also available. All samples for DEA 1.1 and 1.X typing with reactions ranging from negative to 1+ after the 15-minute 37°C incubation phase must also be taken through the antiglobulin phase of testing before a valid interpretation is rendered. This procedure is technically demanding and more appropriate for high volume testing centers with experienced technical staff. Currently the procedure for DEA 3, 4, 5, and 7 determinations differs from DEA 1.1 and 1.X typing, and does not require the Coombs phase. Note that antisera are not available for every known blood type,

regardless of species, so sensitization can still occur when administering transfusions of apparently type-specific blood.

A rapid typing protocol for detecting Aa and Ca erythrocyte antigens in horses has recently been developed.[69] This discovery may prompt the development of commercially available typing reagents. The protocol's usefulness in the practice setting is limited at this time without such reagents, and with the inability to detect Qa, a hemolysin. Since commercial typing products are not currently available, samples from horses, cattle, sheep, and goats may be sent to the Veterinary Genetics Laboratory (Davis, CA) for typing (530-752-2211; www.vgl.ucdavis.edu) Preparation methods for sending typing samples to reference laboratories are described in Appendix 17.1.

Crossmatching blood

General principles

Crossmatching tests for agglutinating and/or hemolytic reactions between a donor and recipient and is classified as either "major" or "minor." The agglutination technique is adequate for the dog and cat.[70] Since the horse has both agglutinating and hemolytic antibodies, procedures testing for both are warranted. The use of a hemolytic test with complement is necessary for cattle, sheep, and goats since they have minimal agglutinating antibodies.[26,35,50,71]

The major crossmatch evaluates for the presence (positive findings) or absence (negative findings) of detectable levels of antibodies, whether naturally occurring or induced, in the recipient against donor erythrocyte antigens. A major crossmatch should always be performed in animals that have strong naturally occurring antibodies, as in cats, or in those that may have induced antibodies as from prior transfusions. The latter is true even if the same donor blood is intended for repeated transfusion beyond a span of several days. While the 37°C crossmatch is fairly standard for dogs and cats, recommendations for additional phases are under review. Naturally occurring antibodies (anti-DEA 3, 4, 5, and 7) known to result in shortened erythrocyte survival in dogs previously sensitized to these antigens,[2] might be identified using a 4°C step, and more of these reactors could be identified by adding the antiglobulin (Coombs) phase to the tube typing procedure (see Appendix 17.1).[2,5,72] The time required to mount an antibody response to transfused cells appears to vary slightly between species and author's opinions. Erring on the side of caution, repeat crossmatching is recommended when there is a span of greater than two days in horses and cattle,[35,73] and 4 or more days in dogs and cats[1,74] since a prior transfusion.

The minor crossmatch procedure follows the same steps as the major crossmatch but evaluates for the presence or absence of detectable antibodies in donor plasma against recipient erythrocytes. The minor crossmatch is considered less important, purportedly because the plasma volume is small in the donated product compared to the recipient, and is ultimately diluted, particularly when packed erythrocytes are transfused.[65] Exceptions have been documented when transfusing dogs[75] and horses.[19] Administration of packed erythrocytes may contain sufficient antibodies against recipient erythrocytes to induce adverse reactions in these species.

For exotic species in which test volumes are limited and also for cats, a simplified crossmatch procedure can be performed on a clean glass slide (see Appendix 17.1). Grossly visible agglutination indicates incompatibility. Note that this method will not detect potentially fatal hemolytic antibodies.[1,30,76]

An ethylenediaminetetraacetic acid (EDTA) tube and a clot tube from the recipient are preferred for use in crossmatch testing. The same tubes may be collected from the donor or alternatively, blood from the donor unit may be used to provide plasma and cells for the procedure. If these options do not exist, erythrocytes teased from the clot and serum may be used for the entire procedure. The EDTA plasma should not be used in place of serum because this contributes to increased rouleaux formation and difficult interpretation of agglutination, particularly in the horse. Ideally, samples should be free of autoagglutination, hemolysis, and lipemia to aid in the interpretation of the reactions. When autoagglutination is present, or when no compatible units are available, transfusing the least incompatible unit may be a necessity, albeit not without significant risk. Test-transfusing even a small volume of unmatched blood is an unsafe practice, and never recommended.[1]

The interfering effects of hemolysis may be minimized by setting up a control tube with an equal number of drops of serum alone; determine whether hemolysis is increased during the procedure by comparison to the control. Specific procedures for blood typing and crossmatching are presented in Appendix 17.1.

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Appendix 17.1

Procedures

Reagents and equipment described here are used by the Colorado State University Veterinary Teaching Hospital.

Tube typing

This tube typing protocol is used to type dogs for the presence or absence of DEA 1 antigen, and if present, to distinguish between DEA 1.1 and 1.2/1.3. (Adapted from Animal Blood Resources International, Stockbridge, MI.) Fresh EDTA-anticoagulated whole blood free of hemolysis is preferred, but clotted blood can be used.

Typing procedure

1. Make a 4% suspension of erythrocytes:
   a. Add 0.04 mL of packed erythrocytes to 0.96 mL of PBS in a labeled test tube or estimate by adding PBS to erythrocytes until the suspension appears similar to dilute tomato juice. It must have adequate erythrocytes to go through a set of washes later.

2. For each sample to be tested, label three 12- × 75-mm, disposable glass tubes with the animal identification. Label one tube "C" for control, one tube "D1", and another "DX."
3. Add two drops (100 µL) of 0.9% PBS to the control tube.
4. Add two drops (100 µL) of DEA 1.1 and DEA 1.X typing sera (Animal Blood Resources International, Stockbridge, MI) to appropriately labeled tubes.
5. Add one to two drops (70 µL) of the 4% suspension of erythrocytes to each tube, swirl the tubes to mix, and incubate at 37°C for 15 minutes.
6. Centrifuge the tubes at 3400 rpm using a standard blood bank centrifuge (Sero-fuge II, Clay-Adams/Becton Dickinson, Sparks, MD) for 15 seconds.
7. Read for hemolysis by observing the supernatant.
8. Gently rock the tubes and read for macroscopic (Figs. 17.6, 17.7) and microscopic agglutination (Fig. 17.8).

Interpretation

Hemolysis or agglutination reactions of 2+ or greater are considered positive. The control tube should read negative for hemolysis and/or agglutination. A positive control tube indicates autoagglutination and negates the interpretation of positive results in any of the typing tubes. Any blood that is positive in the DEA 1.1 tube is DEA 1.1, and should also be positive in the 1.X tube. Any blood that is negative in the DEA 1.1 tube but positive in the DEA 1.X tube is either DEA 1.2 or 1.3; DEA 1.2 is most likely except in the case of a German shepherd dog from Australia. Dogs positive for either type are not considered universal donors. No further testing is necessary. Negative, trace or 1+ reactions for hemolysis or agglutination with Anti-DEA 1.1 and 1.X are considered negative and must be taken through the Coombs phase, including the control tube, to detect incomplete antibodies as described in the following section.

Figure 17.6 Tube typing. Macroscopic 2+ agglutination (left) and negative control (right). These results could also represent incompatible (left) and compatible (right) macroscopic crossmatch reactions.

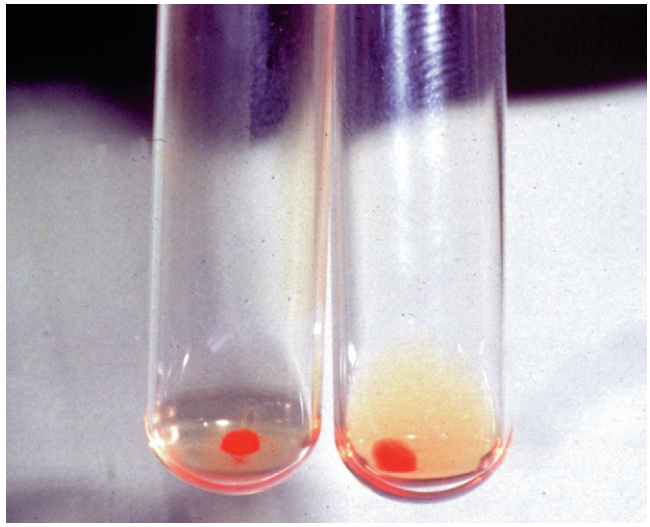

Figure 17.7 Macroscopic 4+ agglutination. Note the tight button of cells. A reaction of this magnitude may be seen with a strong-reacting antibody, such as anti-A, which may be present in type B cats.

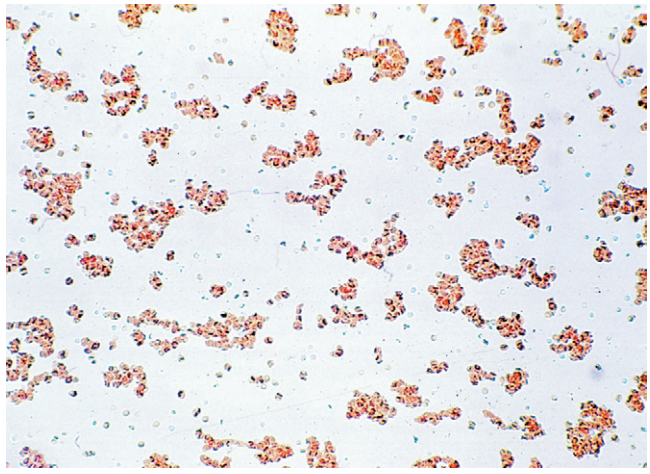

Figure 17.8 Microscopic 4+ agglutination. Note the irregular grape-like clusters. A microscopic reaction of this strength may also be detectable macroscopically as fine agglutination.

Coombs phase to detect false-negative types

The reagent required is Canine Coombs Serum (VMRD, Pullman, WA), stored refrigerated.

Procedure

1. Wash the cells in the "C" and both "D" tubes three times with 0.9% PBS.
   a. Use a squirt bottle of PBS to forcibly add at least 1 mL to each tube, centrifuge for 2 minutes at 3400 rpm (Serofuge II) to tightly pack the cells, then decant supernatant without removing any of the erythrocytes.
   b. Resuspend the button of cells and repeat twice.
   c. After the third wash, invert the tubes and pour off the supernatant, then resuspend the cells in the residual fluid. Insufficient washing results in a false-negative result.
2. Add two drops (100 µL) of Coombs reagent to each tube.
3. Swirl the tubes to mix, and then incubate at 37°C for 15 minutes.
4. Spin at 3400 rpm for 15–30 seconds. Gently mix the tubes, and then read for hemolysis and agglutination.

Interpretation

The interpretation is the same as that described above. If the dog types negative with DEA 1.1 and 1.X at this point, it may be considered a universal donor for the DEA 1 group. Any positive Coombs test result on the control tube using cells from a clot tube should be verified with an EDTA-anticoagulated sample. Calcium is bound by EDTA, which in turn prevents the activation of C_1 (i.e., complement) and, therefore, avoids interference by cold-reacting antibodies.

Sample preparation for sending blood-typing samples to reference laboratories

Large animal blood typing requires ACD–anticoagulated blood for longer cell preservation. Usually, EDTA-anticoagulated whole blood is adequate for small-animal blood typing. Samples collected with an anticoagulant should be gently mixed, not shaken, immediately after collection. The EDTA-anticoagulated whole blood should be shipped cool (with, but not touching, a cold pack). All samples should be protected from temperature extremes and shipped by overnight delivery. The reference laboratory should be contacted to obtain more specific requirements regarding sample type, handling, and shipping. Most laboratories performing parental exclusion typing supply blood collection kits or mailers.

Simplified crossmatch[76]

1. For the major crossmatch, mix two drops of recipient plasma with one drop of blood from the donor on a clean glass slide at room temperature.
2. Observe for macroscopic agglutination within one minute (Fig. 17.9).

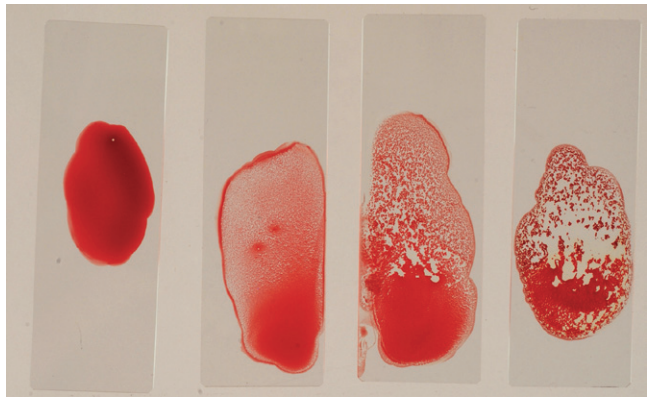

Figure 17.9 Slide crossmatch. Results demonstrate (from left to right) compatible (no agglutination), and 1+ to 3+ incompatible (agglutination) reactions.

3. Repeat steps 1 and 2 for the minor crossmatch, using two drops of donor plasma and one drop of recipient blood.

Agglutination crossmatch test

The agglutination crossmatch test is appropriate for dogs and cats, and it is used in conjunction with the lytic test for horses. In dogs previously transfused with nonuniversal donor blood, an extra autocontrol and major crossmatch tube can be set up and incubated at 4°C to detect cold reacting antibodies (i.e. anti-DEA 3, 4, 5 and 7).[5,72] The following procedure is modified from that described by Jain.[70]

Procedure

1. Centrifuge clotted blood, and place serum into a prelabeled test tube.
2. Wash EDTA-anticoagulated erythrocytes as described in the Coombs phase of the typing procedure above; however, wash cells four times for horses.
3. After the final wash, make a 4% erythrocyte suspension of each specimen in PBS as previously described.
4. Place two drops of the recipient's serum and two drops of the donor's cell suspension in a 12- × 75-mm test tube and mix (i.e., major system). In a second tube, place two drops of the donor's serum and two drops of the recipient's erythrocyte suspension (i.e., minor system). To check for autoagglutination, set up controls in the same manner by mixing the donor's erythrocytes with its own serum, and then follow the same procedure with the recipient's erythrocytes and serum.
5. Shake the rack of tubes, incubate for 15–30 minutes at 37°C, and then centrifuge for 15 seconds at 3400 rpm.
6. Examine the supernatant for hemolysis.
7. Shake the tubes gently by tapping with the finger to detect grossly visible agglutination of erythrocytes (Figs. 17.6 and 17.7).

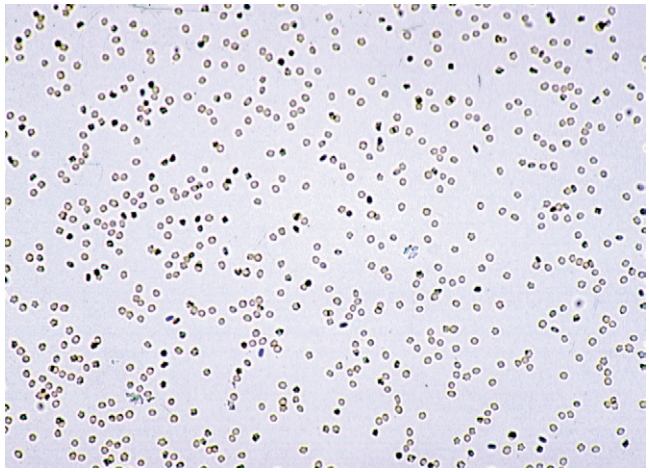

Figure 17.10 Negative microscopic agglutination. All the cells are evenly dispersed.

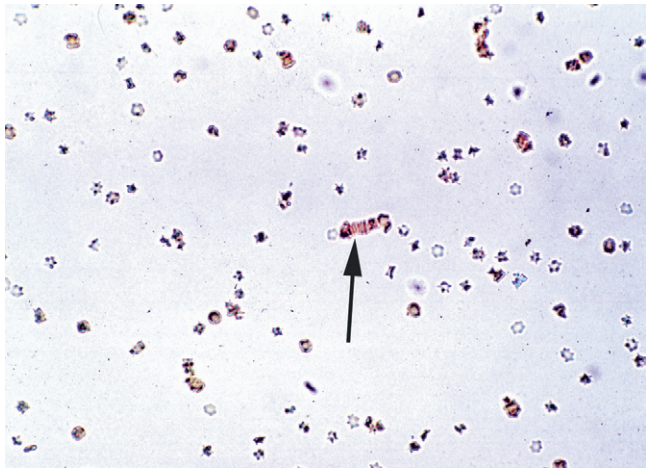

Figure 17.11 Rouleaux (coin-stacking phenomenon). This must not be confused with agglutination, may indicate insufficient cell washing, and is common with equine samples.

8. If no agglutination is observed, transfer a small amount to a glass slide and then examine under low power of the microscope. Lower the condenser to increase the contrast. Erythrocytes are evenly dispersed (Fig. 17.10) if no agglutination is present. If present, agglutination appears as grape-like clusters of erythrocytes (Fig. 17.8). Rouleaux should not be confused with agglutination. Rouleaux formation (Fig. 17.11) is common in horse blood, and it appears as stacks of coins.

Rouleaux and true agglutination may be further differentiated with the saline replacement procedure. First, centrifuge the tube for 15 seconds, remove the serum by pipette and replace with two drops of PBS, mix, and then centrifuge at 3400 rpm for 15 seconds. Next, read for microscopic agglutination. Rouleaux should dissipate, whereas agglutination should remain.

Interpretation

Slight hemolysis in canine blood is nonspecific. Significant hemolysis and/or agglutination in one or both of the crossmatched tubes—but not in the controls—indicate an incompatibility and the need to select a new donor. Incompatibility in the major crossmatch indicates the need to select a new donor. Strong incompatibility in the minor crossmatch, as might be observed in mismatched cats, indicates the need to select a new donor; otherwise, packed or washed erythrocytes minimize transfusion of donor antibodies. In horses, hemolysis in the agglutination test most likely indicates fragile or old cells rather than incompatibility. Therefore, hemolysis in horses should be ignored; however, if all cells are hemolyzed, then agglutination is impossible to detect. Positive control tubes indicate autoagglutination or contaminated reagents, thereby rendering positive crossmatch tubes uninterpretable.

Hemolytic crossmatch test

The hemolytic crossmatch test is required in crossmatching blood from goats, sheep, and cattle, because erythrocytes from these species tend not to agglutinate.[35] Most equine isoantibodies act as hemolysins; thus, performing both the agglutination and hemolytic test in horses is prudent. In this test, fresh rabbit serum is used as the complement source. Because all rabbits possess naturally occurring anti-erythrocyte antibodies, the antibodies must be removed before using the serum as a complement source.

The following procedure is modified from that described by Jain.[70]

Procedure

1. Follow steps 1–4 of the agglutination crossmatch adding serum and cell suspensions to appropriately labeled tubes; if performing the agglutination and hemolytic crossmatches concurrently (as for horses), set up four additional tubes labeled to identify them as major hemolytic and minor hemolytic tubes, and recipient and donor hemolytic control tubes.

2. Add one drop of rabbit complement (absorbed for horses, sheep, and goats; refer to the complement absorption procedure that follows) to the hemolytic phase crossmatch and control tubes.

3. Shake the rack to mix, and then incubate at 37°C for 30 minutes.

4. Centrifuge the tubes at 3400 rpm (1000 g) for 15 seconds. Observe the supernatant in each "hemolytic" tube for hemolysis; there is no need to check these tubes for agglutination.

Interpretation

A positive autocontrol indicates the presence of an autoantibody and may induce uninterpretable results in the crossmatch tubes. The major crossmatch is "incompatible" if the recipient's serum reacts with the donor's erythrocytes, and in this case, the donor blood should not be transfused. The minor crossmatch is incompatible if the donor serum reacts with the recipient's erythrocytes. In this case only packed or washed erythrocytes are generally safe for transfusion.

Absorbed complement preparation;[77] for use in the hemolytic crossmatch in horses, sheep, and goats

Reagents

1. Lyophilized rabbit complement (Pel-Freez), store frozen.
2. $CaCL_2$-$MgCl_2$ solution, store refrigerated.
   a. $CaCL_2$, 14.7 g.
   b. $MgCl_2$, 20.35 g.
   c. Distilled H_2O, approximately 2 L.
3. Na_2-EDTA solution.
   a. 74.4 g per 2 L of distilled H_2O.
   or
   b. Sequester-Sol (Baxter).
4. PBS (Sigma Diagnostics, catalog no. 1000-3), store refrigerated.
5. Normal erythrocytes from the species to be tested i.e. horse, sheep, or goat. (Two lavender-top (EDTA), 10-mL tubes from one donor are adequate.)

Procedure of the University of California-Davis veterinary genetics laboratory

1. Dilute each of three vials of thawed rabbit complement with 1 mL of distilled water.
2. Add 1 part EDTA (0.3 mL) to 10 parts (3 mL) complement.
3. Centrifuge the EDTA-anticoagulated whole blood, and remove the plasma. Wash two aliquots of erythrocytes three times with PBS; refer to the Coombs phase of the typing procedure for washing instructions. (Make enough for two absorptions.)
4. Add 1.1 mL of packed, washed erythrocytes to the complement solution. Incubate at room temperature for 30 minutes.
5. Harvest the complement solution, and repeat the absorption using fresh erythrocytes and incubating on ice instead of at room temperature for 30 minutes.
6. Centrifuge, collect absorbed rabbit complement (C′) into a flask on ice, and add 0.3 mL of $CaCL_2$-$MgCl_2$.
7. Aliquot the C′ in volumes of 0.5 mL in bullet tubes, and freeze at once. Label with the date and contents. Do not refreeze complement once thawed; use immediately or discard. (Do not store in a self-defrosting freezer, because the C′ thaws with each defrost cycle.)

Jaundiced foal agglutination test (colostrum crossmatch)

The jaundiced foal agglutination test correlates highly with hemolytic blood group tests at 1:16 dilutions. Lower dilutions have poorer correlations because of the viscosity of colostrum.[78]

Materials

1. Centrifuge (300–600 g)
2. Test tube rack
3. Disposable glass test tubes (12 × 75 mm)
4. Saline
5. Colostrum (or serum) and EDTA whole blood from mare (erythrocytes teased from clotted blood may be used)
6. EDTA whole blood from foal (erythrocytes teased from clotted blood may be used).

Method

1. Label tubes for 1:2, 1:4, 1:8, 1:16, and 1:32 dilutions for the foal.
2. Add 1 mL of saline to all tubes.
3. Add 1 mL of colostrum to the tubes labeled as foal 1:2. Mix and remove 1 mL of the dilution and then add to the next consecutive tube. Repeat the procedure, discarding 1 mL from the 1:32 tube. Discard tube 8.
4. Add one drop of foal's whole blood to each tube and mix.
5. Centrifuge the tubes for 2 to 3 minutes at medium speed (300–600 g).
6. Invert the tubes and hold upside down, pouring out the liquid contents, and observe the status of the button of erythrocytes at the bottom of each tube. It is easier to compare reactions if all four tubes are poured out at the same time. Grade for macroscopic agglutination by observing how the cells flow down the side of the tube as follows:
   0 No agglutination; cells flow easily.
   1 Weak agglutination; cells in small clumps.
   2 Strong agglutination; cells in large clumps.
   3 Complete agglutination; cells remain packed in a button.
7. If no agglutination is present, report the test as being negative at all dilutions.
8. If agglutination is present, grade the reactions and continue with controls. (Controls may be set up along with the patient tubes during step one to save time.)

Controls

1. Foal autocontrol with 1 mL of saline and one drop of foal whole blood.
2. Mare colostrum/autocontrol, prepared by repeating steps 1 through 7 described earlier using colostrum and mare erythrocytes.

Interpretation

If all controls are negative, report the reactions of colostrum versus foal erythrocytes at all dilutions. A reaction at a dilution of 1:16 or greater is considered to be a high titer, and the colostrum should not be used. A positive foal autocontrol indicates autoagglutination and the possibility that the foal has already nursed. A positive mare colostrum/autocontrol indicates interference from the viscosity of the colostrum or a technical problem. Reaction grades for the same dilution should be compared between the mare autocontrol and the foal crossmatch tubes.

III

Hematology of Common Nondomestic Mammals, Birds, Reptiles, Fish, and Amphibians

18 Mammalian Hematology: Laboratory Animals and Miscellaneous Species

Terry W. Campbell
Colorado State University

Blood collection and handling

Only decreases greater than 10% in the erythrocyte parameters (red blood cell count, RBC; hemoglobin concentration, Hb; and hematocrit, HCT) have biological significance. Blood collection studies in rats (*Rattus norvegicus*) used as laboratory animals have demonstrated that removal of 7.5% of the total blood volume over a 24 hour period did not have a biological effect with complete recovery within 48 hours.[1] The total blood volume of a rat has been determined to be 7.2 ± 1.19 mL/100 g body weight.[2] Removal of blood volumes that exceed 7.5% will have a biological effect, the degree of which and time of complete recovery depend upon the amount removed. Blood collected up to 20% of the total blood volume of healthy rats does not affect the health of the rat.

In the clinical setting and for the ease of calculation, the maximum amount of blood that can be safely withdrawn from a single draw is presumed to be 1% of the animal's body weight or up to 10% of the total blood volume. This practice, however, may overestimate the safe amount that should be withdrawn during blood sampling. For example, on the average, a healthy 300 gram rat has a total blood volume (based upon 7.2 mL/100 g body weight) of 21.6 mL. Removal of 7.5% of the total blood volume (considered to be a safe amount) represents 1.62 mL. If the same rat was sampled based upon 1% of the body weight, then the blood volume collected would be 3.0 mL or nearly twice the recommended safe amount. If up to 20% of the total blood volume can be safely removed from healthy rats, then a 4.32 mL blood sample could be obtained in the example above without causing ill effects. With this consideration the 1% of the body weight rule for blood sample collection would indeed be within safe limits. This assumes the animal is healthy and smaller blood volumes should be sampled when dealing with an unhealthy animal.

Total blood volumes reported for the mouse (*Mus musculus*) vary with some reporting a range of 5–12 mL/100 g body weight and others a range of 7–8 mL/100 g body weight.[3] The same guidelines recommended for laboratory rats likely apply to the laboratory mouse. Therefore, a 20 gram mouse is likely to have a blood volume of 1.44 mL with a safe blood withdrawal volume of 0.1 mL. Using the 1% of the body weight guideline, the sample volume would be twice as much.

Although the blood volume will be restored within 48 hours of blood collection in most healthy mammals, it may take two weeks or longer for all of the blood constituents to return to normal and likely much longer in unhealthy animals. If blood collection is required more frequently than every two weeks, a smaller sample size such as 0.5% of the body weight (if using the 1% body weight rule) each week should be drawn.

Blood collected from small mammals is typically placed in lithium heparin because the blood sample volume is small. The heparinized blood can then be used for hematologic studies and clinical chemistries.

The choice of blood collection method to be used is dependent upon the amount of blood required, frequency of sampling, technical skill of the one obtaining the sample, and parameters measured. A number of collection sites are used to obtain blood from small mammals and listed in Table 18.1.[4] Blood samples are often difficult to obtain from small mammals; they lack superficial vessels, and the deeper vessels may be covered with fat. In some cases, chemical restraint may be necessary to safely handle mammals for blood collection.

Use of a general anesthetic may have an effect on test results. For example, it has been demonstrated that five minutes of 4% isoflurane anesthesia results in a slight decrease in the erythrocyte parameters and potassium and increase in glucose.[1] Similar results have been reported in

Veterinary Hematology and Clinical Chemistry, Second Edition. Edited by Mary Anna Thrall, Glade Weiser, Robin W. Allison, and Terry W. Campbell.

Table 18.1 Sites for blood collection in laboratory animals and miscellaneous species.

	Retro-orbital Plexus	Tail Vein	Jugular Vein	Cranial Vena Cava	Lateral Saphenous Vein	Femoral Vein	Cephalic Vein	Ear Vein	Cardio-centesis
Mice	X	X							X
Rats	X	X			X				X
Hamsters	X								X
Gerbils	X								X
Guinea pigs	X		X	X	X	X	X		X
Chinchillas	X		X	X	X	X	X		X
Rabbits			X		X		X	X	X
Ferrets		X	X	X	X		X		X
Hedgehogs			X	X	X	X	X		

the ferret.[5] Coagulation times may also be affected by anesthesia. As a result, a limit of three minutes of 4% isoflurane anesthesia is recommended to avoid these changes.

In the clinical setting, the tail vein is site of choice for blood collection from small mammalian patients with tails (e.g. mice and rats). Veins are located on either side of the tail of mice and can be dilated by placing the tail in warm water or under a heat lamp prior to blood collection. A tourniquet is placed at the base of the mouse's tail and a 25 gauge needle is used to enter the lateral tail vein. Blood is collected into a microcollection device, such as a microhematocrit or Microtainer (Becton-Dickenson, Rutherford, NJ) tube as it drips from the needle hub. Blood for hematology is collected into tubes containing an anticoagulant, such as EDTA or heparin. Blood collected for clinical biochemistry analysis is collected into tubes containing heparin or no anticoagulant. A blood sample can be obtained from the ventral tail artery of a rat placed in dorsal recumbency and under a general anesthetic. A 22-gauge or smaller needle attached to a 3 mL syringe with plunger removed is used to collect the sample. The artery is located slightly off the ventral midline of the tail. The needle is inserted at a point one-third the length from the base of the tail using a 30 degree angle. Blood fills the syringe from the pressure of the artery once the vessel has been penetrated. Pressure is applied to the collection site to control hemostasis following blood sampling. Although rarely needed, puncture of the tail artery can also be attempted for blood collection in ferrets. The artery is approached along the ventral midline of the tail with a 22- or 21-gauge needle directed toward the caudal vertebrae. The artery is usually located 2–3 mm under the skin.

Blood may be collected from the lateral saphenous vein in small animals by applying a tourniquet above the stifle after clipping the hair. Shaving the hair off the lateral aspect of the tibia exposes the vein. The leg is extended, a 5/8 inch, 25-gauge needle is used to cannulate the vein, and blood is collected as it drips from the needle hub into a microcollection device. The lateral saphenous vein is typically small and will easily collapse, making the collection of large sample volumes difficult.

Blood collection from the retroorbital venous plexus is commonly performed in rodents and requires technical skill and general anesthesia. A heparinized microhematocrit tube is placed in the medial canthus of the eye and directed under the globe to the orbital venous plexus. With the rodent in lateral recumbency, the microhematocrit tube is rotated along its long axis as it is advanced toward the venous plexus along the caudal one-half to two-thirds of the orbit. Following blood collection, pressure on the area is required for hemostasis.

Blood can be collected from the ear of some animals (e.g. rabbits). Blood collection from the vessels in the ear of rabbits is performed by a simple drip method, vacuum ear bleeder, or a Vacutainer (Becton-Dickinson Co.) method. Ear vessels can be dilated prior to blood collection by either wrapping the ear in a warm towel or applying a small amount of oil of wintergreen to the vessel to be punctured. The skin is cleaned with an alcohol wipe prior to venipuncture. A 25-gauge needle can be used to puncture the vessel and the blood is collected as it drips from the needle hub into a microcollection device. This procedure minimizes hematoma formation during sampling. Aspiration into a syringe or Vacutainer tube often results in collapse of the vessel in small rabbits. The vacuum ear bleeder method is performed by lacerating an ear vessel and placing the ear inside a flask with a side arm that is attached to a vacuum line and held firmly against the rabbit's head. This method is generally used for research rabbits where large sample volumes are needed.

Jugular venipuncture can be attempted in small mammals, although the jugular veins may be difficult to locate and positioning the animals for the procedure can be stressful to them. Jugular venipuncture may require the use of sedation or general anesthesia is some mammals (e.g. ferrets, hedge-

hogs, and rabbits). Blood collection in ferrets is commonly performed by jugular venipuncture and simply allowing the ferret to lick food at the time of the procedure may be adequate to limit movement without the need of anesthesia. After the neck has been shaved and extended, blood is collected from the jugular vein using a 22- to 25- gauge needle and a 3 mL syringe. The jugular vein of ferrets is usually more lateral than those of dogs and cats and generally runs between the thoracic inlet and the angle of the mandible when the head and neck are extended. Often, the vein cannot be visualized, especially in large males. Blood collection from the jugular vein of hedgehogs usually requires sedation or anesthesia to prevent the animal from balling and to protect the handler from the animal's quills. Because the jugular vein is protected by thick skin in the ventral neck area, it can be difficult to sample blood from hedgehogs using this technique.

Blood is frequently collected from the cranial vena cava in small mammals, but may result in bleeding into the thoracic cavity. Venipuncture of the cranial vena cava is commonly performed in ferrets, where the ferret is held in dorsal recumbency with the forelimbs held along its sides and the head and neck extended. A 23-gauge or smaller needle attached to a 3-mL syringe is inserted into the thoracic cavity between the first rib and the manubrium, advanced caudally at a 45 degree angle to the body, and directed toward the opposite rear limb. The plunger is pulled back as the needle is slowly advanced or withdrawn, to allow blood to enter the syringe. Depending upon the depth of penetration of the needle, jugular venipuncture may actually occur using this technique in the ferret as the jugular vein lies just under the skin in the area of the sternal notch. The cranial vena cava is the most commonly used site for blood collection from hedgehogs and other small mammals using the same technique as described for the ferret. The heart in these animals; however, has a more cranial location compared to the ferret and the phlebotomist must take this into consideration when using this approach.

Cardiocentesis can also be used to collect blood from small mammals, but should be reserved for terminal procedures because of the risk of death during the procedure. Cardiocentesis requires general anesthesia because the heart often moves away from the needle during the procedure. The small mammal is placed in dorsal recumbency and the heart is located by palpation. The needle is inserted slightly to the left of and under the manubrium and advanced toward the heart, which is stabilized by a thumb and forefinger.

General hematologic features of small mammals

The hematology of laboratory and other small mammals is similar to that of domestic mammals. However, obtaining meaningful reference values can be difficult because of variations associated with blood collection, environmental factors, and laboratory procedures. Blood collection often causes stress or requires chemical restraint. The hemogram can vary with age, environmental conditions, diet, gender, and reproductive status. Also, laboratory procedures and sample handling are not standardized, creating variability between data sets. Tables 18.2 and 18.3 provide suggested reference intervals for erythrocyte and leukocyte parameters, respectively for small mammals.

The erythrocytes of mammals are small, compared to the nucleated erythrocytes of other vertebrates.[4,6,7] The small, nonnucleated, biconcave shape minimizes the hemoglobin to surface distance during gas exchange and increases cell plasticity to improve movement through blood vessels, increasing oxygen delivery to tissues. The hemoglobin content and packed cell volume remain relatively constant among the mammals, but the total erythrocyte count and mean cell size varies. An inverse relationship between cell size and number exists.

The granulocytes of nondomestic mammals vary in appearance but can be classified as neutrophils or heterophils, eosinophils, and basophils.[4,6,7] The heterophils of rabbits and some rodents were previously called pseudoeosinophils because their granules do not stain neutral with Romanowsky stains but are distinctly eosinophilic.[8] Neutrophils of mice often have nonlobed nuclei and those of normal primates appear hypersegmented. Cytochemical and ultrastructural features of cells differ between species. For example, lysozyme activity is lacking in the neutrophils of hamsters and alkaline phosphatase activity is less in neutrophils of mice.[9] Neutrophils of mammals are phagocytic; one of their primary functions is to destroy microorganisms. Circulating neutrophil concentration increases with inflammation especially that associated with invading microorganisms, such as bacteria.

The granules of eosinophils become intensely eosinophilic with maturation as a result of the basic protein content. The ultrastructure of the granules in mammalian eosinophils reveals a distinct crystalline shape that varies with species; for instance, a trapezoidal pattern is found in the eosinophils of guinea pigs and true rodents and a needle-shaped pattern is found in rabbit eosinophils.[9] Mammalian eosinophils have phagocytic activity similar to that of neutrophils, but are less effective. Mammalian eosinophils respond to metazoan infections (especially those involving helminth larvae), allergic inflammation (especially those associated with mast cell and basophil degranulation), and antigen-antibody complexes. Therefore, eosinophilia suggests one of these processes.

Mammalian basophils have characteristic cytoplasmic granules that are strongly basophilic in Romanowsky stained blood films. Unlike basophils of lower vertebrates, those of mammals tend to have lobed nuclei. The ultrastructural

Table 18.2 Erythrocyte parameters for laboratory animals and miscellaneous species (mean and reference interval).

	PCV	RBC	Hb	MCV	MCHC	MCH	Reticulocytes
	%	$\times 10^6/\mu L$	g/dL	fL	%	pg	%
Mice †	40.4	8.3	13.1	49.1	32.3	15.9	4.7
	32.8–48.0	6.5–10.1	10.1–16.1	42.3–55.9	29.5–35.1	13.7–18.1	0–11.3
Rats †	46.1 ± 2.5	7.8	14.8	59.0 ± 6.4	32.4	18.9	2.2
	41.1–51.1	6.6–9.0	13.2–16.4	52.6–65.4	30.2–34.6	16.5–21.3	0–4.6
Guinea	42.1	5.1	12.9	83.0	30.6	—	2.3
pigs †	35.9–48.3	4.1–6.1	10.5–15.3	75.0–91.0	28.2–33.0		0–6.1
Hamsters †	52.5	7.5	16.8	71.2	—	—	—
	47.9–57.1	2.7–12.3	13.4–19.2	64.8–77.6			
Chinchilla †	38.3	6.6	11.7	58.0	—	—	—
	25.0–52.0	5.2–9.9	8.8–15.4	—			
Rabbits †	42.0	6.0	13.3	70.4	31.7	22.3	3.7
	36.6–47.4	5.2–6.8	11.5–15.1	64.6–76.2	29.5–33.9	21.1–24.5	1.1–6.3
Ferrets ‡	49.2	8.11	16.2	47.1	32.0	15.0	5.3
	42–55	6.8–9.8	14.8–17.4	42.6–51.0	30.3–34.9	13.7–16.0	2–14
Hedgehogs*	36	6	12.0	67	34	22	—
	22–64	3–16	7.0–21.1	41–94	17–48	11–31	

†Modified from Jain NC. *Essentials of Veterinary Hematology*. Philadelphia, Lea & Febiger, 1993, pp. 54–71.
‡Modified from Fox JG. Normal clinical and biologic parameters. In Fox JG ed. *Biology and Diseases of the Ferret*. Philadelphia, Lea & Febiger, 1988, pp. 159–73.
*Ivey E, Carpenter JW. African hedgehogs. In Quesenberry KE and Carpenter JW eds. *Ferrets, Rabbits, and Rodents; Clinical Medicine and Surgery*, 2nd ed. St. Louis, Saunders, 2003, p. 345.

Table 18.3 Leukocyte parameters of laboratory animals and miscellaneous species (mean and reference intervals).

	WBC		Bands	Neutrophils	Lymphocytes	Mono-cytes	Eosinophils	Baso-phils	Platelets
	$\times 10^3/\mu L$								$\times 10^3/\mu L$
Mice†	6.33	$\times 10^3/\mu L$	0	1.20	4.86	0.14	0.08	0	1.16
	2.61–10.05		0–0.02	0.4–2.0	1.27–8.44	0–0.29	0–0.17	0–0.02	0.78–1.54
Rats†	9.98	$\times 10^3/\mu L$	0	2.48	7.07	0.25	0.17	0	1.04
	7.30–12.66		0-0.02	1.25–3.71	5.07–9.07	0.05–0.44	0.04–0.30	0–0.03	0.84–1.24
Guinea	11.11	$\times 10^3/\mu L$	0	2.50	8.01	0.31	0.27	0	0.55
pigs†	8.22–14.0		0–0.01	1.35–3.65	5.47–10.55	0.06–0.56	0–0.69	0–0.02	0.39-0.71
Hamsters†	7.62	%	—	29.9 ± 8.0	73.5 ± 9.4	2.5 ± 0.8	1.1 ± 0.02	0	—
	6.32–7.92								
Chinchilla†	8.0	%	—	44.6	53.6	1.2	0.5	0.4	—
	2.2–45.1			10.0–78.0	19.0–98.0	0.0–5.0	0.0–9.0	0.0–11.0	
Rabbits†	8.18	$\times 10^3/\mu L$	0	2.35	5.18	0.25	0.08	0.21	0.43
	6.30–10.06			1.49–3.21	3.36–7.00	0.05–0.45	0.01–0.15	0.06–0.36	0.25–0.61
Ferrets‡	10.5	%	—	59.5	33.4	4.4	2.6	0.2	545
	4–18			43–84	12–50	2–8	0–5	0–1	310–910
Hedgehogs*	11	$\times 10^3/\mu L$	—	5.1	4.0	0.3	1.2	0.4	229
	3–43			0.6–37.4	0.9–13.1	0.0–1.6	0.0–5.1	0.0–1.5	60–347

†Modified from Jain NC. *Essentials of Veterinary Hematology*. Philadelphia, Lea & Febiger, 1993, pp. 54–71.
‡Modified from Fox JG. Normal clinical and biologic parameters. In Fox JG ed. *Biology and Diseases of the Ferret*. Philadelphia, Lea & Febiger, 1988, pp. 159–73.
*Ivey E, Carpenter JW. African hedgehogs. In Quesenberry KE and Carpenter JW eds. *Ferrets, Rabbits, and Rodents; Clinical Medicine and Surgery*, 2nd ed. St. Louis, Saunders, 2003, p. 345.

appearance of the granules varies with species; for instance, a coiled threaded pattern is observed in basophil granules from primates and rabbits and a homogenous pattern is observed in rodents.[9] Basophils participate in allergic and delayed hypersensitivity reactions.

Mammalian monocytes generally are the largest leukocytes in peripheral blood films and do not vary grossly in appearance between species. The monocyte nucleus varies in shape and the moderately abundant cytoplasm is typically light blue-gray. The granules, when present, are very fine and appear azurophilic in Romanowsky stained preparations. Monocytes engulf and degrade microorganisms, abnormal cells, and cell debris. Monocytes also regulate immune responses and myelopoiesis.

The appearance of mammalian lymphocytes varies depending upon the species, lymphocyte type, and degree of activation. Mammalian lymphocytes vary in size, color of cytoplasm (light to dark blue), and degree of nuclear chromatin condensation. Variability depends on the degree of antigenic stimulation and type of lymphocyte.

In general, the leukocyte morphology of nondomestic mammals is a reliable indication of disease. The presence of immature cells, toxic neutrophils, and Döhle bodies are more reliable criteria for infectious diseases than are total leukocyte and differential counts, given the amount of information known regarding various strains and breeds.

Hematologic features of rodents

Mice (*Mus musculus*) and rats (*Rattus norvegicus*)

Hematologic parameters of mice and rats are influenced by a variety of factors, including site of sample collection, age, gender, strain, anesthesia, method of restraint, temperature, and stress.[1,5,10–12] In rats, collection of blood from the heart resulted in a significant decrease in the erythrocyte and leukocyte counts, hemoglobin concentration, and hematocrit compared to samples taken from the retroorbital venous sinus and tail.[11] A distinct circadian rhythm affects peripheral leukocyte concentrations with an increase in circulating leukocyte concentration occurring during the light phase and a decrease during the dark phase. A distinct decrease in the total leukocyte count associated with a decrease in lymphocytes occurs in mice following the stress, such as occurs during transportation.[10,13] Thus, it is difficult to establish reference hematologic values for mice and rats because of the large number of strains and variations in blood collection methods, handling techniques, and environmental conditions. Published reference intervals for several strains of rats and mice are available and the reader should refer to the references at the end of this chapter (Table 18.2).[2,3,14] Erythrocytes of healthy rats and mice vary in size with a range between 5 to 7 µm in diameter; therefore, a marked anisocytosis is common. Polychromasia is also common with

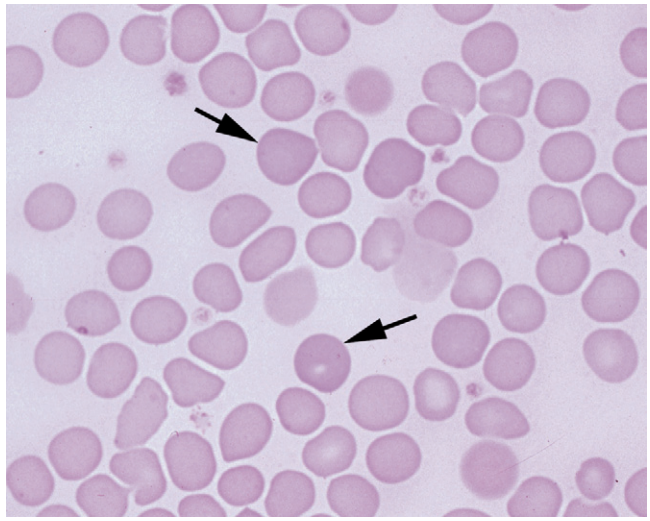

Figure 18.1 Polychromatic erythrocytes (arrows) in the blood of a rat. Wright-Giemsa stain.

polychromatic cells representing 1–18% of the erythrocyte population (Fig. 18.1).[15] This is likely related to the relative short erythrocyte half-life of 45–68 days for rats and 40–50 days for mice.[2,3] Adult rats and mice normally have a high degree of reticulocytosis with means that average between 2 and 7%; the young have even higher numbers that ranging between 10 and 20%. Erythrocyte concentrations in females tend to be less than those of males. The normal PCV is 39–54% for rats and 35–45% for mice. The hemoglobin concentration generally ranges between 13.4 and 15.8 g/dL with a mean of 14.6 g/dL. Howell-Jolly bodies are found in small numbers of erythrocytes in normal rats and mice. Rouleaux formation of erythrocytes is rarely seen, even with inflammatory disease.

Granulocytes of mice and rats often have nuclei without distinct lobes and typically have a horseshoe, sausage, or ring (doughnut) shape (Fig. 18.2).[4,16] The ring shape results from a gradually increasing hole that develops in the nucleus during maturation of the granulocyte. Nuclear segmentation occurs as the ring breaks during maturation and begins to form constrictions. Neutrophils usually have a colorless cytoplasm but may contain few dustlike red granules, thus appearing diffusely pink with Romanowsky stains (Fig. 18.3). Rat neutrophils measure 11 µm in diameter. In general, the neutrophil represents 12–38% of the leukocyte differential. Eosinophils have a ring- or U-shaped nucleus, a basophilic cytoplasm, and numerous small round eosinophilic cytoplasmic granules that may be arranged in small clumps (Fig. 18.4). Eosinophils generally comprise 0–7% of the leukocyte differential. Basophils are present in small numbers (0–1%) and contain numerous basophilic granules (Fig. 18.5). Basophils should be differentiated from mast cells that may appear in peripheral blood, especially when

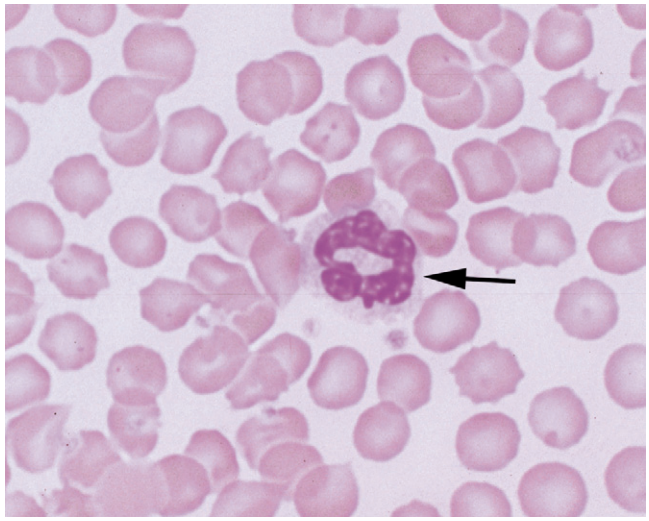

Figure 18.2 A neutrophil (arrow) with a nucleus forming a ring in the blood of a rat. Wright-Giemsa stain.

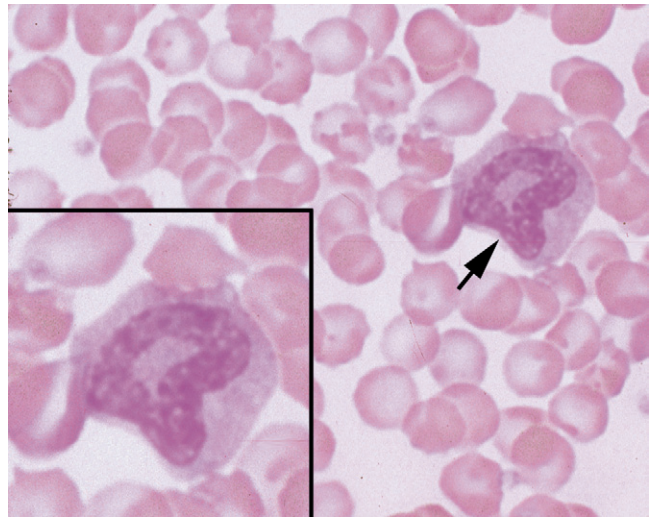

Figure 18.4 An eosinophil (arrow) with a nucleus forming a ring in the blood of a rat. Wright-Giemsa stain.

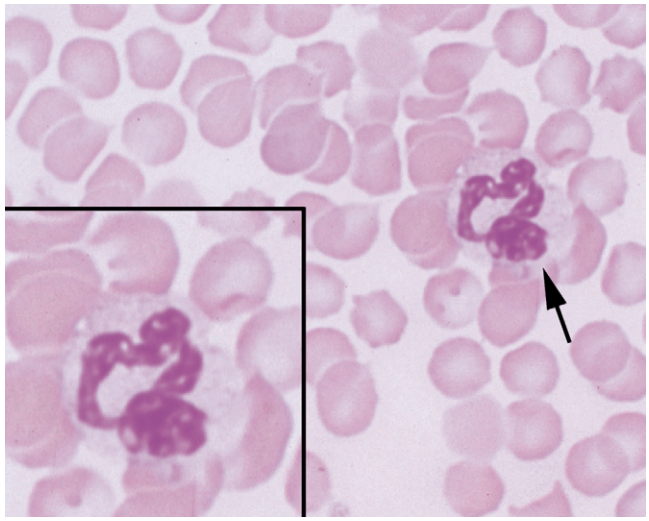

Figure 18.3 A neutrophil (arrow) with fine, pink cytoplasmic granules in the blood of a rat. Wright-Giemsa stain.

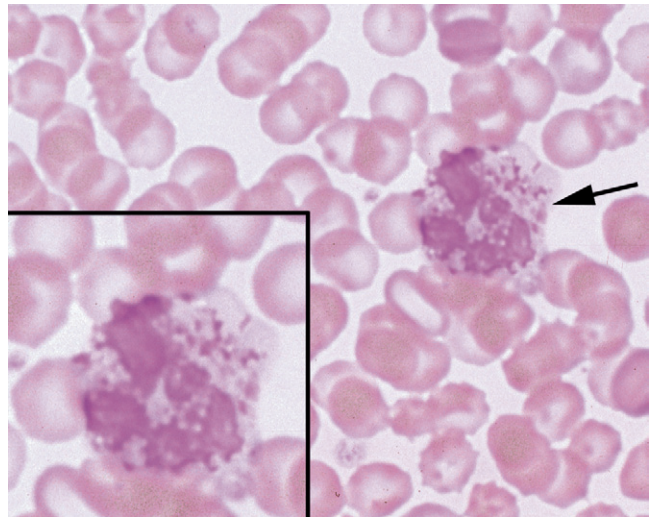

Figure 18.5 A basophil (arrow) in the blood of a rat. Wright-Giemsa stain.

cardiocentesis is performed. Basophil numbers appear higher in blood collected from the tail of mice and rats when excessive trauma is involved, such as laceration technique and compressing the tail to facilitate blood flow.[2]

Lymphocytes are the predominant leukocyte in the peripheral blood of rats and mice where they represent 60–75% and 70–80% of the leukocyte population, respectively. The size of lymphocytes ranges from the size of erythrocytes to the size of neutrophils. The cytoplasm of lymphocytes stains light blue and azurophilic cytoplasmic granules are occasionally found in large lymphocytes (Fig. 18.6).

Monocytes (17 µm diameter) are the largest leukocyte found in the peripheral blood of rats and mice. They account for 1–6% of the leukocyte population in rats and 0–2% in mice. Monocytes have a variably shaped nucleus with the kidney-bean shape being the most common form. The abundant blue-gray cytoplasm often contains fine azurophilic granules and occasional vacuoles.

Leukocyte concentrations of mice and rats not only demonstrate a distinct diurnal variation, but also vary markedly between strains.[12] There is an age-dependent variation in the neutrophil to lymphocyte ratio, with the lymphocyte

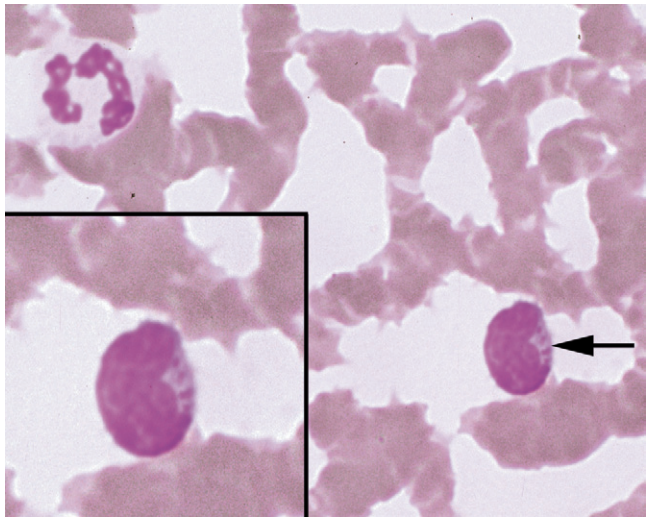

Figure 18.6 A lymphocyte (arrow) with azurophilic granules in the blood of a rat. Wright-Giemsa stain.

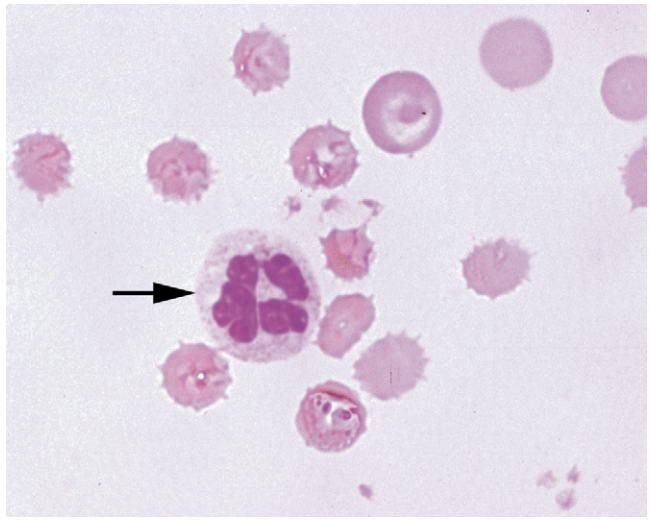

Figure 18.7 A neutrophil (arrow) in the blood of a guinea pig. Wright-Giemsa stain.

concentration decreasing and neutrophil concentration increasing as a rodent ages.

Platelet concentrations in rodents tend to be high compared to those of larger domestic mammals. Platelet concentrations greater than 1×10^6 per µL are common.

Guinea pigs (*Cavia porcellus*)

Guinea pigs have larger erythrocytes than other rodents with a range of 6.6–7.9 µm.[17] The total erythrocyte count and hemoglobin concentration of guinea pigs are generally lower than those of true rodents. Polychromasia and a macrocytosis characterize regenerative responses to anemia. The neutrophils of guinea pigs measure 10–12 µm in diameter, have a pyknotic segmented nucleus, and contain cytoplasmic granules that stain eosinophilic that often cause them to be referred to as heterophils or pseudoeosinophils (Fig. 18.7). Although they stain differently than the neutrophils of domestic mammals with Romanowsky stains, the neutrophils of guinea pigs are equivalent in function. Guinea pig eosinophils (10–15 µm in diameter) are larger than the neutrophils and have larger round to rod-shaped bright red cytoplasmic granules, making eosinophils easy to differentiate from neutrophils (Fig. 18.8). The granules of basophils are reddish-purple to black. Like those of rats and mice, lymphocytes are the predominant leukocyte in the differential of healthy guinea pigs and small lymphocytes (approximately the size of erythrocytes) are the most common form. Large lymphocytes are almost twice as large as small lymphocytes, have a slightly smaller nucleus: cytoplasmic ratio, and often contain azurophilic granules. Approximately 3–4% of the leukocytes in the peripheral blood of adult guinea pigs are large mononuclear cells that contain a single, large cytoplasmic inclusion referred to as a Kurloff body (Fig. 18.9). These Foa-Kurloff cells are unique to cavies, such as guinea pigs. The finely granular and occasionally vacuolated Kurloff bodies stain homogeneously red with Romanowsky stains and stain positive with toluidine blue and PAS.[8] They appear to be influenced by sex hormones and occur in low numbers in immature male guinea pigs. The exact function of these cells is not known, but many speculate that they may function as killer cells.[17]

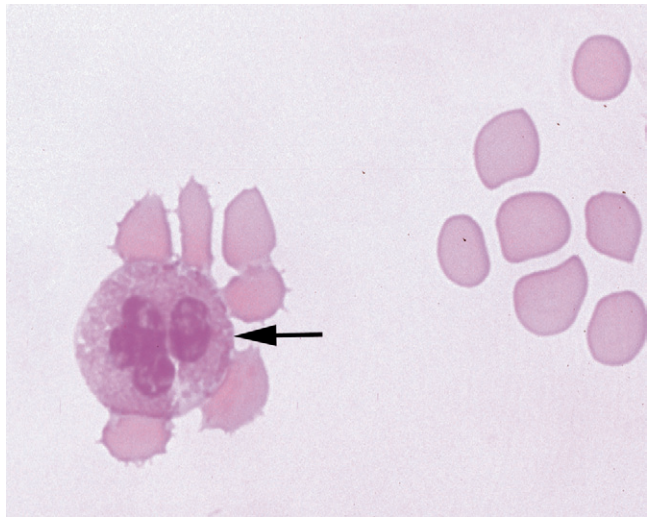

Figure 18.8 An eosinophil (arrow) in the blood of a guinea pig. Wright-Giemsa stain.

Other rodents

The hematologic features of hamsters (*Mesocricetus auratus*), gerbils (*Meriones unguiculatus*), and chinchillas (*Chinchilla*

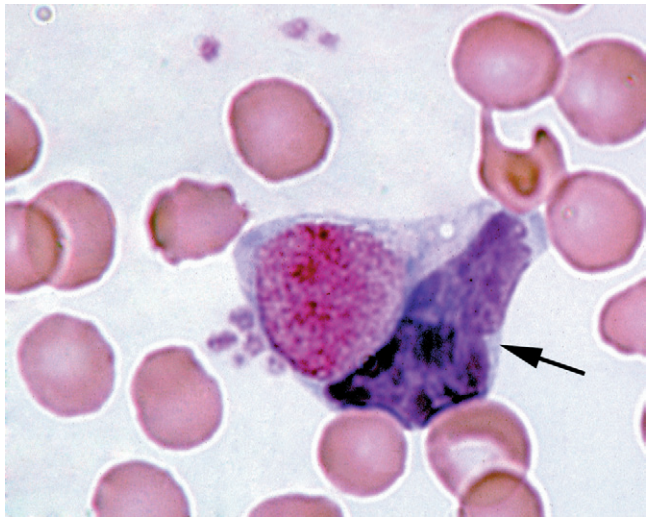

Figure 18.9 A lymphocyte (arrow) containing a Kurloff body in the blood of a guinea pig. Wright-Giemsa stain.

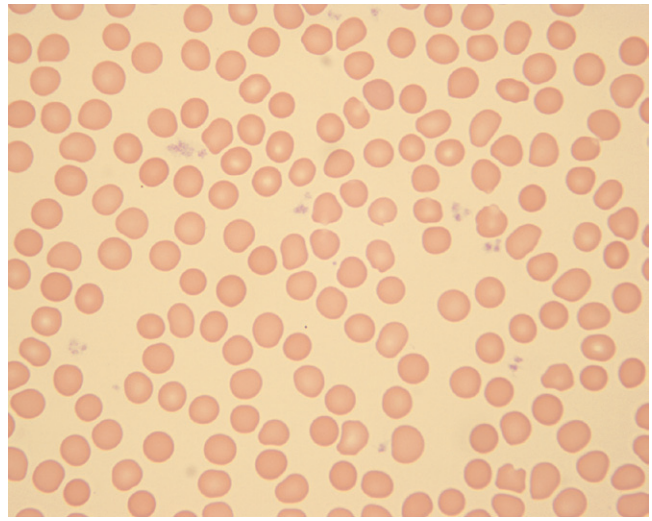

Figure 18.10 Erythrocytes in the blood of a rabbit. Wright-Giemsa stain, 500x.

lanieger) resemble those of mice and rats.[18,19] As with rats and mice, polychromasia is normal finding in blood film from hamsters and gerbils and Howell-Jolly bodies are common. Nucleated red blood cells may account for up to 2% of the erythrocytes in blood films of normal hamsters.

The neutrophils of chinchillas are typically hyposegmented and resemble neutrophils of dogs with the Pelger-Huet anomaly. Hamster neutrophils contain prominent round to rod-shaped eosinophilic cytoplasmic granules and are frequently referred to as heterophils. Lymphocytes predominate the leukocyte differential of gerbils; however, a diurnal variation occurs in the numbers and types of leukocytes in the blood of hamsters. Leukocyte numbers of hamsters, a nocturnal animal, increase significantly at night when they are most active with neutrophils rather than lymphocytes being responsible for the increase.[20] Small and large lymphocytes are found in the blood of hamsters, but unlike other rodents, neutrophils tend to predominate the leukogram differential.

Hematologic features of rabbits (*Oryctolagus cuniculus*)

The PCV of healthy rabbits generally range between 30 and 50%. The rabbit erythrocyte is a biconcave disk with an average diameter of 6.8 µm; however, the presence erythrocytes with a range of 5.0–7.8 µm makes reporting of a significant anisocytosis a common finding in the hemogram of normal rabbits (Fig. 18.10). Like rodents, polychromatic erythrocytes and reticulocytes are common in blood films of normal rabbits. Polychromasia is commonly observed in 2–4% of the erythrocyte population of normal rabbits. Nucleated erythrocytes and Howell-Jolly bodies are occasionally observed. The estimated half-life of rabbit erythrocytes is between 57 and 67 days.[20] Male rabbits tend to have higher erythrocyte counts and hemoglobin concentrations than females. The total erythrocyte count, hemoglobin concentration, and hematocrit values can be significantly lower in the pregnant rabbits in the third trimester compared to nonpregnant rabbits; however, the MCV value increases.[21] Use of a general anesthetic does not appear to have an effect on the hematologic test results in rabbits.[22] Erythrocyte fragility studies in rabbits based upon the sodium chloride concentrations indicate the first detectable hemolysis at 0.5–0.3% NaCl.[23] As with most other species of mammals, a regenerative response to an anemia is characterized by increased anisocytosis, polychromasia, nucleated erythrocytes, and presence of Howell-Jolly bodies. Anemia is commonly associated with a variety of diseases in rabbits. Infectious diseases often result in increases in the number of nucleated erythrocytes.

The rabbit neutrophil is between 10 and 15 µm in diameter. The polymorphic nucleus stains light blue to purple with Romanowsky stains. The cytoplasm of rabbit neutrophils typically stains diffusely pink with Romanowsky stains due to the fusion of the many small acidophilic granules (primary granules) (Figs. 18.11, 18.13, and 18.17). These cells are often referred to as pseudoeosinophils or heterophils in the literature because of the larger eosinophilic cytoplasmic granules (secondary granules) that stain dark pink to red with Romanowsky stains. Rabbit neutrophils are ultrastructurally, functionally, and biochemically equivalent to neutrophils from other domestic mammals and humans.[9] An occasional neutrophil with characteristics of the Pelger-Huet anomaly may be observed in blood films from normal rabbits. Rabbit neutrophils are easily distinguished from the eosinophils, which have large eosinophilic granules.

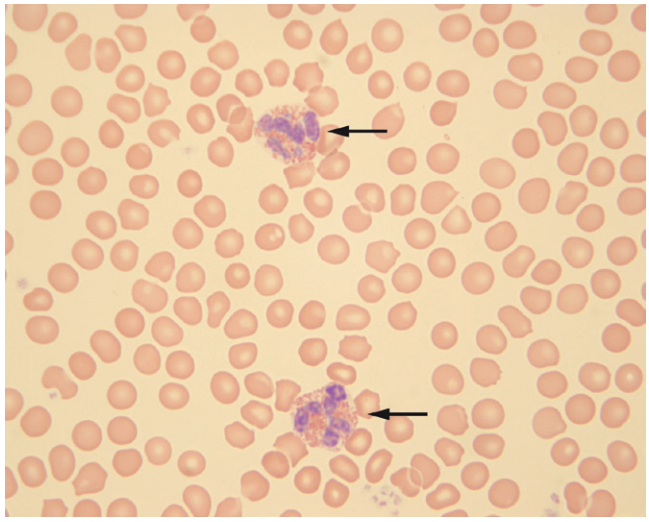

Figure 18.11 Neutrophils (heterophils; arrows) in the blood of a rabbit. Wright-Giemsa stain.

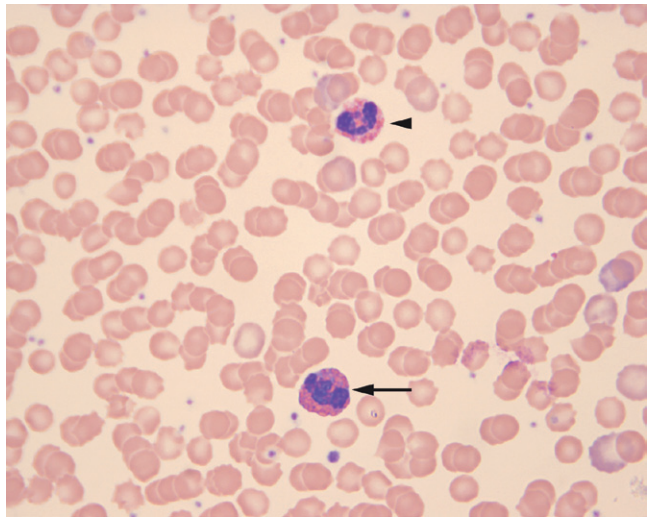

Figure 18.13 An eosinophil (arrow) and a neutrophil (heterophil; arrowhead) in the blood of a rabbit. Wright-Giemsa stain.

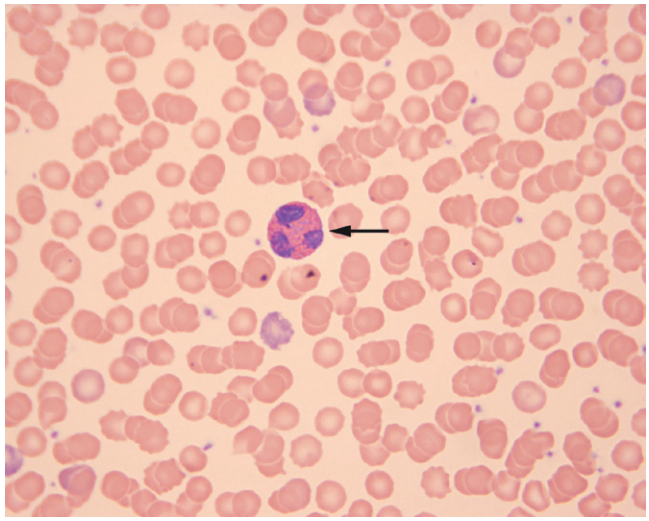

Figure 18.12 An eosinophil (arrow) in the blood of a rabbit. Wright-Giemsa stain.

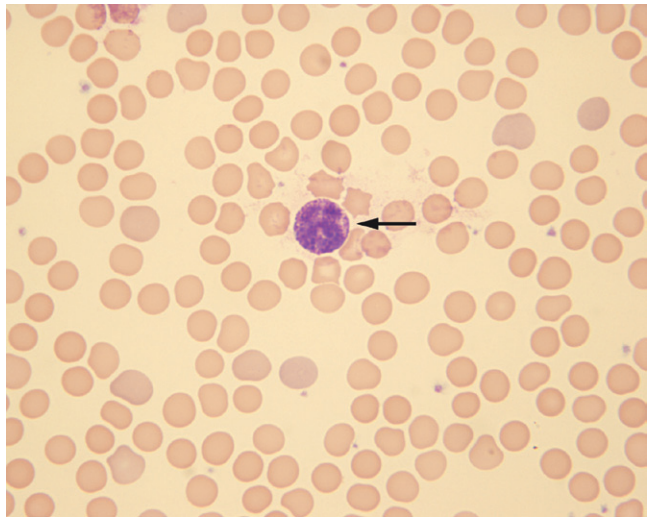

Figure 18.14 A basophil (arrow) in the blood of a rabbit. Wright-Giemsa stain.

The eosinophils of rabbits are larger than the neutrophils and are between 12 and 16 µm in diameter (Figs. 18.12, 18.13, and 18.17). The large cytoplasmic granules of the eosinophil are more numerous than those of neutrophils. Eosinophil granules are poorly defined and stain intensely pink to a dull pink-orange with Romanowsky stains creating a tinctorial quality that differs from the neutrophil granules. The nucleus of the eosinophil is often bi-lobed to U-shaped.

Rabbits typically have more basophils than do other species; commonly five percent of the leukocytes are basophils, but they can be as high as 30% in rabbits with no apparent abnormalities.[4] The basophils of rabbits resemble those of domestic mammals (Figs. 18.14 and 18.17).

Rabbit lymphocytes are morphologically similar to those of other domestic mammals and humans (Fig. 18.15). The majority of lymphocytes are small, between 7 and 10 µm in diameter; however, large lymphocytes between 10 and 15 µm in diameter may also be present. Azurophilic granules are often commonly present in the cytoplasm of the large lymphocytes.

Rabbit monocytes are similar to those found in other domestic mammals (Figs. 18.16 and 18.17). The nucleus varies from lobulated to bean-shaped and the cytoplasm stains blue and may contain a few vacuoles.

The normal leukocyte concentration of rabbits is typically reported to range between 7000 and 9000/µL. Variations

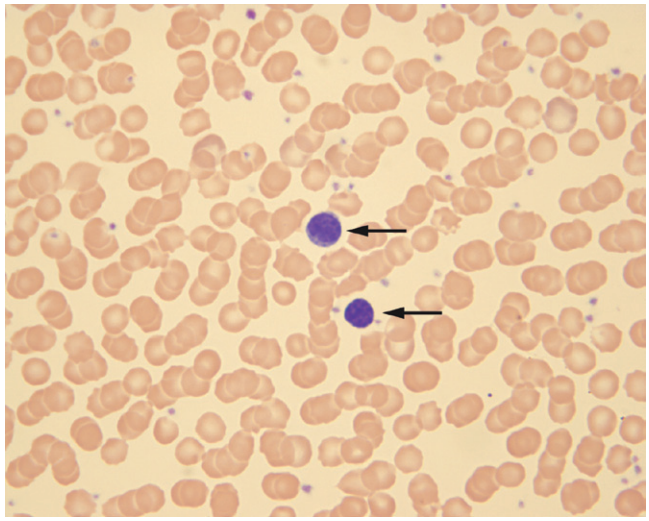

Figure 18.15 Lymphocytes (arrows) in the blood of a rabbit. Wright-Giemsa stain.

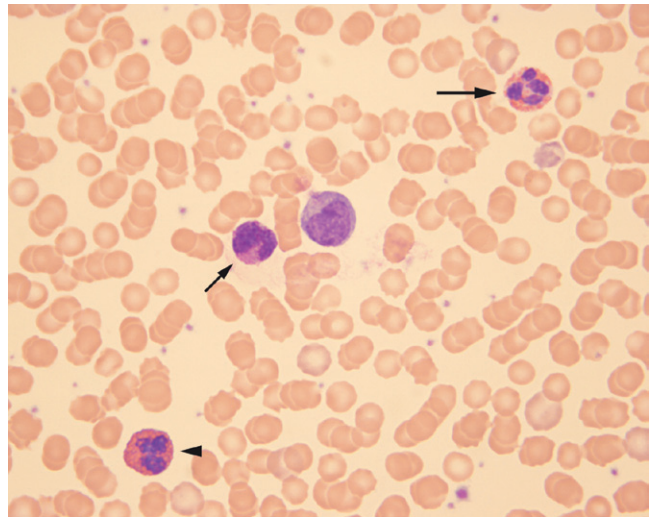

Figure 18.17 A monocyte (large cell in center), neutrophil (heterophil; long arrow), eosinophil (arrowhead), and basophil (short arrow) in the blood of a rabbit. Wright-Giemsa stain.

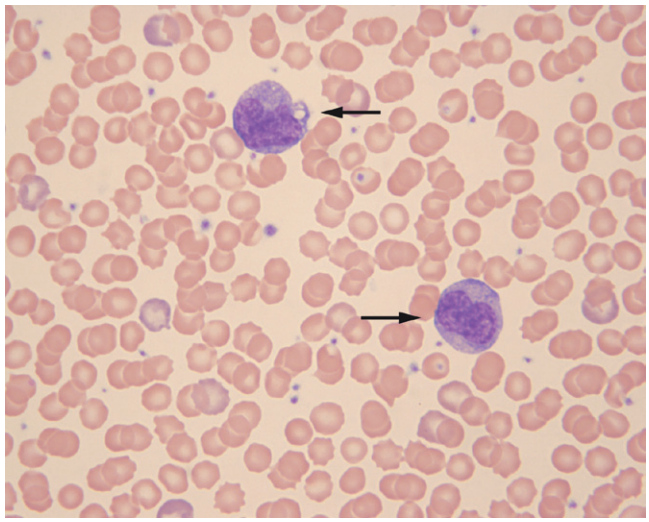

Figure 18.16 Monocytes (arrows) in the blood of a rabbit. Wright-Giemsa stain.

occur with age, restraint methods, methods of blood collection which may alter the neutrophil : lymphocyte (N:L) ratio, and pregnancy. A bimodal increase in the leukocyte concentration is seen with increasing age, with highest lymphocyte concentration occurring at three months of age then slowly declining, and highest neutrophil concentrations occurring in older animals.[20] The normal neutrophil: lymphocyte ratio of 33:60 at 2 months of age changes to 45:45 by 12 months of age. Therefore, rabbits younger than twelve months of age are expected to have lower N:L ratios than do older rabbits, which typically have equal numbers of neutrophils and lymphocytes. A stress response associated with restraint during blood collection procedures can result in as much as a 15–30% decrease in the total leukocyte concentration.[10] A mature neutrophilia and lymphopenia characterize glucocorticoid-mediated changes in the leukogram. Pregnant rabbits demonstrate a slight increase in total leukocyte counts during the first half of gestation owing to an increase in lymphocyte numbers; however, a significant decrease can occur in the second half owing to a decrease in lymphocytes and/or neutrophil numbers.[21]

Rabbits generally do not develop a leukocytosis with bacterial infections, but will have a reversal of the N:L ratio; N:L ratio reversal is also associated with increases in serum cortisol concentrations.[24] Therefore, evaluation of the N:L ratio appears to be the more reliable indicator of inflammatory disorders than are total leukocyte concentrations.

Hematologic features of ferrets (*Mustela putorius*)

The hematology of ferrets resembles that of domestic carnivores.[25] Ferrets are commonly anesthetized to restrain them for blood collection. The use of inhalant anesthetics such as isoflurane, enflurane, and halothane result in significant and rapid decreases in the red blood cell count, hematocrit, and hemoglobin concentration. As much as a 33% decrease in the hemoglobin concentration occurs with the use of these inhalant anesthetics.[5] Splenic sequestration and anesthetic-induced hypotension are possible causes for this response in ferrets. The erythron returns to normal within 45 minutes of recovery from the anesthetic. Either the use of manual restraint or injectable anesthesia such as ketamine or rapid blood collection following anesthetic induction (less than 3 minutes) is required to avoid this effect in the erythron.

The hemogram of domestic ferrets is influenced by gender and age. Young hobs (males) have lower red blood cell counts, hematocrits, and hemoglobin concentrations than adult hobs and young jills (females).[26] Jills have a decrease in the hematocrit with age.

Common causes of nonregenerative anemia in domestic ferrets include malignant neoplasia such as lymphoma, systemic infections, and hyperestrogenism in intact females. Gastrointestinal ulcers are a common cause of blood loss anemia.

The morphology of ferret leukocytes is similar to that of dogs (Figs. 18.18 and 18.19). The ranges in size for the various ferret granulocytes are 10–13 µm for neutrophils in males and 9–10 µm in females; and 12 µm and 14 µm for eosinophils and basophils respectively regardless of gender. The size of small lymphocytes ranges between 6 and 9 µm in male ferrets and 8 and 10 µm in females. Large lymphocytes and monocytes measure 11 to 12 µm and 12 to 18 µm in both sexes respectively. Neutrophil concentrations are higher than lymphocyte concentrations in normal ferrets.[27] Ferrets have an increase in neutrophil concentration and decrease in lymphocyte concentration with increasing age. The total leukocyte count of healthy ferrets can be as low as 3000/µL. Ferrets are unable to develop a marked leukocytosis with inflammatory disease and concentrations greater than 20,000/µL is unusual and a left shift is rare.[28]

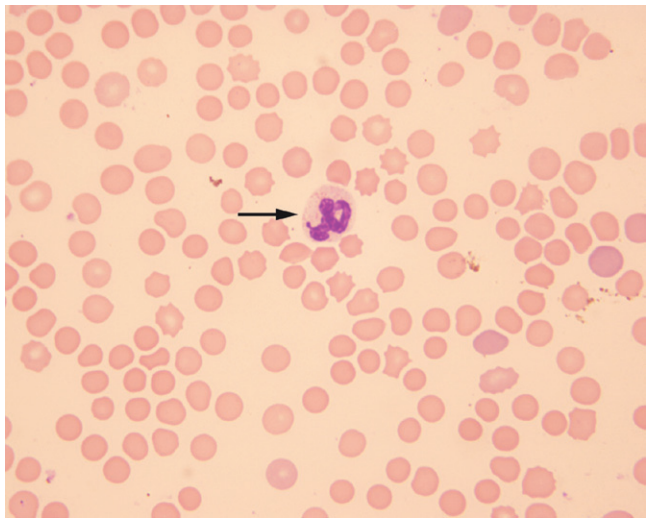

Figure 18.18 A neutrophil (arrow) in the blood of a ferret. Wright-Giemsa stain.

Results of coagulation studies in ferrets vary depending upon methodology. For example, a significant prothrombin time was obtained using a manual method (12.3 ± 0.3 seconds) compared to an automated method (10.9 ± 0.3 seconds).[29] Activated partial thromboplastin time; however was not significantly different between the two methods (18.7 ± 0.9 for the manual method and 18.1 ± 1.1 seconds for the automated method). Fibrinogen concentration for ferrets is reported as 107.4 ± 19.8 mg/dL and antithrombin activity is 96 ± 12.7%.

Hematologic features of African hedgehogs (*Atelerix albiventris*)

The hematology of African hedgehogs resembles that of domestic carnivores. The morphology of erythrocytes and leukocytes is similar to that of other small mammals (Figs. 18.20 through 18.26). Likewise, interpretation of changes in the hemogram are based upon those same changes in other small mammals.

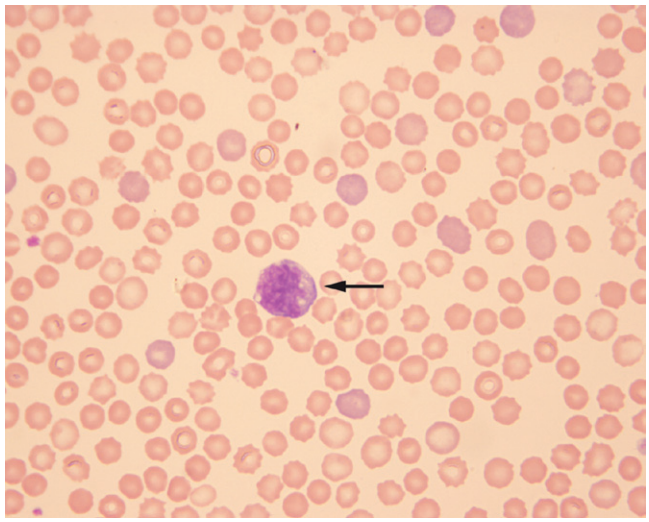

Figure 18.19 A monocyte (arrow) in the blood of a ferret. Wright-Giemsa stain.

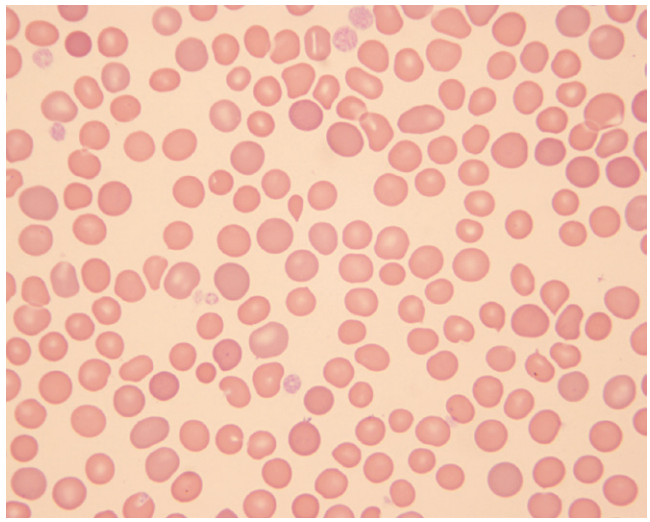

Figure 18.20 Erythrocytes in the blood of a hedgehog. Wright-Giemsa stain.

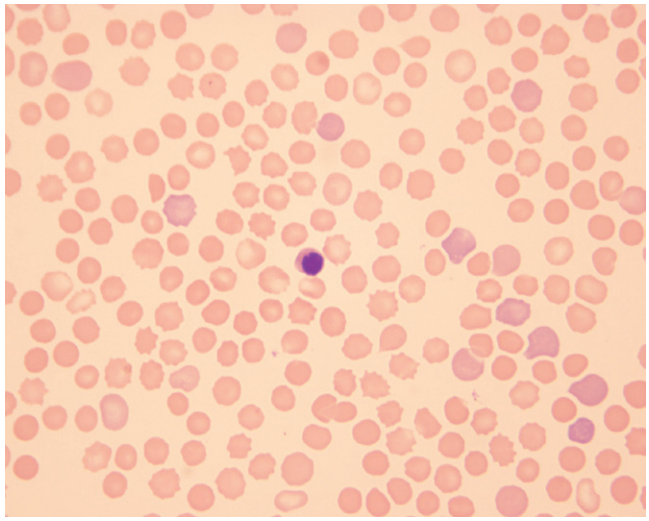

Figure 18.21 A nucleated erythrocyte in the blood of a hedgehog. Wright-Giemsa stain.

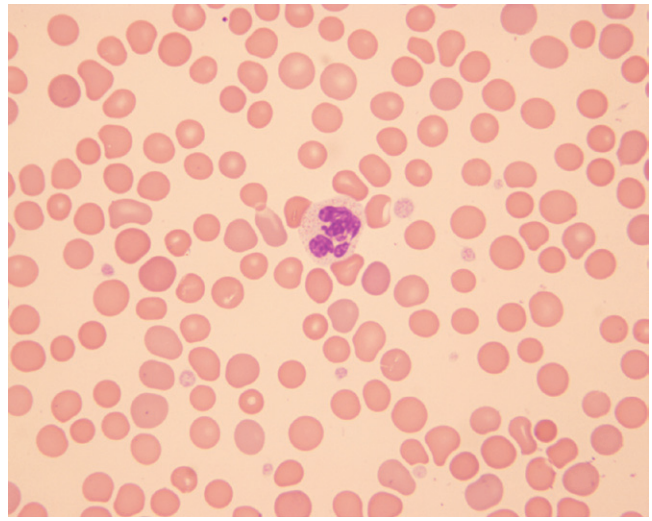

Figure 18.22 A neutrophil in the blood of a hedgehog. Wright-Giemsa stain.

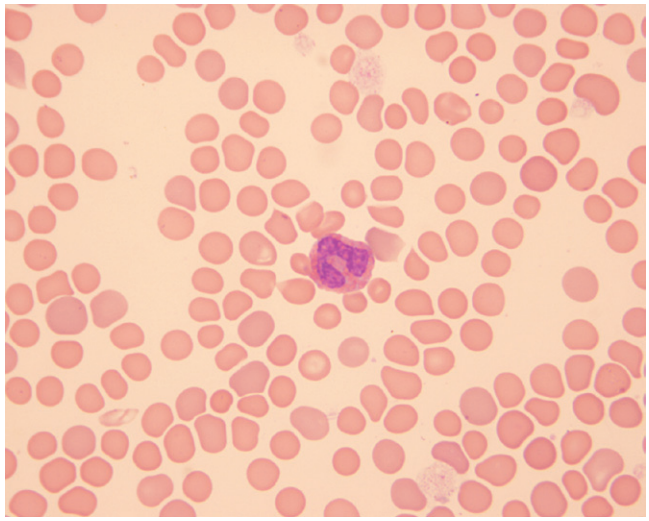

Figure 18.23 An eosinophil in the blood of a hedgehog. Wright-Giemsa stain.

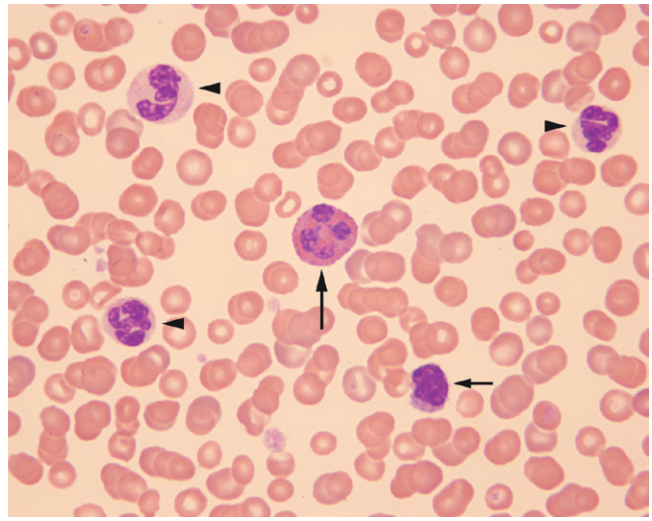

Figure 18.24 An eosinophil (long arrow), a lymphocyte (short arrow), and a neutrophil (arrowhead) in the blood of a hedgehog. Wright-Giemsa stain.

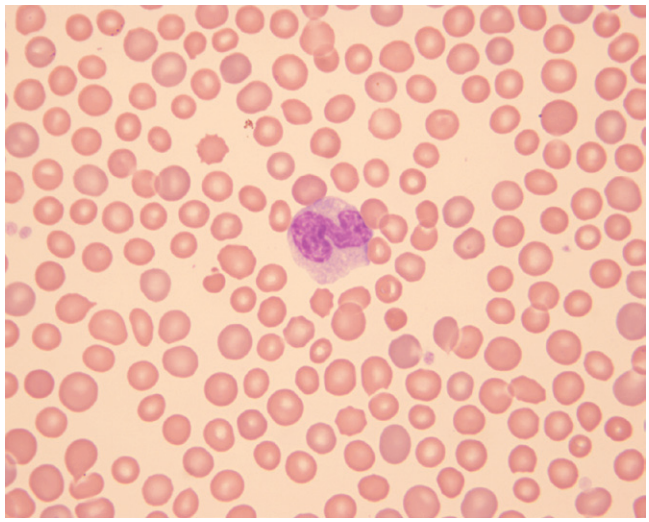

Figure 18.25 A monocyte in the blood of a hedgehog. Wright-Giemsa stain.

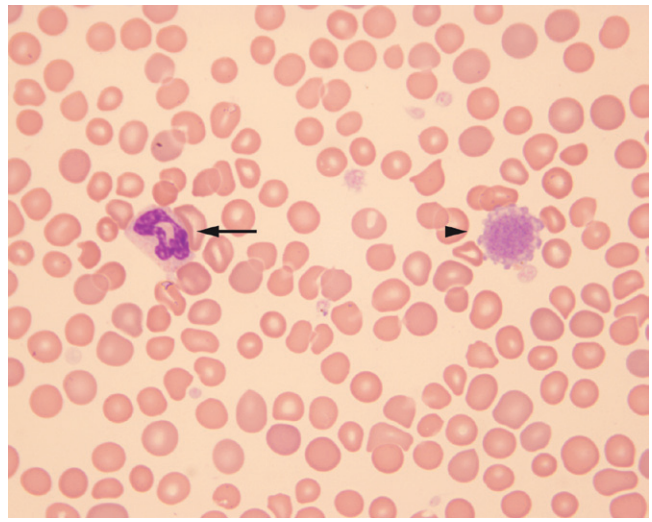

Figure 18.26 A neutrophil (arrow) and giant platelet (arrowhead) in the blood of a hedgehog. Wright-Giemsa stain.

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19 Hematology of Birds

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The normal values for the hematologic parameters of each species of bird have a broad range because of the influences of various intrinsic and extrinsic factors. In general, avian hematological values are subject to extensive variability resulting from different environment and management practices, which can affect physiological responses. For example, heterophil numbers are altered by seasonal changes, diurnal rhythm, gender and age, and diet. Normal hematologic values vary between the species as well. The different avian species from which blood samples are submitted to veterinary laboratories creates a significant logistical challenge to the development of clinically relevant normal values. Thus, published references should only be used as guidelines.[1]

Avian hematology is approached in a manner similar to that of human and mammalian hematology, but a few differences require modification of the hematologic procedures. Major differences include the presence of nucleated erythrocytes, thrombocytes, and heterophil granulocytes in the peripheral blood of birds.

Collection and handling of blood samples

The amount of blood that can be safely removed from a bird depends on its body size and health status. A blood volume representing 1% or less of body weight usually can be withdrawn from healthy birds without detrimental effects.[1] For example; a healthy, 80 g cockatiel (*Nymphicus hollandicus*) can easily tolerate removal of a 0.8 mL blood sample. The sample size taken from severely ill birds, however, must be reduced. For routine hematologic evaluations in birds, a sample size of 0.2 mL usually is adequate. A variety of collection methods have been used to obtain blood from birds, and the method chosen depends on the size of the bird, peculiarities of the species, preference of the collector, volume of blood needed, and physical condition of the patient.

Venous blood provides the best sample for hematologic studies. Blood collected from capillary beds (i.e., clipping of a toenail) usually results in abnormal cell distributions and contains both cells and other substances not found in venous blood, such as tissue fluid, macrophages, and cellular debris. Veins commonly used for venipuncture include the jugular, basilic (cutaneous ulnar, wing or brachial), and medial metatarsal (caudal tibial). Blood can be collected using a needle and syringe when performing venipuncture on the jugular or other large veins. A short (1 inch or smaller) 25–22 G needle attached to a 3–6 mL syringe commonly is used for jugular venipuncture. A needle with an extension tube, such as a butterfly catheter (Abbott Hospitals, North Chicago, IL) aids in stabilization of the needle during sample collection. Blood also can be collected after venipuncture by allowing it to flow through the needle and drip into a microcollection device. Collecting blood by allowing it to flow through the needle, rather than by aspirating it into a syringe, minimizes hematoma formation. A variety of these devices (Microtainer tubes, Becton-Dickinson, Rutherford, NJ) are available. Microcollection tubes containing ethylenediaminetetraacetic acid (EDTA) are available for hematologic studies, but they also are available as plain tubes, with or without a serum separator, and as tubes containing heparin (lithium heparin is preferred) for studies of blood chemistry.

Jugular venipuncture most commonly is used for collecting blood from birds, because most small birds do not have other veins that are large enough for venipuncture.[2,3] Jugular venipuncture involves lightly wetting the feathers with alcohol to expose the featherless tract (apterla) of skin that overlies the jugular furrow. The right jugular vein is the vein of choice for this procedure, because it either is the only jugular vein present or is the larger of the two jugular veins.

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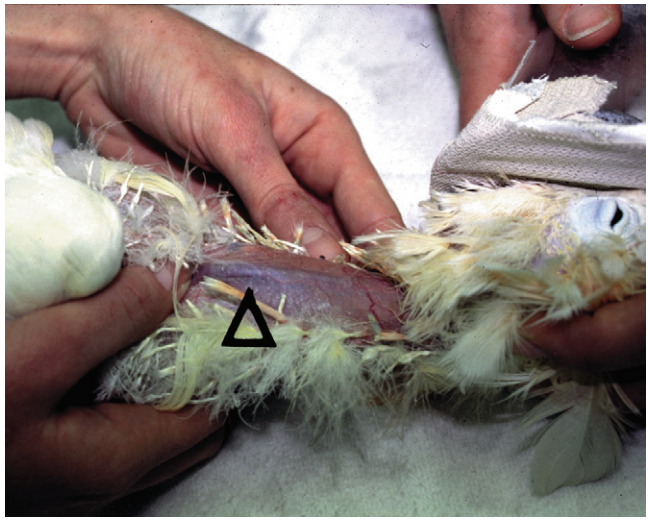

Figure 19.1 Location of the right jugular vein in a cockatoo (*Cacatua moluccensis*).

Figure 19.3 Location of the basilic vein in a parrot (*Amazona* sp.). Image courtesy of Dr. Krystan Grant, Colorado State University.

Figure 19.2 Jugular venipuncture in a parrot (*Amazona* sp.). Image courtesy of Dr. Krystan Grant, Colorado State University.

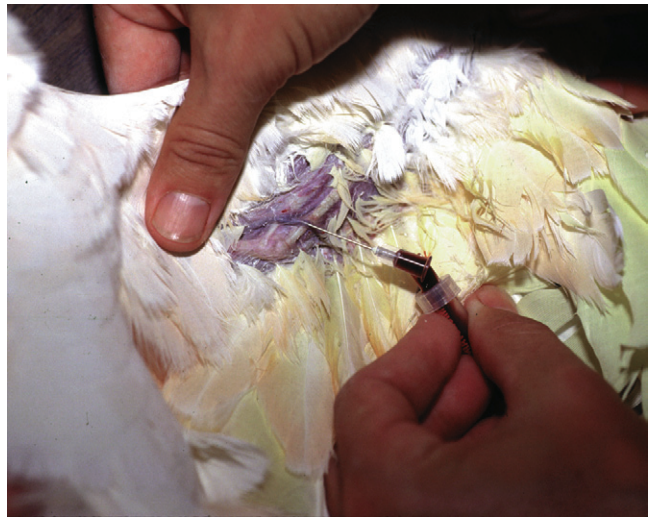

Figure 19.4 Cannulation of the basilic vein of a cockatoo (*Cacatua moluccensis*) using the drip method of blood collection.

Jugular venipuncture is performed by using an appropriate-sized needle and providing proper restraint, with the bird's head and neck extended to allow the jugular vein to fall into the jugular furrow (Figs. 19.1 and 19.2). Jugular venipuncture provides a rapid collection time and the ability to easily collect adequate amounts of blood, even in small birds. The tendency for hematoma formation can be minimized with proper attention to technique and hemostasis.

Another common procedure for blood collection in medium to large birds is venipuncture of the basilic (cutaneous ulnar or brachial) vein.[2] This vein crosses the ventral surface of the humeroradioulnar joint (elbow), and it is easily visualized by wetting the area lightly with alcohol. Using an appropriate-sized needle, blood can be collected after cannulation of this vein either by aspiration into a syringe or by allowing it to drip from the needle hub into a microcollection tube (Figs. 19.3 through 19.5).

Blood also can be collected by aspiration or the drip technique from the medial metatarsal (caudal tibial) vein, which is located on the caudomedial aspect of the tibiotarsus just above the tarsal joint. Because this vein is protected by the surrounding muscles of the leg, hematoma formation is minimal (Figs. 19.6 through 19.8).

Clipping the toenail and lancet wounding are two other methods of blood collection, but these should be reserved for very small birds or for when attempts at venipuncture have failed. After alcohol cleansing, the toenail is clipped until blood flows freely for collection into a microcollection tube. After blood collection from the cut nail, hemostasis is accomplished by applying a hemostatic agent such as silver

Figure 19.5 Blood collection from the basilic vein in a parrot (*Amazona* sp.). Image courtesy of Dr. Krystan Grant, Colorado State University.

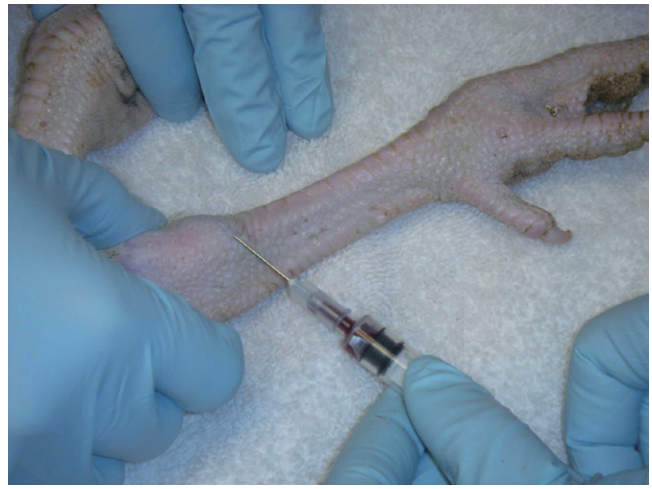

Figure 19.7 Blood collection from the medial metatarsal vein in a turkey poult (*Meleagris gallopavo*). Image courtesy of Dr. Krystan Grant, Colorado State University.

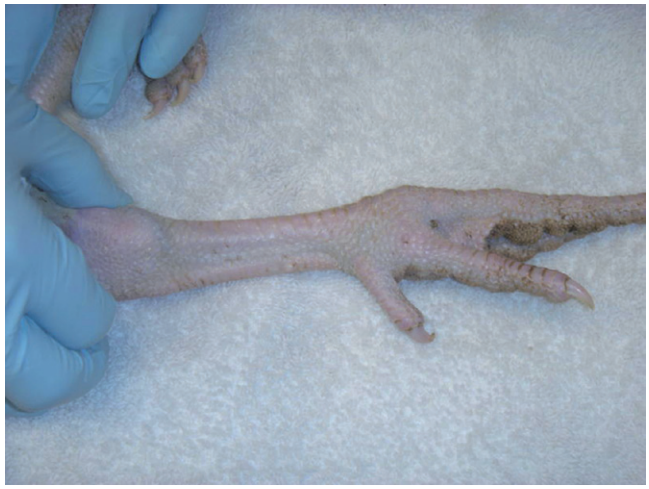

Figure 19.6 Location of the medial metatarsal vein in a turkey poult (*Meleagris gallopavo*). Image courtesy of Dr. Krystan Grant, Colorado State University.

Figure 19.8 Cannulation of the medial metatarsal vein in a macaw (*Ara macao*) using the drip method of blood collection.

nitrate or ferrous subsulfate. Blood collection by this technique yields a poor sample for hematologic studies, however, because the blood is from the capillary bed and usually contains microclots, which interfere with cell counts. Capillary blood also frequently is contaminated with tissue fluid that affects hematologic data. Toenail clipping may result in temporary lameness because of nail damage. An alternative to nail clipping for blood collection from small birds is to collect blood after lancet wounding of vascular structures, such as the cutaneous ulnar vein, medial metatarsal vein, and external thoracic vein.[4] After alcohol cleansing of the skin over lying the vein, the vessel is punctured through the skin using a lancet (i.e., needle), and the blood is allowed to drip into a microcollection tube.

Large volumes of blood can be collected from birds by cardiac puncture or occipital venous sinus puncture. These procedures are potentially dangerous, however, and should be reserved for birds that are used for research or are to be euthanized. Cardiac puncture can be performed using an anterior or a lateral approach.[5,6] The heart is approached anteriorly by inserting a needle along the ventral floor of the thoracic inlet with the bird in dorsal recumbency. Care should be taken to avoid the ingluvies (crop) in some avian species. The needle is inserted near the "V" that is formed

by the furcula, and it is directed toward the bird's dorsum and caudal toward the heart. Once the heart is penetrated, the vibration can be felt to ensure proper needle placement, and blood is then aspirated. In galliforme birds, the heart can be approached laterally by inserting the needle in the fourth intercostal space near the sternum (keel) with the bird held in lateral recumbency. This approach, however, may vary with the species.

Blood collection from the occipital venous sinus requires use of evacuated glass tubes with appropriate needles and needle holders.[7] The occipital venous sinus is located at the junction of the dorsal base of the skull and the first cervical vertebra, and it can be located by palpation while holding the bird's head firmly flexed and positioned in a straight line with the cervical vertebrae. A needle is inserted through the skin at a 30–40° angle to the vertebrae. As soon as the needle penetrates the skin, the rubber stopper of the evacuated tube is perforated gently and the needle advanced until the sinus is reached (Fig. 19.9). Penetration of the sinus results in a rapid flow of blood into the tube. Blood collection by puncture of either the heart or the occipital venous sinus requires proper restraint and technique to avoid permanent damage to the heart or brainstem—and even possible death of the patient.

The method of storage and handling of blood samples can have a significant influence of the hematological results. Blood samples collected without use of an anticoagulant require immediate processing. Dilutions for cell counting and preparation for blood films must be quickly performed with such samples. Because of the urgency for rapid processing of nonanticoagulated blood, most avian blood samples are collected into tubes containing an anticoagulant. Ethylenediaminetetraacetic acid, heparin, and sodium citrate are commonly used, and each has both advantages and disadvantages. The anticoagulant of choice for avian hematology is EDTA, because it allows for proper staining of cells and does not tend to clump leukocytes.[2,8] Hematologic testing, however, should be performed soon after blood collection to avoid artifacts, such as increased cell smudging, which is created by prolonged exposure to any anticoagulant. Excessive liquid anticoagulants dilute the blood sample, thereby resulting in artifactually decreased hematocrit and total cell concentrations, and excessive dry anticoagulants may cause shrinkage of red blood cells, thus affecting the hematocrit. Blood from certain avian groups, such as crows and jays, may show incomplete anticoagulation or partial hemolysis when collected in EDTA. Heparin has the advantage of providing anticoagulated blood for hematology and plasma for evaluations of blood chemistry. Heparinized blood, however, may result in improper staining of cells, thereby resulting in erroneous leukocyte counts and poor cellular morphology in stained blood films. Heparin also causes clumping of leukocytes and thrombocytes and resultant, inaccurate cell counts. A 3.8% sodium citrate solution, used in a ratio of one part citrate solution to nine parts blood, is the anticoagulant of choice for coagulation studies; however, it should not be used for other hematologic evaluations. Consistent use of the same anticoagulant is an important consideration with serial evaluation of the hemogram in the avian patient. For example, it has been demonstrated that although most hematologic parameters were in agreement between blood samples collected into heparin and EDTA, plasma protein and the PCV are significantly lower and lymphocyte counts were significantly higher in the heparinized samples.[9]

Figure 19.9 Blood collection from the dorsal occipital venous sinus in a puffin (*Fratercula cirrhata*).

Blood sampled during field studies often does not have the advantage of immediate processing following blood collection; therefore, artifactual changes may occur during storage. The period of stability of the sample is influenced by temperature, time, and species. In general, avian blood samples collected into EDTA can be stored at 4°C for up to 72 hours and provide reliable results for packed cell volume (PCV), hemoglobin concentration (Hb), total red blood cell count (RBC), mean corpuscular hemoglobin (MCH) values, and mean corpuscular hemoglobin concentration (MCHC) and up to 30 hours with reliable results for mean corpuscular volume (MCV) and the total white blood cell count (WBC).[10]

Blood films can be made using a variety of techniques. The standard two-slide wedge or push-slide method that is commonly used for preparing human and mammalian blood films also can provide adequate blood films for avian hematology.[2,11] This method usually provides good cellular distribution and adequate monolayer fields for proper slide evaluation. Use of precleaned, bevel-edged microscope slides is advised to minimize cell damage during preparation of blood films. To minimize cell damage, a drop of commercially available, purified bovine albumin can be applied to a glass slide, followed by the addition of an equal amount of

Table 19.1 Semiquantitative microscopic evaluation of avian erythrocyte morphology.[a]

	1+	2+	3+	4+
Anisocytosis	5–10	11–20	21–30	>30
Polychromasia	2–10	11–14	15–30	>30
Hypochromasia	1–2	3–5	6–10	>10
Poikilocytosis	5–10	11–20	21–50	>50
Erythroplastids	1–2	3–5	6–10	>10

[a]Based on the average number of abnormal cells per 1000× monolayer field.

blood before making the blood film. The albumin should not be allowed to dry before making the blood film. Alternately, blood films can be prepared by using a slide and coverslip or by using two coverslips. With proper attention to technique, these methods minimize cellular disruption while maintaining good cellular distribution with monolayered areas for examination. The coverslip is pulled across a drop of blood that has been placed on a glass microscope slide or coverslip. A disadvantage of the coverslip method, however, is the inability to use automatic staining methods.

Wright, Wright-Giemsa, Wright-Leishman, and May Grünwald-Giemsa stains have been used for staining air-dried, avian blood films for hematologic examination. Quick stains or modified Wright stains (Diff-Quik, American Scientific Products, Division of American Hospital Supply Corporation, McGraw Park, IL; Hemacolor, Miles Laboratories, Elkhart, IN) also can be used to stain avian blood films. Use of automatic slide stainers (Hema-Tek, Ames Division of Miles Laboratories, Elkhart, IN; Harleco Midas II, EM Diagnostic Systems, Gibbstown, NJ) simplifies the staining procedure and provides a means for consistency and high-quality staining of blood films. Automatic stainers remove much of the staining variation that occurs with hand-staining methods.

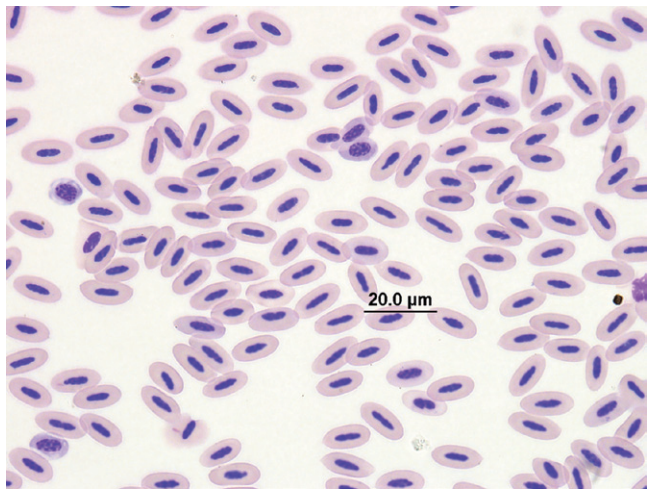

Figure 19.10 Normal erythrocytes in the blood film of a cockatoo (*Cacatua alba*). Wright-Giemsa stain.

Erythrocytes

Morphology

Evaluation of avian erythrocyte morphology involves observation of the cells in a monolayer ×1000 field in which approximately half the erythrocytes are touching one another. In general, such fields represent approximately 200 erythrocytes in most species of birds. Monolayer fields may be difficult to achieve, however, in severely anemic birds (i.e., films are too thin) or in poorly prepared blood films (i.e., films made too thick or thin). Avian erythrocytes should be evaluated on the basis of size, shape, color, nucleus, and presence of cellular inclusions. A semiquantitative scale can be used to estimate the number of abnormal erythrocytes based on the average number per monolayer ×1000 field (Table 19.1).

Mature avian erythrocytes generally are larger than mammalian erythrocytes but smaller than reptilian erythrocytes. Avian erythrocytes vary in size depending on the species, but they generally range between $10.7 \times 6.1\,\mu m$ and $15.8 \times 10.2\,\mu m$.[12] Erythrocytes of adult Coturnix quail for example measure $11.06 \pm 0.70\,\mu m$ in length and $6.80 \pm 0.67\,\mu m$ in width in males and $11.40 \pm 0.63\,\mu m$ in length and $6.73 \pm 0.45\,\mu m$ in width in females.[13] Mature avian erythrocytes are elliptical and have an elliptical, centrally positioned nucleus. Nuclear chromatin is uniformly clumped and becomes increasingly condensed with age. In Wright-stained blood films, the nucleus stains purple, whereas the cytoplasm stains orange-pink with a uniform texture (Fig. 19.10).

Changes in the size of avian erythrocytes include microcytosis, macrocytosis, and anisocytosis. A significant change in the mean size of the erythrocyte is reflected in the mean corpuscular volume (MCV). The presence of macrocytes or

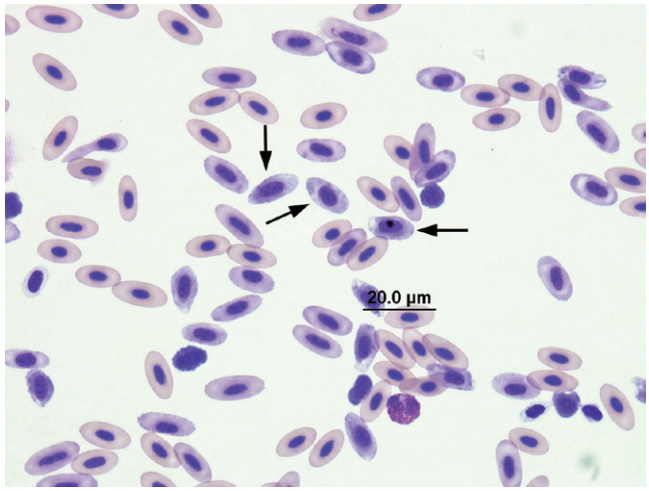

Figure 19.11 Polychromatic erythrocytes (arrows) in the blood film of a hawk (*Buteo jamaicensis*). Wright-Giemsa stain.

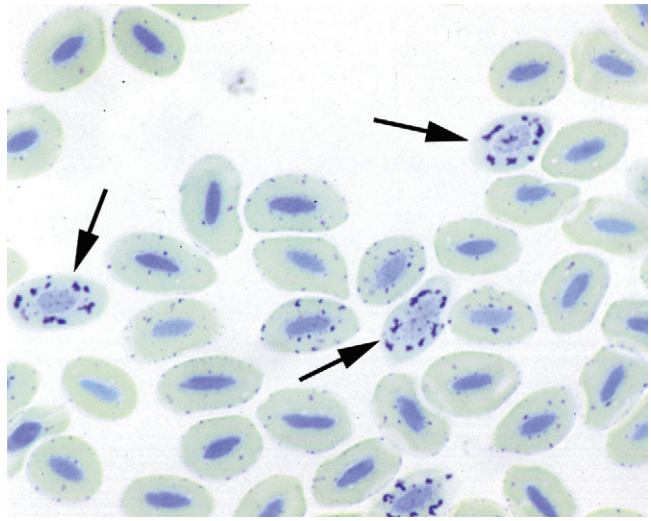

Figure 19.12 Reticulocytes with a distinct ring of aggregated reticulum encircling the red-cell nucleus in the blood film of an eagle (*Aquila chrysaetos*). Brilliant cresyl blue stain.

microcytes also should be noted during assessment of the blood film. The degree of variation in the size of erythrocytes (anisocytosis) can be scored from 1+ to 4+ based on the number of variable-sized erythrocytes in a monolayer field (Table 19.1). Erythrocyte subpopulations have been reported in ducks, in which larger erythrocytes (MCV, 308 fL/cell) most likely represent those most recently released from the hematopoietic tissue and smaller cells (MCV, 128 fL/cell) most likely represent the older, aging cells.

Variations in erythrocyte color include polychromasia and hypochromasia. Polychromatophilic erythrocytes occur in low numbers (usually <5% of erythrocytes) in the peripheral blood of most normal birds. The degree of polychromasia can be graded according to the guideline presented in Table 19.1. The cytoplasm of polychromatophilic erythrocytes is weakly basophilic, and the nucleus is less condensed than in mature erythrocytes (Fig. 19.11). Polychromatophilic erythrocytes are similar in size to mature erythrocytes, and they appear as reticulocytes when stained with vital stains such as new methylene blue.

Reticulocytes are the penultimate cell in the erythrocyte maturation series, and their presence in the peripheral blood of normal birds suggests that the final stages of red-cell maturation occur in circulating blood. Reticulocytes tend to be smaller in size and less elongated compared to the mature erythrocyte. For example reticulocytes of adult Coturnix quail measure 9.80 ± 0.77 µm in length and 8.23 ± 0.72 µm in width in males and 9.80 ± 0.77 µm in length and 7.73 ± 0.70 µm in width in females.[12] Determination of the reticulocyte concentration can be made by staining erythrocytes with a vital stain such as new methylene blue. Reticulocytes have a distinct ring of aggregated reticular material that encircles the nucleus (Fig. 19.12).[8,14] As the cells mature, the amount of aggregated reticular material decreases and

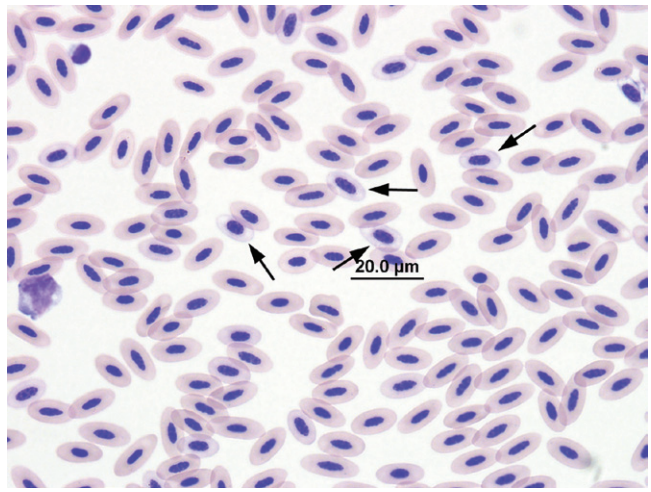

Figure 19.13 Hypochromatic erythrocytes (arrows) in the blood film of a parrot (*Eclectus roratus*). Wright-Giemsa stain.

becomes more dispersed throughout the cytoplasm. With further maturation, the reticular material becomes nonaggregated, thereby resembling the "punctate" reticulocytes of felids. Most mature avian erythrocytes contain a varying amount of aggregate or punctate reticulum. Reticulocytes that reflect the current erythrocyte regenerative response, however, are those with a distinct ring of aggregated reticulum that encircles the red-cell nucleus.

Hypochromatic erythrocytes are abnormally pale in color compared with mature erythrocytes, and they have an area of cytoplasmic pallor that is greater than half the cytoplasmic volume (Fig. 19.13). They also may have cytoplasmic

vacuoles and round, pyknotic nuclei. A significant hypochromasia is reflected as a decrease in the mean corpuscular hemoglobin concentration (MCHC) and mean corpuscular hemoglobin (MCH) values. The degree of hypochromasia can be estimated using the scale presented in Table 19.1.

In most species of birds, the shape of erythrocytes is relatively uniform. The degree of poikilocytosis can be estimated using the scale outlined in Table 19.1.

Atypical erythrocytes occasionally are present in the peripheral blood of normal birds, and such erythrocytes may represent artifacts associated with preparation of the blood film. Careful examination of erythrocyte morphology may reveal significant clues in the detection of disorders affecting avian erythrocytes. As mentioned, the degree of polychromasia and reticulocytosis and the presence of immature erythrocytes in the peripheral blood aid in the assessment of red-blood-cell regeneration. The presence of many hypochromatic erythrocytes (i.e., 2+ hypochromasia or greater) indicates an erythrocyte disorder such as iron deficiency.

Atypical erythrocytes may vary in both size and shape. A slight variation in the size of erythrocytes (1+ anisocytosis) is considered to be normal for birds. A greater degree of anisocytosis, however, usually is observed in birds with a regenerative anemia and is associated with polychromasia. Likewise, minor deviations from the normal shape of avian erythrocytes (1+ poikilocytosis) are considered to be normal in the peripheral blood of birds, but marked poikilocytosis may indicate erythrocytic dysgenesis. Round erythrocytes with oval nuclei occasionally are found in the blood films of anemic birds and suggest a dysmaturation of the cell cytoplasm and nucleus, which may be a result of accelerated erythropoiesis.

The nucleus may vary in its cellular location and contain indentions, protrusions, or constrictions. Anucleated erythrocytes (erythroplastids) or cytoplasmic fragments occasionally are found in normal avian blood films (Fig. 19.14).[2] The nucleus may contain chromophobic streaking, which suggests chromatolysis, or achromic bands, which indicate nuclear fracture with displacement of the fragments. Mitotic activity associated with erythrocytes in blood films suggests a marked regenerative response or erythrocytic dyscrasia (Fig. 19.15). Perinuclear rings are common artifacts of improper slide preparation (e.g., exposure to solvent or formalin fumes, or allowing the slide to dry too slowly), and they represent nuclear shrinkage. Clear, irregular, refractile spaces in the cytoplasm occur when blood films are allowed to dry too slowly. This artifact, which is a form of erythrocyte crenation, should not be confused with avian blood parasites, such as gametocytes of *Hemoproteus* and *Plasmodium*. Disruption or smudging of avian erythrocytes is the most common artifact of slide preparation. Severely ruptured cells result in the presence of purple, amorphous nuclear material in the blood film.

Binucleate erythrocytes rarely occur in the blood films of normal birds. Large numbers of binucleated erythrocytes plus other features of red-blood-cell dyscrasia, however, suggest neoplastic, viral, or genetic disease.[15]

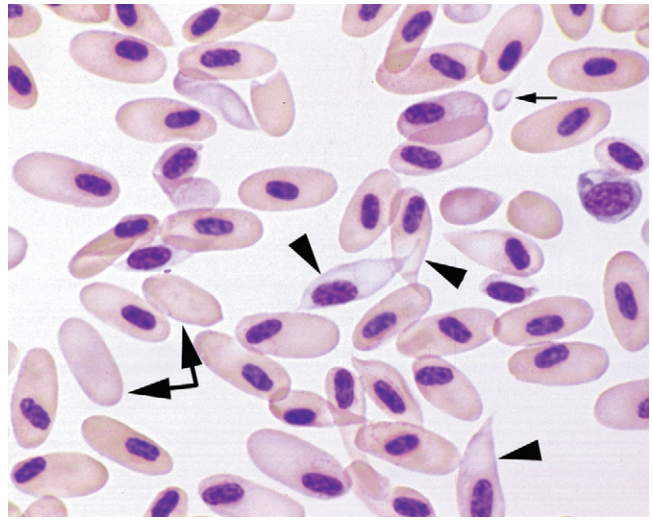

Figure 19.14 Erytrhoplastids (large arrows), erythrocyte fragments (small arrow), and dactylocytes (arrowhead) in a blood film with marked anisocytosis and poikilocytosis from a lovebird (*Agapornis roseicollis*). Wright-Giemsa stain.

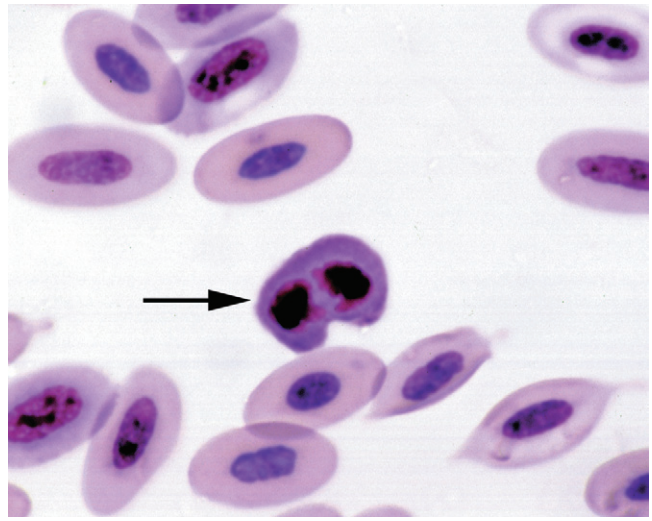

Figure 19.15 Dividing erythrocytes (arrow) in the blood film of an owl (*Nyctea scandiaca*), Modified Wright-Giemsa stain, 500x.

Punctate basophilia is characterized by punctate aggregations of small, irregular, basophilic-staining granules throughout the cytoplasm of erythrocytes in Wright-stained smears. As in mammalian hematology, punctate basophilia is most likely associated with degenerative changes in ribosomal ribonucleic acid and is indicative of a response to anemia or, rarely, lead poisoning. Basophilic stippling can be affected by preparation and staining of the blood film. Using fresh blood without an anticoagulant or rapid drying of

blood films made from EDTA-anticoagulated blood provides the best films for demonstrating basophilic stippling.[16] Such stippling is less apparent when alcohol fixation of blood is used. Heinz bodies rarely are reported in birds and are the result of hemoglobin denaturation (oxidized hemoglobin).[17] Heinz bodies appear as round to irregularly shaped, pale-blue, cytoplasmic inclusions with new methylene blue stain; as round to irregular inclusions of densely stained hemoglobin with Wright stain; or as refractile inclusions in unstained erythrocytes. Agglutination of erythrocytes in blood films also is a rare, abnormal finding.

Laboratory evaluation

Laboratory evaluation of avian erythrocytes involves the same routine procedures as that used in mammalian hematology, but with a few modifications.[18] The standard manual technique for using microhematocrit capillary tubes and centrifugation (12,000 g for 5 min) can be used to obtain a PCV (hematocrit). Hemoglobin concentration is measured using the same methods for mammalian blood samples; however, removal of free erythrocyte nuclei by centrifugation is required.[19]

The total erythrocyte concentration in birds can be determined using the same automated or manual methods as those used for obtaining the total erythrocyte counts in mammalian blood. Automated cell counters (Coulter counter, Coulter Corporation, Miami, FL) provide a rapid, reliable method for obtaining total red-blood-cell concentrations. Two manual methods for obtaining total red-blood-cell count in birds are the erythrocyte Unopette (Becton-Dickinson) method used in mammalian hematology and the Natt-Herrick method, which involves preparation of Natt-Herrick solution to be used as a stain and diluent (Table 19.2).[20] A 1:200 dilution of the blood is made using the Natt and Herrick solution and red-blood-cell diluting pipettes. After mixing, the diluted blood is discharged into a hemacytometer counting chamber, and the cells are allowed to settle for 5 minutes, to the ruled surface, before counting. Erythrocytes located in the four corner and the central squares of the hemacytometer chamber are counted when using either of the manual methods (Figs. 19.16 and 19.17). The number obtained then is multiplied by 10,000 to calculate the total red-blood-cell count per cubic millimeter (mm^3) of blood.

The MCV, MCH, and MCHC also can be calculated for avian hematology using the same formulas as those used for mammals.

Normal erythrocyte physiology

Normal reference values vary among species of birds; however, with a few exceptions, psittacine birds in captivity have similar erythrocyte parameters: PCV of 35–55%; RBC count of 2.4–5.0 × 10^6/μL; Hemoglobin concentration of 11–16 mg/dL; MCV of 90–200 fl; and MCHC of 22–33% (Table 19.3).

Table 19.2 Natt and Herrick's solution and stain.[a]

Sodium chloride [NaCl]	3.88 g
Sodium sulfate [Na SO_4]	2.50 g
Sodium phosphate [Na_2HPO_4]	1.74 g
Potassium phosphate [KH_2PO_4]	0.25 g
Formalin [37%]	7.50 mL
Methyl violet	0.10 g

[a]Bring to 1000 mL with distilled water and filter through Whatman #10 medium filter paper.

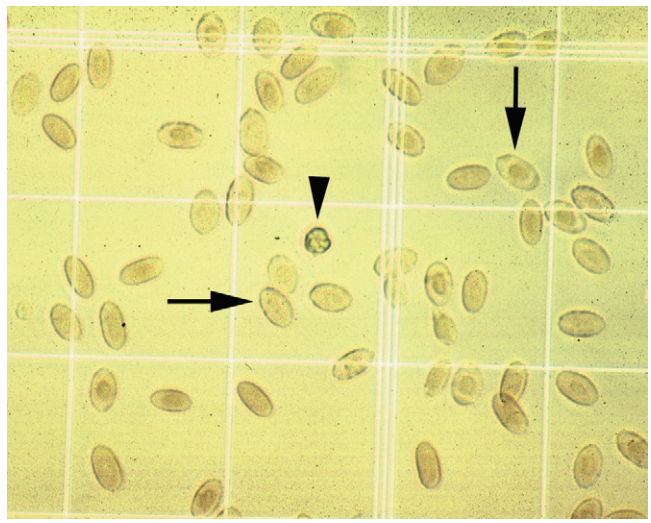

Figure 19.16 Appearance of erythrocytes (arrows) and leukocytes (arrow head) in a hemocytometer using the erythrocyte Unopette method.

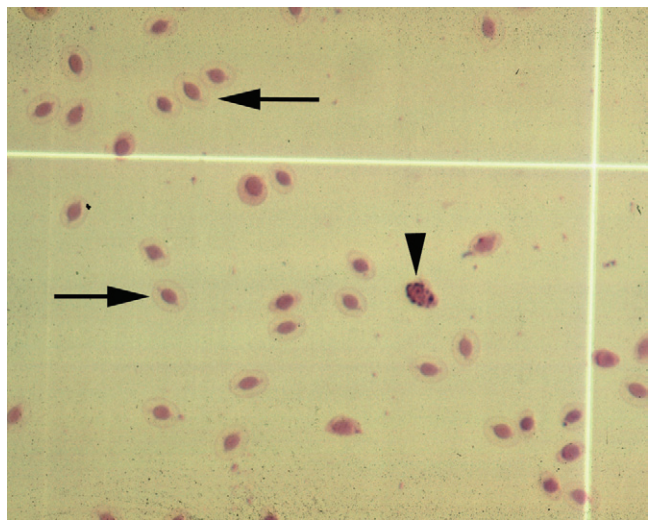

Figure 19.17 Appearance of erythrocytes (arrows) and granulocyte (arrowhead) in a hemocytometer using Natt and Herrick method.

Table 19.3 Erythrocyte parameters for selected birds.

	PCV (%)	RBC (x 10^6/µL)	Hb (g/dL)	MCV (fl)	MCHC (%)
Psittacines					
African Grey parrot [1,3]	43–55	2.4–4.5	11.0–16.0	90–180	23–33
Amazon parrots [1,2]	45–55	2.5–4.5	12.5–25	160–175	29.1–31.0
Blue-fronted Amazon[5]	44–58	2.1–3.5	16.0–18.4	163–209	31.7–37.8
Cuban Amazon[5]	44–54	3.1–3.5	15.2–17.7	142–162	31.4–37.2
Festive Amazon[5]	47–53	3.1–3.8	16.1–17.4	135–164	31.5–34.5
Orange-wing Amazon[5]	46–51	2.8–3.3	15.5–17.5	151–166	32.1–36.0
Vinaceous Amazon[5]	46–52	3.0–3.3	15.0–17.5	145–174	31.7–35.6
Yellow Amazon[5]	38–51	2.1–3.5	12.1–17.4	135–175	31.0–34.1
Budgerigar[1,3]	44–58	2.3–3.9	13–18	90–190	22–32
Cockatiel [1,3]	45–54	2.5–4.7	11–16	90–200	22–33
Cockatoos [1,3]	42–54	2–4	12–16	120–175	28–33
Black cockatoo[5]	40–46	2.4–2.7	12–17	154–184	32–37
Goffin's cockatoo[5]	37–47	2.4–3.4	12–16	119–175	33–39
Palm cockatoo[5]	36–47	2.0–3.6	13–17	131–235	31–36
White cockatoo[5]	37–48	2.8–3.2	14–18	132–171	30–39
Conures [1,3]	42–54	2.9–4.5	12–16	90–190	23–31
Golden conure[5]	50–54	3.6–4.0	17.6–20.4	126–144	33.9–40.7
Patagonian conure[5]	45–52	3.2–4.1	14.3–16.2	127–146	30.9–32.3
Eclectus parrot [1,3]	45–55	2.7–3.8	13.5–16.0	125–175	29–32
Jardine's parrot[1]	35–48	2.4–4.0	11–16	90–190	21–33
Lovebird [1,3]	44–57	3.0–5.1	13–18	90–190	22–32
Macaw [1,3]	47–55	2.7–4.5	15–17	125–170	29–35
Green-wing macaw[5]	39–54	2.7–4.1	9.6–18.7	116–177	21.9–34.9
Military macaw[5]	37–55	2.7–5.2	11.1–19.6	106–173	33.9–40.7
Scarlet macaw[5]	40–54	2.3–3.7	13.1–19.9	135–169	29.7–37.3
Pionus parrot[1]	35–54	2.4–4.0	11–16	85–210	24–31
Quaker [1,3]	30–58	2.8–3.9	11–15	90–200	22–32
Senegal parrot[1]	36–48	2.4–4.0	11–16	90–200	23–32
Others					
Canary [1,3]	37–49	2.5–3.8	12–16	90–210	22–32
Pigeon[3]	38–50	3.1–4.5	13–17.5	85–200	22–33
Chicken [1,3]	23–55	1.3–4.5	7.0–18.6	100–139	20–34
Turkey[1]	30.4–45.6	1.74–3.70	8.8–13.4	112–168	23.2–35.3
Quail[1]	30.0–45.1	4.0–5.2	10.7–14.3	60–100	28.0–38.5
Canada goose[1]	38–58	1.6–2.6	12.7–19.1	118–144	20–30
*Mallard duck[4]	46–51	3.05–3.65	14.8–16.4	134 –162	31.4–31.8
**Mallard duck[4]	34–44	1.61–2.41	11–13	172–227	27–31
Peregrine falcon[1]	37–53	3–4	11.8–18.8	118–146	31.9–35.2
Red tailed hawk[1]	31–43	2.41–3.59	10.7–16.6	150–178	29.7–34.5
Tawny owl[6]	29–47	1.5–2.4	8.0–13.3	154–221	33.1–62.1
White-back vulture[7]	35–54	21–3.0	1632–23.0	186–208	36.2–42.3

*January.

**June.

[1] Pollack C, Carpenter JW, Antinoff N. In: *Birds*, 3rd ed. J Carpenter ed. *Exotic Animal Formulary*. St. Louis, MO: Elsevier Saunders, 2005.

[2] Tell, LA, and SB Citino. Hematologic and serum chemistry reference intervals for Cuban A mazon parrots (*Amazona leucocephala leucocephala*). *J Zoo and Wildlife Med* 1992; 23: 62–4.

[3] Cray C. Blood and chemistry tables. In: *Manual of Avian Medicine*. G Olsen, S Orosz eds, St. Louis, MO: Mosby, 2000.

[4] Campbell, TW. Normal hematology of waterfowl. Edited by B. Feldman, J. Zinkl and M. Jain. 5 ed, *Schalm's Veterinary Hematology*. Philadelphia: Lippincott Williams & Wilkins, 2000.

[5] Polo FJ, Peinado VI, Viscor G, Palomeque J. Hematologic and plasma chemistry values in captive psittacine birds. *Avian Diseases* 1998; 42: 523–35.

[6] Spagnolo V, Crippa, V, Marzia A, Alberti I, Sartorelli P. Hematologic, biochemical, and protein electrophoretic values in captive tawny owls [*Strix aluco*]. *Vet Clin Pathol* 2008; 37: 225–8.

[7] Naidoo V, Diekmann M, Wolters K, Swan GE. Establishment of selected baseline blood chemistry and hematologic parameters in captive and wild-caught African white-backed vultures [*Gyps africanaus*]. *J Wild Dis* 2008; 44: 649–54.

The total erythrocyte concentration and PCV of birds are influenced by species, age, sex, hormonal influences, hypoxia, environmental factors, and disease.[21–29] In general, the total erythrocyte count, PCV, and MCV increase with age. The Hb does not appear to be affected by age; therefore, a decline in MCHC is a function of the increase in MCV.

Total erythrocyte counts and PCV tend to be higher in male compared to female birds. The reason for this may be a hormonal effect where estrogen depresses erythropoiesis, whereas androgens and thyroxin stimulate erythropoiesis. Variations in erythrocyte parameters associated with gender in birds are generally not statistically significant; however, reported differences between males and females have been reported and reflect a seasonal variation, where females tend to have higher PCV, Hb, TRBC, and MCHC values compared to males in the pre-nesting period.

Studies with free-ranging ducks and geese have shown that averages for PCV, Hb, TRBC, and MCHC values tend to be higher in the winter and pre-nesting period in adults regardless of gender compared to the postnesting period and fall.[28] During migration, ducks tend to have slightly lower erythrocyte counts compared to wintering ducks and post-nesting MCV averages for these birds tend to be higher in the winter or pre-nesting periods.[30] Interestingly, these changes also occur in captive ducks that are not able to migrate. Feather molting, a seasonal event, has an effect on the hemogram where the PCV, RBC, and Hb have been shown to decrease in ducks during and after remige molt.

The normal erythrocyte parameters vary among avian species. For example, in Anseriformes, mallard ducks (*Anas platyrhynchos*), a dabbling duck, have higher average PCV and TRBC values in the winter and pre-nesting period compared to diving ducks (*Aythya* spp and *Oxyura jamaicensis*); whereas, diving ducks have higher MCV values during the winter and pre-nesting period compared to mallards.[28] In general, ducks tend to have higher TRBC values than geese, but geese have higher MCV and Hb than ducks during the winter.

Birds, like mammals, respond to blood loss and blood destruction by increasing erythropoietin production, which stimulates erythropoiesis. Avian erythropoietin (a glycoprotein produced by the kidney) acts directly on the bone marrow to increase erythrocyte production. Avian erythropoietin does not stimulate mammalian erythropoiesis, however, and mammalian erythropoietin has no effect on avian hematopoiesis.[12]

Avian hemoglobin has four iron-containing heme subunits, as with mammalian hemoglobin, but the protein moieties (i.e., globulins) are different.[31] In avian erythrocytes, the phosphate compounds influencing the affinity of hemoglobin for oxygen also differ from those in mammals. The hemoglobin of mature birds contains myoinositol pentophosphate, not the 2,3-diphosglycerate as in mammals. Inositol pentophosphate causes hemoglobin to have a lower affinity for oxygen, and it shifts the oxygen dissociation curve to the right of the mammalian curve. Therefore, avian tissues can extract oxygen more readily from hemoglobin than mammalian tissues can.

Responses in disease

The PCV is the quickest and most practical method for evaluating the red-cell mass of birds. As in mammals, the PCV in birds is affected by the number and size of the erythrocytes as well as by changes in the plasma volume that do not affect the actual cell concentrations. These include increased plasma volume (hemodilution), decreased plasma volume (hemoconcentration), improper blood sampling (hemodilution), and epinephrine administration and hypothermia, which may result in hemoconcentration. The normal PCV for many species of birds ranges between 35% and 55%. Therefore, a PCV of less than 35% suggests anemia, and a PCV of greater than 55% suggests dehydration or erythrocytosis (polycythemia). The latter condition can be differentiated by the total serum protein: increased total protein indicates dehydration, whereas normal or low total protein indicates erythrocytosis.

Typically, polychromatic erythrocytes make up 5% or less of the erythrocyte population in blood films from normal birds. The degree of erythrocyte polychromasia and reticulocytosis indicates the degree of erythrogenesis. Anemic birds with greater than 10% polychromasia (3+ and 4+ polychromasia) are exhibiting an appropriate regenerative response to their anemia. Those with a smaller response, however, are not. The number of reticulocytes also indicates a bird's current response to anemia. Therefore, the reticulocyte count can be used in conjunction with assessment of the degree of polychromasia to determine the bird's current erythropoietic response.

Other evidence of active erythropoiesis is the presence of binucleate, immature erythrocytes and an increased number of normal, immature erythrocytes in the peripheral blood. Immature erythrocytes (i.e., rubricytes) in peripheral blood films in addition to increased polychromasia indicate a marked erythrocyte response (Fig. 19.18). In cases of non-anemic birds, however, these cells indicate abnormal erythropoiesis. Immature erythrocytes also may suggest early release from the hematopoietic tissue after anoxic insult or toxicity (i.e., lead poisoning).

The causes of anemia in birds include blood loss (hemorrhagic anemia), increased red cell destruction (hemolytic anemia), and decreased red cell production (depression anemia). The most common causes of hemorrhagic anemia in birds include traumatic injury, bloodsucking parasites, coagulopathies, and hemorrhagic lesions of internal organs, such as ulcerated neoplasms, gastric ulcerations, and rupture of the liver or spleen. Heavy infestation with bloodsucking ectoparasites such as ticks or mites (i.e., *Dermanyssus* mites) or with gastrointestinal parasites such as coccidia can lead to severe blood loss anemia in birds. Coagulopathies that result in

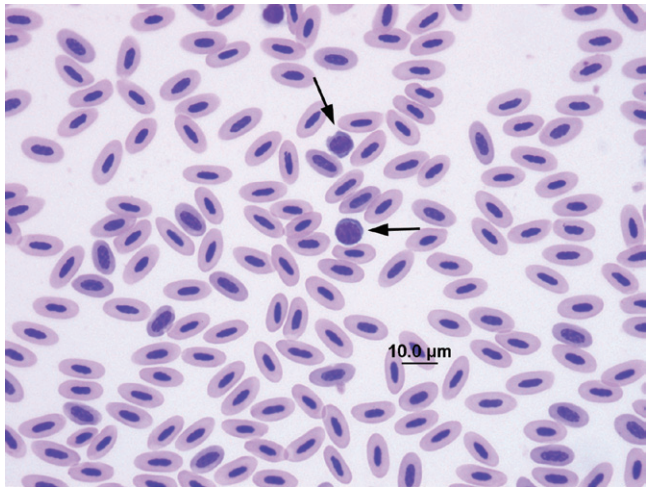

Figure 19.18 Immature erythrocytes (arrows) represented by mid-polychromatic rubricytes in the blood film of a conure (*Aratinga solstitialis*). Wright-Giemsa stain.

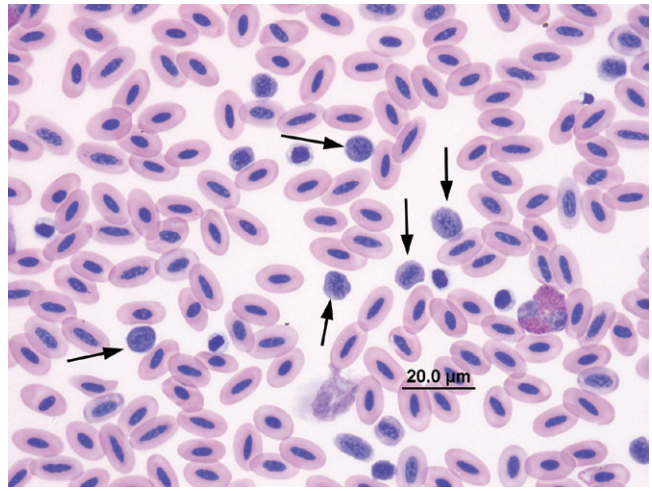

Figure 19.19 Marked numbers of immature erythrocytes (arrows) in a blood film from a vulture (*Cathartes aura*) with a normal packed cell volume (46%). The vulture has lead poisoning. Wright-Giemsa stain.

blood loss anemia usually are acquired and often are associated with toxicities such as aflatoxicosis or coumarin poisoning or severe liver disease such as papovavirus infections.[32–35] Birds can tolerate acute blood loss better than mammals and diving and flying birds are more resistant to blood loss than nondiving birds such as galliformes.[36] The mobilization and restoration of fluid during the first 90 minutes after bleeding in chickens is approximately 13% to 17% of the initial blood volume per hour, which is twice that of dogs.[37]

Hemolytic anemia can result from parasitemias, septicemia, and toxicities. Most avian blood parasites have the potential to cause anemia in the host; however, the two parasites that most frequently are associated with hemolytic anemia are *Plasmodium* and *Aegyptianella*. Salmonellosis or spirochetoses commonly cause bacterial septicemia that result in severe hemolytic anemia.[38] Toxicoses that lead to increased erythrocyte destruction include aflatoxins, certain plant chemicals (i.e., mustards), drugs, and petroleum products.[39–43] Ingestion of petroleum products may produce a Heinz body anemia. Hemolytic anemia occurs in marine birds associated with oil pollution and is characterized by low red-cell indices and numerous immature erythrocytes.[44–47] Although rare, immune-mediated anemia may result in hemolysis, with red-cell agglutination being present in the blood film. Hemolytic anemias typically are characterized by a marked regenerative response. Although hemochromatosis usually does not affect the hemogram, one report in a psittacine with hemochromatosis indicated a severe anemia with a marked regenerative response (4+ polychromasia and immature erythrocytes as early as prorubricytes). The hemochromatosis may have altered the maturation of erythrocytes as a result of defective iron uptake.

A nonregenerative, normocytic, normochromic anemia indicates decreased erythropoiesis (depression anemia), which can develop rapidly in birds with inflammatory diseases, especially those involving infectious agents. Birds appear to develop anemias from a lack of erythropoiesis more quickly than mammals, perhaps because of the relatively short erythrocyte half-life in birds compared with that in mammals.[48] Although the avian erythrocyte life span varies with the species, it is generally shorter than those in mammals. For example, the erythrocyte life span is 28–35 days in chickens, 42 days in pigs, 35 to 45 days in pigeons, and 33–35 days in quail. The degree of polychromasia or reticulocytosis is poor to absent in birds with depression anemias. Infectious diseases frequently associated with depression anemia in birds include tuberculosis, aspergillosis, and chlamydiosis. Chronic hepatic and renal disease and hypothyroidism can also result in a depression anemia.[49] Neoplasia resulting in infiltration of neoplastic cells into the bone marrow can also cause a depression anemia.[50–52]

Hypochromasia can be seen with iron deficiency, chronic inflammatory diseases, and lead toxicosis. Hypochromasia can also be associated with nutritional deficiencies, especially iron deficiency anemia. Hypochromatic erythrocytes frequently appear in the blood films from birds with chronic inflammatory diseases, presumably related to iron sequestration as part of the bird's defense against infectious agents. In such cases, hypochromatic cells often are observed in blood films before the red-cell indices (MCHC and MCH) suggest hypochromasia (Fig. 19.13).

Heavy metal poisoning, especially with lead and zinc intoxication, can result in the appearance of immature and abnormal erythrocytes in the peripheral blood.[53–56] Chronic lead toxicosis also may be associated with an in appropriate release of normal-appearing, immature erythrocytes into the peripheral blood of nonanemic birds (Fig. 19.19). In

this condition, the blood film reveals small, senescent, mature erythrocytes with pyknotic nuclei and immature erythrocytes (usually rubricytes) without the presence of normal, mature erythrocytes. This hematologic response resembles the inappropriate release of nucleated erythrocytes in the blood of nonanemic dogs affected by chronic lead poisoning. Basophilic stippling in the cytoplasm of erythrocytes may be seen with lead poisoning in birds, but can also be associated with erythrocyte regeneration and hypochromic anemia. Changes in PCV, Hb, and MCHC occur in a predictable manner with increasing blood lead concentrations in birds.[53,54] Erythrocyte morphological changes tend to occur when blood lead concentrations are greater than 3 mg/L and therefore are associated with severe lead toxicosis. A decreased MCHC is a more sensitive indicator of lead poisoning in birds than is hypochromasia.

A macrocytic, normochromic anemia occurs in birds with food restriction or folic acid deficiency.[57] Folic acid deficiency causes defective DNA synthesis, thereby causing nuclear maturation to be out of step with hemoglobinization of the cytoplasm. Food restriction anemia also is associated with leukopenia, thrombocytopenia, abnormal erythrocyte shapes (marked poikilocytosis), and hypersegmentation of granulocytes.

Erythrocytosis (polycythemia) rarely is reported in birds.[58] The conditions associated with polycythemia in mammals most likely cause polycythemia in birds as well. A primary erythrocytosis is a myeloproliferative disorder resulting in an absolute erythrocytosis. Most reported cases of an absolute erythrocytosis (PCV, usually >70%) in birds are secondary and associated with chronic pulmonary disease. Cardiac insufficiency, however, also may result in an erythrocytosis. An increase in erythropoietin associated with renal disease is another causative consideration for this disorder. A relative erythrocytosis associated with dehydration is responsible for most avian cases with an erythrocytosis.

The presence of numerous immature erythrocytes (especially rubriblasts) and abnormal-appearing immature erythrocytes in the peripheral blood of birds indicates erythrocytic neoplasia. Erythroblastosis in poultry with avian leukosis complex is an example of this condition.

The genotoxicity of cyclophosphamide and mitomycin-C cause a decrease in the PCV and the formation of erythrocytes with micronuclei and nuclear budding.[41,59,60] The micronuclei are seen in erythrocytes at the rubriblasts and prorubricytes stages of development. These effects are seen up to day 8 following secession of cyclophosphamide therapy. Recovery is signaled by a large increase in immature erythrocytes with complete resolution by day 13. Thus, detection of nuclear budding and micronuclei in avian erythrocytes in blood films is an indication of exposure to a therapeutic or environmental genotoxin.

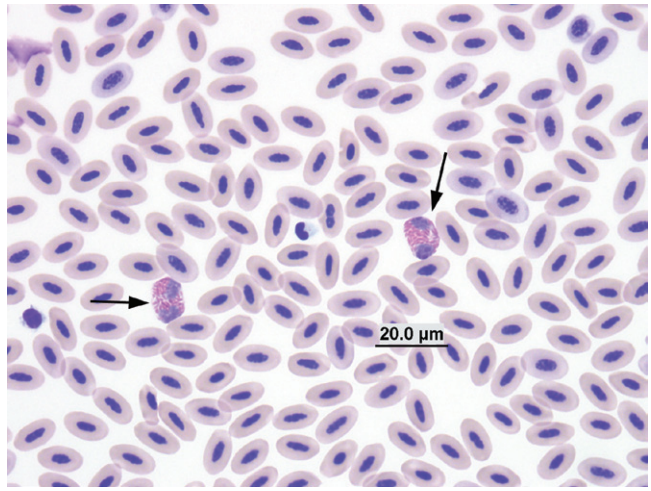

Figure 19.20 Normal heterophils (arrows) in the blood film of a parrot (*Eclectus roratus*). Wright-Giemsa stain.

Leukocytes

Morphology

Leukopoiesis in normal birds appears to be similar to that in mammals, in that leukocytes are released into the peripheral circulation only when they are mature. Leukocytes in avian blood include lymphocytes, monocytes, and granulocytes. The granulocytes are classified as heterophils, eosinophils, and basophils. Heterophils are the most abundant granulocyte in most birds. The cytoplasm of normal, mature heterophils appears colorless and contains eosinophilic granules (dark orange to brown-red) with Romanowsky stains (Fig. 19.20). The cytoplasmic granules typically are elongate (rod or spiculated shaped), but they may appear oval to round in some species. Heterophil granules frequently have a distinct central body that appears to be refractile. The granules may be affected by the staining process and appear atypical (i.e., poorly stained, partially dissolved, or fused). The nucleus of mature heterophils is lobed (usually two to three lobes) with coarse, clumped chromatin that stains purple. The nucleus often is partially hidden by the cytoplasmic granules.

Avian heterophils are considered to be functionally equivalent to mammalian neutrophils; however, there are differences. They actively participate in inflammatory lesions, and they are phagocytic. The cytoplasmic granules of heterophils contain lysozyme and proteins needed for bactericidal activity, although some avian species, such as chickens, have heterophils that lack peroxidase activity.[31,61–67] Heterophils phagocytize microorganisms and destroy them by oxygen-dependent and -independent mechanisms.[68,69] Although chicken heterophils lack the alkaline phosphatase, catalase, and myeloperoxidase needed for oxygen-dependent killing of microorganisms, they do consume oxygen and produce oxygen radicals and hydrogen peroxide, but to a lesser

extent than in mammalian neutrophils.[61,68] Therefore, avian heterophils rely more heavily on oxygen-independent mechanisms, lysozyme, and cationic proteins (i.e. acid hydrolases and cathepsin) to destroy microorganisms. Avian (chicken and turkey) heterophils do not respond to the chemoattractant formyl-methionyl-leucyl-phenylalanine (fMLP) as do mammalian neutrophils.

Ultrastructural studies of avian heterophils reveal primary, secondary, and tertiary granules.[31,63] Primary granules are the most numerous, and they appear as electron-dense, fusiform rods (1.5 × 0.5 µm) with a circular central body. Secondary granules (diameter, 0.5 µm) are less dense and contain eccentric inclusions composed of loose, filamentous material. Tertiary granules (0.1 µm) have a dense core that is separated from a membranous envelope of an electron-luscent area. Based on the results of biochemical evaluations of chicken heterophils, myeloperoxidase and alkaline phosphatase also are absent. Chicken heterophil granules do not stain with alkaline phosphatase, peroxidase, Sudan black B, acid phosphatase, naphthol AS-D chloroacetate esterase methods, or periodic acid-Schiff. Small and medium granules may be seen ultrastructurally in avian heterophils, and these probably represent maturation stages of the cytoplasmic granules.

Abnormal appearing heterophils in blood films include both immature and toxic heterophils. Immature heterophils have increased cytoplasmic basophilia, nonsegmented nuclei, and immature cytoplasmic granules compared with normal, mature heterophils (Fig. 19.20). Immature heterophils most frequently encountered in the blood are myelocytes and metamyelocytes. Heterophil myelocytes are larger than mature heterophils, and they have blue cytoplasm as well as secondary, rod-shaped granules, which occupy less than half the cytoplasmic volume, and a round to oval, nonsegmented nucleus. Heterophil metamyelocytes resemble myelocytes, except that the nucleus is indented and the rod-shaped granules occupy more than half the cytoplasmic volume. Band heterophils resemble mature heterophils, except that the nucleus is not lobed. It often is difficult to recognize a band cell, because the nucleus is hidden by the cytoplasmic granules. Therefore, a true assessment regarding the concentration of band cells in avian blood films requires use of a nuclear stain, such as hematoxylin, which stains only the nucleus and not the cytoplasmic granules.

In response to severe systemic illness, avian heterophils exhibit toxic changes similar to those in mammalian neutrophils. Toxic changes in avian heterophils are subjectively quantified as to the number of toxic cells and the severity of toxicity, as in mammalian hematology. Toxic heterophils have increased cytoplasmic basophilia, vacuolization, abnormal granulation (degranulation, granules that appear deeply basophilic, and granules that appear to coalesce into large, round granules), and degeneration of the cell nucleus (Figs. 19.21 through 19.25). The degree of heterophil toxicity can be rated subjectively on a scale of 1+ to 4+. A 1+ degree of toxicity or mild toxicity is assigned when heterophils exhibit increased cytoplasmic basophilia. A 2+ or mild to moderate degree of toxicity is assigned when heterophils have deeper cytoplasmic basophilia and partial degranulation. A 3+ degree of toxicity or moderate toxicity is assigned when heterophils exhibit deep cytoplasmic basophilia, moderate degranulation, abnormal granules, and cytoplasmic vacuolization, and a 4+ or marked degree of toxicity is assigned when heterophils exhibit deep cytoplasmic basophilia, moderate to marked degranulation with abnormal granules, cytoplasmic vacuolization, and karyorrhexis or karyolysis. The number of toxic heterophils are graded as few (5–10%), moderate (11–30%), and marked (>30%).

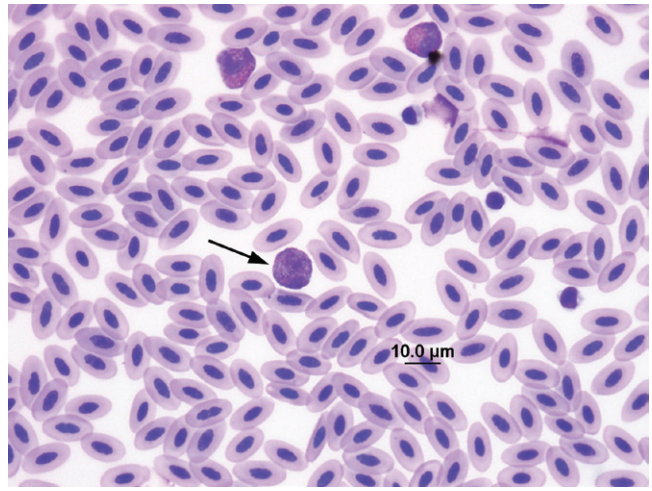

Figure 19.21 A heterophil metamyelocyte (arrow) in the blood film of a parrot (*Amazona oratrix*). Wright-Giemsa stain.

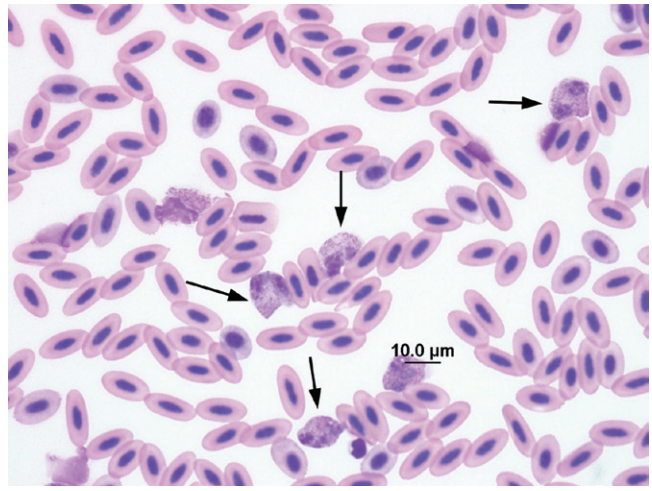

Figure 19.22 Mildly toxic (1+) heterophils in the blood film of an owl (*Bubo virginianus*). Wright-Giemsa stain.

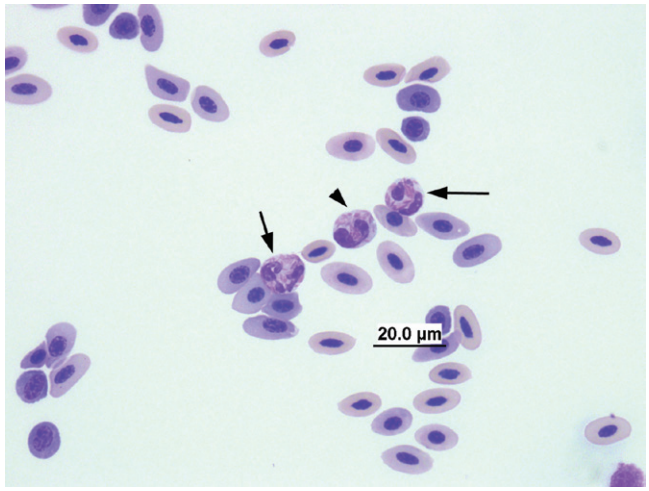

Figure 19.23 Mildly toxic (1+) heterophils (arrows) and a toxic (1+) band heterophil (arrowhead) in the blood film of a hawk (*Buteo regalis*). Wright-Giemsa stain.

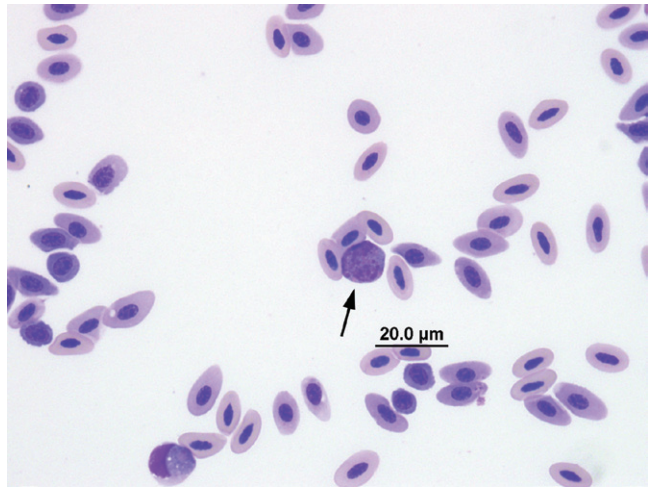

Figure 19.25 A heterophil exhibiting marked (4+) toxicity in the blood film of a hawk (*Buteo regalis*). Wright-Giemsa stain.

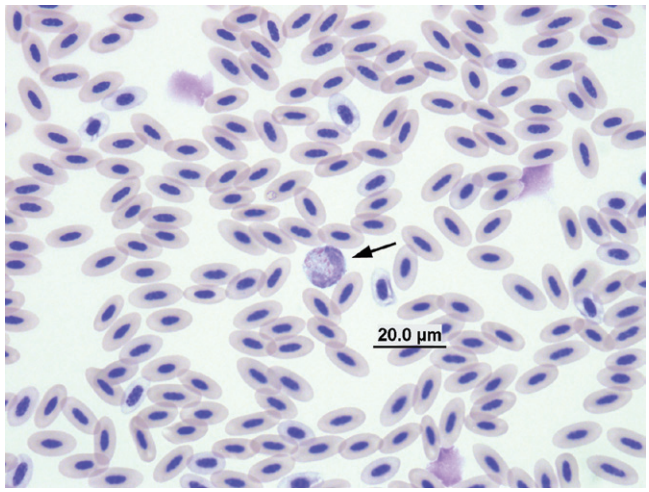

Figure 19.24 Moderate to marked toxic (3+) band heterophil in the blood film of a parrot (*Eclectus roratus*). Wright-Giemsa stain.

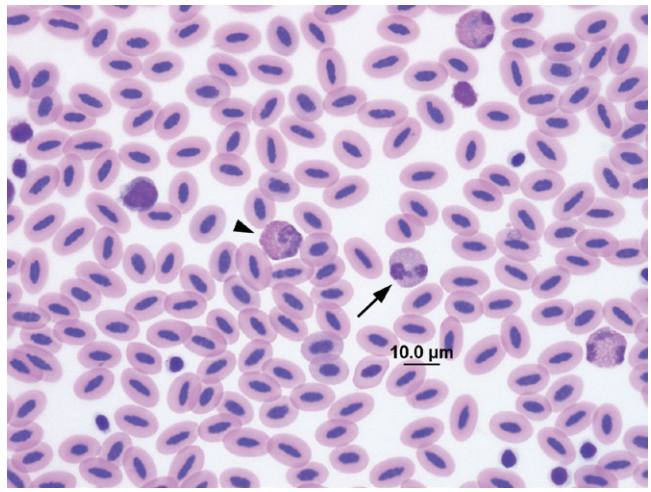

Figure 19.26 An eosinophil (arrow) and a heterophil (arrowhead) in the blood film of an owl (*Strix varia*). Wright-Giemsa stain.

Most avian eosinophils are nearly the same size as heterophils in most blood films.[8] For example, heterophils of adult Coturnix quail measure 10.22 ± 1.20 µm in diameter for males and 9.80 ± 1.14 µm in diameter in females compared to eosinophils that measured 9.76 ± 1.13 µm in diameter for males and 9.55 ± 1.23 µm in diameter in females.[13] In contrast to mature heterophils, avian eosinophils in general have round, strongly eosinophilic cytoplasmic granules, although the granules in some species are oval to elongate. In general, eosinophil granules stain more intensely that heterophil granules (Figs. 19.26, 19.27, and 19.28). The cytoplasmic granules of eosinophils lack the central, refractile body seen in many avian heterophils. The cytoplasm of eosinophils stains clear blue, in contrast to the colorless cytoplasm of normal, mature heterophils. The nuclei of eosinophils are lobed and usually stain darker than heterophil nuclei. The cytoplasmic granules of eosinophils frequently are affected by Romanowsky stains. The granules may appear to be large, swollen, and round, and they also may appear colorless or to stain pale blue (Fig. 19.29). Eosinophils vary in appearance species of birds.

Avian eosinophils have some features in common with mammalian eosinophils.[70,71] The ultrastructure of avian eosinophils reveals large, spherical, primary granules and mature, rod-shaped, specific granules. In some birds, the specific granules possess a crystalline core, a prominent feature of mammalian eosinophils; however, this feature is missing in other species (e.g. chicken and duck).[31] The larger

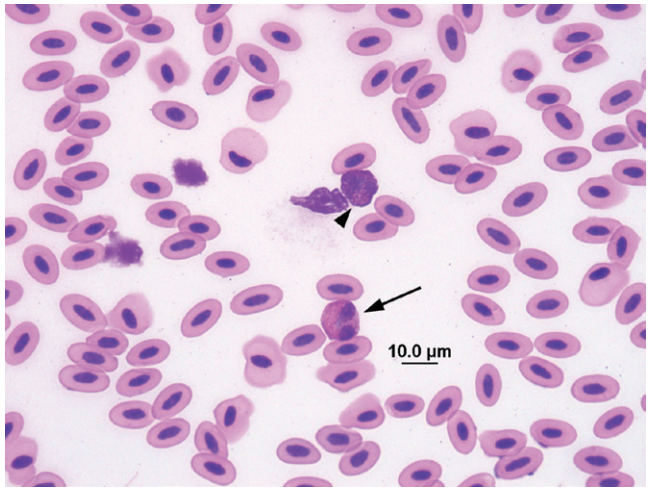

Figure 19.27 An eosinophil (arrow) and a heterophil (arrowhead) in the blood film of an eagle (*Aquila chrysaetos*). Wright-Giemsa stain.

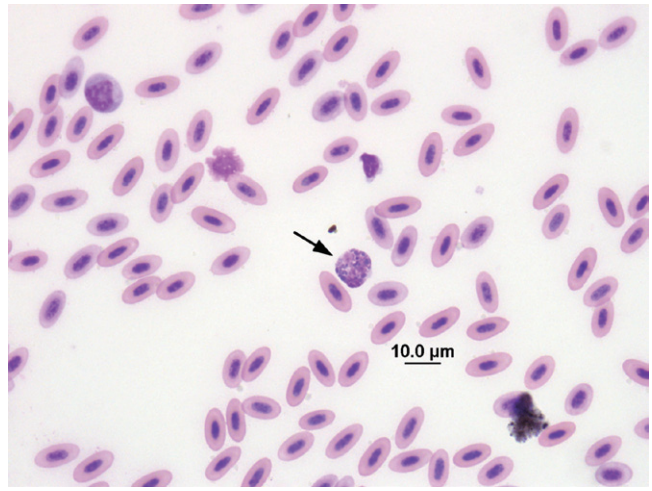

Figure 19.29 An eosinophil with blue-staining granules in the blood film of a parrot (*Psitticus erithacus*). Wright-Giemsa stain.

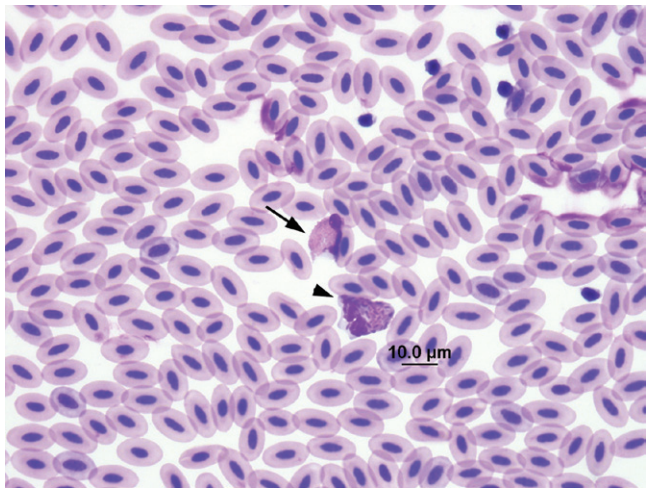

Figure 19.28 An eosinophil (arrow) and a mildly toxic (1+) heterophil (arrowhead) in the blood film of a hawk (*Buteo jamaicensis*). Wright-Giemsa stain.

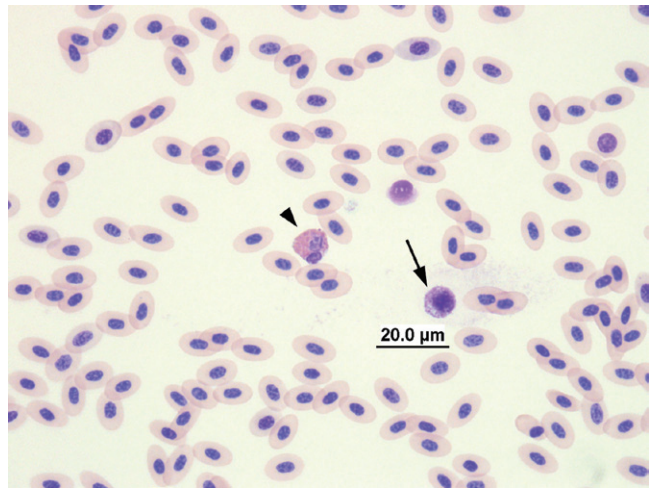

Figure 19.30 A basophil (arrow) and a heterophil (arrowhead) in the blood film of a chicken (*Gallus gallus domesticus*). Wright-Giemsa stain.

primary granules most likely are precursors to the smaller, specific granules. Similar to mammalian eosinophils, specific granules possess a high concentration of arginine and enzymes, such as peroxidase, acid phosphatase, and arylsulfatase. Cytochemical staining of chicken eosinophils indicate a positive reactivity for peroxidase, acid phosphatase, and Sudan black B. Avian eosinophils (chicken and duck) contain major basic protein, a principal protein in mammalian eosinophils, but not neutrophils. Therefore, these reactions can be used to distinguish eosinophils from heterophils. Unlike heterophils, avian eosinophils, although they are immotile and nonphagocytic, do respond to fMLP by forming surface projections and clumping to form large aggregates. Avian eosinophils have been shown to participate in delayed hypersensitivity reactions, a feature not seen with mammalian eosinophils.

Avian basophils tend to be smaller than heterophils and eosinophils. For example, basophils of adult Coturnix quail measure 9.23 ± 1.35 µm in diameter for males and 9.55 ± 1.26 µm in diameter in females.[13] Avian basophils contain deeply metachromic granules that often obscure the nucleus. The nucleus usually is nonlobed, thereby causing avian basophils to resemble mammalian mast cells (Figs. 19.30 and 19.31). The cytoplasmic granules of basophils frequently are affected by alcohol-solubilized stains, and they may partially dissolve or coalesce and appear abnormal in blood films stained with Romanowsky stains. Avian baso-

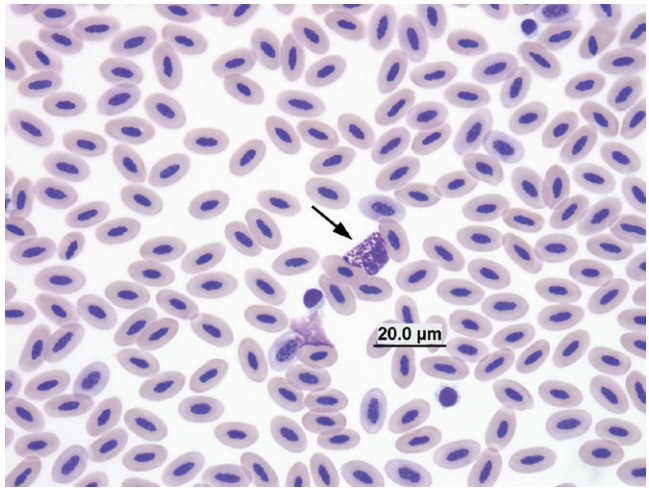

Figure 19.31 A basophil (arrow) in the blood film of a parrot (*Eclectus roratus*). Wright-Giemsa stain.

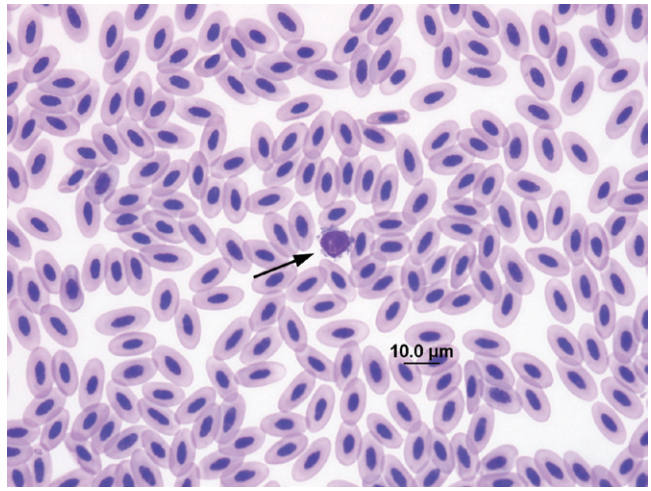

Figure 19.33 A small lymphocyte (arrow) in the blood film of a parrot (*Amazona oratrix*). Wright-Giemsa stain.

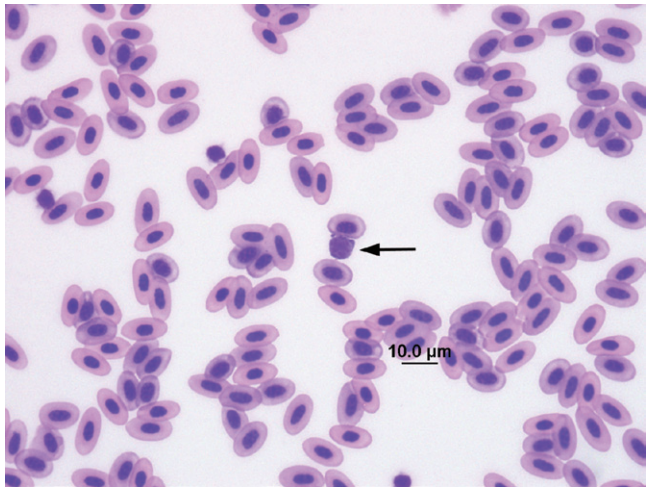

Figure 19.32 A small lymphocyte (arrow) in the blood film of a quail (*Colinus virginianus*). Wright-Giemsa stain.

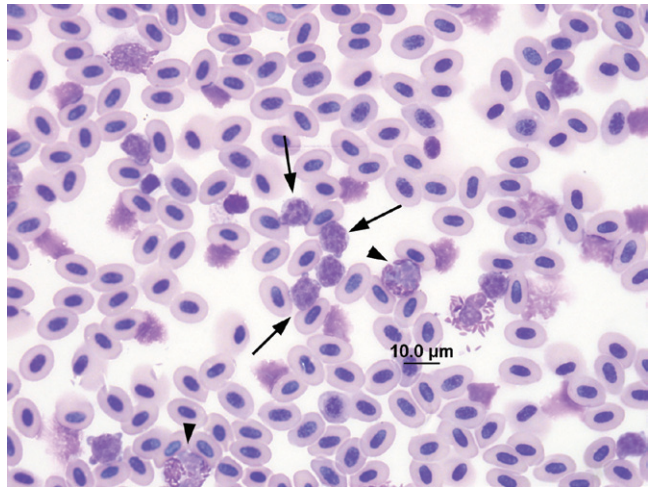

Figure 19.34 Medium lymphocytes (arrows) and an intact heterophil (arrowhead) in the blood film of a parrot (*Amazona aestiva*). Wright-Giemsa stain.

phils frequently are found in the peripheral blood, in contrast to mammalian basophils, which rarely are found in the blood films of normal animals. The function of avian basophils is not known. However, it is presumed to be similar to that of mammalian basophils and mast cells, because their cytoplasmic granules contain histamine. They also participate in acute inflammatory and type IV hypersensitivity reactions.

Avian lymphocytes resemble mammalian lymphocytes and generally come in two sizes, small and medium (Figs. 19.32 through 19.36).[72,73] Small lymphocytes of adult Coturnix quail measure 4.83 ± 0.24 µm in diameter for males and 4.86 ± 0.22 µm in diameter in females, whereas medium lymphocytes measure 7.73 ± 1.33 µm in diameter for males and 8.53 ± 1.40 µm in diameter in females.[13] Typically, they are round cells that often show cytoplasmic irregularity when they mold around adjacent erythrocytes in the blood film. Lymphocytes have a round, occasionally slightly indented, centrally or slightly eccentrically positioned nucleus. The nuclear chromatin is heavily clumped or reticulated in mature lymphocytes, and the cytoplasm typically is scant, except in large lymphocytes, thereby giving lymphocytes their high nucleus:cytoplasm (N:C) ratio. Large lymphocytes that resemble those found in bovine blood films can also be found in the blood of normal birds. Large lymphocytes can be confused with monocytes, however, because of their size, cytoplasmic volume, and pale-staining nuclei. The lymphocyte cytoplasm usually appears to be homogenous and weakly basophilic (pale blue), and it lacks both vacuoles and granules. Cytoplasmic features are

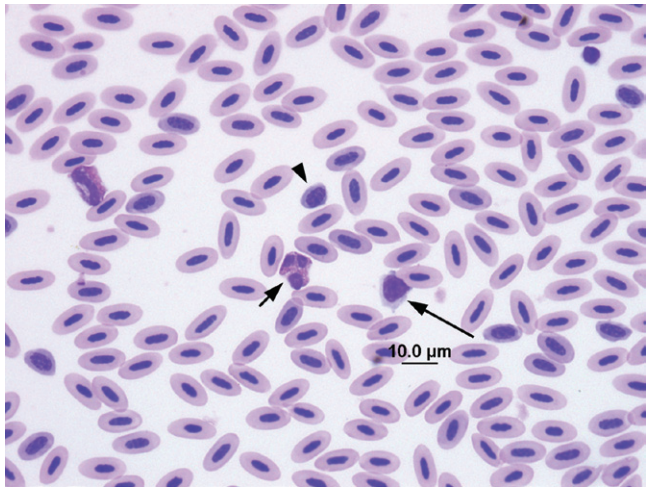

Figure 19.35 A large lymphocyte (long arrow), heterophils (short arrow), and immature erythrocyte (arrowhead) in the blood film of a conure (*Aratinga solstitialis*). Wright-Giemsa stain.

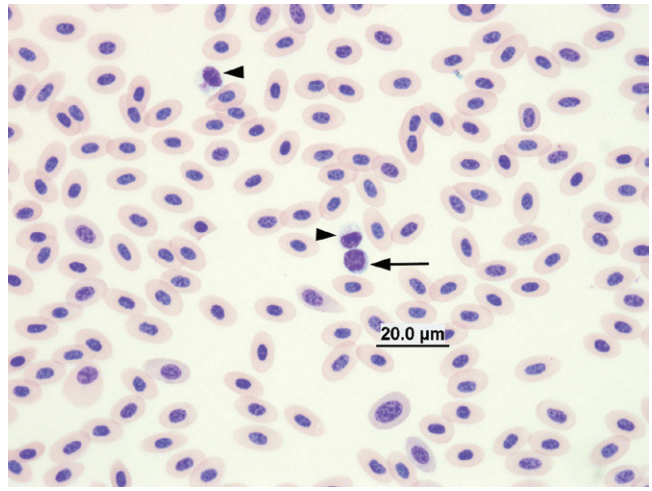

Figure 19.37 A lymphocyte (arrow) and thrombocytes (arrowheads) in the blood film of a chicken (*Gallus gallus domesticus*). Wright-Giemsa stain.

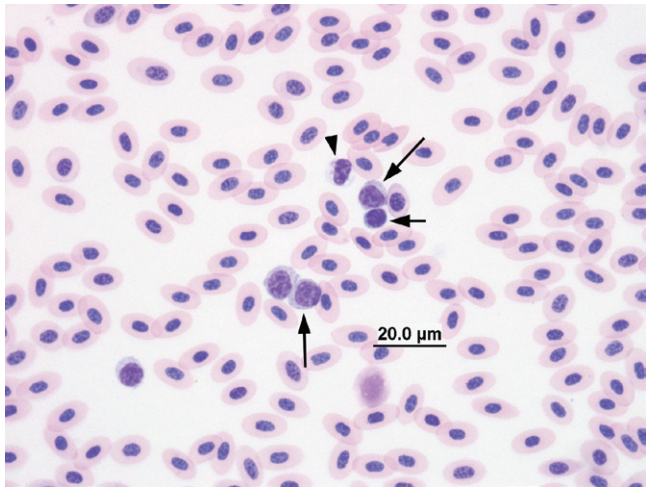

Figure 19.36 Medium lymphocytes (long arrows), a small lymphocyte (short arrow), and a thrombocyte (arrowhead) in the blood film of a chicken (*Gallus gallus domesticus*). Wright-Giemsa stain.

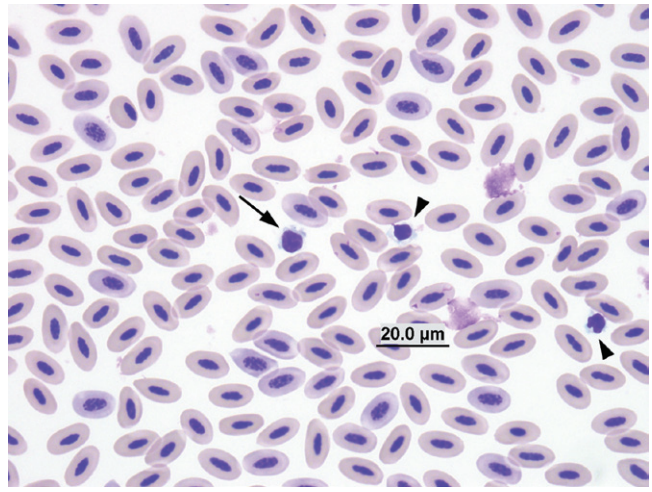

Figure 19.38 A lymphocyte (arrow) and a thrombocyte (arrowhead) in the blood film of a parrot (*Eclectus roratus*). Wright-Giemsa stain.

important when differentiating small lymphocytes from thrombocytes (Figs. 19.36 through 19.39). The latter have clear, colorless cytoplasm that often appears to be vacuolated, with a few distinct specific granules. Occasionally, cells in the blood films of birds have features of both thrombocytes and lymphocytes. These intermediate cells have small, round to oval nuclei with coarsely clumped chromatin and moderately abundant, blue-tinged cytoplasm that lacks both vacuoles and granules. Cytochemical properties indicate these cells to be lymphocytes.[74]

Occasionally, lymphocytes may contain large distinct azurophilic granules or irregular cytoplasmic projections (Fig. 19.40). Although sometimes considered to be natural killer cells, the significance of lymphocytes with azurophilic granules is not known. Irregular cytoplasmic projections are indicative of cellular degeneration, a significant finding if represented by the majority of lymphocytes.

Abnormal lymphocytes are classified as either reactive or blast-transformed lymphocytes. Reactive lymphocytes are small to medium lymphocytes with heavily clumped nuclear chromatin and deeply basophilic cytoplasm. Lymphocytes develop into reactive lymphocytes when antigenically stimulated. Blast-transformed lymphocytes are large lymphocytes with dispersed, smooth nuclear chromatin, which may contain nucleoli (Figs. 19.41). They have basophilic cytoplasm that may exhibit a prominent, clear, perinuclear halo

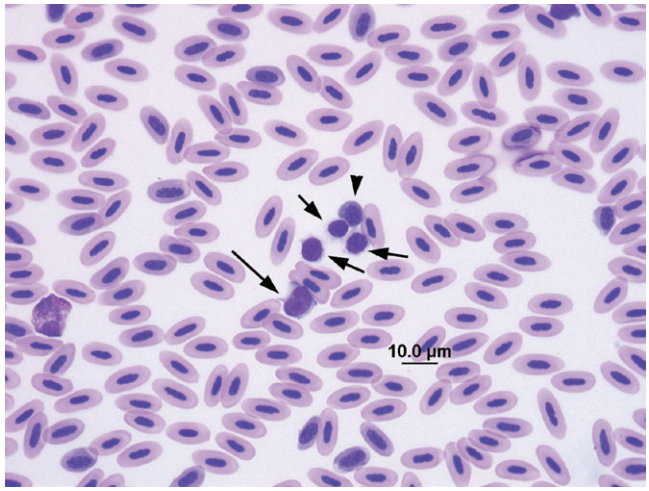

Figure 19.39 A lymphocyte (long arrow), thrombocytes (short arrows), and an immature erythrocyte (arrowhead) in the blood film of a conure (*Aratinga solstitialis*). A heterophil is also present in the lower left hand corner of the image. Wright-Giemsa stain.

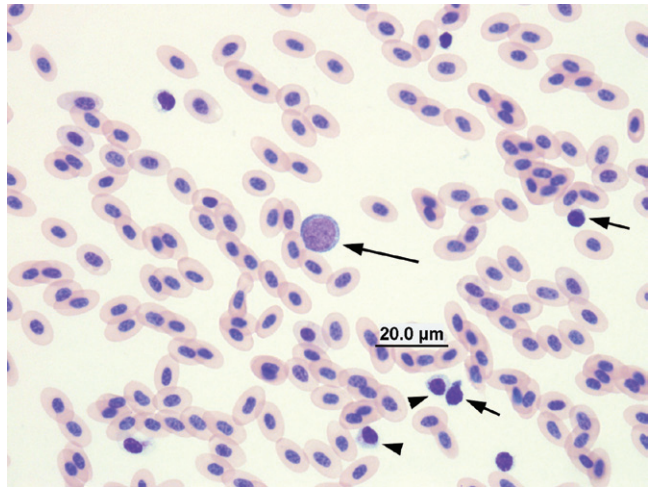

Figure 19.41 A reactive lymphocyte (long arrow), small mature lymphocytes (short arrows), and thrombocytes (arrowheads) in the blood film of a chicken (*Gallus gallus domesticus*). Wright-Giemsa stain.

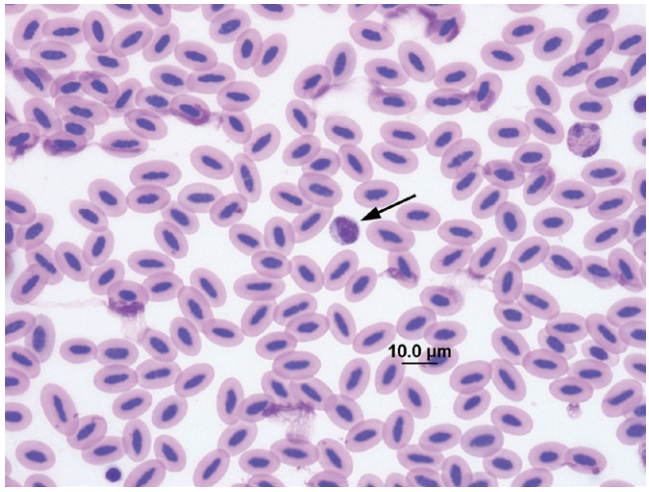

Figure 19.40 A lymphocyte with azurophilic granules (arrow) in the blood film of an owl (*Strix varia*). Wright-Giemsa stain.

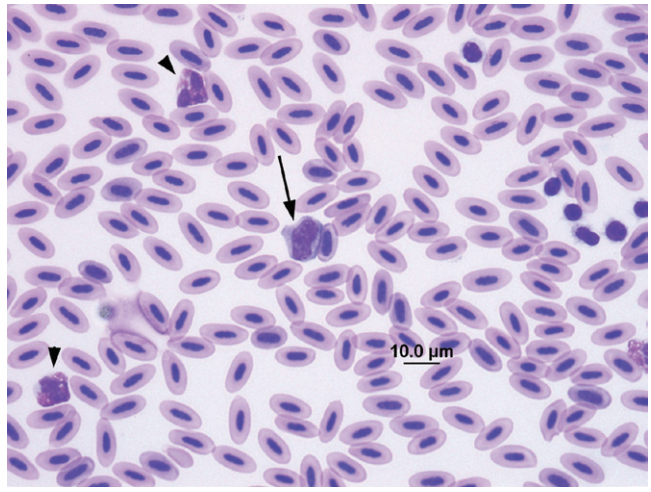

Figure 19.42 A monocyte (arrow) and heterophils (arrowhead) in the blood film of a conure (*Aratinga solstitialis*). A thrombocyte is also present in the upper right hand corner of the image. Wright-Giemsa stain.

or Golgi zone. These lymphocytes have anaplastic features and may be neoplastic, but they also may result from immunologic stimulation.[75] Plasma cells also can be found in the peripheral blood of birds. These are large B lymphocytes with eccentrically positioned mature nuclei; abundant, deeply basophilic cytoplasm; and a distinct Golgi zone. Lymphocytes that contain prominent azurophilic granules are also considered to be reactive.

Avian monocytes typically are the largest leukocyte, and they resemble their mammalian counterpart, varying in shape from round to ameboid. For example, monocytes of adult Coturnix quail measure 13.53 ± 0.74 µm in diameter for males and 13.26 ± 0.45 µm in diameter in females.[13] Monocytes have abundant, blue-gray cytoplasm that may appear to be slightly opaque, and they contain vacuoles or fine, dustlike eosinophilic granules (Figs. 19.42 through 19.44). Avian monocytes frequently exhibit two distinct zones in the cytoplasm: a light-staining perinuclear area, and a darker-staining area. The monocyte nucleus can vary in shape and is relatively pale, with less chromatin clumping compared with lymphocyte nuclei. The ultrastructure of avian monocytes and macrophages reveals a cytoplasmic membrane that is composed of blebs or filaments, a prominent Golgi apparatus, many ribosomes, and a variable

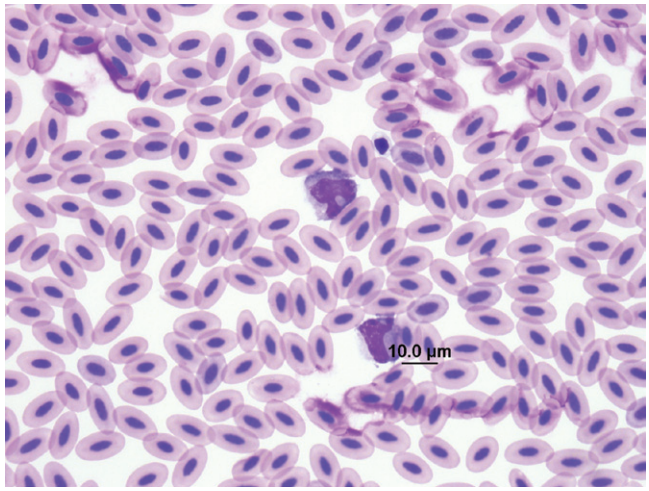

Figure 19.43 Two monocytes in the blood film of a hawk (*Buteo jamaicensis*). Wright-Giemsa stain.

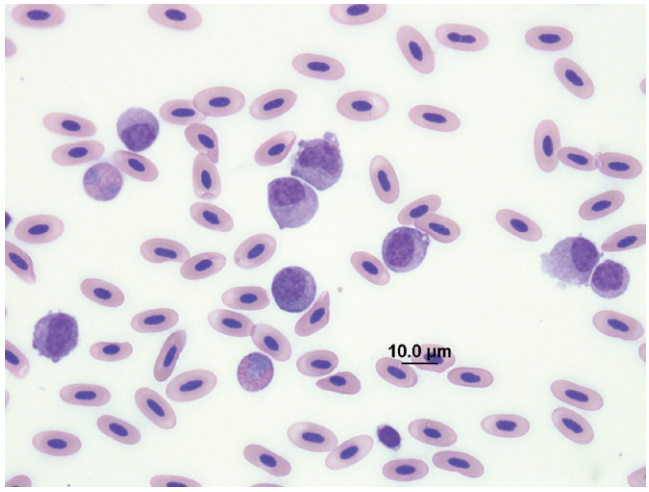

Figure 19.44 Eight monocytes and two heterophils in the blood film of a gannet (*Morus* sp) with a marked monocytosis. Wright-Giemsa stain.

number of pinocytic vesicles and lysosomes.[31] Monocytes exhibit phagocytic activity and migrate into tissues to become macrophages.[76] They possess biologically active chemicals that are involved in inflammation and oxidative destruction of invading organisms. Monocytes also have an important immunologic role in antigen processing.[31]

Laboratory evaluation

The presence of nucleated erythrocytes and thrombocytes in avian blood precludes use of the routine methods used to count leukocytes in mammalian blood. Automated methods for counting white blood cells in mammalian blood produce erroneous results when applied to avian blood, because all the cells in the peripheral blood of birds are nucleated.[77] Also, the size of the erythrocytes is similar to the size of many of the leukocytes; thrombocytes and small lymphocytes also are similar in size. Therefore, direct and semidirect, manual methods for obtaining total leukocyte concentrations in birds have been developed. A commonly used semidirect method involves the staining of avian heterophils and eosinophils with phloxine B as the diluent.[2,78] Phloxine B commonly is used as a specific stain for eosinophils in mammalian blood. The procedure is simplified by using the Eosinophil Unopette 5877 system (Becton-Dickinson), which was developed for determining total eosinophil concentrations in mammalian blood.[79] The blood is diluted 1: 32 with the phloxine B solution in the Unopette vial using the 25-µL pipette that is provided. After a Neubauer-ruled hemacytometer chamber has been properly loaded with the blood–phloxine mixture and allowed to stand in a humid chamber for a minimum of 5 minutes, the eosin-stained heterophils and eosinophils are counted in both sides of the chamber (18 large squares) (Fig. 19.45). The hemacytometer should be loaded immediately after proper mixing of the blood and phloxine diluent, because red blood cells also may stain after prolonged exposure. The total heterophil and eosinophil concentration per cubic millimeter of blood (heterophils + eosinophils/mm^3) is calculated using the formula for obtaining a total eosinophil count in mammalian blood:

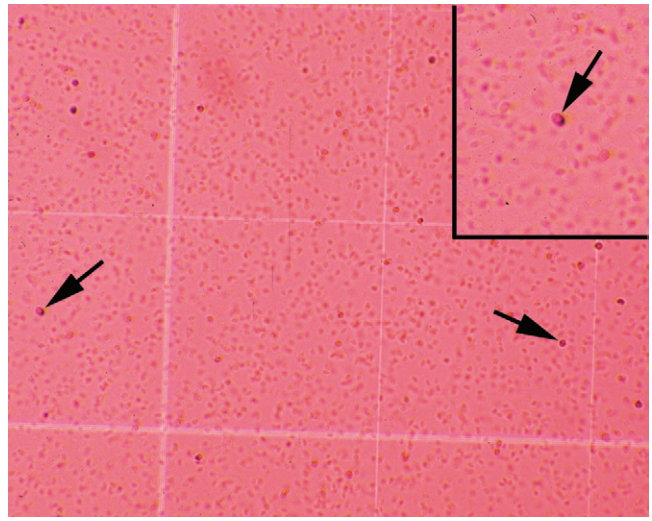

Figure 19.45 The appearance of acidophils stained with phloxine dye in a hemocytometer chamber, 100×.

The total leukocyte concentration (TWBC/mm^3) is calculated after completing a leukocyte differential using the following formula:

The TWBC/mm^3 can be obtained using one calculation with the following formula:

$$\text{TWBC/mm}^3 = \frac{\text{Eosin-stained cells} \times 1.11 \times 16 \times 100}{\%\ \text{Heterophils and eosinophils}}$$

where the number of eosin-stained cells are counted in both sides of the hemacytometer (18 large squares).

A direct method for obtaining total leukocyte concentrations in avian blood is to make a 1:200 dilution with Natt-Herrick solution (Table 19.2) using a standard red blood cell–diluting pipette or by adding 20 µL of blood to 4 mL of the Natt-Herrick solution (Fig. 19.17).[20] The total leukocyte concentration is obtained by counting all the leukocytes (dark-blue cells) in the nine large squares in the ruled area of the hemacytometer chamber using the following formula:

$$\text{TWBC/mm}^3 = (\text{Total cells in nine large squares} + 10\%) \times 200$$

The advantage to this method is that a total erythrocyte and thrombocyte count also can be obtained using the same charged hemacytometer. A disadvantage is that differentiating thrombocytes from small lymphocytes often is difficult, thus creating errors in the counts. Staining for 60 minutes in the Natt-Herrick solution, however, improves the differentiation between small lymphocytes and thrombocytes.

A second method for obtaining a direct total leukocyte count in birds is to dilute the anticoagulated blood 1:100 with 0.01% toluidine blue in phosphate-buffered saline before charging a Neubauer-ruled hemocytometer.[80] Cells that are equal to or larger than the width of the erythrocytes are counted in the nine large squares of the hemacytometer. The total leukocyte count is calculated using the standard formula:

$$\text{TWBC/mm}^3 = \frac{\text{No. cells} \times 10 \times 100}{9}$$

Or, to simplify the math,

$$\text{TWBC/mm}^3 = (\text{Number of cells} + 10\%) \times 100$$

Toluidine blue stains leukocytes blue, erythrocytes pale orange, and thrombocytes pale blue. Counting cells that are equal to or larger than the width of erythrocytes should rule out thrombocytes, which tend to be smaller in width than erythrocytes. Small lymphocytes tend to be equal to or larger than the width of erythrocytes. Immature erythrocytes are distinguished from small lymphocytes by their round to irregular shape; their round, centrally positioned nucleus with dark, irregularly clumped chromatin; and their moderate volume of basophilic hyalin cytoplasm. A corrected total leukocyte concentration can be obtained when a large number of immature erythrocytes are present by using the following formula:

$$\text{Corrected TWBC/mm}^3 = \frac{\text{TWBC} \times 100}{100 + \text{No. immature RBCs per 100 leukocytes}}$$

With counting methods requiring use of a hemocytometer, the difference between the counts obtained from each chamber should not exceed 10% to ensure accuracy between the two sides. If the difference does exceed 10%, the procedure should be repeated. The semidirect method using the phloxine stain is easier to perform and is more precise for hemocytometer counting than the Natt and Herrick method. To our knowledge, no comparisons have been made for the toluidine blue method; however, the results should be similar to those with the Natt and Herrick method. Because the semidirect method using phloxine B stain for determination of total leukocyte counts in birds depends on the leukocyte differential, especially the number of heterophils and eosinophils, it becomes less accurate as the level of mononuclear leukocytes exceeds that of the granulocytes.

Crude estimation of the cell numbers from blood films is an inappropriate method for obtaining a total leukocyte count in birds. Estimated leukocyte counts should be used only when quantitative counts (i.e., phloxine B, Natt and Herrick, or toluidine blue methods) are unavailable or as a means of detecting submission or laboratory error (e.g., if the number of leukocytes observed in a blood film appears to be less than that reported from a quantitative count).[81]

Crude estimation of the leukocyte concentration in a well-prepared blood film is made by obtaining the average number of leukocytes in five monolayer ×1000 (oil-immersion) fields and using the following formula:

$$\text{Estimated WBC/mm}^3 = \frac{\text{Number of WBC in 5 fields} \times 3{,}500{,}000}{1000}$$

The number 1000 is the average number of erythrocytes in five monolayer ×1000 fields, and 3,500,000 is the approximate number of erythrocytes per cubic millimeter in birds with normal PCVs. If the PCV is outside the normal range of 35% to 55%, then the estimated count should be corrected for the PCV using the following formula:

$$\text{Corrected Estimated WBC/mm}^3 = \frac{\text{Estimated WBC} \times \text{Observed PCV}}{\text{Normal PCV (45\%)}}$$

Less experienced observers may wish to obtain an estimated total leukocyte count by determining the average number of leukocytes per field in ten monolayer ×40 (high-dry) fields and then multiplying by 2000.[82]

Accurate interpretation of leukocyte counts, especially when determined by the semidirect method, depends on the

accurate identification and differentiation of leukocytes in the blood film.

Responses in disease

Avian leukograms often vary widely between normal birds of the same species (Table 19.4). Because birds often become excited when handled, the blood collection process usually results in a physiologic leukocytosis, and this physiologic response increases the concentration of heterophils and lymphocytes in the peripheral blood. Normal total leukocyte reference intervals obtained from birds generally are broader than those obtained from domestic mammals.[2,29,83] Thus, avian leukogram values must differ greatly from the normal reference intervals to have diagnostic significance.

In general, gender differences in the normal leukogram of birds are not clinically significant; however, age differences can be. Generally, percentages of heterophils and lymphocytes and absolute lymphocyte counts vary significantly between adult and juvenile birds where younger birds tend to have higher lymphocyte counts.[27,32] The leukogram may also be affected by seasonal influences, especially in free-ranging birds. For example, the absolute leukocyte, heterophil, and lymphocyte counts of ducks decrease during and after the remige molt, a seasonal event.[84]

Table 19.4 Leukocyte parameters for selected birds.

	WBC × 10^3/μL	Heterophils %	Lymphocytes %	Monocytes %	Eosinophils %	Basophils %
Psittacines						
African grey parrot[1,3]	5–15	45–75	20–50	0–3	0–2	0–22
Grey parrot[5]	4.0–20.0	29–83	16–68	1–6	0–3	0
Amazon parrots[1,2]	6–11	30–75	20–65	0–3	0–1	0–5
Blue-fronted Amazon[5]	4.7–11.0	12–47	52–84	1–3	0–1	0–1
Cuban Amazon[5]	1.9–24.7	19–28	71–75	0–5	0–5	0–1
Festive Amazon[5]	2.2–7.0	22–32	66–76	0–4	0–2	0
Orange-wing Amazon[5]	1.2–10.1	22–41	56–73	2–5	0–5	0–2
Yellow Amazon[5]	2.2–7.2	12–52	48–80	0–8	0–1	0–1
Budgerigar[1,3]	3–8	40–65	20–45	0–1	0–1	0–1
Caique[1]	8–15	39–72	20–61	0–2	0–2	0–2
Cockatiel[1,3]	5–13	40–70	25–55	0–2	0–2	0–6
Cockatoos[1,3]	5–10	55–80	20–45	0–2	0–1	0–3
Black cockatoo[5]	3.7–22.1	7–61	33–90	3–7	0	0–2
Palm cockatoo[5]	1.4–17.6	24–75	24–69	1–7	0–1	0–1
White cockatoo[5]	1.3–18.7	18–83	15–80	0–4	0–1	0–1
Conures[1,3]	4–13	40–70	20–50	0–3	0–3	0–5
Golden conure[5]	4.2–8.0	22–49	49–69	1–3	0–2	0
Patagonian conure[5]	2.5–8.7	24–63	35–66	0–3	0–1	0
Eclectus parrot[1,3]	9–20	35–50	45–65	0–2	0–1	0–3
Grey-cheek parakeet[1]	4.5–12.0	40–75	20–60	0–3	0–1	0–5
Jardine's parrot[1]	4–10	55–75	25–45	0–2	0–1	0–1
Lory[1]	8–13	40–60	22–69	0–2	0–1	0–1
Red Lory[5]	0.8–9.0	26–79	19–70	0–5	0–5	0–1
Lovebird[1,3]	3–16	40–75	20–55	0–2	0–1	0–6
Macaw[1,3]	7–22	40–60	35–60	0–3	0–1	0–1
Blue and gold macaw[5]	1.7–36.0	13–60	36–84	0–2	0–2	0–2
Green-wing macaw[5]	3.8–30.0	14–62	35–84	0–8	0–3	0–2
Hyacinthine macaw[5]	1.5–19.2	52–89	10–77	0–2	0–4	0
Military macaw[5]	13.7–18.0	12–63	43–80	0–8	0–2	0–1
Scarlet macaw[5]	4.7–22.0	26–67	36–68	0–8	0–4	0–2
Pionus parrot[1]	4.0–11.5	50–75	25–45	0–2	0–2	0–1
Quaker[1,3]	4–10	55–80	20–45	0–4	0–2	0–6
Senegal parrot[1]	4–14	55–75	25–45	0–2	0–1	0–1
Others						
Canary[1,3]	4–9	50–80	20–45	0–1	0–2	0–1
Finch[1,3]	3–8	20–65	20–65	0–1	0–1	0–5

Table 19.4 (*Continued*)

	WBC × 10^3/μL	Heterophils %	Lymphocytes %	Monocytes %	Eosinophils %	Basophils %
Mynah[1]	6–11	25–65	20–60	0–3	0–3	0–7
Toucan[1]	4–10	35–65	25–50		0–4	0–5
Pigeon[1,2]	1.3–2.3	50–60	20–40	0–3	0–3	0–3
Chicken [1,3]	0.9–3.2	15–50	29–84	0–7	0–16	0–8
Ringneck pheasant[1]	1.8–3.9	12–30	63–83	2–9	0–1	0–3
Turkey[1]	1.6–2.5	29–52	35–48	3–10	0–5	0–9
Quail[1]	1.3–2.5	25–50	50–70	0–4	0–15	0–2
Canada goose[1]	1.3–1.9					
*Mallard duck [4]	2.3–2.5	35–40	52–56	0–6	0–1	0–4
**Mallard duck[4]	2.3–2.5	27–31	64–68	0–3	0–1	0–3
Golden eagle[1]	1.2–1.5	81–86	14–22	0–1	2–5	0–1
Peregrine falcon[1]	3.3–11.0	1–9	1–3	0–1	0–1	0–1
Tawny owl[6]	4.0–59.0	1.6–9.6 × 10^3/μL	2.1–7.2 × 10^3/μL	0–0.5 × 10^3/μL	0.2–3.0 × 10^3/μL	0.1–0.4 × 10^3/μL
White-back vulture[7]	1.3–2.0	1.5–25.9 × 10^3/μL	0–4.8 × 10^3/μL	0–3.7 × 10^3/μL	0–2.2 × 10^3/μL	0–0 × 10^3/μL

*January.

**June.

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The general causes of a leukocytosis in birds include inflammation, which may be associated with infectious or noninfectious causes, toxicities (i.e zinc), hemorrhage into a body cavity, rapidly growing neoplasms, and leukemia. A leukocyte differential aids in the assessment of a leukocytosis. Because leukocytosis often is caused by inflammation, a heterophilia usually is present as well. The magnitude of the heterophilia depends on both the cause and the severity of the inflammation: the greater the degree of heterophilia, the greater the severity of the inflammation. A leukocytosis and heterophilia can be associated with inflammation in response to localized or systemic infections caused by a spectrum of infectious agents (i.e., bacteria, fungi, *Chlamydophila*, viruses, and parasites) and noninfectious causes (i.e., traumatic injury, foreign bodies, or toxicities).[17,75,85–88] A marked leukocytosis and heterophilia often are associated with diseases produced by common avian pathogens, such as *Chlamydophila*, *Mycobacterium*, and *Aspergillus*.[89–91] A slight to moderate leukocytosis in birds also can occur with excess endogenous or exogenous glucocorticoids (stress leukogram). A corticosteroid-induced leukocytosis reveals a slight to moderate, mature heterophilia and lymphopenia.[92,93] The heterophil:lymphocyte (H:L) ratio has been used as an index of stress in birds.[94,95] The H:L ratio appears to be an unreliable indicator of stress owing to the lack of correlation between that ratio and plasma corticosterone concentrations. The magnitude of leukocytosis and heterophilia during disease or corticosteroid excess varies with the H:L ratio, with greater responses being seen in species with normal H:L ratios of 3.0:1 versus those with ratios of 0.5:1. Initially, species that normally have high numbers of circulating lymphocytes (e.g., Anseriformes) may show a leukopenia but, later (i.e., up to 12 hours) demonstrate typical leukocytosis, heterophilia, and lymphopenia. Species that normally have greater numbers of circulating heterophils (e.g., galliformes) show a less dramatic change in the stress leukogram.

Immature heterophils rarely are present in the peripheral blood of normal birds. When they do occur, however, their presence usually results from excessive peripheral utilization of mature heterophils, with depletion of the mature storage pool in the hematopoietic tissue that indicates a severe inflammatory response, especially when associated with a

leukopenia. Marked increases in the concentration of immature heterophils also may result from granulocytic leukemia, which is a rare condition in birds.

Toxic heterophils are associated with severe, systemic illness such as septicemia, viremia, chlamydophilosis, mycotic infections, and severe tissue necrosis. The degree of heterophil toxicity usually indicates the severity of the bird's condition, and a marked number of 4+ toxic heterophils indicates a grave prognosis.

Hematological indicators of inflammation are species and etiologic dependent; however, the presence of a mild to moderate anemia, heterophilia, monocytosis, and heterophil morphological atypia appears to be the most consistent hematologic changes associated with inflammation in birds. Interpretive guidelines have been developed for some species of birds. For example, in free-ranging black cockatoos (*Calyptorhynchus* spp), anemia can be graded based upon a PCV of 30–35% as a mild anemia, 20–30% as moderate anemia, and less than 20% as severe anemia.[87] A mild leukocytosis in black cockatoos is represented by a leukocyte count less than 25,000/μL, whereas a count between 25,000 and 40,000/μL and greater than 40,000/μL represent moderate and severe responses respectively. Significant heterophilias in black cockatoos are represented by counts less than 20,000/μL as mild, 21,000–30,000/μL as moderate, and greater than 30,000/μL as severe. Toxic heterophils were not a common finding in black cockatoos with inflammatory disorders.

In contrast to black cockatoos, the inflammatory response in falcons (*Falco* spp) appears different. Normal falcons in general are reported to have a PCV between 37% and 53%; hemoglobin concentration between 12 and 21 g/dL; total leukocyte count between 3000 and 11,000 μL; and an absolute heterophil count greater than the absolute lymphocyte count.[85] Falcons rarely exhibit a leukocytosis greater than 17,000/ μL in response to inflammation regardless of the etiology. Aspergillosis, a common mycotic disease of birds, frequently causes a severe inflammatory disease in birds; however that response is variable between the species. For example, falcons (*Falco* spp) with aspergillosis develop a relatively mild leukocytosis compared to Buteo hawks that demonstrate a severe leukocytosis.[96–98]

Leukopenia is associated with either consumption of peripheral leukocytes or decreased production. Heteropenia results from decreased survival of mature heterophils or from decreased or ineffective production. Leukopenias associated with heteropenias can occur with severe bacterial infections or certain viral diseases (e.g., Pacheco's parrot disease).[99] Leukopenia and heteropenia with the presence of immature heterophils suggest exhaustion of the mature heterophil storage pool because of excessive peripheral demand for heterophils, as seen with severe inflammation. A degenerative response is reflected by a leukopenia, heteropenia, immature heterophils, and toxic heterophils. Degenerative responses and depletion are differentiated by the presence of toxic heterophils or by following the decreasing leukocyte count with serial leukograms. Bone marrow evaluation also may be helpful. In general, a degenerative response in the leukogram of a bird indicates a poor prognosis for survival. As discussed, leukopenia and lymphopenia can occur as an early, corticosteroid-induced leukogram response in some species of birds. Leukopenias and lymphopenia also may suggest a viral cause, although such causes have been poorly documented in birds.[100] Leukopenia and lymphopenia has been associated with mycotoxins poisoning and other toxicities in birds.[40,64,101,102]

Lymphocytosis may occur with antigenic stimulation. An occasional reactive lymphocyte may be found in blood films from normal birds; however, many reactive lymphocytes suggest antigenic stimulation associated with infectious disease (Fig. 19.41). Lymphocytosis also can occur with lymphocytic leukemia (e.g., avian leukosis).[102,103] In some cases of lymphocytic leukemia, immature lymphocytes may be present in the blood film. A marked lymphocytosis in which most lymphocytes appear as small, mature lymphocytes with scalloped cytoplasmic margins also has been associated with lymphoid neoplasia.[104]

Lymphopenia can occur with glucocorticosteroid excess, which may be more pronounced in some avian species than others. Immunosuppressive drugs also may cause lymphopenia. Lymphopenia may also be associated with toxicities, such as zinc intoxication.[105]

Monocytosis often is associated with infectious diseases caused by organisms that typically cause granulomatous inflammation, such as *Mycobacterium* and *Chlamydophila*, and fungi, such as *Aspergillus*. Chronic bacterial granulomas and massive tissue necrosis also may result in monocytosis. In most situations, a monocytosis is most commonly associated with a concurrent heterophilia. A monocytosis has been seen in certain nutritional deficiencies, such as zinc deficiency, as well.[105,106]

Because the exact functions of avian eosinophils are not known, interpreting the cause of peripheral eosinophilia is difficult in birds. Although this avian granulocyte was given the name "eosinophil," avian eosinophils may behave differently than mammalian eosinophils. Studies have shown that avian eosinophils may participate in delayed (type IV) hypersensitivity reactions, a participation that does not occur with mammalian eosinophils.[107] Experiments using parasite antigens have failed to induce peripheral eosinophilias, although eosinophilias associated with gastrointestinal nematode infestations have been reported.[108] The responses of avian eosinophils to inflammation are variable and have not been reliably associated with a specific etiology. Despite limited knowledge regarding the function of avian eosinophils, peripheral eosinophilia in birds can be loosely interpreted as being a response to internal or external parasitism or exposure to foreign antigens (i.e., hypersensitivity response).

Eosinopenia may be difficult to document in birds. If present, it is expected to be associated with a stress response or with administration of glucocorticosteroids.

Basophilia is rare in birds. Because avian basophils produce, store, and release histamine, they may have a function similar to that of mammalian basophils. Therefore, avian basophils may participate in immediate hypersensitivity reactions, release mediators for thrombocyte activation, cause smooth muscle contractions, initiate edema, and affect coagulation.[16] Basophils appear to participate in the initial phase of acute inflammation in birds; however, this usually is not reflected as a basophilia on the leukogram.[109] Peripheral basophilia may suggest early inflammation or an immediate hypersensitivity reaction in birds. A stress-related basophilia occurs in chickens subjected to food restriction, but this response may be age or duration dependent.[57,110]

Thrombocytes and hemostasis

Morphology

Thrombocytes are nucleated cells that are found in the peripheral blood of birds. They tend to be round to oval cells with a round to oval nucleus that contains densely clumped chromatin. They are generally the smallest cell in the peripheral blood, but only slightly smaller than small mature lymphocytes. For example, thrombocyte measurements for adult Coturnix quail are $4.10 \pm 0.30\,\mu m$ in diameter for males and $4.06 \pm 0.32\,\mu m$ in diameter for females.[13] The nucleus is more rounded than an erythrocyte nucleus, and cells have a high N:C ratio. Normal, mature thrombocytes have a colorless to pale-gray cytoplasm, which often has a reticulated appearance. The appearance of the cytoplasm is an important feature in differentiating thrombocytes from small, mature lymphocytes (Figs. 19.36 through 19.39 and 19.41). Cytoplasmic vacuolation can occur in activated or phagocytic thrombocytes. Thrombocytes frequently contain one or more distinct eosinophilic (specific) granules, which usually are located in one area of the cytoplasm. Thrombocytes participate in the hemostatic process and, like mammalian platelets, tend to clump in blood films. Activated thrombocytes occurring in aggregates may have indistinct cellular outlines or cytoplasmic pseudopodia.

Ultrastructurally, the cytoplasm resembles that of mammalian platelets. The granules that frequently are seen in thrombocytes at light microscopy appear as aggregates of many small granules at electron microscopy.[31] The specific granules contain primarily 5′-hydroxytryptamine, and they are an unlikely source of thromboplastin.[48] Thrombocytes aggregated in clumps show degranulation of specific granules, cellular degeneration, and nuclear pyknosis. Avian thrombocytes contain a large amount of serotonin, and some studies suggest that they are capable of phagocytosis and may participate in removing foreign materials from the blood.[72,111]

Laboratory evaluation

The thrombocyte concentration of most avian species studied ranges between 20,000 and 30,000 cells/mm^3, or 10–15 thrombocytes per 1000 erythrocytes. The actual thrombocyte concentration is difficult to determine, because thrombocytes tend to clump. Therefore, their concentration often is reported as either normal, increased, or decreased, based on estimates made from peripheral blood films. Approximately one to five thrombocytes can be seen in a monolayer ×1000 (oil-immersion) field in a blood film from a normal bird, unless the thrombocytes clump excessively during preparation. Thrombocytopenia is suggested by thrombocyte numbers less than one per monolayer ×1000 field, and thrombocytosis is suggested by numbers greater than five in an average monolayer ×1000 field. A thrombocyte concentration can be obtained with the same hemacytometer used for obtaining total leukocyte and erythrocyte counts with the Natt and Herrick method. The number of thrombocytes counted in the central large square on both sides of the Neubauer-ruled hemocytometer is multiplied by 1000 to obtain the number of thrombocytes per microliter of blood. An estimated thrombocyte count can be obtained from the blood film using the same formula as that for estimation of the total leukocyte count by counting the average number of thrombocytes in 5 oil immersion [×1000] fields:

Again, if the PCV is outside the normal range of 35–55%, then the estimated thrombocyte count should be adjusted for the PCV as follows:

Responses in disease

Avian thrombocytes are derived from mononuclear precursors in the bone marrow. Immature thrombocytes occasionally are present in the peripheral blood of birds. They are round to oval cells with round to oval nuclei and basophilic cytoplasm compared with mature thrombocytes (Fig. 19.46). The mid and late immature thrombocytes most commonly are seen when immature cells are present (see the discussion of avian hematopoiesis). The presence of immature thrombocytes usually indicates a regenerative response to excessive utilization of thrombocytes. Young birds tend to have relatively higher numbers of circulating thrombocytes than adult birds.

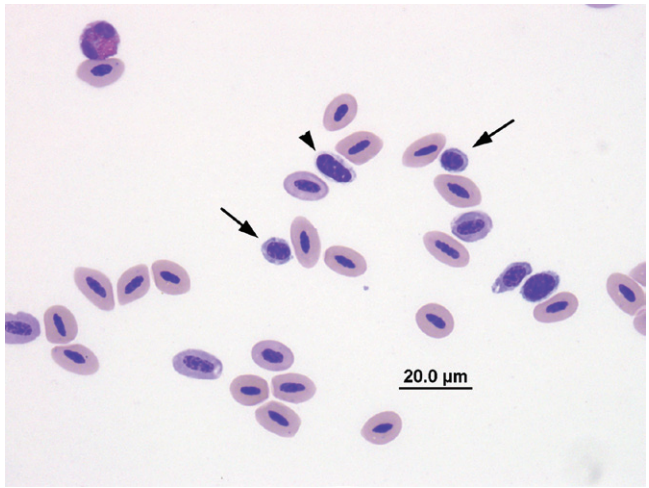

Figure 19.46 Immature thrombocytes (arrows) and a mature thrombocyte (arrowhead) in the blood film of a parrot (*Eclectus roratus*). Wright-Giemsa stain.

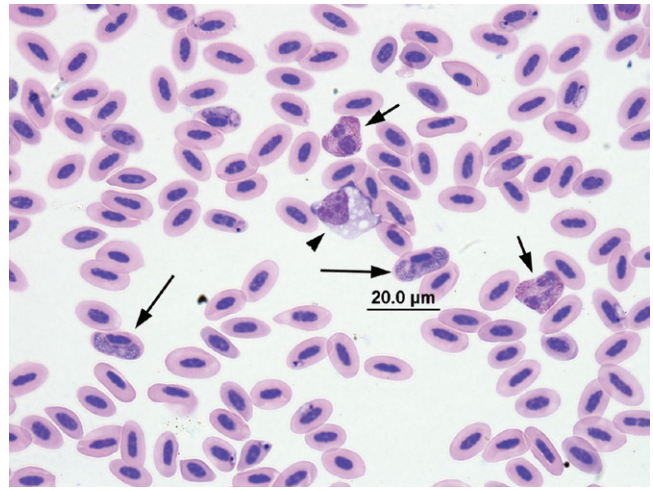

Figure 19.47 Haemoproteus gametocytes (long arrows), heterophils (short arrows), and a monocyte (arrowhead) in the blood film of an owl (*Bubo virginianus*). Wright-Giemsa stain.

Thrombocytopenia results from either decreased bone marrow production or excessive peripheral utilization or destruction. Decreased thrombocyte concentrations often are associated with severe septicemia and, possibly, diffuse intravascular coagulation.

The initial hemostatic plug of birds is formed through the adhesion and aggregation of thrombocytes, and the secondary hemostatic plug develops through the coagulation cascade after injury to a blood vessel wall. Most clotting factors involved in avian blood coagulation are similar to those in mammals. Although evidence suggests an intrinsic clotting mechanism in some avian species, coagulation of avian blood appears to depend on the extrinsic clotting system, which involves the release of tissue thromboplastin (i.e., factor III).[112] The extrinsic and common pathways can be evaluated using a one-step prothrombin time test. Avian brain thromboplastin is required for avian prothrombin time testing, because commercially available rabbit-brain thromboplastin and other mammalian sources give unreliable results in birds.[12,113] The normal prothrombin time for most birds is 13 seconds or less. Studies suggest that the source of thromboplastin should be from the brain of the same species of bird as the patient for accurate prothrombin time determinations.[112]

Whole blood (capillary) clotting times in birds usually are less than 5 minutes; however, normal values appear to range between 2 and 10 minutes. The whole-blood clotting time is more variable than the prothrombin time.

Blood parasites

The three genera of hemosporidian parasites in the phylum Apicomplexa in the family Plasmodiidae: *Haemoproteus* and *Plasmodium* and the family Leucocytozoidae: *Leukocytozoon* are the most commonly encountered parasites in avian blood films.[2,114–119] Their identification usually can be made using the stains commonly used for evaluating blood cells. Films made from fresh blood, without addition of an anticoagulant, provide samples with fewer artifacts affecting the parasite.

Haemoproteus

Protozoan blood parasites of the genus *Haemoproteus* are common in many species of wild birds. The only forms of the parasite in the peripheral blood of birds are gametocytes, which range in size from small, developing, ring forms to the elongate, crescent-shaped, mature gametocyte that partially encircles the erythrocyte nucleus to form the characteristic "halter shape" (Fig. 19.47). The mature gametocyte typically occupies greater than half the cytoplasmic volume of the host erythrocyte, and it causes minimal displacement of the host cell nucleus: the nucleus is never pushed to the cell margin. *Haemoproteus* gametocytes contain refractile, yellow to brown pigment granules that represent iron pigment deposited as a result of hemoglobin utilization. Macrogametocytes stain dark blue with Romanowsky stains and have iron pigment dispersed throughout the cytoplasm of the parasite, whereas microgametocytes stain pale blue to pink and have iron pigment aggregated into a spherical mass. Occasionally, extraerythrocytic macrogametes and microgametes can be found in blood films, especially those made from blood collected several hours before the film was actually prepared (Fig. 19.48). Extraerythrocytic macrogametes are round and resemble those within erythrocytes. Microgametes are small, spindle-shaped structures scattered throughout the blood film (Fig. 19.49). Usually, these forms are found in the midgut of the insect host after a blood meal.

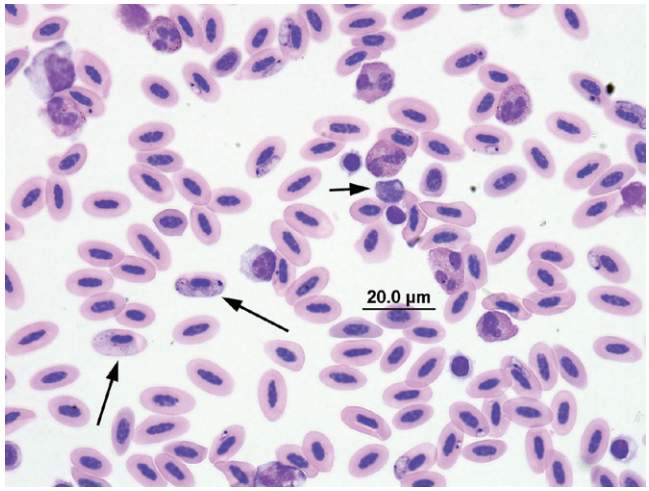

Figure 19.48 *Haemoproteus* gametocytes (long arrows) and an extracellular macrogametocyte (short arrow) in the blood film of an owl (*Bubo virginianus*). Wright-Giemsa stain.

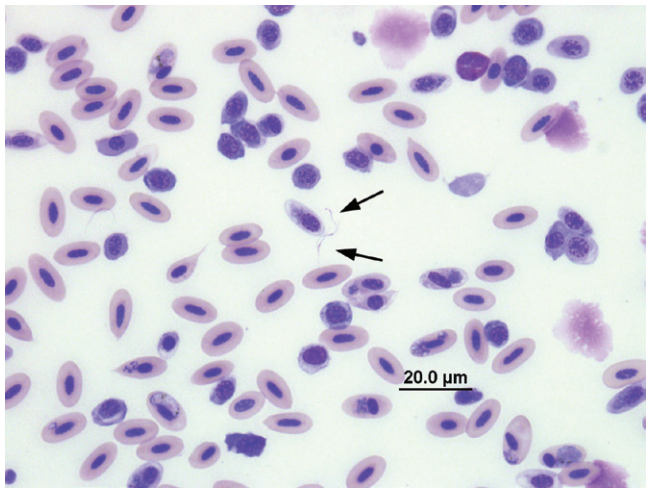

Figure 19.49 *Haemoproteus* gametocytes and an extracellular microgametocyte (arrows) in the blood film of a kestrel (*Falco sparverius*). Wright-Giemsa stain.

Bloodsucking insect vectors, such as hippoboscid flies (Hippoboscidae) and midges of the genus *Culicoides* (Ceratopogonidae), transmit *Haemoproteus*.[120] The insect host ingests gametocytes when it feeds, and the parasites then undergo a series of developmental stages to become sporozoites within the salivary gland. Sporozoites are injected into the new avian host when the insect feeds. The sporozoites enter the bird's vascular endothelial cells in various tissues (primarily the lung, liver, bone marrow, and spleen) and then undergo schizogony.[121] *Haemoproteus* schizonts occasionally are found in cytologic or histologic samples of infected tissue, and they appear as large, round cysts containing numerous multinucleated bodies or cytomeres. Each cytomere produces numerous merozoites that escape into the bloodstream when the endothelial cell and cytomeres rupture. Merozoites enter erythrocytes to become gametocytes, which then are ingested by insect hosts to complete the cycle.

The pathogenicity of *Haemoproteus* generally is low, and parasitized birds rarely show evidence of disease. Clinical disease, however, can occur in certain avian species, such as pigeons, jays, and quail, nestlings, and in birds suffering from other diseases that, perhaps, result in immunodeficiencies.[2,122–124] Mortalities associated with this parasite may also be the result of infections in aberrant hosts. The clinical signs include hemolytic anemia, anorexia, and depression. Hepatomegaly and splenomegaly may be observed at postmortem evaluation. Death may be associated with severe anemia and hepatic necrosis resulting in hemorrhage related to megaloschizont-associated lesions (preerythrocytic stage) rather than the intraerythrocytic gametocytes.

The degree of parasitemia associated with *Haemoproteus* can be used as an index to assess the recovery of birds, especially raptors, from traumatic injuries or diseases. For example, an injured raptor may present with marked *Haemoproteus* parasitemia; with greater than 15% of the erythrocytes being affected. As the bird recovers from its injuries, however, the parasitemia decreases dramatically. Presumably, this represents an improved immune status of the bird. The intensity of the hematozoan infection can be calculated from manual quantification of 2000 erythrocytes; however, this method is significantly lower than the intensity calculated from digital quantification of 50,000 erythrocytes.[21] The later allows for a precise method to quantify infections of low to moderate intensity.

Plasmodium

Parasites of the genus *Plasmodium* can be pathogenic and responsible for malaria, which affects certain species of birds (e.g., canaries, penguins, ducks, pigeons, raptors, and domestic poultry). Many avian species appear to be asymptomatic carriers of the parasite, however, and do not develop the clinical disease. Outbreaks of avian malaria occur sporadically in endemic areas, especially during seasons associated with increased mosquito populations. Clinical signs associated with avian malaria include anemia, anorexia, depression, and acute death. The hemogram often reveals hemolytic anemia, leukocytosis, and lymphocytosis. Hemoglobinuria or biliverdinuria also may occur. Splenomegaly and hepatomegaly often are seen on postmortem examination.

Detection of *Plasmodium* is based on presence of the organism in blood films. Unlike *Haemoproteus*, stages other than the gametocyte, such as schizonts and trophozoites, can be found within erythrocytes, thrombocytes, and leukocytes (Figs. 19.50 and 19.51). Certain *Plasmodium* sp. have round to irregular gametocytes that cause marked displacement of

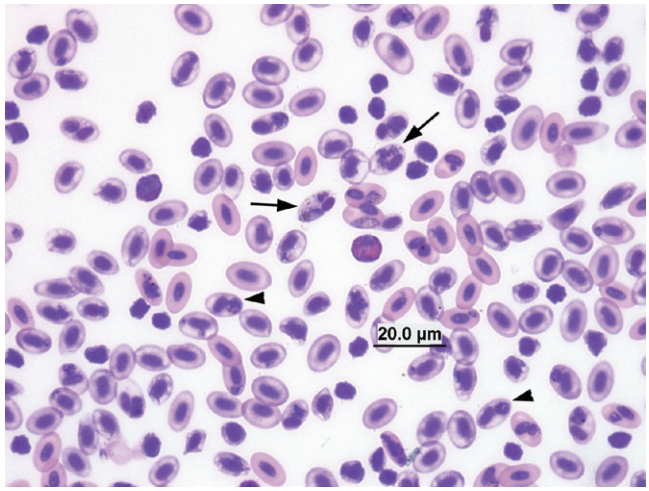

Figure 19.50 *Plasmodium* gametocytes (arrows) and schizogony (arrowhead) in the blood film of a skua (*Stercorarius skua*). Wright-Giemsa stain.

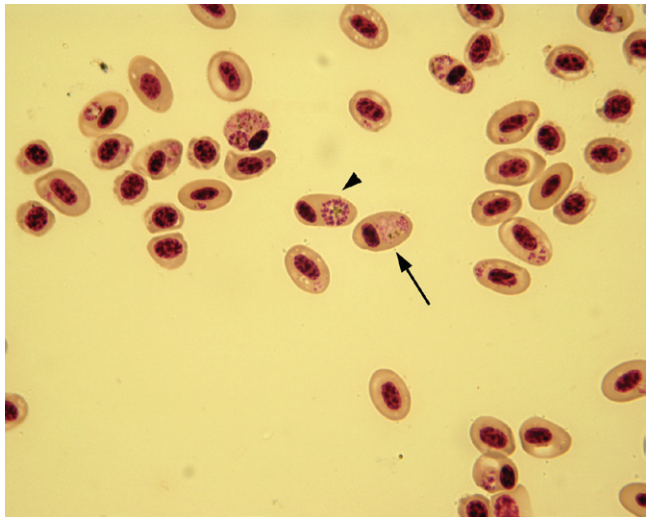

Figure 19.51 *Plasmodium* gametocytes (arrow) and schizogony (arrowhead) in the blood film of a skua (*Stercorarius skua*). Wright-Giemsa stain.

the host-cell nucleus, whereas other species have elongate gametocytes that do not displace the host-cell nucleus.[121] Like those of *Haemoproteus*, *Plasmodium* gametocytes contain refractile, yellow to brown, iron pigment granules, which tend to be scattered, and macrogametocytes stain deeper blue than microgametocytes. *Plasmodium* trophozoites are small, round to oval, ameboid forms containing a large vacuole that pushes the parasite nucleus to one edge, thereby giving the trophozoite a "signet-ring" appearance. Schizonts are round to oval inclusions that contain several deeply staining merozoites; the number of merozoites is used to determine the *Plasmodium* species. Schizonts with developing merozoites exhibit clusters of merozoites that

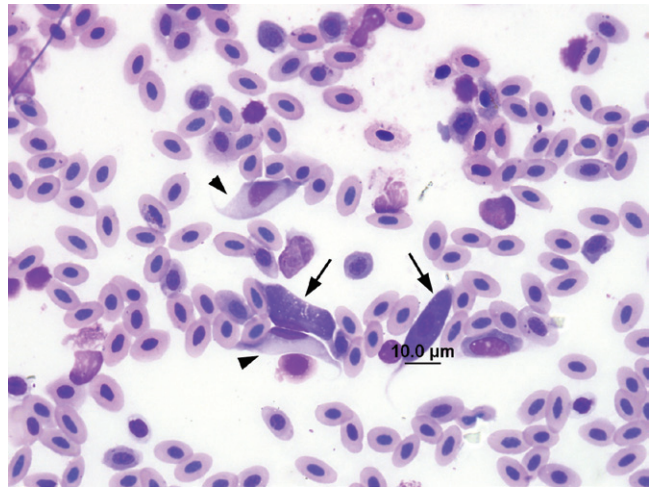

Figure 19.52 *Leukocytozoon* macrogametocytes (arrows) and microgametocytes (arrowheads) in the blood film of a hawk (*Buteo jamaicensis*). Wright-Giemsa stain.

appear to be fused, which is in contrast to mature merozoites, which appear to be distinct bodies and separate from each other. Identification of the *Plasmodium* species depends on the location and appearance of the schizonts and gametocytes.

The life-cycle of *Plasmodium* is similar to that of *Haemoproteus*, except that mosquitoes (Culicidae) act as intermediate hosts and schizogony occurs in the red blood and endothelial cells of various organs.[67] The key features used to differentiate *Plasmodium* from *Haemoproteus* are the presence of schizogony in the peripheral blood, parasite stages within thrombocytes and leukocytes, and gametocytes causing marked displacement of the erythrocyte nucleus.[2]

Leukocytozoon

Leukocytozoon, which is a protozoan parasite commonly found in the blood of wild birds, is identified by large, dark-staining macrogametocytes or light-staining microgametocytes. The large gametocytes grossly distort the infected host cell, thereby elongating and distending the cell and making the identification of the cell difficult (Fig. 19.52).[125] Some parasitologists believe that immature erythrocytes rather than leukocytes, as suggested by the name of the parasite, serve as the host cell for *Leukocytozoon*.[121] As with *Haemoproteus*, only the gametocytes of *Leukocytozoon* occur in the peripheral blood. Parasitized cells appear to have two nuclei: a dark-staining, host-cell nucleus that lies along the cell membrane; and a pale pink–staining, parasite nucleus that lies adjacent to the host-cell nucleus. *Leukocytozoon* gametocytes do not contain the refractile pigment granules seen in the gametocytes of *Haemoproteus* and *Plasmodium*.

Leukocytozoon is transmitted by black flies (Simuliidae), which act as intermediate hosts and inject sporozoites into

the blood of susceptible avian species. The sporozoites invade the endothelial and parenchymal cells of various tissues such as the liver, heart, and kidney, in which schizogony occurs. Schizonts mature and then rupture to release merozoites that infect erythrocytes and, possibly, leukocytes. Merozoites become gametocytes in the peripheral blood or are ingested by macrophages to become megaloschizonts in tissues such as the liver, lung, and kidney. Megaloschizonts also release merozoites that develop into gametocytes.

The pathogenicity of *Leukocytozoon* usually is low; however, certain species can be highly pathogenic for some birds, such as young waterfowl and turkeys.[126,127] The clinical signs associated with this parasite include anemia, anorexia, and depression. Clinical laboratory evaluation may reveal a hemolytic anemia, leukocytosis, and elevated serum enzymes such as aspartate aminotransferase or alanine aminotransferase, thereby suggesting hepatocellular necrosis. Postmortem findings may include splenomegaly and hepatomegaly with hepatic necrosis.

Microfilaria

Microfilaria of filarial nematodes commonly are found in the peripheral blood of many species of birds (Fig. 19.53). The adult filarial nematodes usually are not seen unless they occur in peripheral locations, such as in the fluid of distended joints. Adult filarial nematodes may occur anywhere within the body of birds, but they most frequently are seen in the air sacs, subcutaneously, or in the body cavities. Most of these parasites are considered to be nonpathogenic and cause little harm to their host.

Less common avian blood parasites

Other parasites that are seen less frequently in the peripheral blood of birds include *Atoxoplasma*, *Aegyptianella*, *Trypanosoma*, and *Borrelia*. *Atoxoplasma* is a coccidian parasite that often is found in passerine birds, which can be highly pathogenic, especially to canaries.[128] It is transmitted directly via oocysts in the feces. Atoxoplasmosis is diagnosed on the basis of demonstrating characteristic sporozoites within the lymphocytes on peripheral blood films or cytologic imprints of the liver, spleen, or lung. The sporozoites appear as pale, eosinophilic, round to oval, intracytoplasmic inclusions within lymphocytes, monocytes, or macrophages in Romanowsky-stained preparations (Fig. 19.54). The sporozoites indent the host lymphocyte nucleus, thereby resulting in a characteristic crescent shape. Sporozoites of *Atoxoplasma* lack pigment granules, but detection of *Atoxoplasma* in the peripheral blood can be improved by using a preparation of a buffy-coat film to concentrate the leukocytes for examination.

Aegyptianella is a minute parasite of avian erythrocytes that lacks pigment granules. It is a piroplasma that can affect several avian species, usually those originating in tropical or subtropical climates. *Aegyptianella pullorum* occurs in chickens, geese, ducks, and turkeys. The organism is detected by demonstrating the developing forms within erythrocytes in blood films (Fig. 19.55). Three forms can occur. One form, the initial body, is a small, anaplasma-like structure that is less than 1 mm in diameter and appears as a round, basophilic, intracytoplasmic inclusion. A second form is a round- to piriform-shaped inclusion with pale-blue cytoplasm and a chromatin body at one pole resembling those of *Babesia*. The third form is a larger (2–4 mm), round to elliptical inclusion. *Aegyptianella* can be pathogenic, resulting in anemia, anorexia, and diarrhea. Postmortem findings include splenomegaly, hepatomegaly, and hepatic as well as renal degeneration.

Trypanosomes (*Trypanosoma*) occasionally are found in the peripheral blood of wild birds, especially passerines,

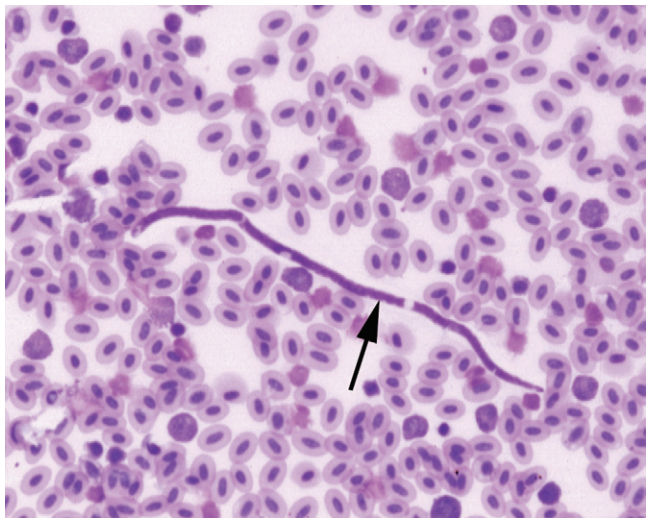

Figure 19.53 A microfilaria in the blood film of a cockatoo (*Cacatua moluccensis*). Wright-Giemsa stain.

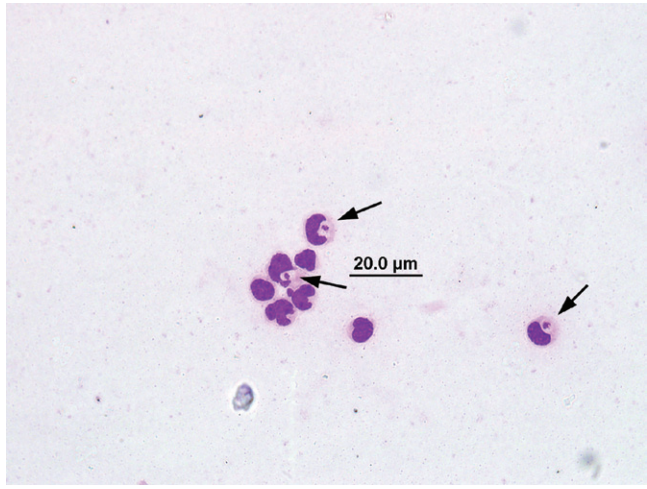

Figure 19.54 *Atoxoplasma* inclusions within lymphocytes from a buffy coat film of a thrush (*Garrulax chinensis*). Wright-Giemsa stain.

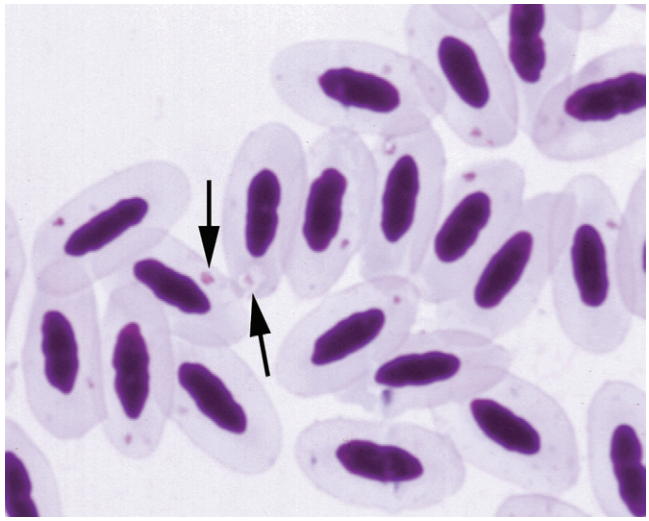

Figure 19.55 *Aegyptianella* inclusions within erythrocytes of a parrot (*Amazona finschi*). Wright-Giemsa stain.

galliformes, waterfowl, and pigeons. They are transmitted by biting insects such as mosquitoes, hippoboscid flies, and blackflies or mites. Avian trypanosomes resemble those found in mammals. They have an undulating membrane; a slender, tapering posterior end; and a short, anteriorly directed flagellum. Trypanosomes usually are considered to be an incidental finding.

Borrelia anserina is the causative agent of avian spirochetosis, which can affect several species of birds, especially galliformes and waterfowl. It is transmitted by arthropod vectors such as ticks and mites. *Borrelia* is a loosely spiraled spirochete that tapers into fine filaments and is found free in the plasma. During the acute stages of the disease, the organism is spiral shaped; however, as the disease progresses and the bird nears death, the organism may appear abnormal or clumped and often is difficult to find. In acute avian spirochetosis, affected birds are depressed, anemic, and weak. Postmortem findings include splenomegaly and hepatomegaly. Birds recovering from the disease exhibit a regenerative anemia.

Hematopoiesis

Bone marrow

The bone marrow is the primary site for erythropoiesis, granulopoiesis, and thrombopoiesis during late embryonic development and post-hatched birds.[129] In some adult birds, such as chickens, the hematopoietic activity of the bone marrow primarily is associated with erythropoiesis and, possibly, thrombopoiesis, with only a small reserve of granulopoiesis compared to that of mammalian bone marrow. Therefore, compared with mammals, granulopoiesis in mature birds is more diffuse and is found in a variety of tissues.[130] During embryonic development, granulocyte stem cells colonize to create foci of granulopoiesis in the spleen, kidney, lungs, thymus, gonad, pancreas, and other tissues, including the bone marrow.[14,131,132] The bone marrow also provides an environment for lymphocyte maturation. Because it is the most readily available source of hematopoietic tissue in birds, the bone marrow is used to evaluate disorders of blood cells. Cytologic evaluation of the bone marrow is indicated in avian patients with nonregenerative anemia, heteropenia, and other unexplained alterations involving the cellular elements in circulating blood.

Bone marrow collection

Marrow samples for cytologic evaluation can be successfully obtained in most avian species via bone marrow aspiration. The best source of bone marrow for most birds is the proximal tibiotarsus, because the procedure at this location is relatively simple.[2] Marrow may also be collected from the sternum (keel), however, and from most of the long bones, except the pneumatic bones. A general anesthetic usually is not required, but a local anesthetic can be used with caution in large birds. The type of biopsy needle used for aspiration depends on the size of the bird, location of the biopsy site, and preference of the cytologist. Biopsy needles commonly used for bone marrow collection in both domestic mammals and humans (Jamshidi bone marrow biopsy–aspiration needles and disposable Jamshidi Illinois-Sternal/Iliac aspiration needles, Kormed Corp., Minneapolis, MN) can be used for marrow collection in birds. The pediatric sizes are preferred, however, because of the relatively small bone size in most birds compared with mammals. Spinal needles containing a stylet can be used for marrow collection in very small birds.

The procedure for collecting bone marrow from the proximal tibiotarsus begins with application of a skin disinfectant, as for any surgical procedure. The medial or cranial aspect of the proximal tibiotarsus just below the femoral-tibiotarsal joint is a suitable location for aspiration, because only a minimal amount of soft tissue overlies the bone in this area. After application of a local anesthetic, a small incision is made using a scalpel blade to facilitate passage of the needle through the skin. The needle with stylet is placed against the bone (Figs. 19.56 and 19.57), and using gentle pressure and rotary movements, the needle then is advanced into the marrow cavity. A perpendicular approach to the bone should be used. The hand not being used to manipulate the needle is used to stabilize the tibiotarsus. Once the needle is positioned into the marrow cavity, the stylet is removed, and a 6- to 12-mL syringe is attached (Figs. 19.58 and 19.59). Marrow is aspirated into the lumen of the needle by applying negative pressure to the syringe using the syringe plunger (Figs. 19.60 and 19.61). Excessive or prolonged negative pressure should be avoided to minimize

Figure 19.56 Placement of a Jamshidi Illinois-Sternal/Iliac aspiration needle (Kormed Corp, Minneapolis, MN) in the proximal tibiotarsus of a flamingo chick (*Phoenicopterus ruber*).

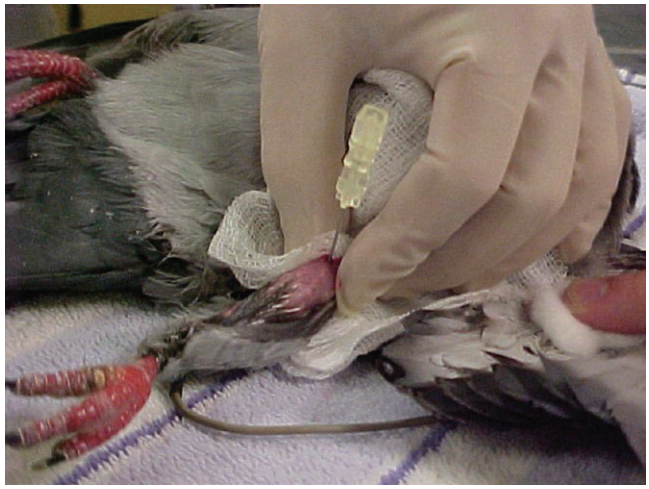

Figure 19.57 Placement of a spinal needle in the proximal tibiotarsus of a pigeon (*Columba livia*).

Figure 19.58 Removal of the stylet from a Jamshidi Illinois-Sternal/Iliac aspiration needle (Kormed Corp, Minneapolis, MN) placed in the proximal tibiotarsus of a flamingo chick (*Phoenicopterus ruber*).

Figure 19.59 Removal of the stylet from a spinal needle placed in the proximal tibiotarsus of a pigeon (*Columba livia*).

Figure 19.60 Aspiration of a bone marrow sample from a Jamshidi Illinois-Sternal/Iliac aspiration needle (Kormed Corp, Minneapolis, MN) placed in the proximal tibiotarsus of a flamingo chick (*Phoenicopterus ruber*).

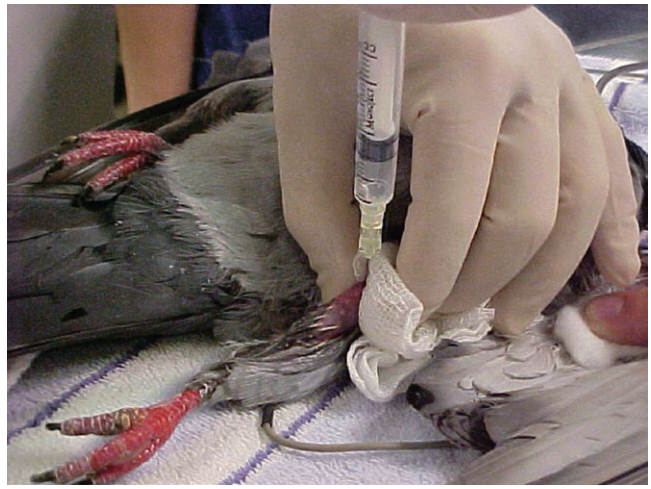

Figure 19.61 Aspiration of a bone marrow sample from a spinal needle placed in the proximal tibiotarsus of a pigeon (*Columba livia*).

blood contamination of the marrow sample. Unlike collection of bone marrow from most mammals, avian marrow should not appear in the syringe (except in very large birds) because of the small marrow volume in most birds. Therefore, the marrow sample is found in the lumen of the biopsy needle.

When aspiration is completed, the needle and syringe are removed from the tibiotarsus while making sure that negative pressure is not being applied to the syringe. The needle is removed from the syringe, and the syringe is filled with air to force the marrow from the lumen onto a glass microscope slide. A second glass microscope slide is placed atop of the marrow sample, and the marrow is allowed to spread between the two slides as they are pulled apart. Bone marrow samples also can be obtained from the keel (sternum) of some birds, such as galliformes; the biopsy needle is introduced into the widest part of the sternal ridge in the manner as described for the proximal tibiotarsus.

Marrow core biopsies for histologic evaluation can be obtained from birds using a technique similar to that of marrow aspiration. Once the biopsy needle is introduced into the bone marrow space, the stylet is removed, and the needle is advanced deeper into the marrow cavity, toward the opposite cortex. Once the opposite cortex has been reached, the needle is twisted and redirected slightly to detach the marrow plug within the lumen of the needle. Gentle vacuum may be applied to the syringe to aid in holding the marrow plug in the needle as the needle is withdrawn from the marrow cavity. The marrow core sample is removed by reinsertion of the stylet (usually beginning at the tip of the needle) to push the sample out of the needle. Imprint films can be made from the core sample for cytologic evaluation before the sample is placed in 10% neutral-buffered formalin. A sample holder often is required to maintain the marrow core while it is being fixed in the formalin solution.

Examination of avian bone marrow

Bone marrow slides are stained with the same Romanowsky stains used for blood films. Interpretation of avian bone marrow begins with scanning of the marrow film using the 10× microscope objective to evaluate both the number and the distribution of cells. Because an actual cell count of a bone marrow sample cannot be obtained, the cellularity is estimated by evaluating the ratio of fat and cells in marrow particles and is compared with the cellularity of normal bone marrow. The degree of cellularity is estimated as poor, normal, or high.

The distribution of cells can be estimated as well. Myeloid, erythroid, and thrombocytic elements may appear to be normal, hypoplastic (decreased), or hyperplastic (increased). A more objective approach is to perform an actual differential count based on 1000 cells or more, but this is more time-consuming and may not provide more information.

In addition to estimating the degree of cellularity and evaluating the distribution of cell types in the marrow sample, the cytologist also should estimate the myeloid: erythroid (M:E) ratio. Any changes involving the maturation sequence of each cell line should be noted as well. The cell lines include erythrocytes, granulocytes (heterophils, eosinophils, and basophils), monocytes, and thrombocytes. Other cells that occasionally are found include lymphocytes, plasma cells, osteoblasts, and osteoclasts. The presence of abnormal cells also should be noted.

The normal M:E ratio varies with species; however, the ratio in most species is approximately 1.0.[133–135] For example, the M:E ratio is 1.23 ± 0.17 in blackheaded gulls (*Larus ridibundus*) where the mean percentage of erythroid cells is 39.91 ± 3.26%; of myeloid cells is 49.37 ± 4.86%; of thrombocyte precursors is 5.95 ± 0.79%; and all other cells is 4.77 ± 0.53%.[136]

An accurate interpretation of the bone marrow response can be made only in conjunction with knowledge regarding the current peripheral blood cellular response. Therefore, a hemogram made from a blood sample collected at the same time as the bone marrow sample should be evaluated.

Erythropoiesis

Avian erythropoiesis occurs within the lumen of the vascular sinusoids in the bone marrow.[48] These sinuses are lined by elongated endothelial cells that are associated with the most immature cells of the erythroid series. The more mature cells are located in the lumen of the sinuses. The vascular sinuses communicate with a central vein.

Avian erythropoietin, which is a glycoprotein that differs structurally from mammalian erythropoietin, is necessary for the multiplication and differentiation of precursor stem cells committed to the erythroid series.[137] Erythropoietin can be obtained from the blood of anemic birds, and the site of its production is considered to be the kidney.

The stages of maturation in normal avian erythropoiesis appear to be similar to those of mammals. The terminology used for the different stages of erythrocyte maturation, however, varies in the literature. In general, seven stages are recognizable in red blood cell development based on findings with Romanowsky stains.[2] These include rubriblasts (proerythroblasts), prorubricytes (basophilic erythroblasts), basophilic rubricytes (early polychromatic erythroblasts), early polychromatic rubricytes (late polychromatic erythroblasts), late polychromatic rubricytes (orthochromic erythroblasts), polychromatic erythrocytes, and mature erythrocytes. As erythroid cells mature, the nuclear size decreases, the chromatin becomes increasingly condensed, the nuclear shape changes from round to ellipsoid, the amount of cytoplasm increases, the hemoglobin concentration increases (resulting in increasing eosinophilia), and the cell shape changes from round to ellipsoid. Unlike mam-

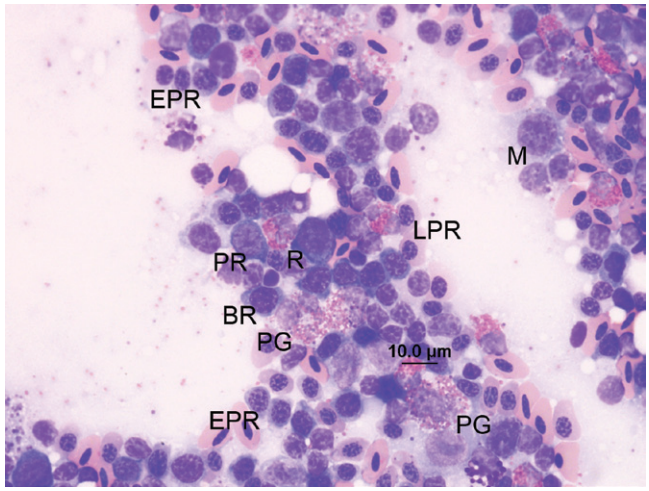

Figure 19.62 A rubriblast (R), prorubricyte (PR), basophilic rubricyte (BR), early polychromatic rubricytes (EPR), late polychromatic rubricyte (LPR), myeloblast (M), and progranulocyte (PR) in the bone marrow aspirate of a conure (*Aratinga solstitialis*). Wright-Giemsa stain.

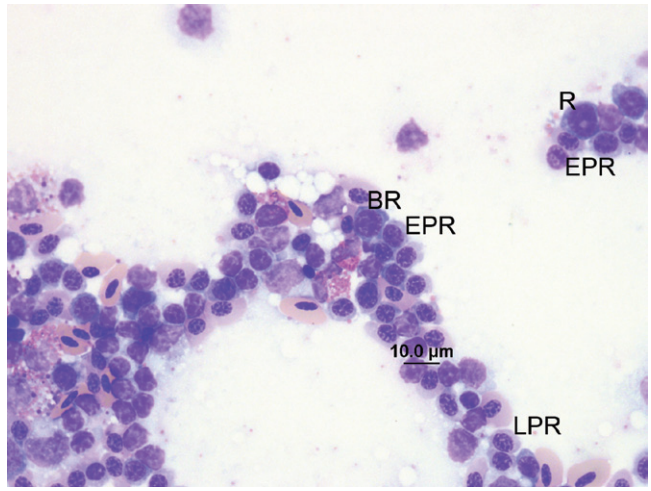

Figure 19.63 A rubriblast (R), basophilic rubricyte (BR), early polychromatic rubricyte (EPR), and late polychromatic rubricyte (LPR) in the bone marrow aspirate of a conure (*Aratinga solstitialis*). Wright-Giemsa stain.

malian erythrocytes, avian erythrocytes normally retain their nucleus.

Rubriblasts

Rubriblasts are large, round, deeply basophilic cells with a large, round, central nucleus that results in a high N:C ratio (Figs. 19.62 through 19.64 and 19.66). The nuclear chromatin typically is coarsely granular, and large, prominent nucleoli or nucleolar rings are present. The cytoplasm is deeply basophilic, with clear spaces most likely representing mitochondria.

Prorubricyte

The prorubricyte resembles the rubriblast, but it lacks the prominent nucleoli (Figs. 19.62 and 19.65). The N:C ratio is high, and the large nucleus usually is surrounded by a narrow rim of blue cytoplasm. The cytoplasm is predominantly basophilic but may contain spots of reddish material, suggesting the beginning of hemoglobin development. The cytoplasm lacks the mitochondrial spaces of the rubriblast.

Rubricyte

Rubricytes are round cells that are smaller than rubriblasts and prorubricytes. They can be divided into three stages, based primarily on the appearance of the cytoplasm. The basophilic rubricyte is the earliest rubricyte stage and is characterized by a homogenous, basophilic cytoplasm and a round nucleus with clumped chromatin (Figs. 19.62 through 19.65). The next stage, the early polychromatophilic rubricyte, is smaller than the basophilic rubricyte and has a gray (basophilic to slightly eosinophilic) cytoplasm because of increased hemoglobin production (Figs. 19.62 through 19.65). The nucleus of early polychromatophilic rubricytes contains clumped chromatin and is small in relation to the amount of cytoplasm. The final rubricyte stage, the late polychromatophilic rubricyte, is ellipsoid and has more eosinophilic (eosinophilic gray to weakly eosinophilic) cytoplasm than earlier stages (Figs. 19.62 through 19.65). The nucleus of late polychromatophilic rubricytes varies from round to slightly ellipsoid, with irregularly clumped chromatin.

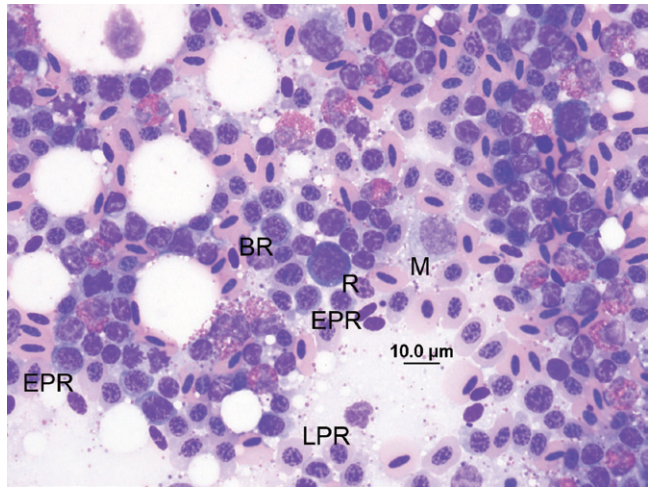

Figure 19.64 A rubriblast (R), basophilic rubricyte (BR), early polychromatic rubricytes (EPR), late polychromatic rubricyte (LPR), and myeloblast (M) in the bone marrow aspirate of a conure (*Aratinga solstitialis*). Wright-Giemsa stain.

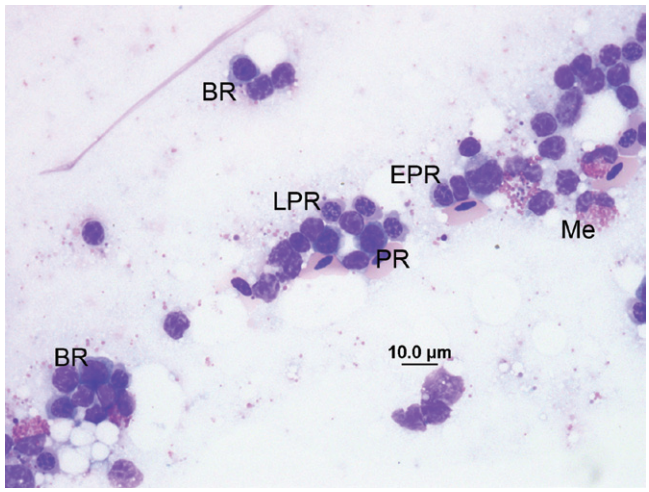

Figure 19.65 A prorubricyte (PR), basophilic rubricytes (BR), early polychromatic rubricyte (EPR), late polychromatic rubricyte (LPR), and metamyelocyte (Me) in the bone marrow aspirate of a conure (*Aratinga solstitialis*). Wright-Giemsa stain.

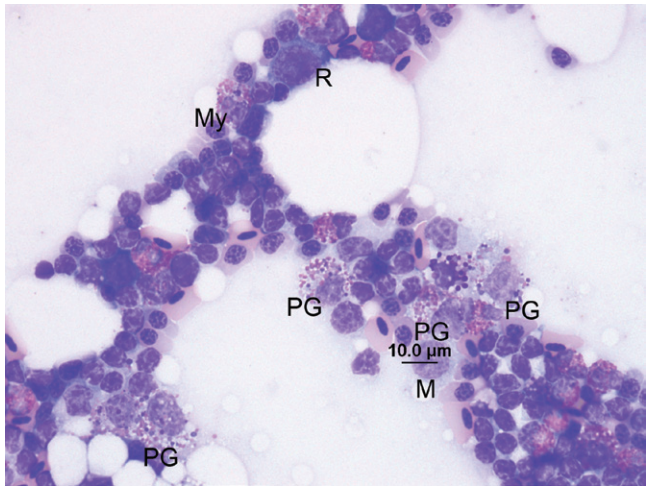

Figure 19.66 A rubriblast (R), myeloblast (M), progranulocytes (PR), and myelocytes (My) in the bone marrow aspirate of a conure (*Aratinga solstitialis*). Wright-Giemsa stain.

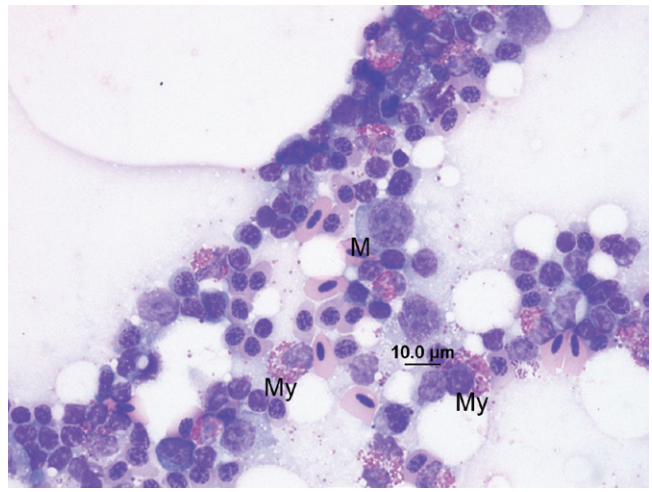

Figure 19.67 A myeloblast (M) and myelocytes (My) in the bone marrow aspirate of a conure (*Aratinga solstitialis*). Wright-Giemsa stain.

Polychromatophilic erythrocytes and mature erythrocytes

Cells in the final stages of erythropoiesis are the polychromatophilic erythrocyte and the mature erythrocyte. These cells are found in the peripheral blood of normal birds and were described earlier. The mature erythrocyte has a flattened, ellipsoid shape. The nuclear chromatin is condensed and transcriptionally inactive.

Granulopoiesis

Avian granulocytes appear to develop in a manner similar to those of mammals. The maturation stages have been described based on their morphologic appearance, primarily in chicken bone marrow. Thus, the study of avian hematopoiesis lags behind research in mammalian hematopoiesis, in which morphologic criteria are only part of the overall evaluation. Avian granulocytes show a progressive decrease in size and cytoplasmic basophilia as they mature, which is similar to the granulocytes of mammals. Specific cytoplasmic granules appear during the later stages of development and then progressively increase in number, until a full complement is reached in the cytoplasm of the mature granulocyte. The nuclei of granulocytes initially are round and progress toward segmentation, except for basophils, which do not segment, and the nuclear chromatin becomes increasingly condensed with maturity. The developmental stages of avian granulocytes include, in order of maturation, myeloblasts (granuloblasts), progranulocytes (promyelocytes), myelocytes, metamyelocytes, band cells, and mature granulocytes.

Myeloblasts

Avian myeloblasts are large, round cells with a high N:C ratio (Figs. 19.62, 19.64, 19.66, and 19.67). The cytoplasm stains a lighter blue than that of rubriblasts. Myeloblast nuclei typically are round, with delicate reticular (fine) chromatin and prominent nucleoli. Myeloblasts do not contain specific cytoplasmic granules and, possibly, represent a stage that is common to all granulocytes. Myeloblasts frequently are associated with other developing granulocytes, especially on imprints of bone marrow core biopsy specimens.

Progranulocytes

Avian progranulocytes are large cells with light blue cytoplasm and slightly eccentric nuclei (Figs. 19.62 and 19.66).

The N:C ratio is smaller than that of myeloblasts because of an increase in cytoplasm. The nuclear chromatin often has a delicate reticular pattern. Nucleoli are absent, and nuclear margins may be indistinct. Progranulocytes contain primary (immature) granules that vary in appearance among the types of granulocytes. Heterophil progranulocytes contain primary granules that vary in color and shape. They often appear as orange spheres (primary granules) and rings or as deeply basophilic spheres and rings. Eosinophil progranulocytes contain only brightly staining, orange, primary granules, and they appear to lack the dark magenta granules and rings found in heterophil progranulocytes. Basophil progranulocytes contain basophilic granules that appear to be smaller than the specific basophilic granules and the immature granules of the heterophil series. Fewer ring forms are seen in basophil progranulocytes.

Myelocytes

Myelocytes are smaller than myeloblasts and progranulocytes, and they contain the secondary or specific granules of the mature granulocytes, thereby making identification of this cell somewhat simple (Figs. 19.66 and 19.67). The round to oval nucleus of the myelocyte appears to be more condensed than the nuclei of myeloblasts and progranulocytes. Heterophil myelocytes typically are round cells, with a light blue cytoplasm that contains a mixture of rod-shaped specific granules and primary granules and rings. The eosinophilic, rod-shaped specific granules occupy less than half the cytoplasmic volume. Eosinophil myelocytes lack the deeply basophilic granules and rings that occasionally are found in early heterophil myelocytes. Basophil myelocytes contain basophilic specific granules that occupy less than half the cytoplasmic volume. The specific basophil granules have a slightly eosinophilic tinge, compared with the deep violet of the smaller primary granules that also may be present.

Metamyelocytes

Metamyelocytes are slightly smaller than myelocytes, have slightly indented nuclei, and possess specific cytoplasmic granules that occupy greater than half the cytoplasmic volume (Fig. 19.65). Heterophil and basophil metamyelocytes have fewer primary granules than myelocytes and progranulocytes.

Band cells and mature granulocytes

Band cells resemble mature granulocytes, except that the nucleus appears as a curved or coiled band rather than segmented. Identifying band cells often is difficult, because the exact shape of the nucleus is obscured by specific cytoplasmic granules. A specific nuclear stain such as hematoxylin usually is required to determine the concentration of band cells. Because mature basophils lack a segmented nucleus, the band stage of basophils is not apparent. Mature granulocytes generally are the most abundant cell of each granulocytic cell line in the bone marrow of normal birds and were described earlier.

Thrombocytes

Avian thrombocytes appear to derive from a distinct line of mononuclear cells in the bone marrow, unlike mammalian platelets, which are cytoplasmic fragments of large, multinucleated megakaryocytes. The thrombocyte series consists of thromboblasts, immature thrombocytes, and mature thrombocytes. Thromboblasts resemble rubriblasts, but they tend to be smaller, with round nuclei having fine to punctate nuclear chromatin and one or more nucleoli. The cytoplasm is scant, stains deeply basophilic, and may contain clear spaces. They tend to be round to oval, with cytoplasmic blebs.

Immature thrombocytes are divided into three groups—early, mid, and late immature thrombocytes—based on their degree of maturity (Fig. 19.46). Early immature thrombocytes are intermediate in size between thromboblasts and more mature stages. They tend to be round to oval and have more abundant cytoplasm than thromboblasts. The cytoplasm is basophilic and may contain vacuoles. The nuclear chromatin is aggregated into irregular clumps. Mid immature thrombocytes are slightly elongate or irregular, with pale blue cytoplasm. Cytoplasmic specific granules and vacuoles occasionally are seen at this stage of development. The nucleus contains heavy chromatin clumping. Late immature thrombocytes are oval and slightly smaller than the mid immature stage. The cytoplasm stains pale blue, with vaguely defined, clear areas. Specific granules frequently are seen at one pole of the cell. The nucleus is oval and has densely packed chromatin. The mature thrombocyte is the definitive cell in the thrombocyte series and was described earlier.

Other cells in avian bone marrow

Monocytes and macrophages

Monocytopoiesis is poorly defined in birds. Granulocytic precursor cells may be similar to—or even the same as—monocytic precursor cells. Monocytes originating in hematopoietic tissues become the monocytes and macrophages found in blood and body tissues, respectively. A variety of tissues, notably bone marrow, embryonic yolk sac, and spleen, can produce macrophage colonies. Mature monocytes are described in the discussion of leukocytes. Macrophages within the bone marrow usually contain iron pigment within the cytoplasm, because they are involved with iron metabolism during hemoglobin synthesis and catabolism. Iron may appear as gray to black granulation or as golden, crystalline material.

Lymphocytes

Aggregates of lymphocytes are found within the bone marrow of birds, although major sites of lymphopoiesis in

adult birds are located in the spleen, liver, intestines, and cecal tonsils. Avian lymphocytes can be classified as B lymphocytes (providing humoral immunity) or T lymphocytes (responsible for cell mediated immunity), but these two cell types usually cannot be differentiated based on morphology alone. B lymphocytes differentiate in the bursa of Fabricius, and T-lymphocytes differentiate in the thymus.

Immature avian lymphocytes are larger than mature lymphocytes, and they are classified as either lymphoblasts or prolymphocytes based on morphology. Lymphoblasts have large nuclei, with fine chromatin, and they contain one or more prominent nucleoli. The cytoplasm is relatively abundant and deeply basophilic. Prolymphocytes resemble lymphoblasts, but their nuclear chromatin is coarser and nucleoli are not present. Mature lymphocytes have coarse chromatin that typically is clumped. Cytoplasm is scant and stains light blue.

Osteoblasts

Avian osteoblasts are large cells found in the bone marrow that resemble those of mammals. They have abundant, foamy, basophilic cytoplasm, with a distinct, clear Golgi apparatus. The nucleus is round to oval and eccentrically located in the cell, contains reticular to coarsely granular chromatin, and possesses one or more distinct nucleoli. Osteoblasts are polygonal to fusiform, and they may have indistinct cytoplasmic margins.

Osteoclasts

Osteoclasts are large, multinucleated, giant cells with an ameboid shape (Fig. 19.68). The cytoplasm is weakly basophilic and vacuolated, and red cytoplasmic granules may be present. Nuclei are round to oval and often contain prominent nucleoli.

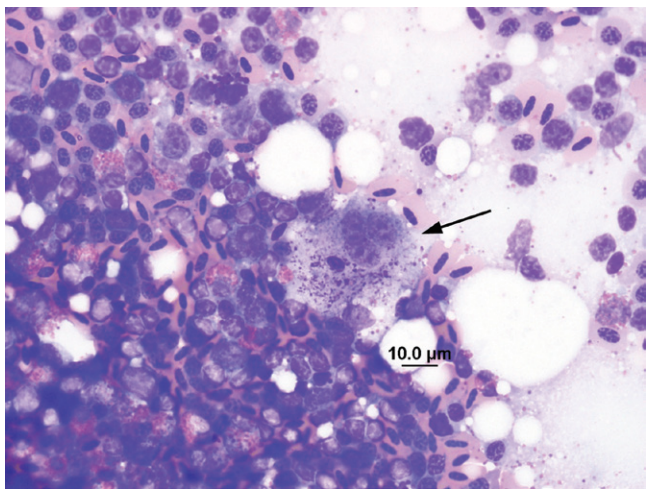

Figure 19.68 An osteoclast (arrow) in the bone marrow aspirate of a conure (*Aratinga solstitialis*). Wright-Giemsa stain.

Hematopoietic tissues other than bone marrow

Bursa of Fabricius

Based on research using domestic chicken and quail embryos, lymphoid cells first appear in the developing bursa of the 13–15 day embryo.[31] Granulopoiesis also occurs in the developing bursa of the 12–13 day chicken embryo, but it disappears either at or just before hatching. The bursa reaches its maximum growth around 4 weeks after hatching then gradually undergoes involution during a 2–3 month period.

During development, the bursa contains numerous deeply basophilic, lymphoid precursor cells. Lymphoid precursors reach a maximum number in the 13–25 day embryo and then decline as lymphoid differentiation progresses. Lymphoid precursors may originate from an external source, such as the yolk sac or bone marrow. Seeding of the bursa with lymphoid precursor cells appears to occur in the 7–14 day embryo, depending on the species. Thus, the sole source of B lymphocytes in the adult bird is the self-regenerating aggregates of B lymphocytes that originated in the bursa and then spread to the spleen, liver, intestines, and cecal tonsils.

Thymus

The thymus is organized into a cortex consisting of densely packed lymphoid cells and a medulla. Lymphoid precursors originating from the yolk sac or bone marrow begin to colonize the thymus during the first 4–8 days of development, depending on the species. The influx of lymphoid precursors appears to last from 24 to 36 hours and then ends abruptly. The invasion of the thymus by precursors is followed by a 4–5 day refractory period before another influx occurs. This cyclic colonization of the thymus by lymphoid stem cells consists of two to three colonization periods, which may extend into the post-hatchling period, depending on the species.[31] This contrasts with colonization of the bursa, which occurs during a distinct, single episode in the embryo before hatching. T lymphocytes acquire their T antigen during a 24-hour period of development around the time of the second wave of colonization, between days 12–15 of embryonic life. T lymphocytes originating in the thymus spread to the spleen, liver, intestines, and cecal tonsils, and they are the predominate lymphoid cell of the spleen and peripheral blood of hatched birds.

Spleen

T and B lymphocytes appear at different locations in the white pulp of the spleen.[31] The central arteries of the white pulp are surrounded by a periarteriolar lymphoid sheath, which is comprised of densely packed T lymphocytes. Capillaries branching at right angles from the central arteries are surrounded by periellipsoid lymphoid tissue consisting of B

lymphocytes. B lymphocytes also are found at the germinal centers located within the periarteriolar lymphoid sheath. During embryonic development, the spleen participates in erythropoiesis and granulocytopoiesis. Granulopoiesis becomes more predominant as the embryo matures. At hatching, however, the granulocytes begin to disappear, and by 3 days, they are replaced by lymphocytes.

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20 Hematology of Reptiles

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Evaluation of the hemogram and blood film is part of the laboratory evaluation of reptilian patients. Hematology is used to detect conditions such as anemia, inflammatory diseases, parasitemias, hematopoietic disorders, and hemostatic alterations. Hematologic evaluation involves examination of the erythrocytes, leukocytes, and thrombocytes in the peripheral blood.

When evaluating the hematologic responses of reptiles, external factors such as environmental conditions that may enhance or inhibit the animal's response to disease should not be overlooked. The cellular responses in the blood of reptiles are less predictable than those in the blood of endothermic mammals and birds whose cellular microenvironments are more stable. A number of intrinsic factors, such as age and gender, also affect the hematologic data from reptiles. In addition, a number of sample-handling factors, such as the site of blood collection, type of anticoagulant used, method of cell counting, and type of stain used, add to the variability of reptilian hemogram values. All these factors complicate the establishment of normal reference values in reptiles. Therefore, total and differential leukocyte counts must differ greatly (i.e., twofold or greater increase or decrease) from normal reference values to be considered significant.

Blood collection and handling

Blood samples for hematologic and blood biochemical studies can be collected from reptiles using a variety of methods, with the choice depending on the peculiarities of the species, volume of blood needed, size of the reptile, physical condition of the patient, and preference of the collector.[1–7] A few venipuncture sites are available for blood sampling from some reptiles; however, because the lymphatic vessels often accompany blood vessels in reptiles, a mixture of blood and lymph frequently occurs with venipuncture of the peripheral vessels.[3,8–10] The mixing of lymphatic fluid with the blood sample is variable, and it dilutes the cellular components of the blood, thereby resulting in a lower packed cell volume (PCV), hemoglobin concentration (Hb), total erythrocyte count (TRBC), and leukocyte count (WBC). Therefore, the collection site chosen for a blood sample influences the hematologic values.

Jugular venipuncture can be used to collect blood from reptiles, especially lizards and chelonians (turtles and tortoises) (Fig. 20.1). An advantage of jugular venipuncture is that it minimizes the chances for the hemodilution of the sample with lymphatic fluid; however, jugular venipuncture may require chemical restraint. Blood collection via jugular venipuncture is recommended in some species of reptiles, such as chameleons, to minimize unwanted effects, such as skin darkening, which occurs with blood collection from other sites. With the head and neck extended, the jugular vein is approached as it lies just under the skin in a line between the angle of the mandible and the thoracic inlet. The right jugular vein may be larger than the left in some species.

The dorsal postoccipital vein or venous plexus (sinus) is a common location for obtaining blood samples in reptiles, especially tortoises, turtles, and crocodilians, but samples from this location commonly are diluted with a variable amount of lymphatic fluid, which can be seen as a clear liquid entering the syringe. The dorsal postoccipital venous plexus is reached by inserting the hypodermic needle along the dorsal aspect of the neck behind the occipital protuberance, just off the midline and lateral to the cervical vertebrae. In some species, this venous plexus is located just off midline and lateral to the cervical vertebrae. To collect blood from sea turtles using this technique, a 20-G, 1.0- to 1.5-inch needle is inserted in an area located one-third of the distance from the dorsal midline to the lateral aspect of the neck and one-third of the distance from the head to the carapace (Fig. 20.2).[2] Blood is collected either into a syringe

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Figure 20.1 Blood collection by jugular venipuncture in a turtle (*Chelonia mydas*).

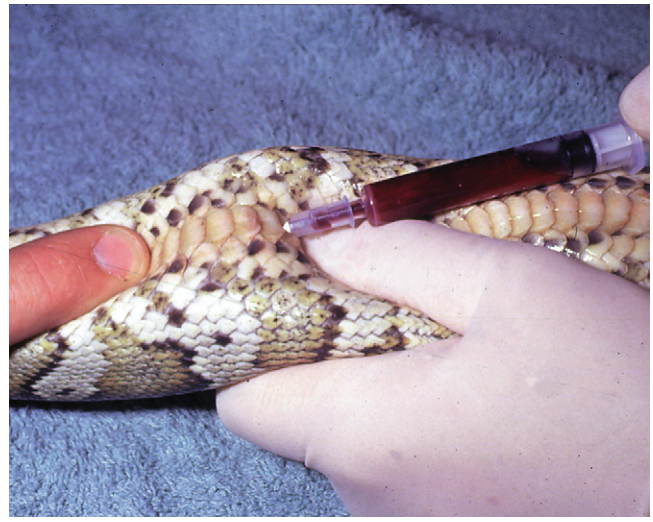

Figure 20.3 Blood collection by cardiocentesis of a snake.

Figure 20.2 Blood collection from the dorsal postoccipital vein of a turtle (*Chelonia mydas*).

or an evacuated tube. This method also may be used for other chelonians, such as tortoises and semiaquatic freshwater turtles. The supravertebral vessel is located in the area similar to the dorsal postoccipital venous sinus and is approached just behind the nuchal crest or occiput along the dorsal midline and just dorsal to the spinal cord in crocodilians for blood collection.

Cardiocentesis commonly is performed for blood collection from reptiles, especially snakes. The heart of a snake is located by observing the heartbeats, which move the ventral scutes overlying the heart, or by palpation. Because the heart will move caudally and cranially, it should be stabilized at the apex and base using a thumb and forefinger during sample collection. A needle (i.e., a thin-wall, 22 or 23 G needle attached to a 3–6 mL syringe) is inserted under the scute—not through the scale—and then advanced into the heart. Usually, the attached syringe fills slowly as the heart pulsates (Fig. 20.3). The heart of small lizards can be located by transillumination of the thoracic cavity. Cardiocentesis may be harmful in lizards, however, and it is not commonly used for blood collection in this group of reptiles. Cardiocentesis of chelonians is performed by passing a needle through the plastron along the midline at the junction of the humeral and pectoral scutes. In small chelonians, a hole is drilled through the plastron using an 18–20 G needle, whereas a sterile drill bit may be required for larger chelonians. After the procedure, the hole drilled through the plastron should be sealed with an epoxy. If multiple samples are required, a larger hole can be drilled into the plastron over the heart and then plugged with a rubber stopper from a blood collection tube, which is sealed into position with epoxy. This provides an access port to the heart for blood collection.

The ventral coccygeal vein (ventral caudal or tail vein) also is a common site for blood collection in reptiles, especially lizards, snakes, and crocodilians. This vein lies just ventral to the caudal vertebrae. To collect blood from the ventral coccygeal vein, a 22–23 G, 1 inch needle is inserted under a ventral scale or the ventral midline and then is directed toward the vertebrae (Fig. 20.4). Slight negative pressure should be applied to the syringe as the needle is being advanced. Often, a vertebra is encountered before blood enters the needle, and in such cases, the needle is withdrawn slowly until blood flows into the syringe. Lizards and crocodilians can be bled from the tail in this manner by extending the tail over the edge of a table and approaching the site from underneath. This method of restraint is better tolerated by most reptiles compared with holding them in dorsal recumbency. Lizards also can be restrained in a verti-

Figure 20.4 Blood collection by venipuncture of the ventral coccygeal vein in the tail of a lizard (*Iguana iguana*) using the ventral approach.

Figure 20.5 Blood collection by venipuncture of the ventral coccygeal vein in the tail of a lizard (*Iguana iguana*) using the lateral approach.

cal position by allowing them to cling to a cage door, thereby permitting access to the ventral tail. In addition, a lateral approach can be used in lizards and crocodilians by inserting the needle along the lateral aspect of the tail in an area where a natural groove or line occurs (Figs. 20.5 and 20.6). The needle tip should be placed just beneath the caudal vertebra and into the vein.

Other, less commonly used procedures for collecting blood from reptiles include sampling from the brachial vein or artery, palatine-pterygoid vein, ventral abdominal vein, and toenails. Blood collection from the brachial vein or artery is a blind approach, but it may be attempted in chelonians or lizards. Samples obtained using this method, however, frequently are diluted with lymph. Blood can be collected from

Figure 20.6 Blood collection from the ventral coccygeal vein of an alligator (*Alligator mississippiensis*) using the lateral approach.

the palatine-pterygoid veins in the oral cavity of medium and large snakes, but this method requires either a cooperative patient or general anesthesia. In addition, these veins are fragile and easily lacerated. Blood can be collected by aspiration into a syringe or by allowing the blood to flow from the needle hub into a microcollection tube. Lizards have large ventral abdominal veins that lie just under the skin on the ventral midline, and although this vein is easily located, it also is easily lacerated. Thus, hemostasis after venipuncture can be a problem. To minimize bleeding, the vein can be cannulated with a needle and blood collected from the needle hub into a microcollection tube. Lastly, although blood from a capillary bed provides a poor sample for hematologic studies, blood from a clipped toenail may be the only collection procedure available in very small reptiles (i.e., <30 g). After cleaning of the toenail, the nail is clipped using nail trimmers, and blood is collected into a microcollection tube. A styptic powder or solution is used to aid clotting.

The blood volume of reptiles is estimated to range between 5% and 8% of the body weight, and most species tolerate withdrawal of as much as 10% of the blood volume (or 1% of body weight) without detrimental effects.[11] A smaller volume representing 0.5% of the body weight has been recommended for smaller animals.[12] Only 0.2–0.3 mL of blood is required for routine hematologic studies; most reptiles tolerate this loss.

Blood should be collected into an anticoagulant for hematologic evaluations. Although ethylenediaminetetraacetic acid (EDTA) generally is the anticoagulant of choice for hematologic studies, blood from many species of reptiles, especially chelonians, often undergoes hemolysis in its presence. Therefore, use of an alternative anticoagulant, such as lithium heparin, is necessary. Heparin creates a blue tinge to blood films and may cause clumping of leukocytes and

thrombocytes, thereby creating difficulties in obtaining accurate cell counts. To minimize these effects of heparin, the blood sample should be processed soon after collection, and slides should be made as soon as possible. A blood film made from a drop of blood that contains no anticoagulant and is taken from the needle immediately after collection can be used to avoid interference with anticoagulants during staining.

Erythrocytes

Morphology

Mature erythrocytes in reptiles generally are larger than those in birds and mammals. Reptilian erythrocytes are ellipsoidal cells with centrally positioned, oval to round nuclei, dense purple chromatin, and often, irregular margins (Fig. 20.7). The cytoplasm generally stains uniformly orange-pink with Romanowsky stains such as Wright stain. Polychromatophilic erythrocytes have nuclear chromatin that is less dense and cytoplasm that is more basophilic than in mature erythrocytes. Immature erythrocytes occasionally are seen in the peripheral blood of reptiles, especially very young animals or those undergoing ecdysis. Immature erythrocytes are round to irregular cells with large, round nuclei and basophilic cytoplasm (Figs. 20.8 and 20.9). The nucleus lacks the dense chromatin clumping of the mature cell. Immature erythrocytes often appear to be smaller than mature erythrocytes, probably for the same reasons as those described for avian hematology (see Chapter 19). Mitotic activity associated with erythrocytes is common in the peripheral blood of reptiles (Fig. 20.10).

Reticulocytes are detected by staining cells with a vital stain such as new methylene blue. Reptilian reticulocytes, like avian reticulocytes, have a distinct ring of aggregated reticulum that encircles the red-cell nucleus. These cells best correspond to the polychromatophilic erythrocytes found in Romanowsky-stained blood films, and they probably are the cells that were recently released from erythropoietic tissues (Fig. 20.11). Basophilic stippling commonly occurs in reticulocytes with Romanowsky stains.

Intraerythrocytic inclusions of unknown etiology are frequently found in the blood films of reptiles. Round to irregular basophilic inclusions frequently are seen in the cytoplasm of erythrocytes in the peripheral blood films from many

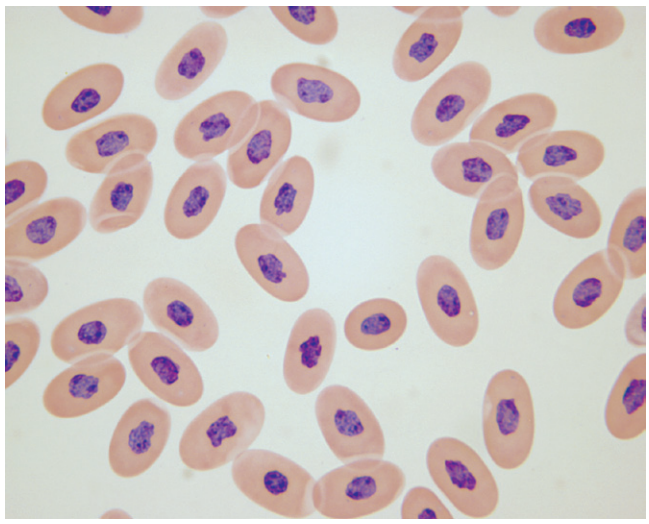

Figure 20.7 Normal erythrocytes in the blood film of a snake (*Lichanura trivirgata*).Wright-Giemsa stain.

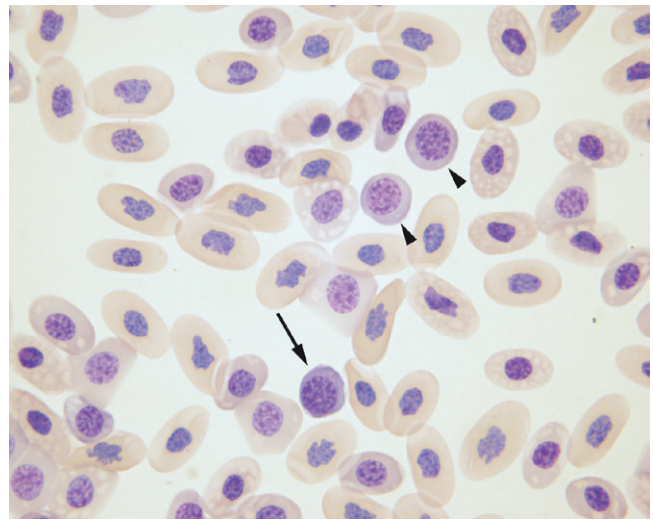

Figure 20.8 Immature erythrocytes in the blood film of a lizard (*Iguana iguana*). A basophilic rubricyte (large arrow) and a midpolychromatic rubricyte (arrowhead) are shown. Wright-Giemsa stain.

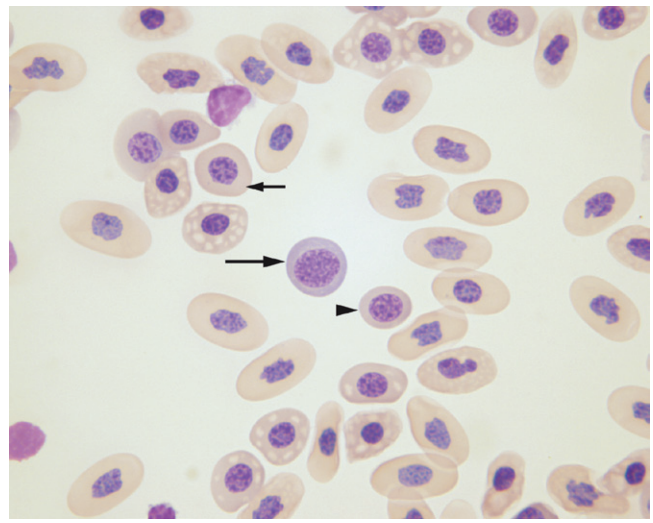

Figure 20.9 Immature erythrocytes in the blood film of a lizard (*Iguana iguana*). A basophilic rubricyte (large arrow), a midpolychromatic rubricyte (arrowhead), and a late polychromatic rubricyte are shown.Wright-Giemsa stain.

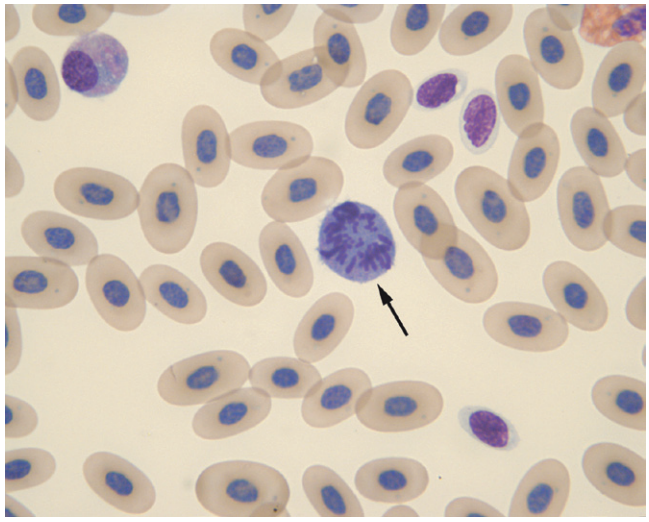

Figure 20.10 An erythrocyte exhibiting mitotic activity in the peripheral blood of a snake (*Boa constrictor*).Wright-Giemsa stain.

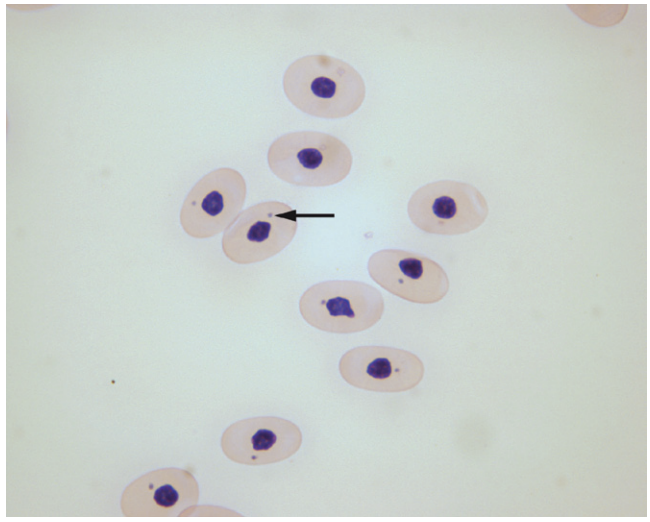

Figure 20.12 Basophilic inclusions (arrow) in the blood film of a turtle (*Trachemys scripta elegans*).Wright-Giemsa stain.

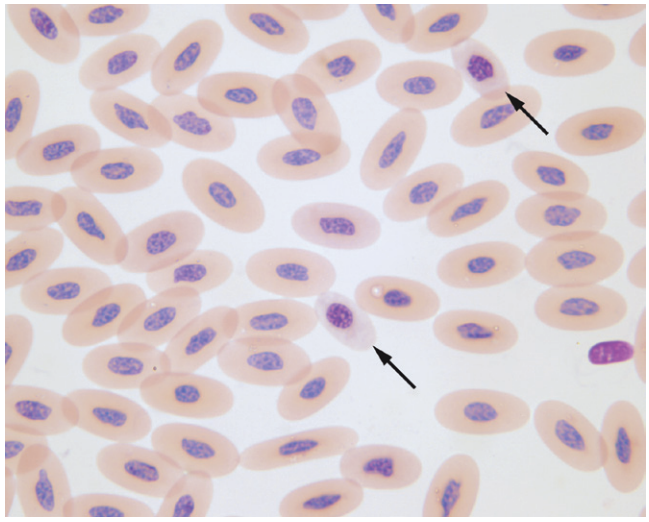

Figure 20.11 Polychromatic erythrocytes (arrows) in the blood film of a snake (*Lampropeltis alterna*).Wright-Giemsa stain.

species of reptiles (Fig. 20.12). These inclusions appear to represent an artifact of slide preparation; because blood films made repeatedly from the same sample often reveal varying degrees of these inclusions. Blue-staining (Romanowsky stains), approximately round, inclusions bodies have been studied in turtles where they measure 0.6–1.3 µm in diameter in painted turtles (*Chrysemys picta picta*) and 0.5–2.0 µm in diameter in loggerhead turtles (*Caretta caretta*).[13] In the painted turtles the inclusions appeared singly in 6–34% of the erythrocytes in blood films and generally adjacent to the cell nucleus. The inclusions were not associated with vacuoles, granules, or crystalline structures as would be expected with viral agents (see Responses in Disease). Electron-microscopic images suggest that these inclusions are degenerate organelles; therefore, it is possible they represent senescent changes in erythrocytes.[14,15] This is supported by the finding that these inclusions were associated with mature cells that tend to be longer and narrower with smaller areas compared to less mature cells and the inclusions become large as the erythrocyte ages. Other artifacts found in the erythrocyte cytoplasm include vacuoles and refractile clear areas.[3] These can be minimized with careful preparation of blood films.

Laboratory evaluation

Laboratory evaluation of the reptilian erythron involves determination of the PCV, TRBC, and Hb of blood. The PCV is obtained by microhematocrit centrifugation. A PCV also can be calculated by electronic cell counters that are accurately adjusted for each species according to differences in erythrocyte sizes. Microhematocrit centrifugation, however, is the most practical method for obtaining PCVs of reptilian blood.

A TRBC (cell/µL) can be determined by a manual hemocytometer counting method or by an automated cell counter. Two manual methods that commonly are used to obtain a TRBC in reptilian blood involve either an erythrocyte Unopette system (Becton-Dickinson, Rutherford, NJ) or Natt-Herrick solution (see Chapter 19 on avian hematology). The erythrocyte Unopette system is the easier method, because the 1:200 dilution of the whole, anticoagulated blood is made using the diluent, pipette, and mixing vial provided with the kit. In contrast, the Natt and Herrick method requires preparation of the diluent/stain solution and use of a red blood cell–diluting pipette. Blood is drawn to the 0.5 mark on the red blood cell–diluting pipette, and Natt-Herrick solution then is drawn to the 101 mark to

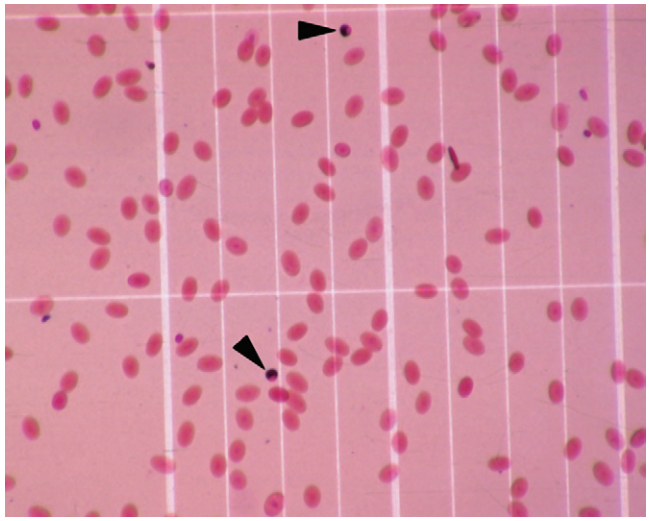

Figure 20.13 Appearance of erythrocytes and dark-staining leukocytes (arrow heads) in the Neubauer-ruled hemocytometer using Natt-Herrick solution, 100×.

prepare the 1:200 dilution. With both methods, the diluted blood is discharged onto the hemocytometer counting chamber and allowed to settle for a minimum of 5 minutes before counting. The total number of erythrocytes in the four corner and central squares of the central, large square of the Neubauer-ruled counting chamber is obtained using ×40 (i.e., high-dry) magnification (Fig. 20.13). The TRBC is calculated by multiplying the number of erythrocytes that are counted by 10,000. Alternately, a 1:100 dilution of the blood sample is made with the Natt-Herrick solution, which is then, diluted 1:100 with saline to produce a 1:1000 dilution for charging the hemocytometer. The TRBC is obtained by multiplying the number of cells counted on both sides of the hemocytometer by 1000.[15]

The Hb is determined by the same technique as that described for avian hematology (see Chapter 19). The cyanmethemoglobin method or automated procedures, such as use of the hemoglobinometer (Coulter Electronics, Hialeah, FL), can determine the Hb of reptilian blood. The cyanmethemoglobin reagent–blood mixture requires centrifugation to remove the free nuclei from the lysed erythrocytes before measuring the optical density to obtain an accurate Hb value.

Responses in disease

Reptiles have lower TRBCs compared with those of mammals and birds, and the TRBC appears to have an inverse relationship with the size of the erythrocytes. Lizards tend to have smaller erythrocytes than other reptiles; therefore, they have higher TRBCs. Snakes have lower TRBC values than lizards but greater values than chelonians. The TRBC, Hb, and PCV values vary with a number of factors, including the environment (TRBC values are highest before and lowest immediately after hibernation), nutritional status; and gender (males tend to have higher TRBCs than females).[16–21]

The normal PCV of most reptiles generally ranges between 20% and 40%.[17,22,23] Therefore, a PCV of less than 20% suggests anemia, a PCV of greater than 40% suggests either hemoconcentration or erythrocytosis (polycythemia) (Table 20.1). The hemoglobin concentration of reptiles generally ranges between 5.5 and 12 g/dL.[21]

The causes of anemia in reptiles are similar to those described for birds and mammals. Anemia can be classified as hemorrhagic (i.e., blood loss), hemolytic (i.e., increased red-cell destruction), or depression anemia (i.e., decreased red-cell production). Hemorrhagic anemias usually result from traumatic injuries or bloodsucking parasites; however, other causes, such as a coagulopathy or an ulcerative lesion, should be considered as well. Hemolytic anemia can result from septicemia, parasitemia, or toxemia. Depression anemia usually is associated with chronic inflammatory diseases, especially those associated with an infectious agent. Other causes that should be considered for depression anemia in reptiles include chronic renal or hepatic disease, neoplasia, chemicals, and possibly, hypothyroidism.

The degree of polychromasia or reticulocytosis in the blood films of normal reptiles generally is low, and it represents less than 1% of the erythrocyte population. This may be associated with the long erythrocyte life span (600–800 days in some species) and, therefore, with the slow turnover rate of reptilian erythrocytes compared with those of birds and mammals.[17,21,24] The relatively low metabolic rate of reptiles also may be a factor. Young reptiles tend to have a greater degree of polychromasia than adults.

Slight anisocytosis and poikilocytosis are considered to be normal for most reptilian erythrocytes. Moderate to marked anisocytosis and poikilocytosis are associated with erythrocytic regenerative responses and, less commonly, with erythrocyte disorders. An increased polychromasia and number of immature erythrocytes is seen in reptiles responding to anemic conditions. Young reptiles or those undergoing ecdysis also may exhibit an increased polychromasia and immature erythrocyte concentration. Erythrocytes exhibiting binucleation, abnormal nuclear shapes (anisokaryosis), or mitotic activity can be associated with marked regenerative responses (Fig. 20.14). These nuclear findings, however, also may occur in reptiles awakening from hibernation or in association with severe inflammatory disease, malnutrition, and starvation. Basophilic stippling usually suggests a regenerative response, but it may also be seen in patients with lead toxicosis. Hypochromatic erythrocytes are associated with iron deficiency or chronic inflammatory disease (presumably in association with iron sequestration).

Erythrocytic intracytoplasmic inclusions in reptilian blood films can be caused by viruses or blood parasites (see section on blood parasites). Viral inclusions caused by an iridovirus

Table 20.1 Erythrocyte parameters for selected reptiles.

	PCV [%]	RBC [$\times 10^6$/μL]	Hb [g/dL]	MCV [fl]	MCHC [g/dL]
Lizards					
Argentine lizard[a] winter	24–28	0.8–1.1	10–14	252–300	36–48
Argentine lizard[a] summer	18–26	0.8–1.1	7–13	198–262	41–49
Adult male iguanas[b]	29–39	1.0–1.7	6.7–10.2	228–303	22.7–28.0
Adult female iguanas[b]	33–44	1.2–1.8	9.1–12.2	235–331	24.9–31.0
Juvenile iguanas[b]	30–47	1.3–1.6	9.2–10.1	—	—
Prehensile-tailed skink[c]	24–60	0.8–1.4	7.4–11.6	152–600	17–56
Snakes					
Boa constrictor[d,e]	24–40	1.0–2.5	3.3–15.3	159–625	21–42
Ball pythons[f]	16–21	0.3–1.3	5.5–7.9	211–540	25–40
Yellow rat snake[g]	9–46	0.2–1.6	2.8–15.2	179–961	26–54
Jungle carpet python[h]	23–37	0.5–1.3	4.0–15.5	178–414	23.5–53.2
Chelonians					
Aldabra tortoise[i]	11–17	0.3–0.7	3.2–8.0	375–537	28–40
Desert tortoise[j]	23–37	1.2–3.0	6.9–7.7	377–607	19–34

[a]Troiano JC, Gould EG, Gould I (2008) Hematological reference intervals in argentine lizard *Tupinambis merianae* [Sauria-Teiidae]. *Comp Clin Pathol* 17: 93–7.
[b]Harr KE, Alleman AR, Dennis PM, *et al.* (2001) Morphologic and cytochemical characteristics of blood cells and hematologic and plasma biochemical reference intervals in green iguanas. *JAVMA* 218[6]: 915–21.
[c]Wright KM, Skeba S (1992) Hematology and plasma chemistries of captive prehensile-tailed skinks [*Corcucia zebrata*]. *J Zoo Wildl Med* 23: 429–32.
[d]Chiodini RJ, Sundberg JP (1982) Blood chemical values of the common boa constrictor [*Constrictor constrictor*]. *Am J Vet Res* 43: 1701–2.
[e]Rosskopf WJ, Woerpel RW, Yanoff SR (1982) Normal hemogram and blood chemistry values for boa constrictors and pythons. *Vet Med Small Anim Clin* May: 822–3.
[f]Johnson JH, Benson PA (1996) Laboratory reference values for a group of captive ball pythons [*Python regius*]. *Am J Vet Res* 57: 1304–7.
[g]Ramsey EC, Dotson TK (1995) Tissue and serum enzyme activities in the yellow rat snake [*Elaphe obsolete quadrivitatta*]. *Am J Vet Res* 56: 423–8.
[h]Centini R, Klaphake E (2002) Hematologic values and cytology in a population of captive jungle carpet pythons, *Morelia spilota cheynei*. *Proc Assoc Rept Amph Vet*, pp. 107–11.
[i]Carpenter JW (2001) *Exotic Animal Formulary*, 3rd ed. St. Louis: Elsevier Saunders, p. 107.
[j]Gottdenker NL, Jacobson ER (1995) Effect of venipuncture sites on hematologic and clinical biochemical values in desert tortoises [*Gopherus agassizii*]. *Am J Vet Res* 56: 19–21.

are represented by snake erythrocyte viruses and lizard erythrocytic viruses (formerly known as *Pirhemocyton*) and are identified as small round (punctate to oval) red-staining (Giemsa stain) inclusions that may be associated with rectangular or hexagonal translucent, crystalline, or albuminoid vacuoles.[15,25–31] The iridovirus inclusion of box turtles appears as round to oval pink granular inclusions in the cytoplasm of leukocytes.[26,28,31]

Leukocytes

Morphology

The granulocytes of reptiles can be classified into two groups, acidophils and basophils, based on their appearance in blood films prepared with Romanowsky stains. The acidophils are further divided into heterophils and eosinophils. Reptilian heterophils generally are round cells with eosinophilic (bright orange), fusiform cytoplasmic granules (Figs. 20.15 through 20.17). The cytoplasm of normal heterophils is colorless. The mature heterophil nucleus typically is round to oval and is eccentrically positioned in the cell, with densely clumped nuclear chromatin. Some species of lizards have heterophils with lobed nuclei. Heterophils range between 10 and 23 μm in size but vary between species and individual blood samples.[20]

The cytoplasmic granules of reptilian heterophils usually are peroxidase negative, except in a few species of snakes and lizards.[18,32,33,34] In addition, reptilian heterophils generally do not stain positive for alkaline phosphatase.[14,18,21,32–35] Therefore, reptilian heterophils are functionally equivalent to mammalian neutrophils, but they most

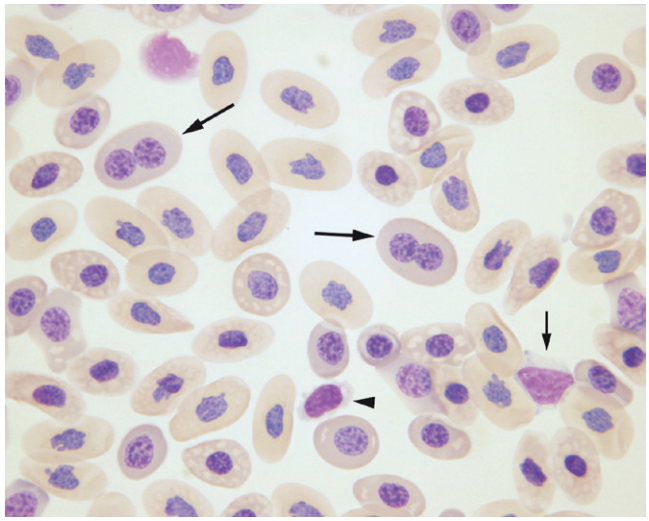

Figure 20.14 Binucleated erythrocytes (arrows) in the peripheral blood of a lizard (*Iguana iguana*). Also shown are a lymphocyte (small arrow) and a thrombocyte (arrowhead). Wright-Giemsa stain.

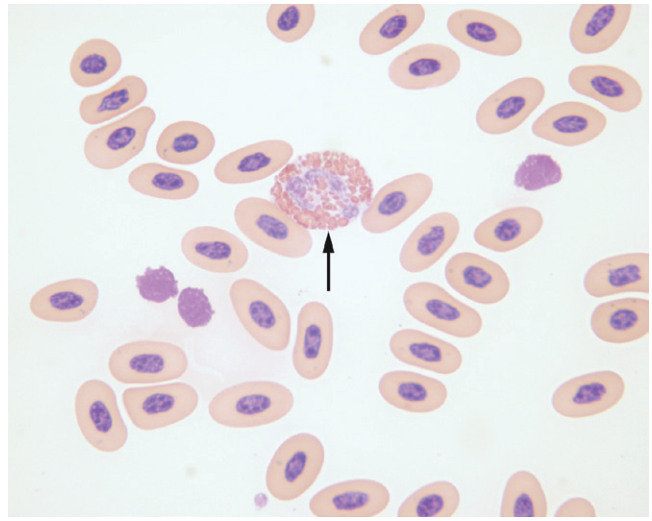

Figure 20.16 A heterophil with a lobed nucleus (arrow) in the blood film of a lizard (*Tupinambis merianae*). Wright-Giemsa stain.

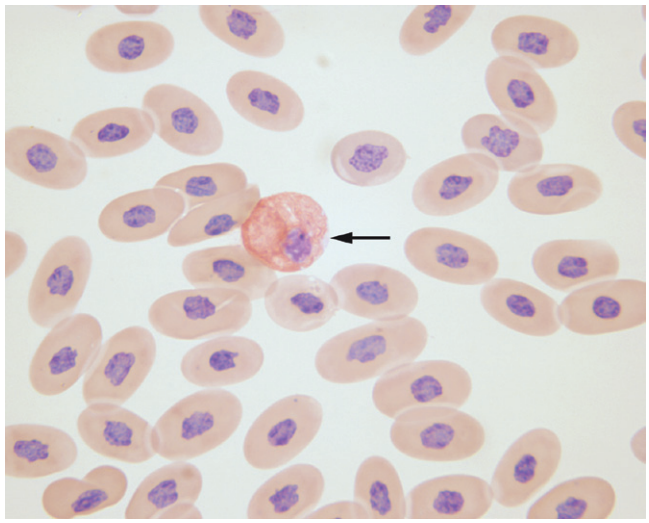

Figure 20.15 A heterophil (arrow) in the blood film of a snake (*Lichanura trivirgata*).Wright-Giemsa stain.

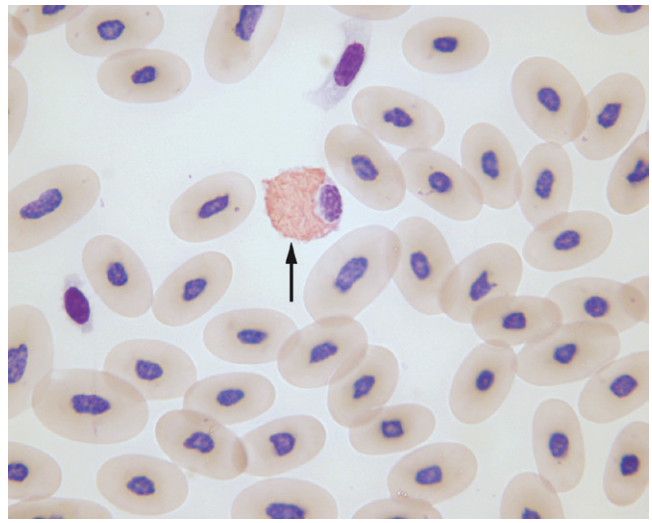

Figure 20.17 A heterophil (arrow) in the blood film of a tortoise (*Testudo graeca*). Wright-Giemsa stain.

likely behave like avian heterophils, in that they rely more heavily on oxygen-independent mechanisms to destroy phagocytized microorganisms.[36]

Eosinophils in most reptilian blood films are large, round cells with spherical, eosinophilic cytoplasmic granules (Figs. 20.18 and 20.19). The granules of some species of reptiles, such as iguanas, stain blue with Romanowsky stains.[3] The cytoplasmic granules of eosinophils also stain positive for peroxidase in some species of reptiles, thereby allowing easy differentiation between eosinophils and heterophils.[14,18] Like heterophils, eosinophils vary in size with the species. For example, snakes have the largest eosinophils, whereas lizards have the smallest. The nucleus typically is central in its cellular position and is variable in shape, ranging from slightly elongated to lobed.

Reptilian eosinophils contain glycogen, myeloperoxidase, and basic proteins.[21] The latter are known potent toxins for parasites (especially helminthes), inactivating leukotrienes, and causing histamine release from mast cells.

Basophils usually are small, round cells that contain basophilic, metachromatic cytoplasmic granules, which often obscure the nucleus (Figs. 20.20 and 20.21). When visible, the cell nucleus is slightly eccentric in position and nonlobed. Basophil granules frequently are affected by water-based stains, which cause them to partially dissolve. Therefore, alcohol fixation and use of Romanowsky stains

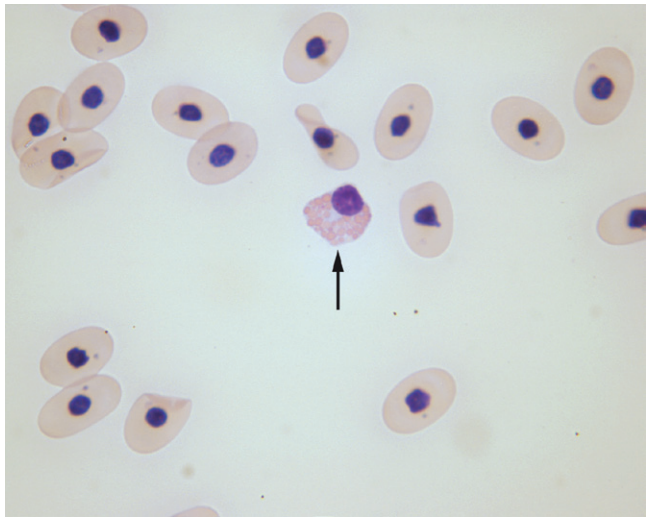

Figure 20.18 An eosinophil (arrow) in the blood film of a turtle (*Trachemys scripta elegans*).Wright-Giemsa stain.

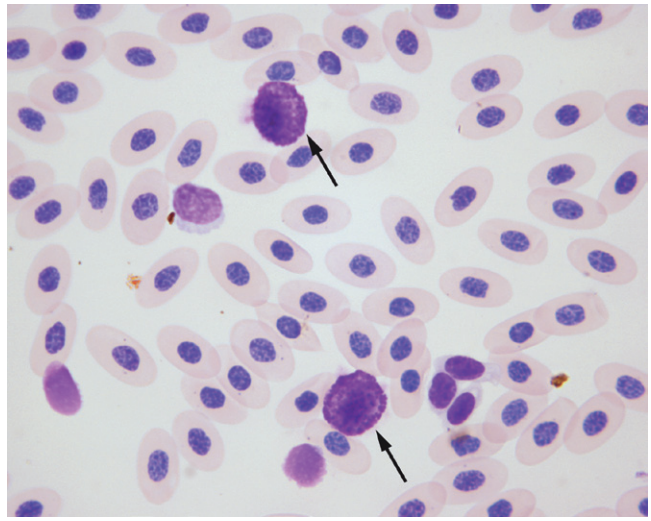

Figure 20.20 Basophils (arrows) in the blood film of a lizard (*Iguana iguana*). Wright-Giemsa stain.

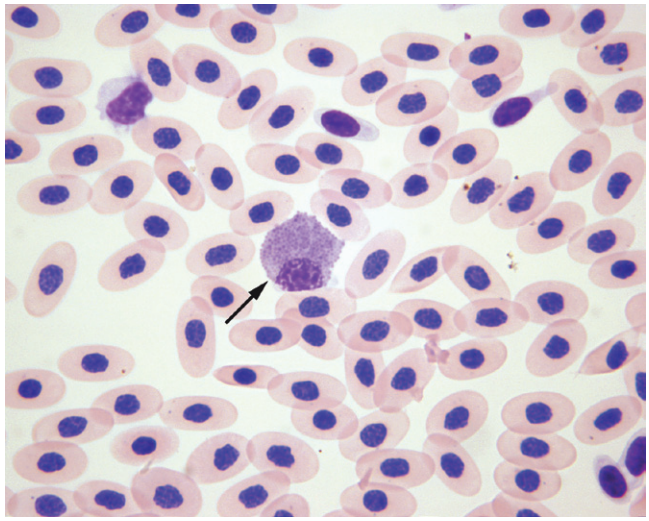

Figure 20.19 An eosinophil with blue cytoplasmic granules (arrow) in the blood film of a lizard (*Iguana iguana*). Wright-Giemsa stain.

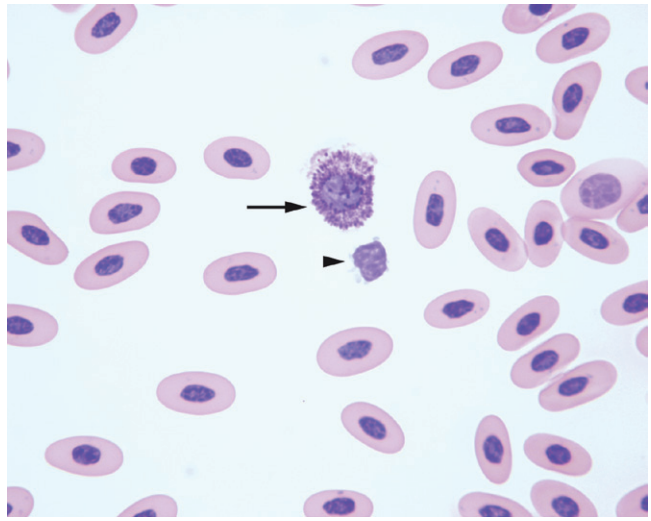

Figure 20.21 A basophil (arrow) and a lymphocyte (arrowhead) in the blood film of a lizard (*Tupinambis merianae*). Wright-Giemsa stain.

provide the best staining results for reptilian basophils. Like acidophils, basophils vary in size according to the species, but they generally range between 7 and 20 µm.[20] Lizards tend to have small basophils, whereas turtles and crocodiles have large basophils.

Reptilian lymphocytes resemble those of birds and mammals. They vary in size from small (5–10 µm) to large (15 µm).[20] Lymphocytes are round cells that exhibit irregularity when they mold around adjacent cells in the blood film or fold at their cytoplasmic margin (Figs. 20.14, 20.21, 20.22, 20.23). They have a round or slightly indented nucleus that is centrally or slightly eccentrically positioned in the cell; nuclear chromatin is heavily clumped in mature lymphocytes. Typically, lymphocytes have a large nucleus: cytoplasm (N:C) ratio. The typical small, mature lymphocyte has scant slightly basophilic (pale blue) cytoplasm. Large lymphocytes have more cytoplasmic volume compared with small lymphocytes, and the nucleus often is pale staining. The cytoplasm of a normal lymphocyte appears to be homogenous and lacks both vacuoles and granules.

Monocytes generally are the largest leukocytes in the peripheral blood of reptiles, and they resemble those of birds and mammals (Figs. 20.24 and 20.25). They vary in shape from round to ameboid. The nucleus also is variable in shape, ranging between round to oval to lobed. The nuclear chromatin of monocytes is less condensed and stains

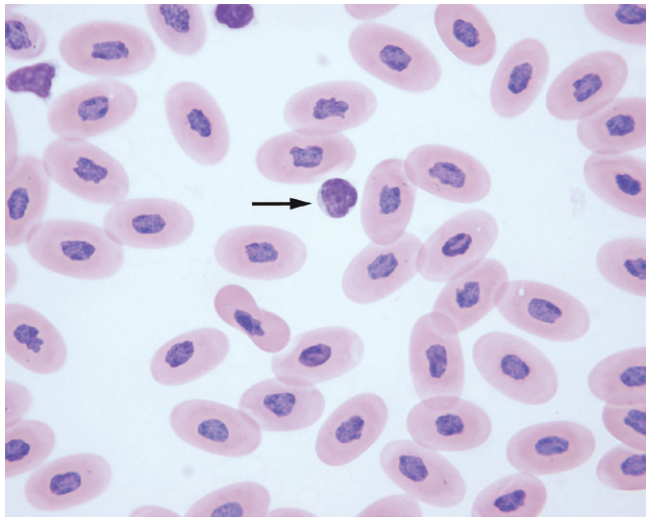

Figure 20.22 A small lymphocyte (arrow) in the blood film of a snake (*Lichanura trivirgata*). Wright-Giemsa stain.

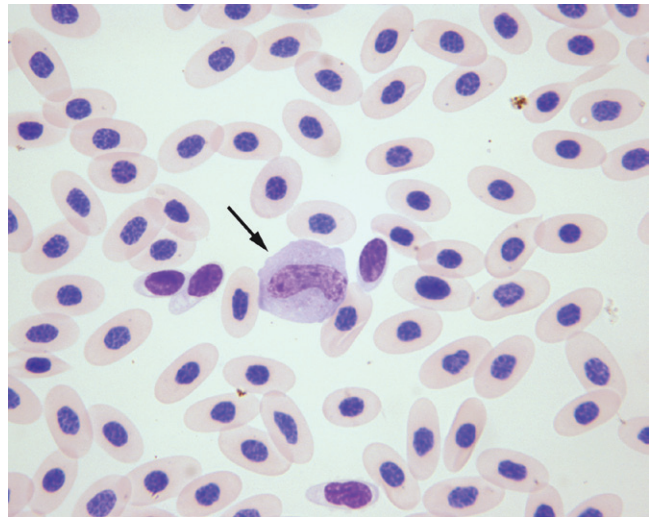

Figure 20.24 A monocyte (arrow) in the blood film of a lizard (*Iguana iguana*). Wright-Giemsa stain.

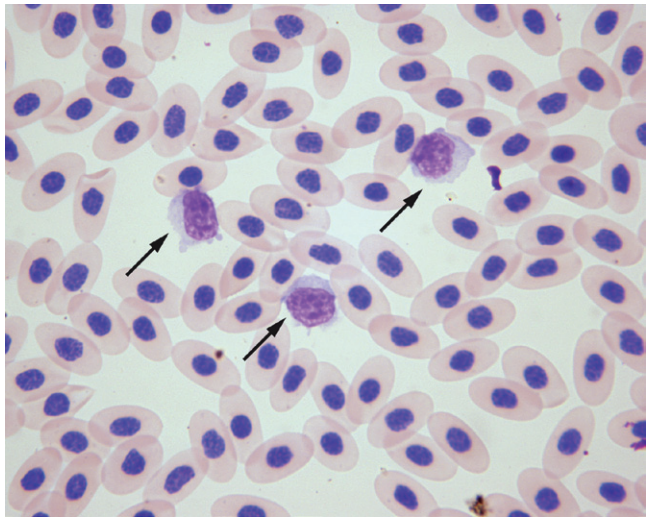

Figure 20.23 Medium lymphocytes (arrows) in the blood film of a lizard (*Iguana iguana*). Wright-Giemsa stain.

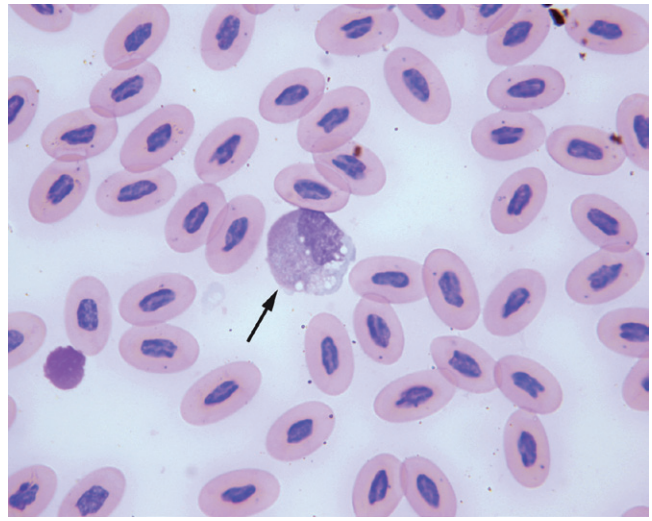

Figure 20.25 A monocyte (arrow) in the blood film of a lizard (*Pogona vitticeps*). Wright-Giemsa stain.

relatively pale compared with the nuclei of lymphocytes. The abundant cytoplasm of monocytes stains blue-gray, may appear to be slightly opaque, and may contain vacuoles or fine, dustlike eosinophilic or azurophilic granules. Some authors refer to monocytes that have an azurophilic appearance to the cytoplasm as a separate cell line referred to as an azurophil.[15] These cells have cytochemical and ultrastructural characteristics similar to those of monocytes and therefore, could be reported as monocytes rather than as a separate cell type (Fig. 20.26).[14,17,18,21,35,37–39] There appears to be little clinical advantage to refer to these cells as a cell other than a monocyte; therefore, the term azurophilic monocyte can be used for these cells.

Laboratory evaluation

Evaluation of the reptilian leukogram involves determination of a total and a differential leukocyte count and examination of the leukocyte morphology on a stained blood film. Manual counting methods are used to obtain total leukocyte concentrations in reptiles for the same reasons they are used in avian hematology: the presence of nucleated erythrocytes and thrombocytes in the blood of reptiles precludes the use of electronic cell-counting procedures. Two manual methods commonly used to obtain a total leukocyte count in reptilian blood are the Natt and Herrick method and the phloxine B method (see Chapter 19 on avian hematology) (Fig. 20.13). In species of reptiles that normally have higher numbers of

circulating lymphocytes than of heterophils, the Natt and Herrick method is preferred, because the accuracy of the phloxine B method relies on large numbers of heterophils and eosinophils.

Responses in disease

The percentage of heterophils in the leukocyte differential of normal reptiles varies with the species. Tables 20.2a and 20.2b Heterophils can represent as much as 40% of the leukocytes in some normal reptilian species. The heterophil concentration in reptiles also is influenced by seasonal factors. For example, the heterophil concentration is highest during the summer months and is lowest during hibernation.[16] Because the primary function of heterophils is phagocytosis, significant increases in the heterophil count of reptiles usually are associated with inflammatory disease, especially microbial and parasitic infections or tissue injury. Noninflammatory conditions that may result in heterophilia include stress (i.e., glucocorticosteroid excess), neoplasia, and heterophilic leukemia.

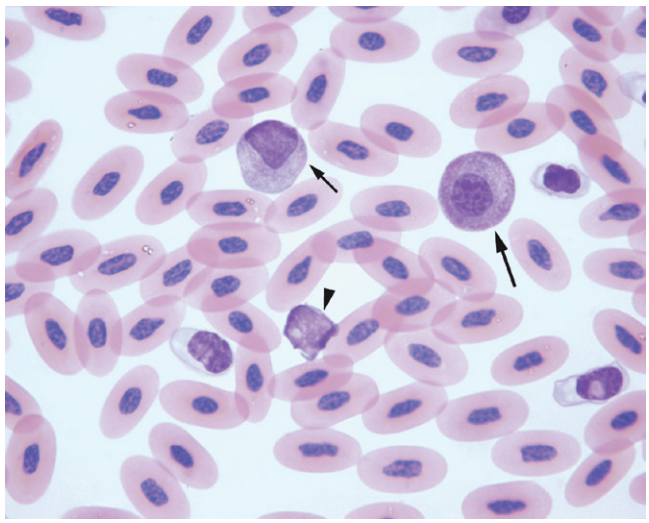

Figure 20.26 An azurophilic monocyte (large arrow), monocyte (small arrow), and lymphocyte (arrowhead) in the blood film of a snake (*Lampropeltis alterna*).The other nonerythrocytic cells are thrombocytes. Wright-Giemsa stain.

Heterophils may appear to be abnormal in reptiles suffering from a variety of diseases. For example, heterophils may exhibit varying degrees of toxicity with inflammatory diseases, especially those involving infectious agents such as bacteria. Toxic heterophils exhibit increased cytoplasmic basophilia, abnormal granulation (i.e., dark blue to purple granules or granules with abnormal shapes and staining), and cytoplasmic vacuolation (Figs. 20.27 through 20.31). Degranulated heterophils may be associated with artifacts of blood-film preparation or represent toxic changes. Nuclear lobation in species that normally do not lobate their heterophil nuclei also is an abnormal finding and suggests severe inflammation.

The number of circulating eosinophils in normal reptiles is variable. In general, lizards tend to have low numbers of eosinophils compared with some species of turtles, which can have as much as 20% eosinophils.[16,20–22,40–43] Like heterophils, the number of eosinophils present in the peripheral blood is influenced by environmental factors, such as seasonal changes. The number of eosinophils generally is lower during the summer months and highest during hibernation in some species. Eosinophilia may be associated with parasitic infections and stimulation of the immune system.

The percentage of basophils in the differential leukocyte count of normal reptiles can range from 0% to 40%.[16,21,22,40,42–45] Seasonal variation in the basophil concentration is minimal, unlike that in the acidophil concentration, which varies with the season. Some species of reptiles normally have high numbers of circulating basophils.[46] For example, some species of turtles typically have circulating basophil numbers

Table 20.2a Leukocyte parameters for selected reptiles.

	WBC × $10^3/\mu L$	Heterophils × $10^3/\mu L$	Lymphocytes × $10^3/\mu L$	Monocytes × $10^3/\mu L$	Eosinophils × $10^3/\mu L$	Basophils × $10^3/\mu L$
Lizards						
Argentine lizard[a] winter	13.1–18.1	1.5–2.2	6.7–7.7	1.8–3.0	3.4–4.4	0.2–0.4
Argentine lizard[a] summer	16.0–20.8	1.9–2.9	7.8–8.5	1.6–2.6	3.8–5.0	0.3–0.5
Adult male iguanas[b]	11.1–24.6	1.0–5.4	5.0–16.5	0.2–2.7	0.0–0.3	0.1–1.0
Adult female iguanas[b]	8.2–25.2	0.6–6.4	5.2–14.4	0.4–2.3	0.0–0.4	0.2–1.2
Juvenile iguanas[b]	8.0–22.0	1.0–3.8	6.2–17.2	0.3–0.6	0.0–0.4	0.1–0.7

[a]Troiano JC, Gould EG, Gould I. Hematological reference intervals in argentine lizard *Tupinambis merianae* [Sauria-Teiidae]. *Comp Clin Pathol* 2008;17:93–97.

[b]Harr KE, Alleman AR, Dennis PM, Maxwell LK, Lock BA, Bennett RA, Jacobson ER: Morphologic and cytochemical characteristics of blood cells and hematologic and plasma biochemical reference intervals in green iguanas. *JAVMA*, 218[6]: 915–921, 2001.

Table 20.2b Leukocyte parameters for selected reptiles.

	WBC × $10^3/\mu L$	Heterophils %	Lymphocytes %	Monocytes %	Eosinophils %	Basophils %
Lizards						
Prehensile-tailed skink[c]	3.9–22.4	16–58	2–40	0–6	0–18	4–26
Snakes						
Boa constrictor[d,e]	4–10	20–65	10–60	0–6	0–3	0–20
Ball pythons[f]	7.9–16.4	56–67	7–21	12–22	—	0–2
Yellow rat snake[g]	0.4–32.0	—	—	—	—	—
Jungle carpet python[h]						
Chelonians						
Aldabra tortoise[i]	1.0–8.3	32–79	2–40	0–8	0–7	0–4
Desert tortoise[j]	6.6–8.9	35–60	25–50	0–4	0–4	2–15

[c]Wright KM, Skeba S: Hematology and plasma chemistries of captive prehensile-tailed skinks [*Corcucia zebrata*]. *J Zoo Wildl Med* 23: 429–432, 1992.
[d]Chiodini RJ, Sundberg JP: Blood chemical values of the common boa constrictor [*Constrictor constrictor*]. *Am J Vet Res* 43: 1701–1702, 1982.
[e]Rosskopf WJ, Woerpel RW, Yanoff SR: Normal hemogram and blood chemistry values for boa constrictors and pythons. *Vet Med Small Anim Clin*, May:822–823, 1982.
[f]Johnson JH, Benson PA: Laboratory reference values for a group of captive ball pythons [*Python regius*]. *Am J Vet Res* 57: 1304–1307, 1996.
[g]Ramsey EC, Dotson TK: Tissue and serum enzyme activities in the yellow rat snake [*Elaphe obsolete quadrivitatta*]. *Am J Vet Res* 56: 423–428, 1995.
[h]Centini R, Klaphake E: Hematologic values and cytology in a population of captive jungle carpet pythons, *Morelia spilota cheynei*. *Proc Assoc Rept Amph Vet* 107–111, 2002.
[i]Carpenter JW. *Exotic Animal Formulary* 3rd ed. St. Louis, Elsevier Saunders 2001; 107.
[j]Gottdenker NL, Jacobson ER. Effect of venipuncture sites on hematologic and clinical biochemical values in desert tortoises [*Gopherus agassizii*]. *Am J Vet Res* 1995;56:19–21.

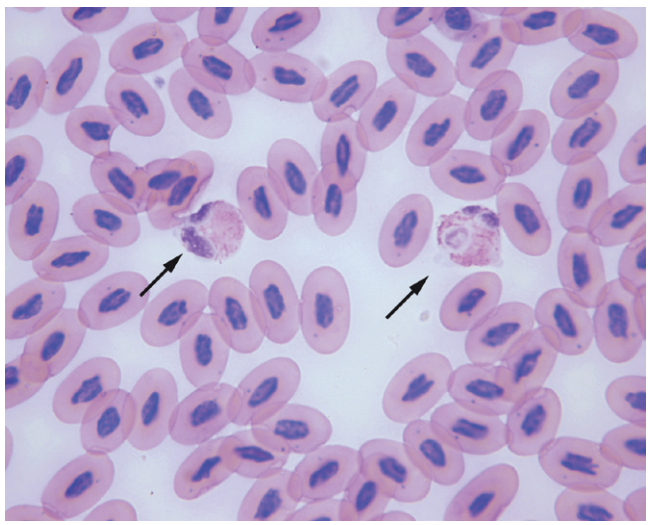

Figure 20.27 Mildly toxic (1+) heterophils (arrows) in the blood film of a lizard (*Pogona vitticeps*). Wright-Giemsa stain.

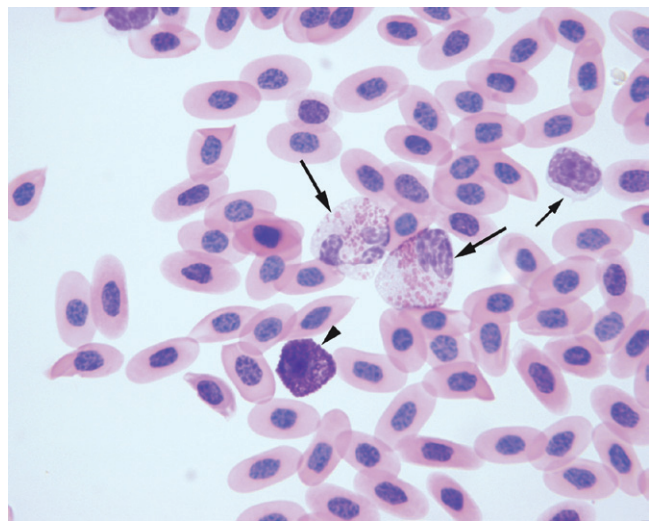

Figure 20.28 Moderately toxic (2 + to 3+) toxic heterophils (arrows), a basophil (arrowhead), and a lymphocyte (small arrow) in the blood film of a lizard (*Iguana iguana*). Wright-Giemsa stain.

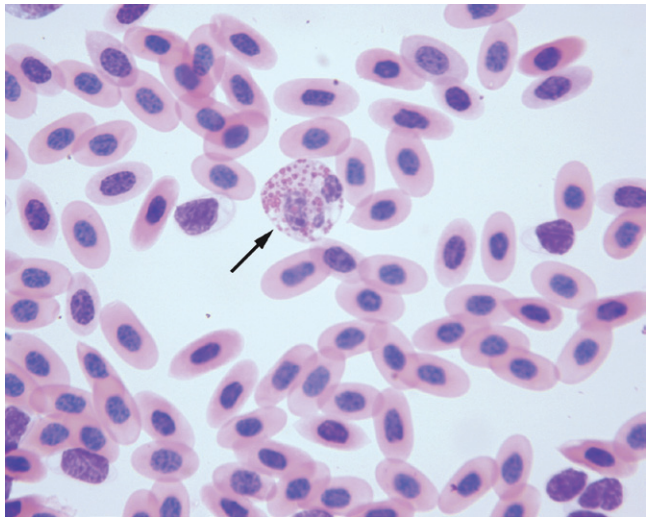

Figure 20.29 A moderately toxic (2 + to 3+) toxic heterophil (arrow) in the blood film of a lizard (*Iguana iguana*). Wright-Giemsa stain.

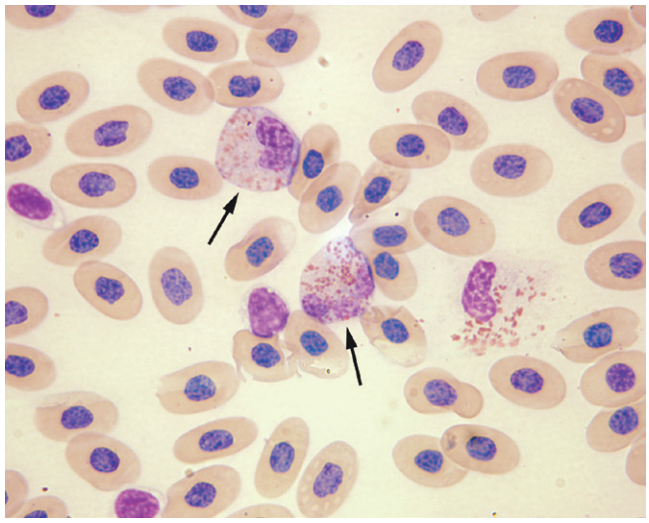

Figure 20.30 A moderately to marked toxic (3 + to 4+) toxic heterophils (arrows) in the blood film of a lizard (*Iguana iguana*). Wright-Giemsa stain.

that represent as much as 40% of the leukocyte differential, although the reason for this is unknown.

Based on the results of cytochemical and ultrastructural studies, reptilian basophils most likely function in a manner similar to that of mammalian basophils.[47,48] They appear to process surface immunoglobulins and to release histamine on degranulation. Basophilias have been associated with parasitic and viral infections.

The lymphocyte concentration in reptilian blood also varies and can represent more than 80% of the normal leukocyte differential in some species.[21] Lymphocyte numbers are influenced by several environmental and physiologic factors. Like heterophils and eosinophils, lymphocytes also are influenced by seasonal change; lymphocyte counts tend to be lowest during the winter months and highest during the summer months.[16,21,40] Temperate reptiles have decreased numbers—or even absent—lymphocytes during hibernation, after which the lymphocyte concentration increases.[49–51] Tropical reptiles also demonstrate decreased numbers of circulating lymphocytes during the winter months despite their lack of hibernation.[21] Lymphocyte numbers also are affected by gender, with the female members of some species having significantly higher lymphocyte concentrations than males of the same species.[16,21]

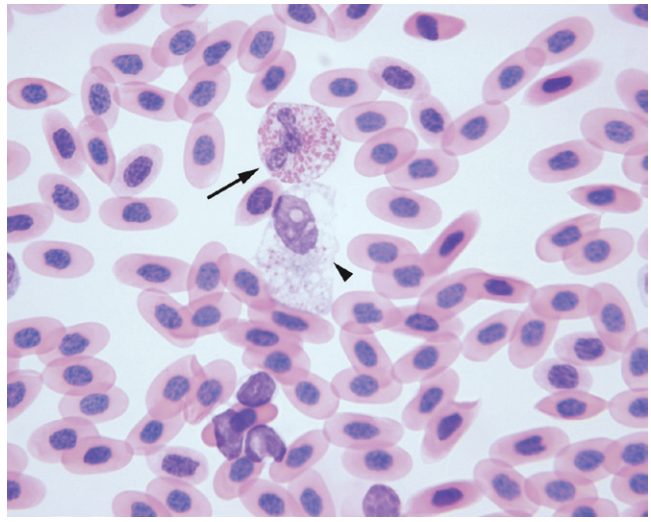

Figure 20.31 A moderately toxic (2 +) toxic heterophil (arrow) and a reactive monocyte (arrowhead) in the blood film of a lizard (*Iguana iguana*). Wright-Giemsa stain.

Reptilian lymphocytes function in a manner similar to those of birds and mammals. They have the same major classes of lymphocytes, B and T lymphocytes, that are involved with a variety of immunologic functions.[52,53] Unlike those in birds and mammals, however, the immunologic responses of ectothermic reptiles are influenced greatly by the environment. For example, low temperatures may suppress or even inhibit the immune response in reptiles.

Lymphopenia often is associated with malnutrition or is secondary to a number of diseases because of stress and immunosuppression. Lymphocytosis occurs during wound healing, inflammatory disease, parasitic infection (e.g., anasakiasis and spirorchidiasis), and viral infections. Lymphocytosis also occurs during ecdysis.[23] The presence of reactive lymphocytes and, less commonly, of plasma cells suggests stimulation of the immune system (Fig. 20.32). These cells resemble those of birds and mammals. Reactive lymphocytes have more abundant, deeply basophilic cytoplasm compared with normal lymphocytes, and their nuclear chromatin may appear to be less condensed. Plasma cells have abundant, intensely basophilic cytoplasm that contains a distinct Golgi zone and an eccentrically positioned nucleus.

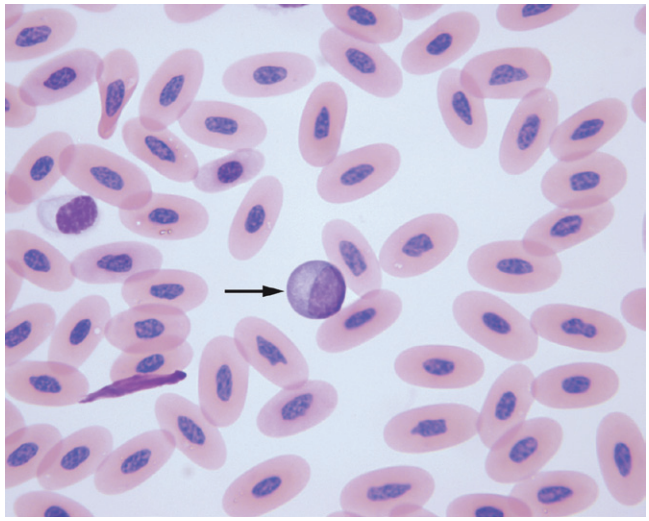

Figure 20.32 A plasma cell (arrow) in the blood film of a snake (*Lampropeltis alterna*). Wright-Giemsa stain.

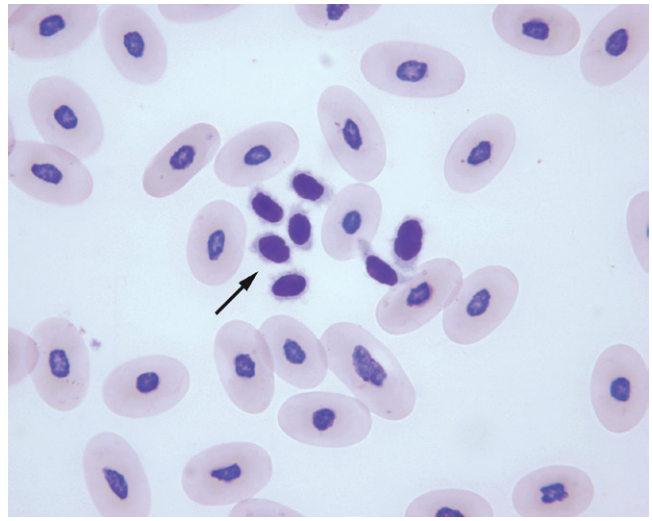

Figure 20.33 Thrombocytes (arrow) in the blood film of a tortoise (*Testudo graeca*). Wright-Giemsa stain.

Monocytes generally occur in low numbers in the blood films of normal reptiles, ranging between 0% and 10% of the leukocyte differential.[16,21,22,40–43,45,54,55] Snakes typically have monocytes with an azurophilic appearance to the cytoplasm, which frequently is referred to as azurophils in the literature (Figs. 20.24 through 20.26). The monocyte concentration changes little with seasonal variation. Monocytosis suggests inflammatory diseases, especially granulomatous inflammation. A progressive increase in monocyte numbers along with the total leukocyte count and heterophil numbers have been associated with an increase in the tumor score of green turtles (*Chelonia mydas*) with fibropapillomastosis.[56]

Circulating siderophagocytes and erythrophagocytes are occasionally found in the blood film of normal reptiles as well as those undergoing a hemolytic disorder.[57] Unlike mammals, reptiles do not have lymph nodes that filter out macrophages involved in erythrophagocytosis occurring in various parts of the body, such as the coelomic cavity. Instead, macrophages undergoing erythrophagocytosis and heme catabolism appear to be transported in the blood to sites such as the spleen for filtering.

Although considered to be rare, several cases of leukemia have been reported in reptiles.[58–63] The myeloproliferative diseases of reptiles can be classified in the same manner as those in mammals. Special cytochemical studies may be required to identify the abnormal cells.

Hematology is most valuable as a tool for assessing the response of reptilian patients to disease or therapy. A favorable response in the leukogram is a shift from a leukocytosis or leukopenia to a normal leukocyte concentration. A normal heterophil, eosinophil, or monocyte count after a heterophilia, eosinophilia, or monocytosis, respectively, usually indicates improved patient status. Disappearance of toxic heterophils, reactive lymphocytes, and plasma cells from the blood film indicates improvement and a favorable response to therapy. Anemic reptiles exhibiting an erythrocytic regenerative response have a better prognosis compared with those exhibiting little or no response. Similarly, a normal thrombocyte concentration after a thrombocytopenia indicates a favorable response. Therefore, hematology can be a valuable tool in the assessment of reptilian patients.

Thrombocytes and hemostasis

Morphology

Thrombocytes of reptiles appear as elliptical to fusiform, nucleated cells (Figs. 20.14, 20.26, 20.33, and 20.34). The centrally positioned nucleus has dense nuclear chromatin that stains purple, whereas the cytoplasm typically is colorless and may contain a few azurophilic granules. Activated thrombocytes are common and appear as clusters of cells with irregular cytoplasmic margins and vacuoles. Thrombocytes appear to be devoid of cytoplasm when aggregated.

Laboratory evaluation

The actual thrombocyte concentration may be difficult to determine, because thrombocytes tend to clump both in vitro and when exposed to heparin, which is a commonly used anticoagulant in reptilian hematology. The thrombocyte concentration can be measured using the Natt and Herrick method for obtained erythrocyte and leukocyte counts. After preparing the 1: 200 dilution of the blood with Natt-Herrick solution and charging a Neubauer-ruled hemocytometer, the number of thrombocytes in the entire central ruled area (i.e., the central, large square) are counted on both sides of the hemocytometer. The number of thrombocytes per microliter of blood is obtained by multiplying that

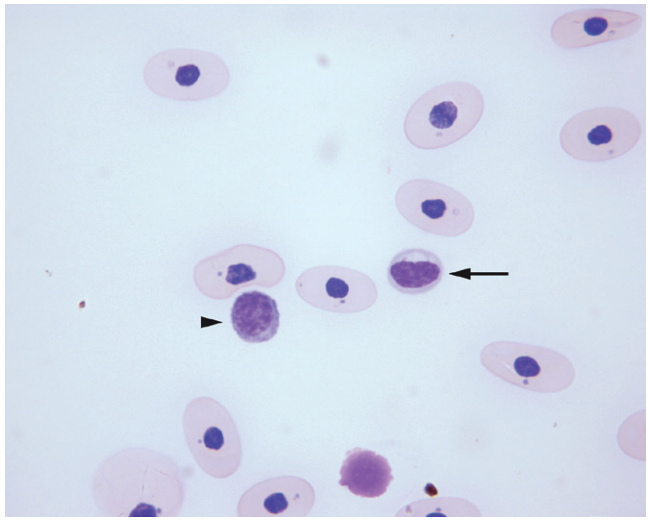

Figure 20.34 A thrombocyte (arrow) and a lymphocyte (arrowhead) in the blood film of a turtle (*Trachemys scripta elegans*). Wright-Giemsa stain.

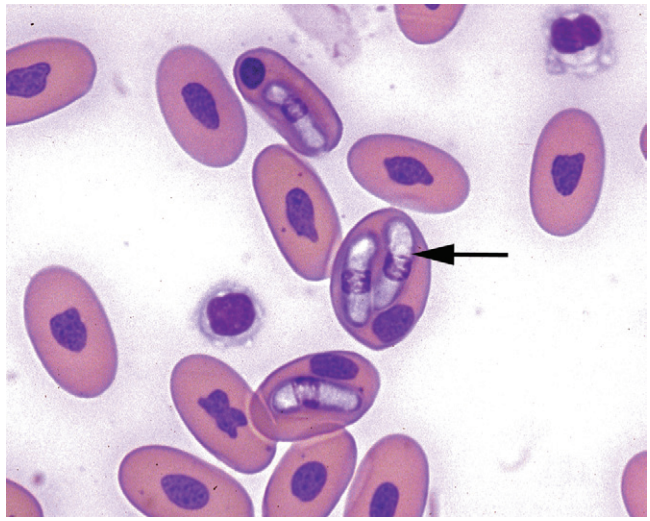

Figure 20.35 A hemogregarine, most likely Hepatozoon, in the blood film of a snake (*Corallus canina*). Wright-Giemsa stain.

number by 1000. A subjective thrombocyte concentration can be determined based on the number of thrombocytes that appear in a stained blood film and it can be reported as either reduced, normal, or increased. Thrombocytes typically occur in numbers that range between 25 and 350 thrombocytes per 100 leukocytes in the blood film of normal reptiles.

Responses to disease

Reptilian thrombocyte numbers vary with species and seasons. For example, the mean absolute thrombocyte count of the argentine lizard (*Tupinambis merianae*) is $7.2 \times 10^3/\mu L$ during the winter months but $9.1 \times 10^3/\mu L$ during the summer.[64]

Reptilian thrombocytes have a significant role in thrombus formation, and they function similarly to avian thrombocytes and mammalian platelets. The ultrastructural features of activated reptilian thrombocytes include pseudopodia with fine, granular material and many fibrin-like filaments radiating both between and around the cells.[21] Immature thrombocytes of reptiles resemble the immature thrombocytes of birds and, when present in blood films, represent a regenerative response. Thrombocytopenias of reptiles most likely result from excessive peripheral utilization of thrombocytes or decreased thrombocyte production. Thrombocytes with polymorphic nuclei are considered to be abnormal, and may be associated with severe inflammatory disease.

Blood parasites

Blood parasites are common in reptiles. Their presence usually is considered to be an incidental finding; however, some have the potential of causing disease, such as hemolytic anemia.

Common hemoprotozoa include the hemogregarines, trypanosomes, and *Plasmodium*.[65] Less commonly encountered hemoprotozoans include *Leishmania*, *Saurocytozoon*, *Haemoproteus*, and *Schellackia*, and the piroplasmids. Microfilaria are commonly found in the peripheral blood films of some reptiles.

Hemogregarines

Hemogregarines are the most common group of sporozoan hemoparasites affecting reptiles, especially snakes.[66–69] The three genera of hemogregarines that are common in reptiles are *Hemogregarina*, *Hepatozoon*, and *Karyolysus*. The hemogregarines are not easily differentiated based on the appearance of their gametocytes within the cytoplasm of erythrocytes or tissue schizonts.[70,71] The hemogregarines found in snakes usually belong to the genus *Hepatozoon* and those of semiaquatic freshwater turtles usually to the genus *Hemogregarina*. *Karyolysus* typically occurs in Old World lizards and, possibly, tree snakes. No cases of hemogregariniasis have been reported in sea turtles, and the parasites are rare in tortoises. Accurate classification of the hemogregarines into their appropriate genus cannot be accomplished based on their appearance in the blood film alone. Therefore, the general term hemogregarine is used when reporting their presence in blood films during hematologic examinations.

Hemogregarines are identified by the presence of intracytoplasmic gametocytes in erythrocytes (Figs. 20.35 and 20.36). The sausage-shaped gametocytes have a colorless to pale purple cytoplasm that lacks the refractile pigment granules found in the gametocytes of *Plasmodium* and *Haemoproteus*, and they distort the host cell by creating a bulge in the cytoplasm.[3,72] Typically, only one gametocyte is found per

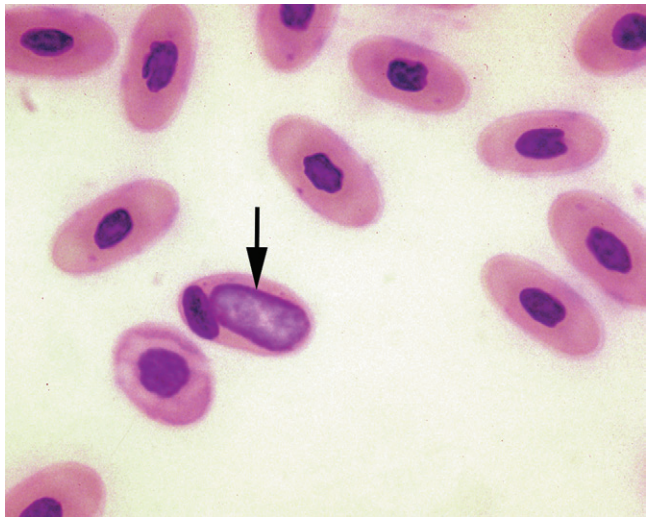

Figure 20.36 An erythrocytic inclusion resembling that of a hemogregarine in the blood film of a tortoise (*Geochelone emys*). Wright-Giemsa stain.

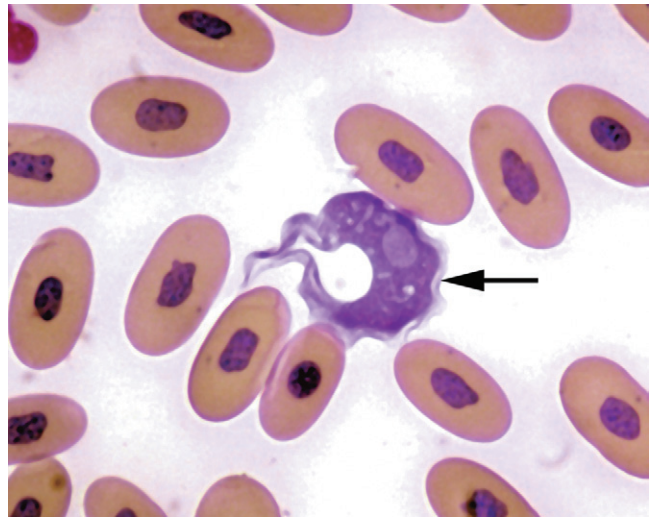

Figure 20.37 A trypanosome in the blood film of a snake (*Corallus canina*). Wright-Giemsa stain.

erythrocyte; however, in heavy infections, two gametocytes may be found in one cell.

Hemogregarines have a lifecycle that involves sexual reproduction (sporogony) in an invertebrate host and asexual multiplication (merogony) in a reptilian host. The parasite infects the reptilian host when the sporozoites are transmitted from the invertebrate host as it feeds on the blood of the reptile or is ingested by the reptile. Several biting, invertebrate hosts (i.e., mites, ticks, mosquitoes, flies, and bugs) can transmit the parasite to terrestrial reptiles, whereas leeches appear to be the primary intermediate host for the hemogregarines of aquatic reptiles. Reptilian hemogregarines are well adapted to their natural host and do not cause clinical disease; however, because they are relatively not host-specific, they can cause significant clinical disease in unnatural or aberrant host species.[69] Such infections result in severe inflammatory lesions associated with schizonts in a variety of organs.

Trypanosomes

The trypanosomes found in reptiles resemble those found in mammals and birds. They are large, extracellular, flagellate protozoa with a bladelike shape, a single flagellum, and a prominent, undulating membrane (Fig. 20.37). For transmission, they require a bloodsucking invertebrate host, such as biting flies for terrestrial reptiles or leeches for aquatic reptiles. Trypanosomes have been found in all orders of reptiles, have a worldwide distribution, rarely cause clinical disease, and often are associated with lifelong infections.[71]

Plasmodium

More than 60 species of *Plasmodium* have been described in reptiles; most have been identified in lizards and a few in snakes.[71,73] *Plasmodium* in reptiles resemble those found in birds (see Chapter 19 on avian hematology). The gametocytes have refractile pigment granules that aid in differentiation between *Plasmodium* and hemogregarines. Also, unlike hemogregarines, *Plasmodium* schizogony (packets of merozoites) can occur in blood cells. The trophozoites are small, signet-ring structures in the cytoplasm of erythrocytes. The lifecycle of *Plasmodium* involves a sporogony stage in an insect host (e.g., mosquito) and schizogony and gametogony in a reptile host. Infections with *Plasmodium* can result in a severe hemolytic anemia.

Sauroleishmania

Sauroleishmania rarely are seen in blood films of reptiles. The organism is related to trypanosomes, and it primarily infects lizards.[70] When present, the organism (i.e., amastigote or leishmanial stage) appears as a round to oval inclusion of 2–4 µm with blue cytoplasm and an oval, red nucleus in the cytoplasm of thrombocytes or mononuclear leukocytes.

Saurocytozoon

Saurocytozoon produce large, round gametocytes that lack pigment granules in the cytoplasm of leukocytes in peripheral blood films. Only the gametocyte stage is found in the peripheral blood, and schizogony occurs in the tissues. The organism resembles *Leukocytozoon* of birds, because it grossly distorts the host cell that it parasitizes (see Chapter 19). As indicated by its name, this is a parasite of lizards and, most likely, is transmitted by mosquitoes.[70]

Lainsonia and *Schellackia*

Lainsonia and *Schellackia* are coccidian parasites of lizards and snakes. They produce schizonts that can be found in the intestinal epithelium and sporozoites that can be found in

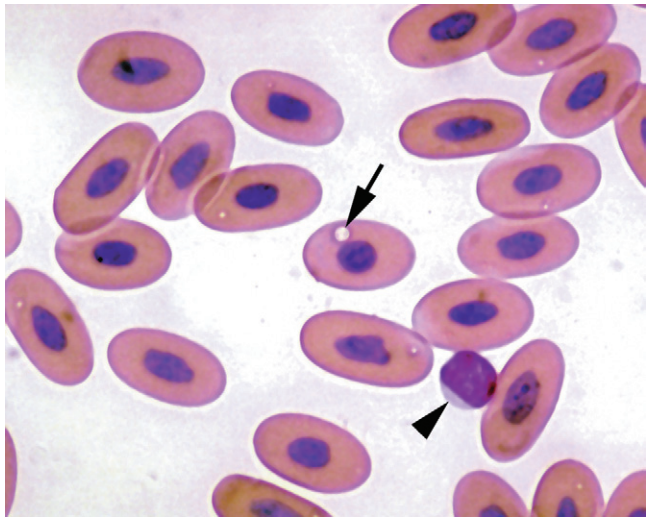

Figure 20.38 An erythrocytic cytoplasmic *Serpentoplasma* inclusion (arrow) in the blood film of a snake (*Corallus canina*). A small lymphocyte is also present (arrowhead). Wright-Giemsa stain.

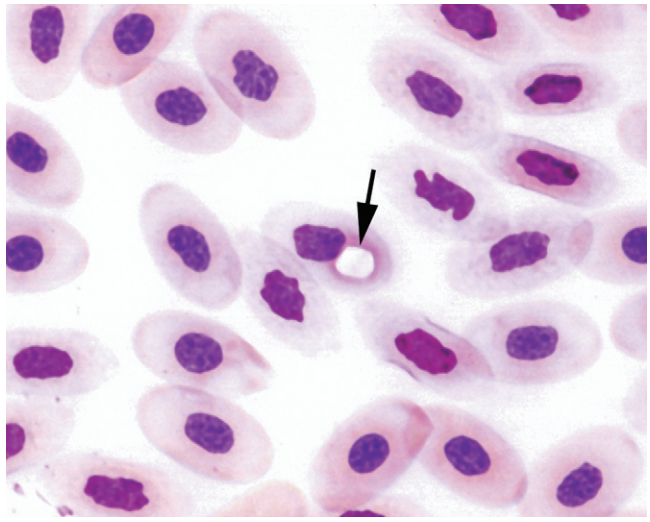

Figure 20.39 A rectangular, vacuole or clear cytoplasmic inclusion in the cytoplasm of an erythrocyte in the blood film of a lizard (*Iguana iguana*). Wright-Giemsa stain.

the peripheral blood.[70] The sporozoites are intracytoplasmic inclusions that are seen in erythrocytes and mononuclear leukocytes, primarily lymphocytes that resemble those of *Atoxoplasma* in birds (see Chapter 19). The parasite is identified by the round to oval, pale-staining, nonpigmented inclusions that deform the host-cell nucleus into a crescent shape. *Schellackia* and *Lainsonia* are transmitted by mites or, possibly, by ingestion of oocysts from feces.

Piroplasmids

The piroplasmids of reptiles include *Babesia*, *Aegyptianella* (*Tunetella*), and *Sauroplasma* or *Serpentoplasma*. They have been reported in chelonians, lizards, and snakes and appear as small, nonpigmented inclusions in the cytoplasm of erythrocytes. The inclusions are small, round to piriform, nonpigmented, and signet ring–like vacuoles measuring from 1 to 2 µm in diameter (Fig. 20.38).[70] Piroplasmids commonly found in the peripheral blood erythrocytes of lizards are referred to as *Sauroplasma*, whereas the same organisms in the blood of snakes are called *Serpentoplasma*. The piroplasmids are transmitted by biting insects or arthropods. They reproduce by either schizogony or binary fission.

Pirohemocyton

Pirhemocytonoisis is characterized by the presence of intraerythrocytic inclusions in lizards. The inclusions appear as red, punctate to oval bodies that may be associated with vacuoles (albuminoid vacuoles) and irregular, pale-staining areas in the cytoplasm of erythrocytes in Giemsa-stained blood films. Similar inclusions have been reported in snakes and turtles.[25,27] These intraerythrocytic inclusions of reptiles were previously considered to be a piroplasm, referred to as *Pirhemocyton*, until ultrastructural studies revealed the presence of a virus consistent with members of the Iridoviridae. One report of pirhemocytonosis in snakes was suggestive of an oncornavirus based on the results of ultrastructural studies. As the infection develops, the inclusions increase in size, measuring between 0.5 and 1.5 µm.[30] A single inclusion per erythrocyte is typical; however, two inclusions per cell may occur on occasion. Natural infections with the lizard erythrocytic virus appear to be nonfatal, even with high viremias (i.e., >85% of the erythrocytes are infected) that result in the appearance of spindle-shaped or thin, elongate erythrocytes.[30]

The square to rectangular (occasionally hexagonal transparent to crystalline-like intracytoplasmic inclusions in erythrocytes of reptiles, especially iguanas may be associated with hemoglobin crystals.[15] These are often referred to as *Pirhemocyton* inclusions and are frequently found in healthy reptiles (Fig. 20.39). They appear to have no clinical significance.

Haemoproteus

Haemoproteus (*Haemocystidium*) has been reported in lizards, turtles, and snakes.[70,73] They resemble the *Haemoproteus* found in birds; only the gametocytes with refractile pigment granules are found in the peripheral blood films (see Chapter 19). The parasite can cause dehemoglobinization of the infected erythrocyte.[73]

Microfilaria

Microfilaremia in reptiles typically is not associated with clinical signs of illness or changes in the hemogram or blood biochemical profile.[74,75] The reptile typically survives for years with these parasites, and microfilaria are detected as an incidental finding on examination of routine Romanowsky-stained blood films. Microfilaria are produced by adult

female filarid nematodes, which can live in various locations in the body of a reptile. Microfilaria are ingested by a suitable bloodsucking arthropod (i.e., tick or mite) or insect (i.e., mosquito), in which they develop into the infective, third-stage larval form. The lifecycle is complete when the infective form enters a new reptilian host during intermediate-host feeding.

Hematopoiesis

The bone marrow appears to be the primary site for erythropoiesis, granulopoiesis, and thrombopoiesis in adult reptiles. The bone marrow of some reptiles, especially turtles and tortoises, is not gelatinous, and hematopoietic cells may be difficult to sample for study. A saline-soak technique can be used for turtles in which a 2 mm thickness of bone is allowed to soak for 18 to 24 hours at 4°C, and then agitated for 30 minutes, and the solution centrifuged to obtain the hematopoietic cells.[76]

Erythropoiesis in the bone marrow occurs within the vascular space of the reticular stroma.[14,76] Foci of extra medullary erythropoiesis, in the liver and spleen, are common. The maturation stages of reptilian erythrocytes appear to be similar to those of birds and mammals. In general, seven recognizable stages are involved in erythrocyte development: rubriblasts, prorubricytes, basophilic rubricytes, early polychromatic rubricytes, late polychromatic rubricytes, polychromatic erythrocytes, and mature erythrocytes. The morphologic features of these cells are similar to those described in birds (see Chapter 19).

As the reptilian erythrocyte matures, the cell becomes larger and the cytoplasm increasingly eosinophilic because of increased hemoglobin synthesis. A clear, size-related progression in erythrocyte development may not be evident in some species, but the shape of the cell changes from spherical to a flattened ellipsoid with maturation. The erythrocyte nucleus also decreases in size, with its shape changing from round to ellipsoid, and the nuclear chromatin becomes increasingly condensed as the cell matures. Sudan black B stain can be used as an erythrocyte marker that stains the cytoplasm of erythrocyte precursors and mature erythrocytes dark gray to black.

Developing granulocytes are morphologically similar to mammalian granulocytes and are associated with the extra vascular spaces of the bone marrow reticular stroma.[14] The maturing granulocytes migrate through the endothelial cells of the sinusoids to enter the bloodstream. The maturation stages of the granulocytes of reptiles also resemble those of birds (see Chapter 19, Avian Hematology) (Fig. 20.40). As the granulocyte matures, the cell decreases in size, and the cytoplasm becomes less basophilic. Specific, characteristic granules appear in the myelocyte and metamyelocyte stages of development, and these increase in number with maturation. The nuclear chromatin becomes increasingly condensed with maturity, and in those species that lobate their nuclei, the nucleus changes from round to segmented. Mature and immature heterophils from some species stain positive with chloroacetate esterase, α-naphthyl butyrate esterase, α-naphthyl acetate esterase, and leukocyte alkaline phosphatase chemical stains.[15,37,76,77] The cytoplasmic granules of the eosinophils of some reptiles typically are large, round, and pink with Romanowsky stains and golden brown with benzedrine peroxidase, which helps in the differentiation of eosinophil precursors from heterophil precursors.

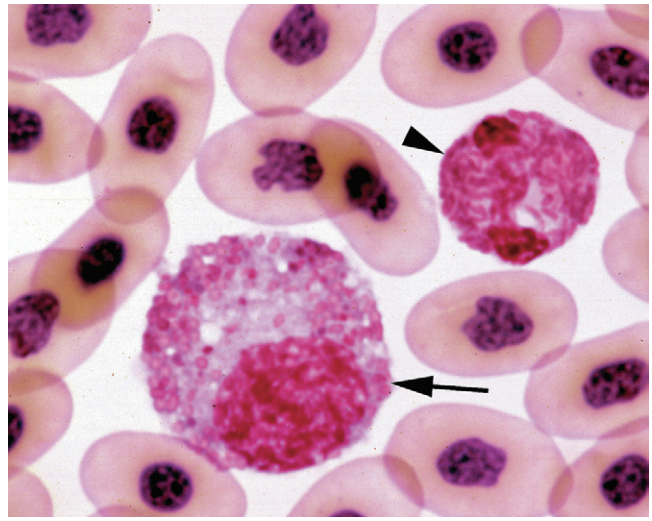

Figure 20.40 A progranulocyte (arrow) and a mature heterophil (arrowhead) in the blood film of a lizard (*Iguana iguana*). Wright-Giemsa stain.

Thrombopoiesis in reptiles is similar to that in birds (see Chapter 19 on avian hematology). The elliptical, mature thrombocytes are derived from round precursor cells. As thrombocytes develop, they become smaller and the cytoplasm becomes less basophilic. The shape of the cell nucleus changes from round to oval with maturity. During the later stages of development, the nuclear chromatin becomes densely packed, and specific cytoplasmic granules may appear. It often is difficult to differentiate thrombocytes from lymphocytes in hematopoietic specimens. Special chemical stains may be used to differentiate the two in some species, in which thrombocytes stain positive with periodic acid-Schiff, acid phosphatase, and α-naphthyl butyrate esterase and lymphocytes do not.[22,37,76]

The thymus is the first lymphoid organ to develop in reptiles. The lymphocytes derive from bloodborne stem cells, which most likely originate from the yolk sac.[14] The origin of the immunoglobulin-producing cells (i.e., B lymphocytes) is unknown, because a reptilian equivalent to the avian bursa of Fabricius has not been found. During the early stages of splenic development, large numbers of granulocytes are present, indicating granulopoiesis. With later development, however, they disappear, and the spleen

becomes primarily involved with lymphopoiesis. Lymphopoiesis of reptiles resembles that of mammals and birds. Reptilian lymphoblasts, prolymphocytes, and mature lymphocytes appear to be identical to those found in birds and mammals, and they can be found in lymphopoietic tissues such as the spleen.

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21 Hematology of Fish

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Hematologic evaluation of fish is not routinely used in establishing the diagnosis of fish diseases, but it can be useful in the detection of diseases affecting the cellular components of blood. Certain diseases of fish result in anemia, leukopenia, leukocytosis, thrombocytopenia, and other abnormal changes of the blood cells. Evaluation of the hemogram also may be useful in following the progress of the disease or the response to therapy.

Blood collection and handling

Blood for diagnostic sampling can be collected safely from fish that are greater than 3 inches (8 cm) in length.[34] The collection procedure itself should be accomplished in less than 30 seconds, however, because fish that are held out of water for longer periods suffer from respiratory distress and electrolyte imbalance. Blood for hematologic evaluation should be collected in either heparin or ethylenediaminetetraacetic acid (EDTA) as an anticoagulant. Disadvantages of heparin include the tendency for leukocytes and thrombocytes to clump and the creation of a blue tinge to blood films with Romanowsky stains. In addition, if the blood sample contains a small clot, heparin may not prevent coagulation once it has started. Disadvantages of EDTA include the hemolysis of erythrocytes in some fish species. Hemolysis also can occur with the use of tricaine sedation or anesthesia, but cooling the blood sample to 25°C and rapidly preparing the film can minimize the hemolysis associated with tricaine.[34]

Blood can be collected from fish via the caudal vertebral vein or artery.[7,31] Venipuncture of these vessels can be accomplished with or without sedation or anesthesia, and the caudal vertebral vein or artery can be approached either ventrally or laterally.[7] The ventral approach involves insertion of the needle under a scale along the ventral midline near the base of the caudal peduncle (Fig. 21.1). The needle is then directed toward the vertebral bodies. After reaching the vertebral bodies, the needle is withdrawn slightly, both ventrally and laterally, while negative pressure is applied to the syringe. Once the vessels have been entered, blood will begin to enter the syringe. The needle may need to be rotated slightly to properly position the needle hub in the vessel to facilitate collection of the blood.

A lateral approach to the caudal vertebral vessels is performed by inserting the needle a few millimeters below the lateral line near the base of the caudal peduncle (Fig. 21.2). The needle is then directed toward the midline and under the vertebral bodies, and blood is aspirated into the syringe as described for the ventral approach.

Blood can be collected from the heart or bulbous arteriosus using a ventral approach. The needle is inserted slightly caudal to the apex of the V-shaped notch formed by the gill covers (opercula) and isthmus, and it is then advanced toward the heart while a slight vacuum is applied to the syringe. Blood will enter the syringe once the heart is penetrated. An anterolateral approach through an opened gill opercular cover also can be used to reach the heart. In this approach, the needle is directed caudally, from a point one-third of the distance between the ventral limit of the cavity (gill chamber) and medial to the bony support of the caudal wall of the opercular cavity. The needle is then advanced toward the heart using a slight vacuum. Cardiocentesis carries a greater risk of damage to the fish than use of the caudal vertebral vessels for blood collection.

Blood can be collected from large sharks using the vein that courses caudal and slightly ventral to the dorsal fins.[7] With the shark restrained in ventral recumbency or in a sling with its back exposed, a needle is inserted through the soft skin just under the caudal aspect of a dorsal fin as it is lifted dorsally (Fig. 21.3). The needle is then directed under the dorsal fin but is kept to the back and slightly off the midline.

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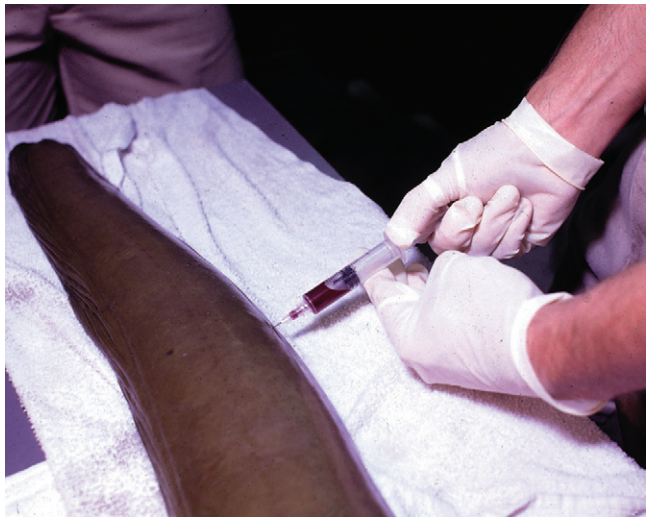

Figure 21.1 Venipuncture of the caudal vein in an eel (*Gymnothorax funebria*) using the ventral approach.

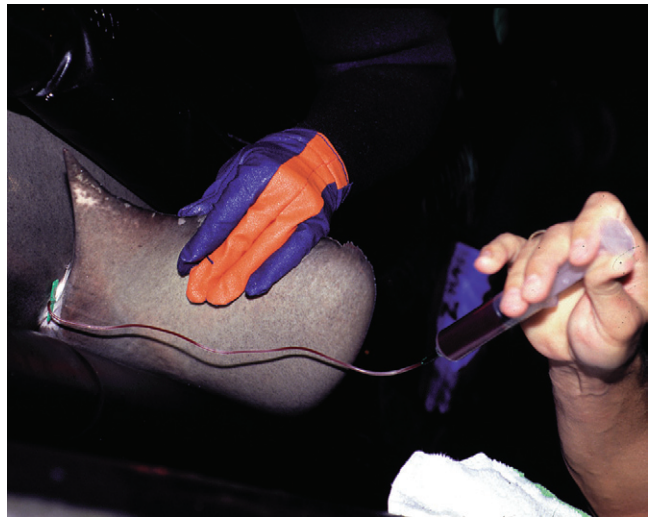

Figure 21.3 Blood collection from a shark (*Negaprion brevirostris*) using the blood vessel under the dorsal fin.

Figure 21.2 Venipuncture of the caudal vein in a grouper (*Epinephellus sp.*) using the lateral approach.

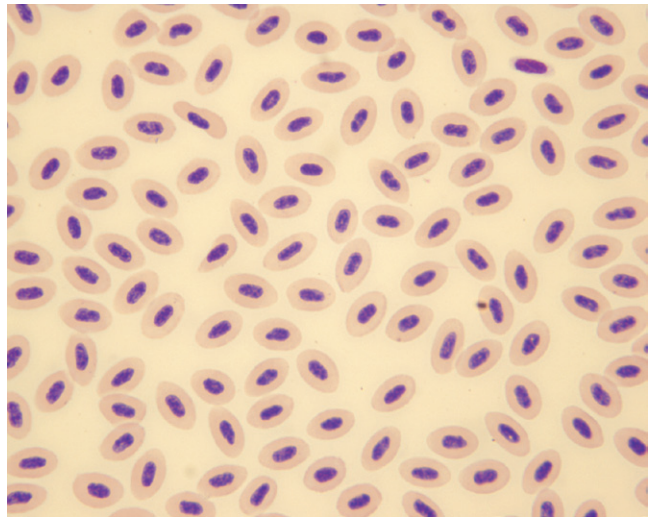

Figure 21.4 Normal mature erythrocytes in the blood film of a bony fish (*Barborymus schwanenfeldii*). Wright-Giemsa stain.

Use of a needle with an extension tube is often helpful for keeping the needle in position should the shark move during the procedure. Advantages of this method compared with venipuncture of the caudal vertebral vessels in large sharks include ease of access to the vessel and restraint of large sharks when using the dorsal fin approach.

Erythrocytes

Morphology

Normal, mature erythrocytes of fish are oval to ellipsoidal, have abundant pale eosinophilic cytoplasm, and include a centrally positioned, oval to ellipsoidal nucleus in Romanowsky-stained blood films (Figs. 21.4 through 21.6). The long axis of the nucleus is parallel to that of the cell, except in a few species with round erythrocyte nuclei. The nuclei of fish erythrocytes can be large, occupying as much as one-fourth (or more) of the cell volume. The nuclear chromatin is densely clumped and stains dark purple. The cytoplasm typically is homogeneous, but it may contain variable amounts of rarefied or pale-staining areas or vacuoles associated with the degeneration of organelles.

Both the size and number of erythrocytes vary between species of fish and, depending on the physiologic conditions, even within a single species. For example, the erythrocytes of fish belonging to the class Chondrichthyes (sharks and rays) generally are larger than those of the class Osteichthyes

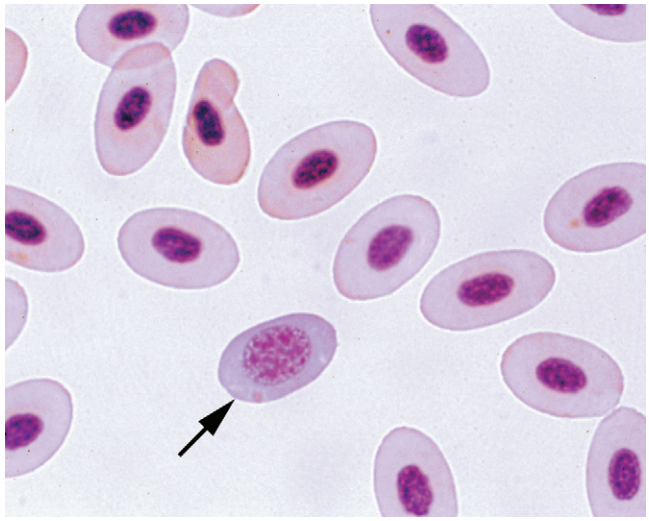

Figure 21.5 Normal mature erythrocytes and a polychromatic erythrocyte (arrow) in the blood of a bony fish (*Gymnothorax funebria*). Wright-Giemsa stain.

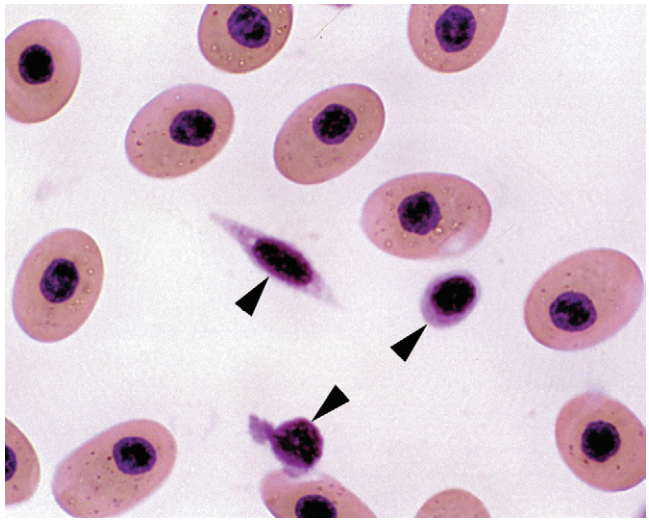

Figure 21.6 Normal mature erythrocytes and thrombocytes (arrowheads) in the blood film of a cartilaginous fish (*Pristis pectinatai*). Wright-Giemsa stain.

(bony fish) (Fig. 21.6).[39] Mature erythrocytes of some fish are biconvex, with a central swelling that corresponds to the position of the nucleus, whereas those of other species are flattened and biconcave.[22]

Slight to moderate anisocytosis and polychromasia are normal in many species of fish. Polychromatic erythrocytes have a pale blue cytoplasm compared with that of mature erythrocytes (Fig. 21.5). They also may appear to be more rounded and to have a less condensed nuclear chromatin.

Because erythropoiesis occurs in the peripheral blood of normal fish, immature erythrocytes may be found in blood films.[39,45,46] Immature erythrocytes have larger, less condensed nuclei and less cytoplasm than mature erythrocytes (Fig. 21.7). Immature erythrocytes (i.e., rubriblasts, prorubricytes, and rubricytes) are round cells with centrally positioned, round nuclei. Depending on the stage of development, the cytoplasmic volume varies in both the amount and intensity of basophilic staining with Romanowsky stains. Erythroid cells in mitosis also may be present in the peripheral blood films from normal fish.

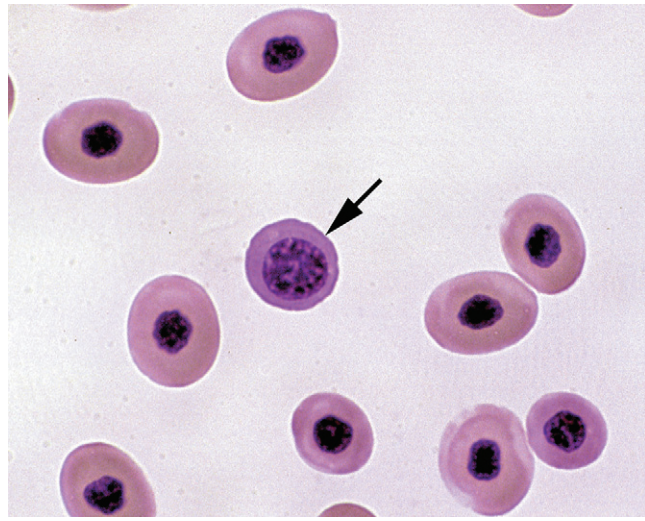

Figure 21.7 An immature erythrocyte (arrow) in the blood film of a cartilaginous fish (*Pristis pectinatai*). Wright-Giemsa stain.

Ultrastructurally, mature fish erythrocytes have a finely granular cytoplasm with no inclusions, whereas immature erythrocytes have a cytoplasm with mitochondria, Golgi complex, and small vacuoles.[23,39]

Laboratory evaluation

Determination of packed cell volume (PCV) is the most commonly used method for evaluating the red cell mass of fish. The microhematocrit method is used for obtaining a PCV of fish blood.

Although a variety of methods have been used to determine the hemoglobin concentration in fish blood, the cyanmethemoglobin method provides the most consistent results.[26] As with avian and reptilian hemoglobin determinations, this procedure requires centrifugation of the blood–cyanmethemoglobin reagent mixture to remove the free erythrocyte nuclei before measurement of optical density.

A total erythrocyte count (TRBC) in fish can be determined by a manual counting method using a hemocytometer or by an electronic cell counter. Three manual methods that can be used to obtain TRBCs in fish blood use the erythrocyte Unopette system (Becton-Dickinson, Rutherford, NJ), Natt-Herrick solution (see Chapter 19 on avian hematology), or modified Dacie's solution (Table 19.1). The erythrocyte Unopette method is the easiest of the three, because the 1:200 dilution of whole anticoagulated blood is made using

Table 21.1 Erythrocyte parameters for selected teleost fish.

	PCV [%]	RBC [×10^6/μL]	Hb [g/dL]	MCV [fl]	MCHC [g/dL]
Bass, hybrid[a]	23–47	3.66–4.96	8–12	81–106	22–30
Channel catfish[b]	40	2.44	—	—	—
Flounder[c]	17–26	1.7–2.6	4.2–6.0	90–126	—
Goldfish[d]	38–40	1.6–1.8	9.7–10.6	241–245	26
Red pacu[e]	25	1.68	—	—	—
Tilapia[f]	27–37	1.91–2.83	7.0–9.8	115–183	22–29
Trout[g]	21–44	0.77–1.67	1.5–7.7	192–420	14.4–70.0

[a]Hrubec TC, Smith SA, Robertson JL, *et al.* (1996) Comparison of hematologic reference intervals between cultured system and type of hybrid striped bass. *Am J Vet Res* 57: 618–23.
[b]Grizzle JM, Rogers WA (1976) *Anatomy and Histology of the Channel Catfish*. Opelika, AL: Craftmaster Printers, 18.
[c]Bridges DW, Cech JJ Jr, Pedro DN (1976) Seasonal hematological changes in winter flounder. *Pseudopleuronectes americanus*. *Trans Am Fish Soc* 105: 596–600.
[d]Burton CB, Murray SA (1979) Effects of density on goldfish blood: I, hematology. *Comp Biochem Physiol* 62A: 555–8.
[e]Tocidlowski ME, Lewbart GA, Stoskopf MK (1997) Hematologic study of the red pacu [*Colossoma brachypornum*]. *Vet Clin Pathol* 26: 119–25.
[f]Hrubec TC, Cardinale JL, Smith SA (2000) Hematology and plasma chemistry reference intervals for cultured Tilapia [*Oreochromis* hybrid]. *Vet Clin Pathol* 29: 7–12.
[g]Miller WR, Hendricks AC, Cairns J (1983) Normal ranges for diagnostically important hematological and blood chemistry characteristics of rainbow trout [*Salmo gairdneri*]. *Can J Fish Aquat Sci* 40: 420–5.

the diluent, pipette, and mixing vial provided with the kit. The Natt-Herrick and modified Dacie's staining methods require preparation of the diluent/stain solution and use of the red blood cell–diluting pipette.[10,32] Blood is drawn to the 0.5 mark on the pipette, and either Natt-Herrick or modified Dacie's stain is drawn to the 101 mark to prepare the 1:200 dilution. The diluted blood is then discharged onto the hemocytometer counting chamber and is allowed to settle for a minimum of 5 minutes before counting. With these stains, the oval erythrocytes show a small, dark blue nucleus that is surrounded by a colorless to faint pink cytoplasm. The total number of erythrocytes in the four corner and central squares in the central, large square of a Neubauer-ruled hemocytometer chamber is obtained using ×40 (high dry) magnification. The TRBC is calculated by multiplying the numbers of erythrocytes by 10,000.

The red blood cell indices (i.e., mean erythrocyte volume (MCV), mean corpuscular hemoglobin concentration (MCHC), and mean cell hemoglobin (MCH)) can be calculated using standard formulas. However, the direct, electronic measurement of MCV appears to be more sensitive for detecting changes in the erythrocyte size in fish and is more reproducible than the calculated MCV.[19] Table 21.1 offers erythrocyte reference values for selected teleost fish.

Responses in disease

The standard practices for collecting, handling, and analyzing blood from mammals and birds can be misleading when applied to fish. Emersion and handling of fish for venipuncture or cardiocentesis can have a marked effect on the hemogram, significantly increasing the hematocrit by as much as 25%.[38] The magnitude of this effect relates directly to the handling and analytic time. Handling of fish for as little as 20 seconds results in the release of catecholamines, which tend to cause hemoconcentration and swelling of the erythrocytes. Therefore, the hematocrit increases, but the hemoglobin concentration remains the same, thereby resulting in a decreased MCHC. The increase in blood catecholamines causes ion exchanges (Na^+/H^+ and Cl^-/HCO^-) across the erythrocyte membrane; thus, as Na^+ and Cl^- enter the cell, water follows osmotically, causing the cell to swell. Cannulation methods have been developed for use in research fish to minimize these effects; however, these methods are impractical for use in clinical studies.[21]

In general, the PCV of fish is lower than that of mammals and birds. Hematocrits vary both between and within fish species, and they appear to correlate with the normal activity of the fish, with less active fish having lower hematocrits than active, fast-swimming fish. Hematocrits also vary during the life-cycle of fish. For example, during prespawning conditions, Atlantic salmon (*Salmo salar*) have high hematocrits compared with those during spawning. Age, sex, water temperature, photoperiod, and seasonal variation also may influence the PCV of fish.[5,20,25,41,47] In fact, the PCV in some species of male fish are large enough to require two reference intervals.

Cartilaginous fish (sharks and rays) and bony fish appear to have different gas transport systems, which affect their erythrocyte parameters. Bony fish exhibit a high cardiac workload and blood pressure, which are associated with a higher PCV and smaller erythrocytes.[11] Sharks and rays, however, exhibit relatively modest cardiac work load, higher cardiac output, higher blood volumes, and increased flow rates, which are associated with lower concentrations of larger cells.

In general, fish with PCVs of greater than 45% usually are considered to be dehydrated, particularly when this finding is supported by increased serum osmolality or total protein. Anemic fish have low PCVs (<20%); however, for some species, such as the Port Jackson shark (*Heterodontus portusjacksoni*), normal PCVs may be as low as 20%.[6]

Fish with regenerative anemia often have an increased concentration of polychromatic and immature erythrocytes in their blood films. Anemic fish that exhibit little or no polychromasia have nonresponsive anemia. A microcytic normochromic anemia has been associated with environmental stresses, such as increased population densities.[5,20,30] A microcytic hypochromic anemia with marked poikilocytosis has been reported in trout (*Salmo gairdneri*) that were fed diets containing yeast, thereby resulting in oxidative damage to erythrocytes.[40] Anemias associated with erythrocytes having pyknotic nuclei, erythroplastids (i.e., erythrocytes without nuclei), and red-blood-cell fragmentation have been associated with conditions that interfere with the splenic removal of senescent red blood cells from the peripheral circulation.[13] Abnormal erythrocyte nuclei (i.e., amitosis, segmentation, and fragmentation) as well as formation of erythroplastids may relate to nutritional disorders, such as deficiency of folic acid or vitamin E and toxicosis from rancid oils and environmental pollutants.[12,22]

Because the immature erythrocytes of fish are smaller than the mature erythrocytes, microcytosis often is associated with marked hemorrhagic or hemolytic anemias, in which the regenerating, immature erythrocytes represent the majority of the peripheral blood erythrocytes.[17] Hemorrhagic anemias of fish are associated with trauma, bloodsucking parasites, vitamin K deficiency, and septicemia (bacteria or viral). For example, enteric red mouth disease (yersiniosis) of fish produces a hemorrhagic septicemia and a hemogram that is characterized by leukocytosis, low PCV, and reticulocytosis.[45] Hemolytic anemias of fish may be associated with toxins (bacterial or environmental), viral infections (erythrocytic necrosis virus), certain nutritional deficiencies, and hemoparasites.[17,34] Cadmium is a calcium channel blocker that impedes normal membrane function in erythrocytes resulting in hemolytic anemia in freshwater teleost fish exposed to toxic levels of cadmium in the water.[37] Nitrite poisoning (brown blood disease or new tank syndrome) of fish also results in severe hemolytic anemia.[7] Nitrite is readily absorbed from the gills and enters into the blood, where it then oxidizes hemoglobin to methemoglobin, which in turn gradually changes the blood from red to brown in color. A hemolytic anemia results as splenic macrophages remove the affected erythrocytes from the circulation.

Several nutritional deficiencies have been produced experimentally in fish. For example, folic acid deficiencies result in normochromic macrocytic anemias, and vitamin B_{12} deficiencies result in hypochromic anemias.[17] Folate deficiency has been suggested as being a cause of the chronic hemolytic anemia that occurs in channel catfish (*Ictalurus punctatus*).[36]

Leukocytes

Leukocytes (especially the granulocytes) exhibit a wide variation in appearance among fish species. This has led to controversy and confusion when applying the nomenclature and classification of piscine leukocytes on the basis of such descriptions from avian and mammalian Romanowsky-stained blood films. Evaluation of the cellular ultrastructure, differential cytochemical staining, immunofluorescence, and function testing of fish leukocytes, however, has helped to alleviate some of this controversy in some species.[2,14,39,51,53]

Leukocytes of commonly studied bony fish

Channel catfish (*Ictalurus punctatus*)

Ultrastructural and cytochemical studies have identified heterophils, basophils, lymphocytes, and monocytes in the peripheral blood of channel catfish. These results support the classification of these cells in Romanowsky-stained blood films.[3,8,15,39,48,52]

Goldfish (*Carassius auratus*)

On the basis of electron microscopy, leukocytes found in the peripheral blood of goldfish can be classified as lymphocytes, monocytes, heterophils, eosinophils, and rarely, basophils.[39] On the basis of cytochemical reaction, goldfish leukocytes can be classified as lymphocytes, heterophils, monocytes, and an atypical, segmented granulocyte.[14,51,53]

Salmonids (trout and salmon, *Salmo* spp.)

On the basis of cytochemical staining, salmonids appear to have three types of leukocytes: lymphocytes, neutrophils, and monocytes.[7]

Striped bass (*Morone saxatulis*)

Striped bass leukocytes are classified as lymphocytes, neutrophils, and monocytes.[7]

White sturgeon (*Acipenser transmontanus*)

Four types of leukocytes—lymphocytes, monocytes, neutrophils, and eosinophils—have been described in white sturgeon.[53]

Summary

Cytochemical studies of piscine leukocytes appear to support the use of mammalian leukocyte terminology as a classification scheme because they are considered to be analogous to mammalian leukocytes based upon cytochemical and ultrastructural studies. In general, neutrophils or heterophils, lymphocytes, and monocytes commonly are reported in the peripheral blood films of fish belonging to the class Osteichthyes (teleost or bony fish). Myeloperoxidase stain is used to differentiate neutrophils from true heterophils, because neutrophils stain positive and heterophils stain negative. In general, fish heterophils are neutrophils, based on myeloperoxidase staining, but are called heterophils because of the presence of prominent eosinophilic cytoplasmic granules with Romanowsky stains.[24,29] Eosinophils and basophils are rare in the peripheral blood of bony fish.[14,42]

Leukocytes of sharks and rays

The peripheral blood of fish belonging to the class Chondrichthyes (cartilaginous fish, such as sharks and rays) contain leukocytes that can be classified as granulocytes, lymphocytes, or monocytes. The granulocytes exhibit marked variation in both numbers and types between species, and the granulocyte classification scheme is based on the results of ultrastructural and cytochemical studies performed in blood samples from the lesser spotted dogfish (*Scliorrhinus canicula*), which has been used as a model for cartilaginous fish. The granulocytes are classified as either G_1 (type I), G_2 (type II), or G_3 (type III).[39] To simplify the identification of these cells using familiar terminology, the G_1 granulocytes resemble the avian or reptilian heterophil, the G_2 granulocyte resembles the mammalian neutrophil, and the G_3 granulocyte resembles the eosinophil based upon the cell's appearance on Romanowsky-stained blood films.[7] Basophils also can be found in the peripheral blood of cartilaginous fish.

Morphology

Neutrophils of bony fish

The neutrophils of bony fish tend to be round to slightly oval cells with eccentric nuclei (Fig. 21.8). The nucleus of mature neutrophils vary in shape, being round, oval, indented (metamyelocyte type), elongated (band cell type), or segmented, and usually with two to three lobes. Nonsegmented nuclei are the most common in the granulocytes of bony fish. The nuclear chromatin is coarsely clumped, and it stains deeply basophilic in Romanowsky-stained blood films. The neutrophils of bony fish have abundant colorless, grayish, or slightly acidophilic-staining (light pink) cytoplasm; small cytoplasmic granules and vacuoles also may be present. The staining of the granules varies, however, and depends on the species or the maturity of the cell. The small, cytoplasmic granules of the neutrophils vary from gray to pale blue or red. Interspecies differences in the cytochemical reactions of bony fish neutrophils are observed; however, in general, they resemble the neutrophils of mammals.[14,17,35]

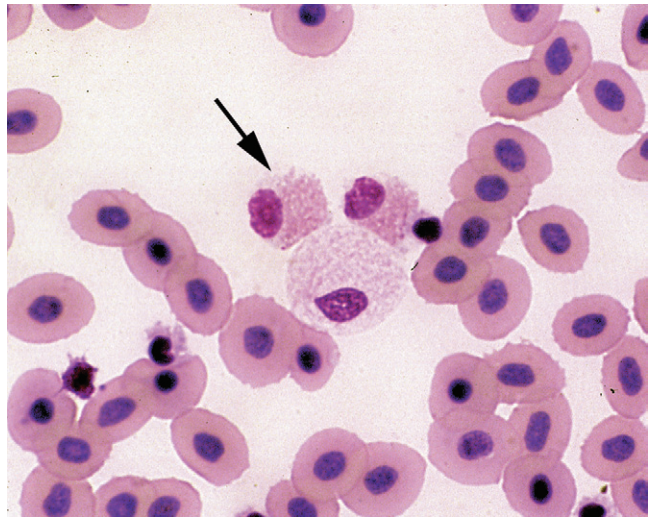

Figure 21.8 Three neutrophils (arrow) in the blood film of a bony fish (*Artromotus ocellatus*). Wright-Giemsa stain.

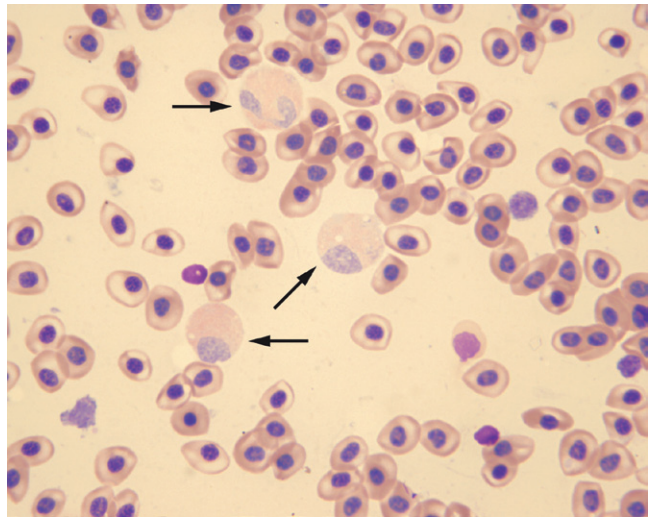

Figure 21.9 Three heterophils (arrows) in the blood film of a bony fish (*Scleropages legendrei*). Wright-Giemsa stain.

Piscine neutrophils that exhibit distinct, rod-shaped cytoplasmic granules on Romanowsky stains often are classified in the literature as heterophils (Fig. 21.9). Some species, such as goldfish and carp (*Cyprinus carpio*), have granulocytes with distinct and slightly acidophilic cytoplasmic granules, colorless cytoplasm, and eccentric, partially lobed nuclei on Romanowsky stains. These cells often are classified as heterophils rather than neutrophils, although they do have cytochemical properties similar to those of neutrophils in other fish. They measure approximately 9–10 in diameter

(some as large as 20 µm). These heterophils are peroxidase and Sudan black B positive when the granules are immature, but they are peroxidase negative in mature granules.[39] Neutrophils from channel catfish and certain species of eel also contain prominent eosinophilic, rod-shaped cytoplasmic granules resembling those of avian heterophils on the basis of Romanowsky stains.[39] The granules of these cells are strongly peroxidase positive. Similar cells have been found in a number of other bony fish as well.[42] In salmonids, such as rainbow trout (*Onchorhynchus mykiss*) and coho salmon (*Onchorhynchus kisutch*), these neutrophils are the predominant granulocyte, as in most bony fish. Piscine neutrophils often reveal artifacts of blood film preparation, causing the cells to appear large and with swollen, pale nuclear chromatin (karyolysis).

Eosinophils of bony fish

Eosinophils rarely are reported in the blood films from bony fish, and some investigators doubt whether they exist at all in some species. When present, however, they appear as intermediate to large granulocytes, with distinct eosinophilic granules and pale blue cytoplasm. The nucleus varies from round (more common) to segmented. They can be distinguished from heterophils on the basis of cytochemistry and ultrastructural findings, although the absence of crystalloids (used as a fingerprint for mammalian eosinophils) often is the rule with piscine eosinophils.[7] Eosinophils have been reported in goldfish, white sturgeon, and channel catfish.[9,14,23,51,52,53] Piscine eosinophils tend to be round, with round to rod-shaped eosinophilic-stained cytoplasmic granules with Romanowsky stain (Fig. 21.10). The granules of piscine eosinophils often are less distinct compared with those of birds and mammals. These granules also have a tinctorial quality that differs from those of heterophils with distinct eosinophilic granules.

Fish eosinophils generally measure 9–14 µm in diameter, for example eosinophils of carp are approximately 7.5 in diameter and have an eccentric nucleus that is indented, sausage-shaped, or bilobate as well as eosinophilic, cytoplasmic granules that are larger than those of the heterophils (neutrophils).[39]

Basophils of bony fish

Basophils are rare in the peripheral blood of bony fish and have been reported only in a few species.[14,42] Basophils are identified as round cells that have round, basophilic cytoplasmic granules that often obscure the cell nucleus (Fig. 21.11). The nucleus is large, eccentric, and round. The nuclear chromatin is homogeneous. The basophils of carp measure between 10 and 20 µm. When present, basophils occur in low numbers.[39]

Granulocytes of sharks and rays

In cartilaginous fish, G_1 (type I) granulocytes typically have an eccentric, irregular, nonlobed nucleus; colorless cytoplasm; and round to oval, eosinophilic cytoplasmic granules (Figs. 21.12 and 21.15). The nucleus may be lobed in some species. These cells resemble avian heterophils, and they often are the most common form of the granulocytes. The G_2 (type II) granulocytes have a lobed nucleus and a colorless cytoplasm that lacks distinct granules (Fig. 21.13). These cells resemble mammalian neutrophils. The G_3 (type III) granulocytes are characterized by a lobed nucleus (in some species), pale blue cytoplasm, and strongly eosinophilic, round to rod-shaped cytoplasmic granules (Fig. 21.12). The

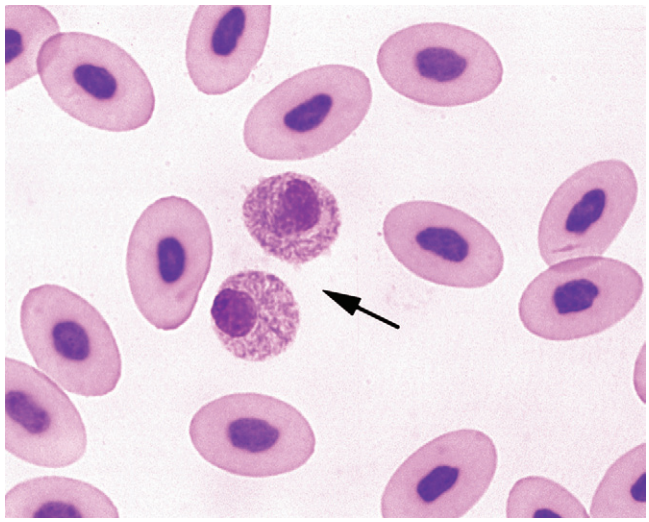

Figure 21.10 Two eosinophils (arrow) with distinct cytoplasmic granules in the blood of a bony fish (*Gymnothorax funebria*). Wright-Giemsa stain.

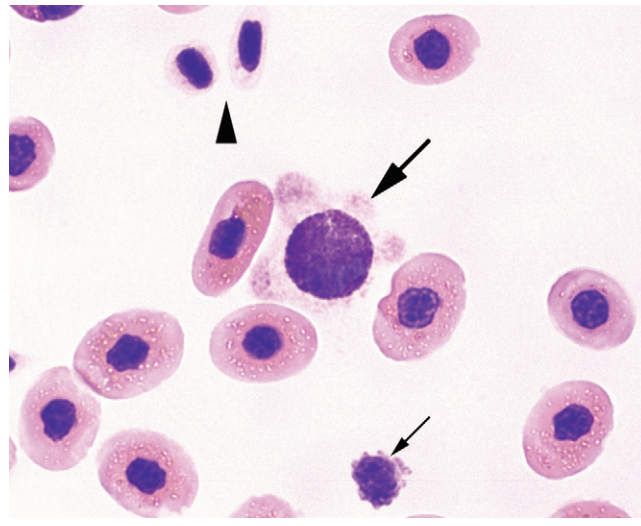

Figure 21.11 A basophil (arrow), lymphocyte (thin arrow), and thrombocyte (arrowhead) in the blood film of a cartilaginous fish (*Pristis pectinatai*). Wright-Giemsa stain.

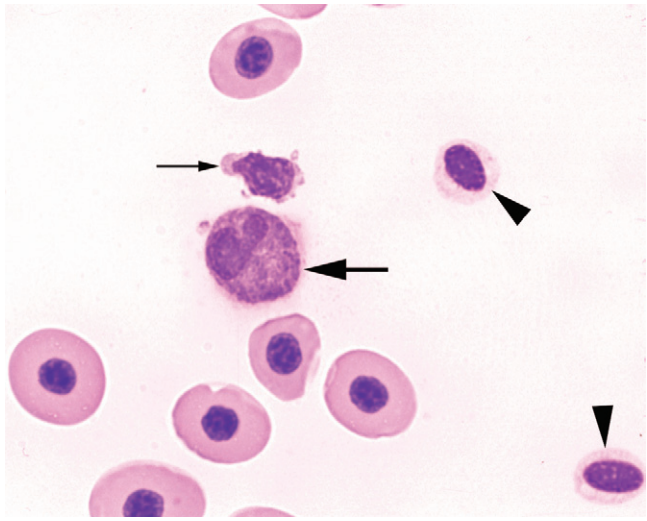

Figure 21.12 A G_1 granulocyte (heterophil; arrow), lymphocyte (thin arrow), and thrombocyte (arrowhead) in the blood film of a cartilaginous fish (*Pristis pectinatai*). Wright-Giemsa stain.

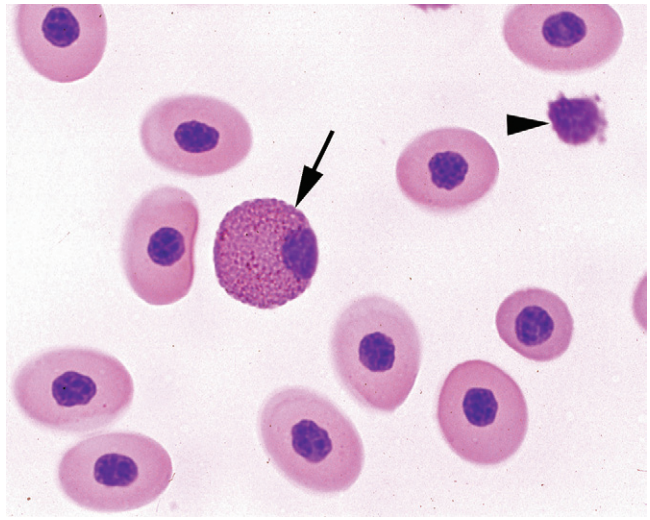

Figure 21.14 A G_3 granulocyte (eosinophil; arrow) and small lymphocytes (arrowheads) in the blood film of a cartilaginous fish (*Pristis pectinatai*). Wright-Giemsa stain.

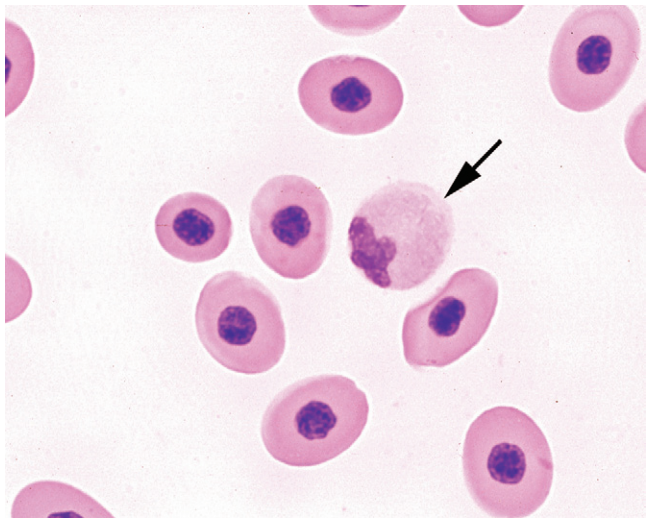

Figure 21.13 A G_2 granulocyte (neutrophil; arrow) in the blood film of a cartilaginous fish (*Pristis pectinatai*). Wright-Giemsa stain.

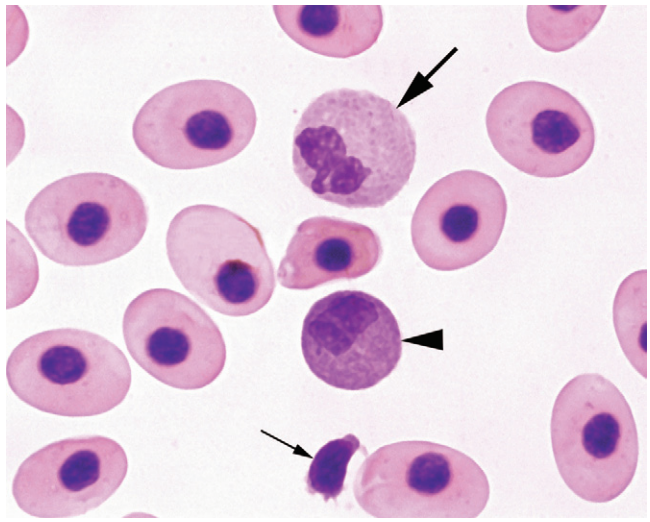

Figure 21.15 A G_1 granulocyte (heterophil; arrow), a G_3 granulocyte (eosinophil; arrowhead), and a lymphocyte (thin arrow) in the blood of a cartilaginous fish (*Negaprion brevirostris*). Wright-Giemsa stain.

cytoplasmic granules in the G_3 granulocytes have tinctorial qualities that differ from those of the G_1 granulocytes in the same blood film (Figs. 21.14 and 21.15). The G_3 granulocytes of cartilaginous fish resemble avian eosinophils.

The granulocytes of sharks and rays tend to stain negatively for peroxidase, β-glucuronidase, and Sudan black B but positive for acid phosphatase, arylsulfatase, and acid naphthyl AS-D chloroacetate esterase.[39] The eosinophilic granulocytes (G_1 and G_3) of the elasmobranchs share few morphologic and cytochemical characteristics with mammalian eosinophils. The function and interrelationships of the granulocytes in cartilaginous fish are not known; however, they appear to be separate cell types rather than intermediate stages of one cell type. Not all species of cartilaginous fish exhibit all the granulocytes described for the lesser spotted dogfish (*Scyliorhinus canicula*). For example, only G_1 and G_3 granulocytes have been found in the rays *Raja clavata* and *Raja microcellata*.[39] Basophils occasionally are found in peripheral blood films of some species of cartilaginous fish.

Lymphocytes of fish (bony and cartilaginous)

Lymphocytes frequently are the most abundant leukocyte in peripheral blood films of fish, and they resemble their counterparts in avian and mammalian blood films (Figs.

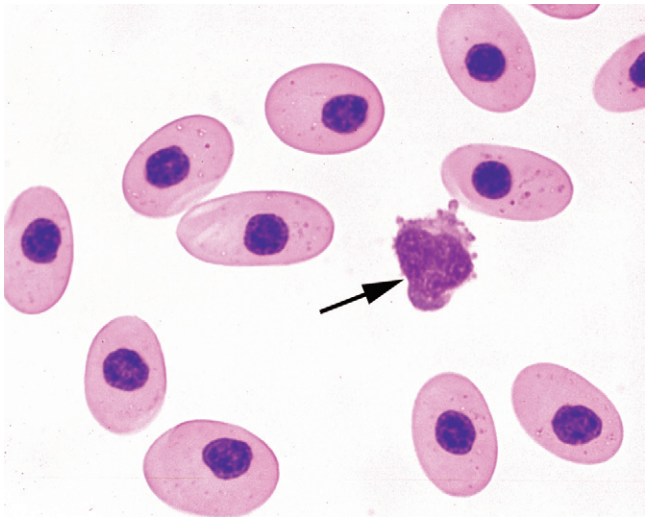

Figure 21.16 A lymphocyte (arrow) in the blood of a cartilaginous fish (*Negaprion brevirostris*). Wright-Giemsa stain.

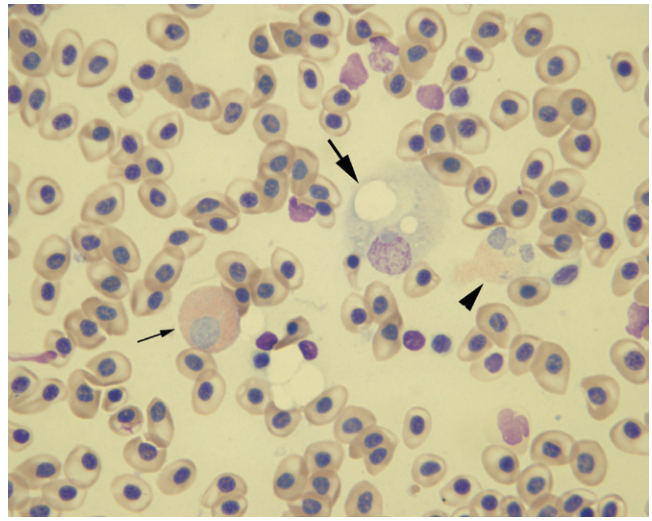

Figure 21.18 A reactive monocyte (large arrow), a heterophil (arrowhead), and an eosinophil (small arrow) in the blood film of a bony fish (*Scleropages legendrei*). Wright-Giemsa stain.

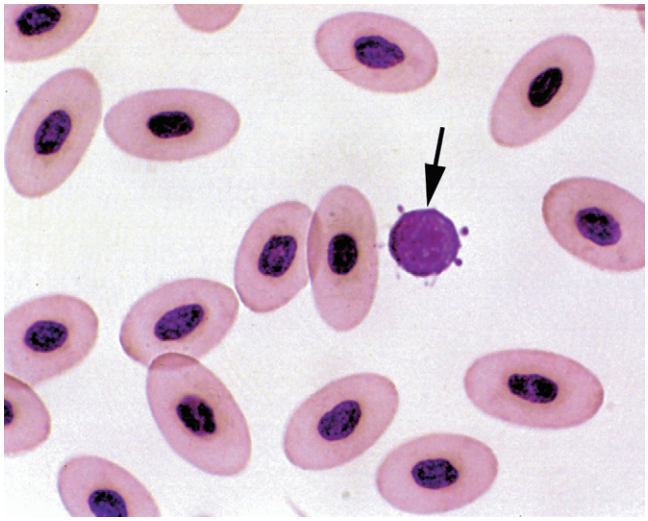

Figure 21.17 A reactive lymphocyte (arrow) in the blood of a bony fish (*Gymnothorax funebria*). Wright-Giemsa stain.

21.11, 21.12, and 21.14 through 21.16).[1] They typically measure between 5 and 10 µm in diameter.[39] Lymphocytes tend to be round, but they may mold around adjacent cells in the blood film. They have a high nucleus:cytoplasm (N:C) ratio, with coarsely clumped, deeply basophilic nuclear chromatin. The scant cytoplasm of small mature lymphocytes stains a homogenous pale blue. An occasional lymphocyte possesses azurophilic cytoplasmic granules. Reactive lymphocytes in blood films from fish resemble those of birds and mammals, with abundant, deeply basophilic cytoplasm and an occasional, distinct Golgi complex (Fig. 21.17). Plasma cells also may be seen in small numbers on the peripheral blood films of many species of fish.

Monocytes of fish (bony and cartilaginous)

Monocytes occasionally are reported in the blood films of most species of fish, and they resemble monocytes of birds and mammals. They are large, mononuclear leukocytes, with abundant blue-gray to blue agranular cytoplasm, which may contain vacuoles. Fish monocytes generally measure 10–20 µm in diameter.[39] The cytoplasmic margins may be indistinct or ragged because of the presence of pseudopodia. The nucleus varies in shape (round to kidney-shaped to bilobate) and generally occupies less than 50% of the cytoplasmic volume. The nuclear chromatin of monocytes generally is more granular and less clumped compared with that of lymphocyte nuclei. Results of ultrastructural studies indicate that monocytes in all species of fish are similar to those in other vertebrates.[39] The term monocyte/macrophage frequently is used to classify piscine monocytes, because cells resembling transformational forms between monocytes and macrophages often are found in peripheral blood films (Fig. 21.18). The term monocyte, however, is reserved for those found in peripheral blood, and the term macrophage is reserved for those found elsewhere. Fish monocytes can be differentiated from immature granulocytes and lymphocytes by the positive, nonspecific esterase reaction in monocytes.

Laboratory evaluation

The same problems associated with obtaining total leukocyte counts in birds and reptiles also apply in fish. Because fish have nucleated erythrocytes and thrombocytes, manual counting methods are used. Direct leukocyte counting methods using a Neubauer-ruled hemocytometer and a variety of staining and diluting solutions have been used.[1,10,32,43] Natt and Herrick's method commonly is used,

and the procedure is the same as that described for obtaining total leukocyte counts in avian and reptilian blood (see Chapters 19, on avian hematology, and 20, on reptilian hematology). The leukocytes appear blue and stain darker than erythrocytes stained with Natt-Herrick. It may be difficult to distinguish small, mature lymphocytes from thrombocytes if the counts are made using a ×10 objective; cells are more accurately identified at higher magnifications. Staining for 60 minutes in Natt-Herrick's solution also may improve the differentiation between small lymphocytes and thrombocytes. Advantages of the Natt and Herrick procedure include the ability to obtain a total erythrocyte, leukocyte, and thrombocyte count using the same charged hemocytometer. In addition, the technique can be applied to blood samples obtained from all lower vertebrates.

A leukocyte differential is obtained from a Romanowsky-stained blood film. Applying a drop of albumin to the slide during preparation of the blood film often is advantageous to minimize smudging of the cells. Quickly drying the blood film using a hair dryer also may help to alleviate cellular artifacts associated with blood film preparation.

Table 21.2 offers leukocyte reference values for selected teleost fish.

Responses in disease

Piscine neutrophils and heterophils participate in inflammatory responses. They are not always phagocytic, however, and little is known regarding their function, including their methods of intracellular killing and digestion of phagocytized organisms. Because the function of fish granulocytes is not known, viewing them as being homologous to the granulocytes of higher vertebrates may be inappropriate. Therefore, interpretation of the changes in granulocyte concentrations of peripheral blood can be difficult. Broad generalizations can be made, however, until the results of further studies indicate the specific functions and responses of these cells to disease. For example, an increased concentration of fish neutrophils or heterophils often is associated with inflammatory diseases, especially those involving infectious agents.[4,15,29] A relative neutrophilia or heterophilia often is associated with lymphopenias, which can be interpreted as a stress response in fish.[28,50]

In response to severe systemic illness, piscine neutrophils and heterophils exhibit toxic changes similar to those in mammalian neutrophils and avian and reptilian heterophils. Toxic neutrophils and heterophils of fish have increased cytoplasmic basophilia, vacuolization, abnormal granulation (degranulation of heterophils, granules that appear deeply basophilic, and heterophils granules that appear to coalesce into large, round granules), and degeneration of the cell nucleus (Fig. 21.19). Toxic neutrophils and heterophils in fish are associated with severe, systemic illness such as septicemia, mycotic infections, and severe tissue necrosis. The degree of toxicity usually indicates the severity of the fish's condition, and a marked number of neutrophils or heterophils exhibiting marked (4+) toxicity indicates a grave prognosis.

Table 21.2 Leukocyte parameters for selected teleost fish.

	WBC × 10^3/μL	Neut/ Heterophils × 10^3/μL	Lymphocytes × 10^3/μL	Monocytes × 10^3/μL	Eosinophils × 10^3/μL	Basophils × 10^3/μL
Bass, hybrid[a]	32.6–115.1	0.4–3.5	22.5–115.1	1.5–7.5	0–0.4	0
Channel catfish[b]	8.9–124.0	4.5–86.8	1.4–23.6	0.7–14.7	0	0–7.1
Flounder[c]	88.0–282.0	2.5–26.6	38.7–154.5	—	—	—
Goldfish[d]	10.1–14.7	—	9.5–13.7	—	—	—
Red pacu[e]	33.5	3.2	21.0	1.2	0.2	0
Tilapia[f]	21.6–154.7	0.6–9.9	6.8–136.4	0.4–4.3	0–1.6	0
Trout[g]	21.0	1.6	18.8	0.6	0	0

[a]Hrubec TC, Smith SA, Robertson JL, *et al.* (1996) Comparison of hematologic reference intervals between cultured system and type of hybrid striped bass. *Am J Vet Res* 57: 618–23.

[b]Tavares-Dias M, de Moraes FR (2007) Leukocyte and thrombocyte reference values for channel catfish [*Ictalurus punctatus* Raf], with an assessment of morphologic, cytochemical, and ultrastructural features. *Vet Clin Pathol* 36: 49–54.

[c]Bridges DW, Cech JJ Jr, Pedro DN (1976) Seasonal hematological changes in winter flounder. *Pseudopleuronectes americanus*. *Trans Am Fish Soc* 105: 596–600.

[d]Murray SA, Burton CB (1979) Effects of density on goldfish blood: II, cell morphology. *Comp Biochem Physiol* 62A: 559–62.

[e]Tocidlowski ME, Lewbart GA, Stoskopf MK (1997) Hematologic study of the red pacu [*Colossoma brachypornum*]. *Vet Clin Pathol* 26: 119–25.

[f]Hrubec TC, Cardinale JL, Smith SA (2000) Hematology and plasma chemistry reference intervals for cultured Tilapia [*Oreochromis* hybrid]. *Vet Clin Pathol* 2000,29: 7–12.

[g]Hunn JB, Wiedmeyer RH, Greer IE, Grady AW (1992) Blood chemistry of laboratory-reared golden trout. *J Aquat Anim Health* 4: 218–21.

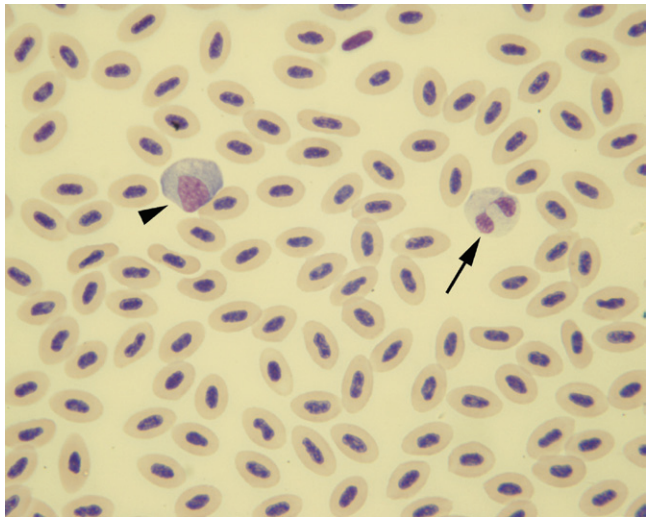

Figure 21.19 Toxic neutrophil (arrow) and a monocyte (arrowhead) in the blood film of a bony fish (*Barborymus schwanenfeldii*) with septicemia. Wright-Giemsa stain.

Eosinophils are found in low concentrations (0–3% of the leukocyte differential) in the peripheral blood of normal fish. Piscine eosinophils participate in inflammatory responses along with neutrophils (heterophils) and macrophages, and they appear to have a limited phagocytic capability.[39] Piscine eosinophils apparently are involved in the control of infections with metazoan parasites, and they participate in the immune responses to antigenic stimulation.[46] Therefore, an increased eosinophil concentration in the peripheral blood of fish suggests an inflammatory response associated with parasitic infections or antigenic stimulation.

The functions of the granulocytes of cartilaginous fish are not known; however, they appear to participate in inflammatory responses. Because the granulocytes account for 20–30% of the leukocytes in sharks and rays, the normal granulocyte: lymphocyte ratio typically is low (<0.5).[7,23] An increase in the granulocyte concentration is indicative of an inflammatory response. A decrease in the lymphocyte concentration results from conditions that reduce the number of circulating lymphocytes, such as stress responses. Both increases in the granulocyte concentrations and decreases in the lymphocyte concentrations of sharks can be associated with bacterial septicemias. The leukogram of cartilaginous fish can be used to follow the progress of these fish during the course of the disease or in response to therapy. For example, an initial increase in the granulocyte concentration or decrease in the lymphocyte concentration that has returned to normal indicates a favorable response to therapy and prognosis.

Piscine monocytes are actively phagocytic cells, and they participate in acute inflammatory responses in fish.[14,39] Monocytes occur in low numbers (<5% of the leukocyte differential) in the peripheral blood of normal fish. Therefore, a monocytosis is suggestive of an inflammatory response in fish that is, perhaps, associated with an infectious agent.

Lymphocytes are the most commonly observed leukocytes in the peripheral blood of most normal fish, in which they typically represent greater than 60% (and as much as 85% in some species) of the leukocyte differential. Lymphocytes play a major role in the humoral and cell-mediated immunity of fish. B lymphocytes in teleost fish function in the same manner as mammalian B-1 cells and produce immunoglobulin M (IgM). Therefore, lymphocytosis is suggestive of immunogenic stimulation, whereas lymphopenia is suggestive of immunosuppressive conditions, such as stress or excess exogenous glucocorticosteroids. Interestingly, B lymphocytes from teleost fish also demonstrate phagocytic and microbicidal activity.[27] Bacterial septicemias commonly affect fish and result in marked leukopenias and lymphopenias. Environmental conditions, such as prolonged photoperiod and elevated water temperature that cause a stress response in fish will also result in a leukopenia associated with a lymphopenia.[49,50]

Thrombocytes and hemostasis

The blood of fish clots in response to injury, as it does in other vertebrates. The speed and effectiveness in fish, however, are variable. Clotting is much more rapid in bony fish compared with sharks and rays. Sharks and rays appear to rely primarily on the extrinsic pathways of coagulation; the addition of skin, high calcium solutions, sea water, or other extrinsic factors enhances clotting. Clot formation in bony fish usually occurs within 5 minutes, whereas clotting in samples taken from sharks and rays can take 20 minutes or longer.[39]

Morphology

Fish thrombocytes are smaller than erythrocytes, vary in shape, and can be round, elongate, or spindle-shaped. In addition, the shape may vary with the stage of maturity or the degree of reactivity. The oval and elongated forms tend to be nonreactive, mature thrombocytes (Fig. 21.20). Immature thrombocytes are round in some species, whereas spindle-shaped thrombocytes appear to be reactive forms and often are found in clumps. The cytoplasm of the piscine thrombocyte is colorless to faint blue; the nucleus is condensed and follows the shape of the cell. Fish thrombocytes also may contain a variable amount of eosinophilic cytoplasmic granules (Fig. 21.21).

Like those in birds and reptiles, thrombocytes in fish often are confused with small, mature lymphocytes. Lymphocytes, however, have slightly more abundant, mildly basophilic cytoplasm compared with thrombocytes (Figs. 21.22 and 21.23). The nucleus of the lymphocyte also usually is larger

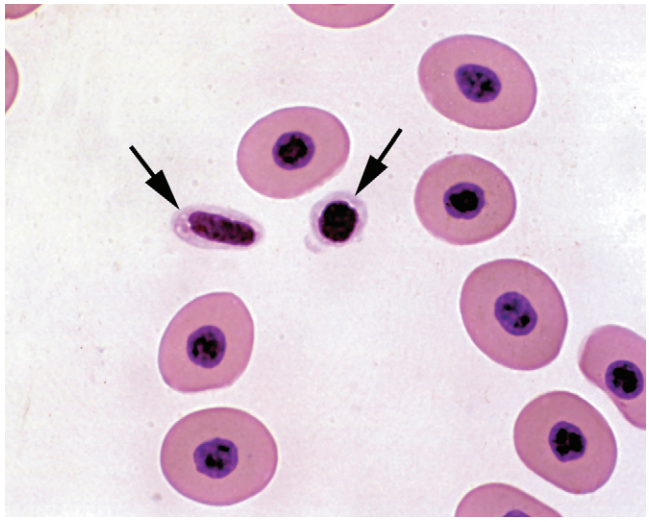

Figure 21.20 Thrombocytes (arrow) in the blood film of a cartilaginous fish (*Pristis pectinatai*). Wright-Giemsa stain.

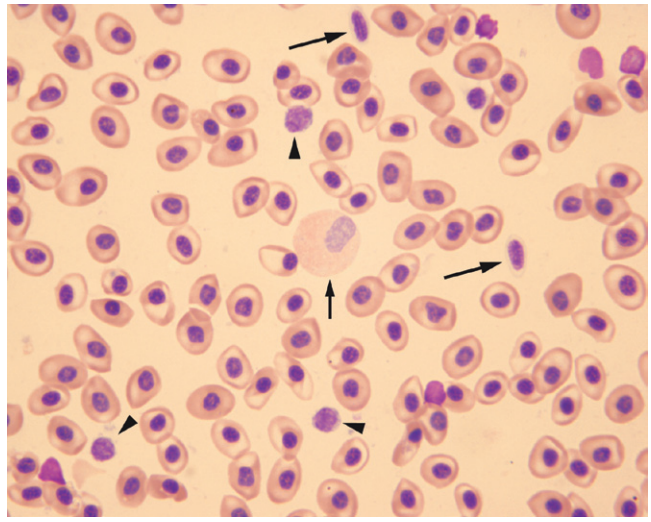

Figure 21.22 Thrombocytes (long arrow), lymphocytes (arrowheads), and a heterophil (short arrow) in the blood film of a bony fish (*Scleropages legendrei*). Wright-Giemsa stain.

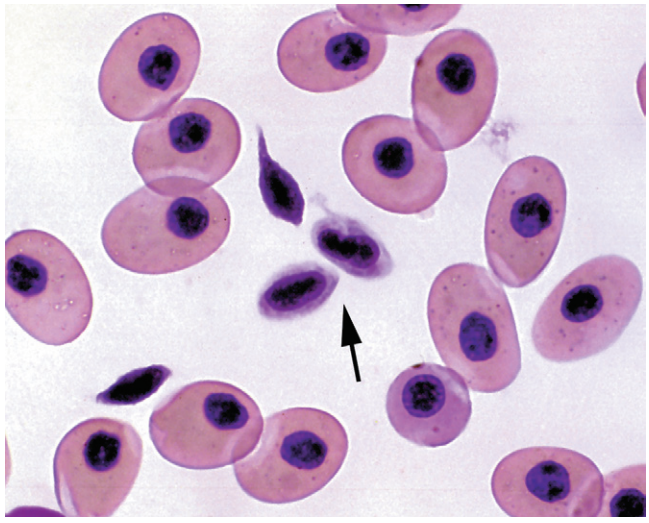

Figure 21.21 Thrombocytes (arrow) with eosinophilic cytoplasmic granules in the blood of a cartilaginous fish (*Negaprion brevirostris*). Wright-Giemsa stain.

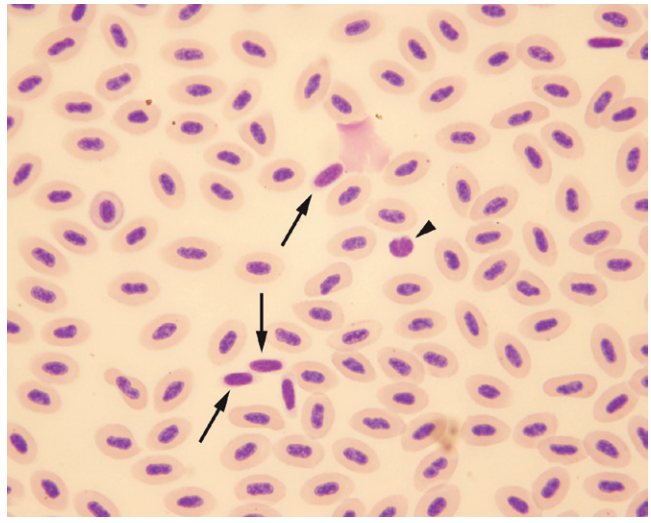

Figure 21.23 Thrombocytes (arrow) and a lymphocyte (arrowhead) in the blood film of a bony fish (*Barborymus schwanenfeldii*) with septicemia. Wright-Giemsa stain.

and less condensed compared with that of the thrombocyte. Fish thrombocytes usually stain weakly positive with periodic acid-Schiff and positive for acid phosphatase.[53]

Laboratory evaluation

The total thrombocyte count can be obtained via the same hemocytometer charged with diluting solutions (i.e., Natt-Herrick solution) used to obtain a total erythrocyte and leukocyte count. The thrombocytes resemble erythrocytes in the hemocytometer, but they are much smaller and appear to be round to oval, with a greater nucleus : cytoplasm (N : C) ratio compared to erythrocytes. All squares in the central, large square of a Neubauer hemocytometer are counted on both sides. The average number of thrombocytes in one large hemocytometer square is calculated and then multiplied by 2000 to obtain the total thrombocyte count per microliter. Because thrombocytes tend to clump, however, accurate counts may be difficult to achieve.

Responses in disease

During the clotting process in fish, fibrinopeptides are formed after the cleavage of fibrinogen, which is under the control of thrombin. These fibrinopeptides differ from those produced

by mammals; however, the basic structure of fibrin in fish, though much larger than its mammalian counterpart, is the same as that in mammals. Fish thrombocyte aggregation differs from mammalian platelet aggregation. For example, fish thrombocytes convert arachidonic acid to prostaglandins with little, if any, thromboxane formation, whereas thromboxane is a potent inducer of platelet aggregation in mammals.[39] Thrombocyte aggregation in sharks is temperature reversible, which is a feature not seen with mammalian platelet aggregation. Shark thrombocyte aggregation also is independent of thrombin and adenosine diphosphate. Therefore, both the control and the outcome of thrombocyte aggregation in fish may not be the same as mammals.

Glucocorticoid excess in fish tends to decrease the thrombocyte concentration and increase the clotting time.[6] Environmental stressors, such as a prolonged photoperiod and elevated water temperature will result in a thrombopenia.[49] Prolonged clotting times also occur with vitamin K deficiency; dietary requirements for vitamin K have been determined for salmonids and channel catfish.

Thrombocytosis and hypercoagulability of whole blood has been associated with exposure to toxic levels of cadmium (126 mg/L) in freshwater teleosts.[44]

Blood parasites

Hemogregarina

Hemogregarina sp. affecting fish resemble those described in the blood films of reptiles, and they are identified by characteristic gametocytes in the cytoplasm of erythrocytes (see Chapter 20 on reptilian hematology). The gametocytes lack refractile pigment granules and may create a bulge in the cytoplasmic membrane. Little is known regarding the life-cycle of fish hemogregarines, but they most likely require a bloodfeeding, intermediate host, such as leeches, copepods, and isopods. Therefore, they more frequently are found in wild-caught fish. Often, the *Hemogregarina* sp. gametocytes in the peripheral blood of fish are considered to be an incidental finding; however, some species can cause anemia, leukocytosis with a marked left shift, and large granulomas in internal organs.[18,45]

Trypanosomes

Trypanosomes occasionally may be found in blood films of fish, especially wild-caught, cold-water species. They can occur in high concentration (1,000,000 organisms/mL) and are especially prevalent in the imprints of kidney tissue.[39] Infections with trypanosomes can result in fatal anemias. Leeches act as the intermediate host for the trypanosomes, and the infective trypomastigotes develop and then enter the fish host when the leech takes a blood meal. Trypanosomes are identified by their slender and serpentine shape, single anterior flagellum, prominent and undulating membrane, nucleus, and kinetoplast. On wet-mount preparations, the trypanosomes exhibit rapid, wriggling movements but have no forward motion.

Trypanoplasms

Trypanoplasms are hemoparasites that resemble trypanosomes morphologically, except that they are more pleomorphic (a slender, serpentine shape is most common), have two flagella (one directed anteriorly and one posteriorly), and kinetosomes. Their life-cycle is similar to that of the trypanosomes. A prepatent period occurs after infection followed by a parasitemia (i.e., cryptobiasis) resulting in either death of the fish or disappearance of the trypanoplasms from the blood.[39] *Trypanoplasma borreli* causes a severe anemia in cyprinids (i.e. Koi, goldfish, and carp), and the disease is referred to as sleeping sickness. Anemia, exophthalmia, ascites, and splenomegaly occur in freshwater salmonids (i.e. trout) with *T. salmositica*; *T. bullocki* infects marine fish, especially flatfish species along the western Atlantic and Gulf of Mexico. On wet-mount preparations, trypanoplasms exhibit flowing, ameboid motility, which aids in their identification.

Piroplasmids

Babesiosoma, *Haemohormidium*, *Haematractidium*, and *Mesnilium* are genera of piroplasmids that have been described in fish.[7] As with the hemogregarines, little is known regarding their life-cycle, which most likely requires a bloodfeeding, intermediate host. Piroplasmids are identified by their intracytoplasmic inclusions in circulating erythrocytes, which can vary from small, ringlike forms to anaplasma-like inclusions. Piroplasmids may cause hemolytic anemia in fish.

Microsporidians

Enterocytozoon salmonis is an intranuclear microsporidium that primarily infects hematopoietic cells of salmonids. The infected cells exhibit intranuclear inclusions. This organism was once considered to be the causative agent of plasmacytoid leukemia of Chinook salmon (*Onchorhynchus tshawytscha*). The presence of high reverse-transcriptase activity in the affected tissues from these fish, however, suggests that an oncogenic retrovirus may be the causative agent for that disease.[33]

Viral inclusions

Intracytoplasmic inclusions occur in the erythrocytes of fish with viral erythrocytic necrosis (i.e., piscine erythrocytic necrosis), erythrocytic inclusion body syndrome, and coho anemia. Viral erythrocytic necrosis occurs in a variety of marine fish, including salmon, cod, and herring. The disease is characterized by marked poikilocytosis, a single intracytoplasmic inclusion (0.3–4.0 µm) within the erythrocytes, and

karyolysis of the red-blood-cell nuclei. Erythrocytic inclusion body syndrome of young salmonids is characterized by progressive, severe anemia, which is caused by a viral agent that creates 0.8–3.0 µm intracytoplasmic inclusions within the erythrocytes.[7,34] A Leishman-Giemsa stain provides the best results for demonstrating the inclusions. An anemia that occurs in seawater-reared coho salmon (*Oncorhynchus kisutch*) results from 0.1–2.0 µm intracytoplasmic inclusions, which often are rod-shaped within the erythrocytes.[34]

Hematopoiesis

Cartilaginous fish (Chondrichthyes) lack bone marrow and lymph nodes, but they do have a lymphoid thymus, spleen, and other lymphomyeloid tissues.[16] Significant hematopoietic activity occurs in the sinusoids of the red pulp area of the spleen, where erythrocytes, thrombocytes, and lymphocytes develop. Little evidence, however, suggests that granulopoiesis occurs in the spleen of these fish. Development of erythrocytes in these fish appears to occur in the same manner as that in mammals. The peripheral blood may be an important component of erythropoiesis, because several stages of erythrocyte development can be found in the routine blood films from cartilaginous fish.[45] The epigonal organ, which is associated with the gonad, and Leydig's organ, which is situated in the submucosa of the alimentary tract, are the major sites for granulopoiesis in cartilaginous fish.[7,39] Myeloblasts, progranulocytes, myelocytes, metamyelocytes, and mature granulocytes have been described in these unique lymphomyeloid tissues.

The principle lymphomyeloid tissues of bony fish (Osteichthyes) are the thymus, spleen, and kidney.[39] The thymus, which is the first lymphoid organ to develop, seeds the spleen and kidney with lymphocytes. The kidney is a major bloodforming organ in bony fish; the pronephric (anterior or head) and opisthonephric (main or trunk) kidneys are the sites of hematopoiesis in these fish. The opisthonephric kidney also functions as an excretory organ. Therefore, the kidney (primarily the pronephros) is the principal site for the differentiation and development of erythrocytes, granulocytes, lymphocytes, monocytes, and possibly, thrombocytes in most bony fish. The typical stages of granulocyte development have been identified for each type of granulocyte in the kidney of bony fish. The spleen of teleost fish is similar to that of elasmobranchs, but it typically has a secondary role in hematopoiesis, except in some species in which it is the only hematopoietic organ.

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22 Hematology of Amphibians

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Compared with other vertebrates seen in veterinary practices, amphibians are unique, because their normal life-cycle includes a metamorphosis from a larval to an adult form. Amphibians have adapted to aquatic, terrestrial, fossorial, and alpine environments, and their normal hematologic parameters vary accordingly. Amphibians are grouped into three orders: Urodela (Caudata) that includes salamanders and newts; Anura (Salientia) that includes frogs and toads; and Gymnophiona (Apoda) that includes the caecilians. Amphibians, especially frogs of the Ranidae family, are often used in research, but hematologic evaluation of amphibians is not routinely used in establishing the diagnosis of amphibian diseases. In fact, establishing reference values and the hematologic interpretation can be challenging because of the various extrinsic and intrinsic factors that influence these results. Extrinsic factors, such as environmental temperature, photoperiod, season, water-quality parameters, diet, and population density, should be noted whenever reference values are reported. Adaptation to a specific environment also influences the hematologic parameters. Important intrinsic factors include gender and age; larval and adult stages should be considered as separate entities, each with their own reference interval.

Collection and handling of blood

The general rule for sampling a safe volume of blood of no more than 1% of the body weight applies to all small exotic animals, including amphibians although some species may have relatively large blood volumes for their size. For example, the aquatic species of amphibians tend to have a blood volume between 13 and 25% of their body mass.[30] This compares to terrestrial species of amphibians like most other terrestrial vertebrates have a blood volume representing 10% of their body weight.

Blood can be collected from frogs and toads by venipuncture of the ventral abdominal or lingual vein or by cardiocentesis. Adequate restraint for blood collection may require sedation or anesthesia, such as submersion of the amphibian in a 0.05% solution of tricaine methanesulphonate. Care must be taken to avoid breaking the fragile mandibular bones while holding the mouth open to collect blood from the lingual vein. Excess saliva is swabbed from below the tongue, after which a large vein of the lingual venous plexus on the ventral aspect of the tongue is punctured with a 25-G needle. Blood is then allowed to flow into a microcollection or hematocrit tube. Venipuncture of the ventral abdominal vein of larger frogs and toads is accomplished by insertion of a 25-G needle through the ventral midline in a craniodorsal direction, midway between the sternum and the pelvis (Fig. 22.1). Blood is collected either by the drip method or by aspiration into a small syringe. Because lymphatic vessels accompany blood vessels in amphibians, a mixture of blood and lymph frequently occurs with venipuncture of the ventral abdominal vein. This mixing of lymphatic fluid with the blood sample is variable, but it will dilute the cellular components of the blood, thereby resulting in lower packed cell volume (PCV), hemoglobin concentration, and erythrocyte and leukocyte concentrations. Cardiocentesis is performed by placing the frog or toad in dorsal recumbency and locating the heart either by visualizing the pulsing heart or by use of a Doppler scan. Visualization of the heart can also be accomplished in some species via transillumination by use of the cool light from a rigid arthroscope inserted into the stomach.[30] Once the heart is located, a 25-G needle is inserted into the ventricle, and blood is aspirated into a syringe.

Blood collection from salamanders and newts can be accomplished by venipuncture of the ventral abdominal vein or by cardiocentesis, in the same manner as that described for frogs and toads. In addition, venipuncture of

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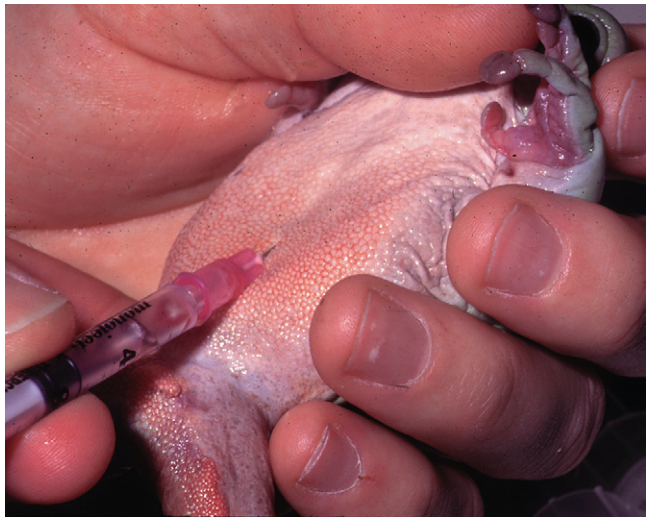

Figure 22.1 Blood collection from a tree frog (*Litoria caterulea*) using the ventral abdominal vein.

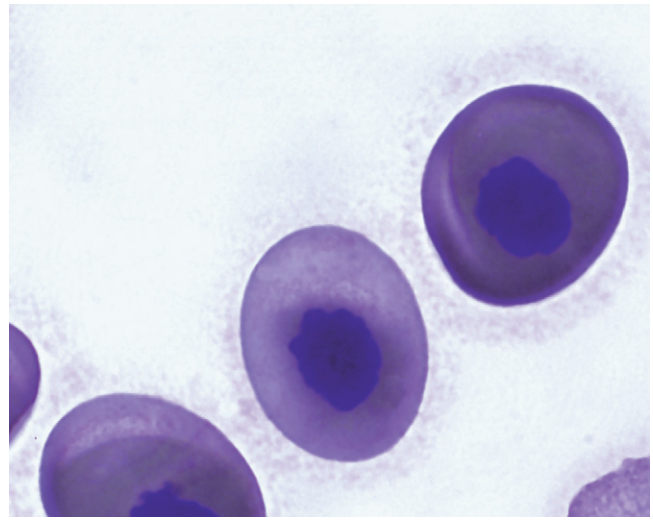

Figure 22.2 Erythrocytes in the blood of a salamander (*Ambystoma tigrunum*). Wright-Giemsa stain.

the ventral coccygeal vein can be performed by insertion of a 25-G needle to a point just below the coccygeal vertebrae, after which blood is aspirated into a syringe. This technique should be avoided, however, in salamanders and newts with tail autotomy (i.e., a natural ability to lose their tail), because the tails may break off during the procedure. Collection of blood after tail amputation has been used in research, but it should not be used in clinical practice.

Blood for hematologic studies in amphibians should be collected using lithium heparin as an anticoagulant. Ethylenediaminetetraacetic acid (EDTA) usually causes hemolysis of amphibian blood and, therefore, should be avoided. Syringes may be pretreated with lithium heparin, or blood can be allowed to drip from the needle hub into a microcollection tube containing lithium heparin.

Erythrocytes

Morphology

Erythrocytes of amphibians are large nucleated, elliptic discs (Fig. 22.2). The cells usually have a distinct nuclear bulge, and the nuclear margins often are irregular. Amphibian erythrocytes are large compared with those of other vertebrates. The mean size of a variety of frog and toad erythrocytes is $22 \times 14\,\mu m$. The average erythrocyte size for salamanders is $37.9 \times 23.9\,\mu m$ for *Ambystoma talpoideum* and $34.5 \times 19.0\,\mu m$ for *A. tigrinum*. The cytoplasm of frog and toad erythrocytes is homogenous and packed with hemoglobin. Ultrastructural analysis reveals rare organelles.[6] Because the erythrocytes of salamanders and newts complete their maturation in the peripheral circulation, the cytoplasm is not homogenous, and ultrastructural examination demonstrates clusters of granular and vacuolar bodies.[26]

There is considerable interspecies variation in the erythrocyte parameters of amphibians and they can be dramatically different from other vertebrates, especially mammals (Table 22.1). For example, the mean values for the fire-bellied toad (*Bombina bombina*) are reported as: red blood cell count = $0.34 \times 10^6\,\mu L$ for males and $0.29 \times 10^6/\mu L$ for females; hemoglobin concentration = 7.1 g/dL; hematocrit = 19.5%; MCV = 607 fl; MCH = 218.5 pg; and MCHC = 36.8 g/dL.[28]

Two forms of erythrocytes differentiated by size and morphology appear to occur in amphibians. One form, a larger elongated form is considered to be the larval form, whereas a smaller, rounded form is considered to be the adult form.[7,16,19] The transition from the larval form to the adult form begins at the onset of metamorphosis and by day 12 a complete transformation to all adult forms occurs.[7]

There is a positive relationship between body size and erythrocyte width in salamanders.[7] As salamanders grow, their erythrocytes become more rounded. This morphological change does not affect the overall area, however.

In some studies, males have higher erythrocyte counts than females. Also the presence of immature erythrocytes (as early as rubriblasts) in the peripheral blood tends to be greater in males than females where they can represent as high as 2% of the erythrocyte population.

The peripheral erythrocyte count of amphibians is affected by seasonal activity. An increase in bone marrow erythropoiesis occurs during the spring and following hibernation; therefore, the highest number of circulating erythrocytes occurs at this time.

Laboratory evaluation

The microhematocrit method is used for obtaining a PCV, which is the most common method for evaluating the red

Table 22.1 Erythrocyte parameters for selected amphibians.

	PCV [%]	RBC [×10^6/μL]	Hb [g/dL]	MCV [fl]	MCHC [g/dL]
American bullfrog[a,b]	39–42	0.450	9.3–9.7	—	21.1–25.9
Cuban tree frog[a]	20–24	—	5.6–6.8	—	25–31
Fire-bellied toad [male][c]	14–26	0.190–0.465	5.0–12.2	412–758	29–55
Fire-bellied toad [female][c]	12–23	0.240–0.355	3.4–8.3	363–917	19–60
Leopard frog [male][a]	19–52	0.227–0.767	3.8–14.6	722–916	23–27
Leopard frog [female][a]	16–51	0.174–0.701	2.7–14.0	730–916	20–28
Mudpuppy[a]	21	0.020	4.6	10,070	22
Tiger salamander[a]	40	1.657	9.4		

[a]Wright KM (2005) Amphibians. In: *Exotic Animal* Formulary, 3rd ed., J. Carpenter (ed.), St. Louis, MO: Elsevier Saunders, p. 46.
[b]Cathers T, Lewbart GA, Correa M, Stevens JB (1997) Serum chemistry and hematology values for anesthetized American bull frogs [*Rana catesbeiana*]. *J Zoo and Wildlife Medicine* 28: 171–4.
[c]Wojtaszek J, Adamowicz A (2003) Haematology of the fire-bellied toad, *Bombina bombina*. *L. Comp Clin Path* 12: 129–34.

cell mass of amphibians. The cyanmethemoglobin method commonly is used to determine the hemoglobin concentration in amphibian blood. As with blood hemoglobin determinations in birds, reptiles, and fish, this procedure requires centrifugation of the blood–cyanmethemoglobin mixture to remove the free erythrocyte nuclei before the optical density is measured.

The total erythrocyte count in amphibians can be determined either by manual counting with a hemocytometer or by an electronic cell counter. Manual counting methods to obtain red-cell concentrations in amphibian blood include the erythrocyte Unopette system (Becton-Dickinson, Rutherford, NJ) and Natt and Herrick's method. These methods are the same as those described for use in avian blood (see Chapter 19).

Responses in disease

The average amphibian erythrocyte life span is considered to be greater than 100 days, which may have an impact on the erythrocytic response. Because newts and salamanders generally are more fishlike than toads and frogs, interpretation of their hemograms may be more like those of fish, whereas hematologic changes in toads and frogs may be more like those of reptiles. In general, amphibian PCVs are lower than those of mammals and birds, and these values vary with species, age, gender, environmental temperature, photoperiod, season, and life style of the amphibian (Table 22.1).[3,22] Normal erythrocytes exhibit a slight anisocytosis; however, increased anisocytosis suggests erythroid regeneration or dyscrasia caused by an increased concentration of large red cells. Because of the stability of their environments, captive amphibians may have erythron parameters that fluctuate less than those of wild amphibians. Because amphibians are ectothermic, the rapidity of their hematologic responses can be manipulated by changes in the environment, such as temperature fluctuation.[15]

Leukocytes

Morphology

The leukocytes of amphibians, like those of most mammals, are classified as being neutrophils, eosinophils, basophils, lymphocytes, and monocytes. Amphibian leukocytes generally are larger than those of mammals.

Amphibian neutrophils resemble those of mammals, and they range from 10 to 25 μm in diameter for most species (Fig. 22.3).[26] They have multilobed nuclei with small cytoplasmic granules that vary in size, shape, and ultrastructure between species. Cells with small eosinophilic cytoplasmic granules often are referred to as heterophils, whereas those cells that do not are referred to as neutrophils.[2,17,22,24,26] Amphibian neutrophils typically are peroxidase positive, but phosphatase activity varies with the species.[14,26]

Eosinophils of amphibians are similar in size to neutrophils, and they have a slightly basophilic cytoplasm, with small to moderate-sized, round to oval, eosinophilic cytoplasmic granules (Fig. 22.4). The nuclei of eosinophils are less lobed than those of neutrophils. Eosinophils are peroxidase negative, and the phosphatase activity varies with species.[14,6] The eosinophils of some species, such as the Colorado River toad (*Bufo alvarus*), are negative for aryl sulfatase and β-glucuronidase activity.[2] Some amphibian eosinophil granules have a crystalloid ultrastructure, but

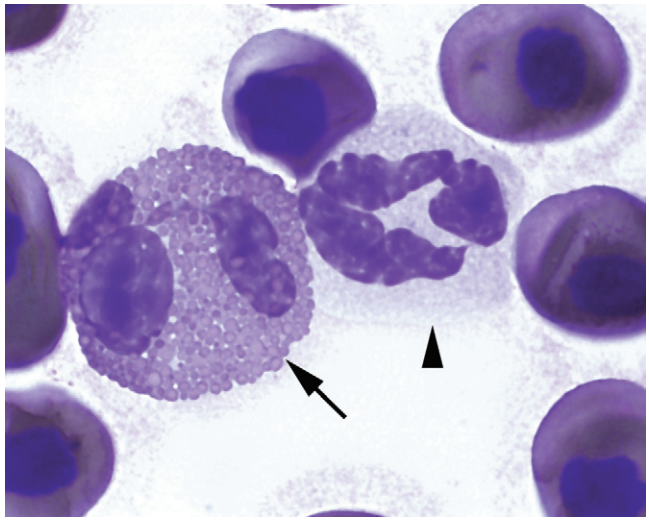

Figure 22.3 A neutrophil (arrowhead) and an eosinophil (arrow) in the blood of a salamander (*Ambystoma tigrunum*). Wright-Giemsa stain.

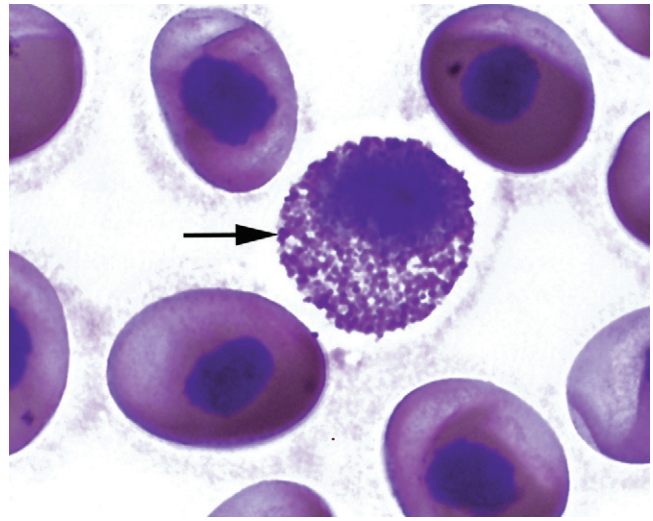

Figure 22.5 A basophil (arrow) in the blood of a salamander (*Ambystoma tigrunum*). Wright-Giemsa stain.

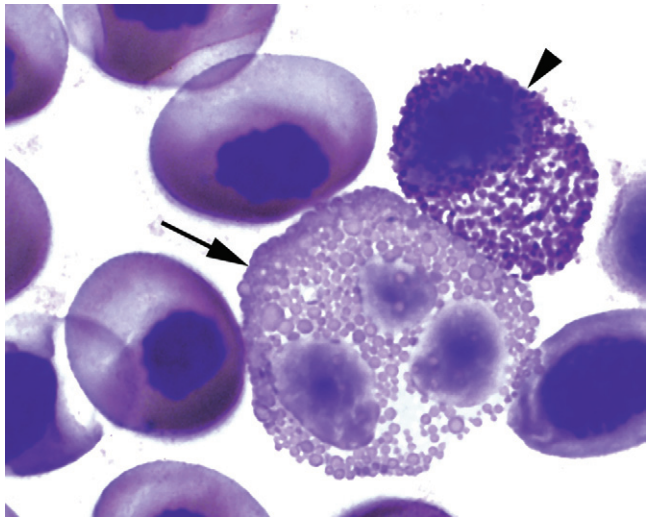

Figure 22.4 An eosinophil (arrow) and a basophil (arrowhead) in the blood of a salamander (*Ambystoma tigrunum*). Wright-Giemsa stain.

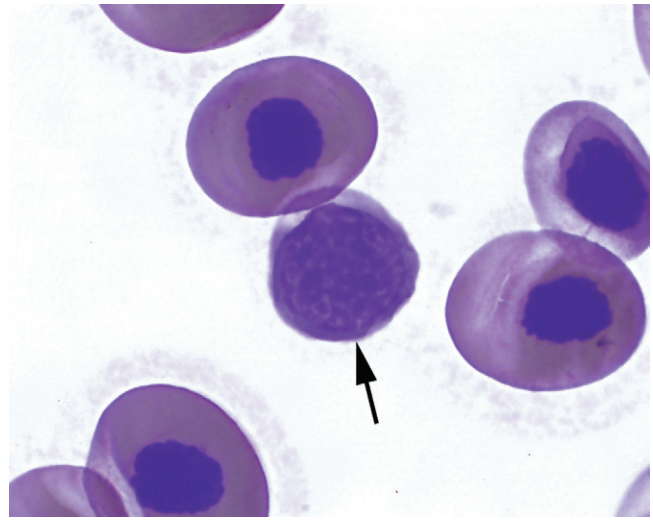

Figure 22.6 A lymphocyte (arrow) in the blood of a salamander (*Ambystoma tigrunum*). Wright-Giemsa stain.

others, lack the crystalloid structures that are typical of the ultrastructural morphology of eosinophils from higher vertebrates.[22]

The size of amphibian basophils varies between species. Typically, these basophils have nonsegmented nuclei and large, metachromatic granules (Figs. 22.4 and 22.5). The granules contain acid mucopolysaccharides (i.e., glycosaminoglycans) that are less sulfated than those of mammals, and the histamine content is lower than that of mammals.[26] Ultrastructural analysis demonstrates large numbers of membrane-bound cytoplasmic granules with small numbers of organelles.

The lymphocytes of amphibians resemble those of other vertebrates. Small lymphocytes are more abundant than larger forms in the blood films of normal amphibians. The lymphocytes are round, with round nuclei, and they have dense chromatin clumping as well as a scant amount of pale-blue cytoplasm (Fig. 22.6). Many of the lymphocytes have distinct azurophilic granules from frogs of the family Ranidae.[2,15,16,26] Amphibian lymphocytes, like mammalian lymphocytes, are nonspecific esterase positive and peroxidase negative but, unlike mammalian lymphocytes, are negative for β-glucuronidase and aryl sulfatase.[26]

Monocytes in amphibian blood films are similar to those of other vertebrates, and they are characterized by their large size; abundant, blue-gray cytoplasm that may be foamy or vacuolated; and a variably shaped nucleus with less chromatin clumping than seen in lymphocyte nuclei (Fig. 22.7).

Amphibian monocytes may contain fine, azurophilic granulation and pseudopodia. They also contain some of the hydrolytic enzymes that are found in mammalian monocytes.

Granulopoiesis occurs in the liver, kidney, and bone marrow of amphibians; however, some species lack bone marrow.[6] Myeloblasts and progranulocytes have not been positively described in amphibians. Immature neutrophils have small granules of various shapes that increase in both size and density with maturation, until the larger, definitive peroxidase-positive granules are formed. Some species do not develop primary granules; rather, they produce a different population of granules. Evidence suggests that in some species, eosinophils begin as round cells, with a round nucleus and scant cytoplasm that contains large, dense, and round primary granules. Further development of eosinophils results in a mixture of the larger primary granules and the smaller secondary granules.[26]

The monocyte is the first leukocyte to appear in the peripheral blood of bullfrog (*Rana catesbeiana*) larvae, in which immature monocytes with linear nuclear chromatin appear 15 days after hatching and mature monocytes with round nuclei, which develop into kidney-shaped or lobed nuclei, appear 22 days after hatching.[24,26] Definitive neutrophils, eosinophils, and basophils in larval bullfrogs appear in the peripheral blood late during development of the frog, but all three appear at the same time.

Lymphopoiesis in amphibians resembles that in other vertebrates. Small lymphocytes are the most common, but larger lymphocytes also may be seen.

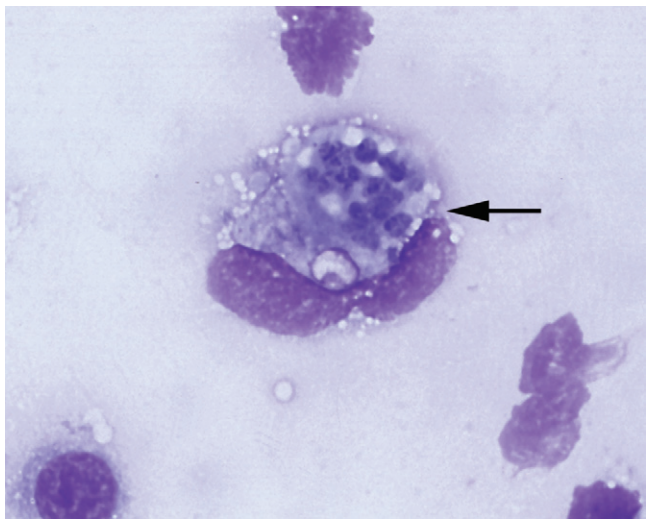

Figure 22.7 A monocyte (arrow) exhibiting leukophagocytosis in the blood of a a tree frog (*Litoria caterulea*). Wright-Giemsa stain.

Laboratory evaluation

As in other nonmammalian vertebrates, amphibians have nucleated erythrocytes and thrombocytes that interfere with automated methods for counting leukocytes; therefore, manual counting methods are used. The Natt and Herrick's or phloxine B method, as described for birds in Chapter 19, can be used to obtain a total leukocyte concentration in amphibian blood.

The leukocyte differential is performed using Romanowsky-stained blood films (Table 22.2a,b). Because most blood samples from amphibians are collected into heparin, making blood films either with blood containing no anticoagulant or immediately after mixing of the blood with the heparin (to decrease cell clumping and improve staining) is best.

Responses in disease

Normal total leukocyte counts exhibit inter- and intraspecies variation.[5,10,12,15,17,21,22,29] The lymphocyte is generally the most numerous leukocyte and the neutrophil is the most numerous granulocyte in the peripheral blood of most amphibian species studied. Little is known regarding the function of the various amphibian leukocytes. The process of interpreting the amphibian leukogram is extrapolated from that used with other vertebrates. Amphibian neutrophils have both migratory and phagocytic activity, and they participate in inflammation. Likewise, amphibian monocytes are phagocytic and, most likely, function in a manner similar to those of other vertebrates. Therefore, increases in the neutrophil and monocyte counts likely suggest an inflammatory response.

Table 22.2a Leukocyte parameters for selected amphibians.

	WBC $\times 10^3/\mu L$	Neut/Heterophils $\times 10^3/\mu L$	Lymphocytes $\times 10^3/\mu L$	Monocytes $\times 10^3/\mu L$	Eosinophils $\times 10^3/\mu L$	Basophils $\times 10^3/\mu L$
Fire-bellied toad [male][a]	2.21–18.48	0.20–5.70	2.30–10.80	0.20–1.80	0–0.90	0.10–4.20
Fire-bellied toad [female][a]	1.04–14.25	0.10–4.40	0.70–7.10	0.10–1.60	0–0.80	0.10–2.30

[a]Wojtaszek J, Adamowicz A (2003) Haematology of the fire-bellied toad, *Bombina bombina*. *L. Comp Clin Path* 12: 129–34.

Table 22.2b Leukocyte parameters for selected amphibians.

	WBC $\times 10^3/\mu L$	Neut/ Heterophils %	Lymphocytes %	Monocytes %	Eosinophils %	Basophils %
African clawed frog[a]	8.2	6.9–9.1	62.6–68.0	0–1	0	7.1–9.9
American bullfrog[a,b]	2.3–8.1	6.8–37.2	47.9–77.9	0–2	2.8–15.0	0–6
Edible frog[a]	6.1	6.7–10.9	48.7–55.3	0–2	18.1–20.7	15.3–17.9
Grass frog[a]	14.4	5.5–7.51.5	65.6–71.4	0–1	11.6–17.4	22–26.4
Japanese newt[c]	1.51–2.09	25.4–30.6	2.6–3.4	5–7	3.3–4.7	53.8–60.2

[a]Wright KM (2005) Amphibians. In: *Exotic Animal* Formulary, 3rd ed., J. Carpenter (ed.), St. Louis, MO: Elsevier Saunders, p. 46.
[b]Cathers T, Lewbart GA, Correa M, Stevens JB (1997) Serum chemistry and hematology values for anesthetized American bull frogs [*Rana catesbeiana*]. *J Zoo and Wildlife Medicine* 28: 171–4.
[c]Pfeiffer CJ, Pyle H, Asashima M (1990) Blood cell morphology and counts in the Japanese newt [*Cynops pyrrhogaster*]. *J Zoo and Wildlife Medicine* 21: 56–64.

Eosinophils have an inferior ability to phagocytize particles or microorganisms compared with that of neutrophils, but they do respond to metazoan parasitic infections.[26] Therefore, peripheral eosinophilia may suggest a parasitic infection.

Amphibian basophils may function in a manner similar to those of mammals. They rarely are found in the peripheral blood of some species, but are abundant in others. For example, the Japanese newt normally has a differential leukocyte count that includes as much as 60% basophils.[22] In this species, basophils are considered to play a significant role in immunosurveillance.

Lymphocytes of frogs and toads demonstrate an immunologic sophistication similar to those of higher vertebrates. The lymphocytes can be classified as B cells that produce immunoglobulins or as T cells with populations of functional diversity, such as helpers and different effectors.[26] In contrast, the lymphocytes of newts and salamanders appear to lack such refinement. Japanese newts demonstrate a transitory lymphocytosis after tail amputation for blood collection.[22]

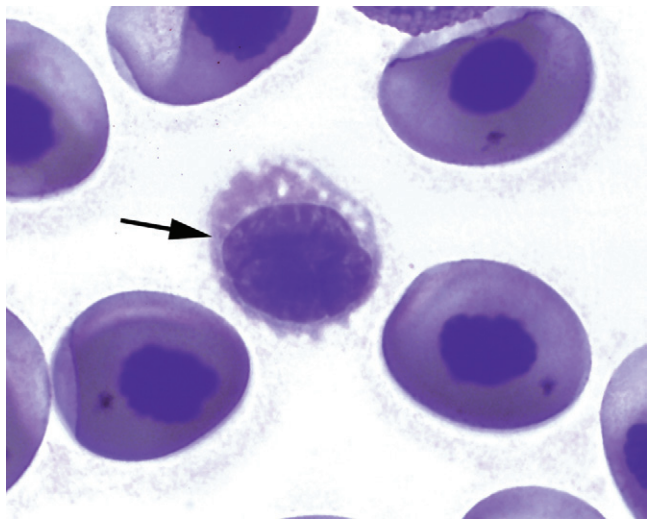

Figure 22.8 A reactive thrombocyte (arrow) with fine eosinophilic cytoplasmic granules in the blood of a salamander (*Ambystoma tigrunum*). Wright-Giemsa stain.

Thrombocytes

Morphology

Amphibian thrombocytes are nucleated cells resembling those described for birds, reptiles, and fish. They tend to resemble small, mature lymphocytes but often are spindle-shaped, with a dense, round to oval nucleus and abundant, colorless cytoplasm (Fig. 22.8). The thrombocytes of some amphibians, such as *Xenops* and *Rana* sp., are alkaline phosphatase positive, whereas the lymphocytes are negative.[26] Anucleated thrombocytes that resemble mammalian platelets have been described in some species.

Laboratory evaluation

The total thrombocyte count can be obtained from the same charged hemocytometer used to obtain the total erythrocyte and leukocyte count. The thrombocytes resemble the erythrocytes in the hemocytometer, but they are smaller and appear to be round to oval, with a greater nucleus:cytoplasm (N:C) ratio compared to the erythrocytes. All squares in the central large square of a Neubauer-ruled hemocytometer are counted on both sides, and the average number of thrombocytes in one large square is calculated and multiplied by 2000 to obtain the total thrombocyte count per microliter. Accurate counts may be difficult to achieve, however, because thrombocytes tend to clump (Table 22.3).

Table 22.3 Thrombocyte counts for selected amphibians.

	African Clawed Frog[a]	Edible Frog[a]	Fire-Bellied Toad [Male][b]	Fire-Bellied Toad [Female][b]	Grass Frog[a]
Thrombocytes $\times 10^3/\mu L$	17.7	16.3	2.76–10.69	1.43–19.47	20.8

[a]Wright KM (2005) Amphibians. In: *Exotic Animal* Formulary, 3rd ed., J. Carpenter (ed.), St. Louis, MO: Elsevier Saunders, p. 46.
[b]Wojtaszek J, Adamowicz A (2003) Haematology of the fire-bellied toad, *Bombina bombina*. *L. Comp Clin Path* 12: 129–34.

Responses in disease

Functionally, thrombocytes are equivalent to mammalian platelets, and they participate in coagulation. Immature forms of thrombocytes (round cells with round nuclei) are not normally found in the peripheral blood of amphibians; therefore, their presence suggests either a regenerative response or dyscrasia. Thromboblasts have fine, nuclear chromatin, with a large, irregular, and eccentric nucleolus and weakly basophilic cytoplasm. Prothrombocytes have elongate nuclei and vacuolated cytoplasm with pale blue granules. Low and high thrombocyte counts are interpreted in the same manner as those described for other nonmammalian vertebrates.

Blood parasites

Microfilaria and trypanosomes commonly are seen in amphibian blood.[1,18,20,25,27] *Toxoplasma*, *Isospora*, and *Leptotheca* are found on occasion. Common differentials for amphibian intraerythrocytic inclusions include hemogregarines such as those described in reptiles (see Chapter 20, Hematology of Reptiles), *Aegyptianella* spp., and a *Pirohemocyton*-like virus. *Lankesterella* spp. may also be found within the cytoplasm of lymphocytes.[8,9,11,13,23] Often, these organisms are considered to be an incidental finding; however, they may be pathogenic when they occur with anemia.

Hematopoiesis

Development of the amphibian erythrocyte is similar to that described for other vertebrates with nucleated erythrocytes. Maturation of the rubriblast to the mature erythrocyte involves a progressive change of cytoplasmic basophilia to eosinophilia, a change from a round to an elongated shape, a decrease in the nuclear and nucleolar size, and an increased chromatin density.

The liver is the predominant erythropoietic tissue of both larval and adult frogs.[26] Larval amphibians may have two populations of morphologically different erythrocytes, which have different origins. One population, originating in the liver, has a centrally positioned nucleus; the other, originating in the kidney, has a peripherally located nucleus. The different erythrocyte populations also have different larval hemoglobins.[4] During metamorphosis, a third population of erythrocytes appears, and this population persists in adults. Dark-field illumination can be used to differentiate larval erythrocytes, which have a white to gray, granular luminescence, from adult erythrocytes, which lack luminescence.

The metamorphosis from larval to adult amphibians is accompanied by the synthesis of hemoglobins with different oxygen affinities and various intracellular modulators of hemoglobin–oxygen affinity.[4] Gilled larval amphibians have blood with a higher affinity for oxygen than that of air-breathing adults. The tetrameric hemoglobin of amphibians consists of two α-like and two β-like globin chains, thereby creating four larval-type and four adult-type hemoglobins. No globin chains are shared between larval and adult amphibians. Adult hemoglobin begins to appear in frogs during tail regression, and it is the only hemoglobin found 3 weeks after metamorphosis. Adult amphibians have higher hemoglobin concentrations and PCVs compared with the larval forms. Metamorphosis results in decreases in adenosine triphosphate (ATP) and guanosine triphosphate concentrations in the erythrocytes, thus suggesting a change in the phosphate regulation of hemoglobin in adults compared with larval forms.

Metamorphosis in newts and salamanders is not always associated with a transition in hemoglobin such as that occurring in frogs and toads. When newts and salamanders change from aquatic to aerobic respiration at metamorphosis, the larval and adult hemoglobins have the same affinity for oxygen. The reduced oxygen affinity of the blood in adults, however, frequently is achieved by an increased erythrocyte concentration of ATP.[4] Even so, some species, such as the tiger salamander (*Ambystoma tigrinum*), experience no decrease in the oxygen affinity of blood at metamorphosis, and the hemoglobin and total erythrocytic organic phosphate concentrations remain unchanged.

Toads, which primarily rely on aerobic respiration, tend to have higher hemoglobin and erythrocyte phosphate concentrations and a lower blood oxygen affinity compared

with frogs, which primarily rely on anaerobic respiration. Aquatic amphibians do not have the same association between high erythrocyte phosphate concentrations and dependence on aerobic production of energy for activity as terrestrial amphibians do. The exchange of gases from the blood to the surrounding water occurs through the skin of aquatic amphibians.

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IV

Clinical Chemistry of Common Domestic Species

23 Laboratory Evaluation and Interpretation of the Urinary System

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General

The primary reason for assessing the urinary system is to recognize failure of that system. Laboratory abnormalities will not appear until so many nephrons are damaged that the remaining nephrons can no longer compensate for the damaged ones. Once identified, renal failure is then categorized as acute or chronic. This identification is further narrowed down to a specific disease diagnosis or, at least, to the renal structure that is diseased, e.g., if the disease is centered on the glomeruli (glomerulonephritis or amyloidosis), tubules (nephrosis), interstitium (interstitial nephritis), renal pelvis (pyelonephritis), or excretory system (cystitis, obstruction, or rupture).

Laboratory analysis of the serum biochemical profile and complete urinalysis are practical means to accomplish the assessment of renal function. The most accurate way to identify renal failure is to directly measure GFR; however, in veterinary medicine this is rarely done. Instead, we use indirect evidence to estimate reductions in GFR, and from this to imply that renal failure is present. Although indirect, it is adequate. If a more precise assessment of renal function is desired, then GFR can be measured by clearance studies.

Fortunately, there are many ways to recognize failure of the urinary system based on the history, biochemical profile, and evaluation of urine. Among other things, these include identification of anuria, polyuria, azotemia, uremia, electrolyte abnormalities, hypoalbuminemia, inappropriate urine specific gravity, casts, cystitis, and hematuria. When renal failure is severe the clinical recognition is easy, when failure is mild and/or the disease is in the earliest stages, then recognition is difficult and may require ancillary diagnostic tests such as creatinine clearance, fractional excretion of sodium, assessment of microproteinuria, or ultrasonography. These ancillary tests are also useful to monitor the patient's response to treatment.

Understanding the physiology of the kidney makes the understanding of clinical pathology simple. If structures are damaged, then functions are lost, and if the remaining nephrons cannot compensate a clinical laboratory or physical examination finding will generally be obvious. For example, glomeruli exclude albumin from the ultrafiltrate, if glomerulonephritis is present, then this exclusionary function is lost. If the disease is severe enough and the remaining nephrons cannot compensate there will be proteinuria, hypoalbuminemia, and potentially, ascites and dependent edema (Table 23.1).

Depending on severity and chronicity of disease, as well as any treatments that have been undertaken to slow disease progression, laboratory abnormalities may be nonexistent, mild, moderate, or marked. The severity of renal disease can be graded as 1, 2, 3, or 4 based on severity of clinical signs, physical examination results, and laboratory abnormalities. The stage of the renal disease correlates with the severity of the renal lesions and is correlated with prognosis. As the stage of the renal disease progresses so does the severity of the laboratory abnormalities and the percentage of animals that have that abnormality. For example, 20% of azotemic dogs have only mild hyperphosphatemia in stage 1 renal disease, but the incidence of hyperphosphatemia increases to 100% in stage 4, at which point serum phosphorus concentration is markedly increased.

Laboratory abnormalities will not appear until enough nephrons are incapacitated (severity) and the remaining nephrons cannot compensate. Two classic examples are dilute urine (isosthenuria) and azotemia. Inability to concentrate urine occurs when approximately 66% of nephrons are not functioning properly. Azotemia is not seen until approximately 75% of nephrons are compromised. However, the finding of azotemia alone does not allow us to narrow

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Table 23.1 The concentration of certain substances measured in serum or plasma are affected by renal function, in that the kidneys play a role in the excretion, conservation or production of those substances. Damage to specific renal structures results in damage to renal function. The abnormalities listed are generalizations and not constants. Their presence will depend on the severity of the lesion, chronicity, treatments, and compensatory ability of surviving nephrons.

Substance	Function Lost	Abnormality
UN Ct	Excretion	Azotemia
Water	Balance	Polyruia, anuria, oliguria
P	Excrete	Hyperphosphatemia
Na Cl	Conserve	Normo- to hyponatremia, hypochloremia
K	Excrete	Hyperkalemia
Ca	Conserve	Hypocalcemia
Acid base	Balance	Acidosis metabolic; alkalosis cow
Albumin	Conserve	Proteinuria, hypoalbuminemia, ascites
Erythropoietin	Produce	Anemia, nonregenerative
Vitamin D	Produce	Hypocalcemia, osteodystrophy
Lipase, amylase	Excrete	Increased one- to threefold
Antithrombin III	Conserve	Decreased AT III, thrombi

down its cause because azotemia can be prerenal, renal, or postrenal.

This chapter will follow the functions of the urinary system, predict clinical pathology results and diagnoses, and provide case examples in the discussion section. The emphasis is on interpretation of laboratory data with less focus on methodology, which is covered exhaustively in many other excellent resources. A glossary of terms is at the end of chapter.

Introduction and Case Examples

Glomerular filtration rate is the best predictor of renal function because it is directly related to total functional renal mass or, in other words, to the number of functioning nephrons. It is the volume of plasma filtered at the glomerular capillaries into Bowman's space per unit of time. A GFR of 3–6 mL/min/kg is normal for a dog and 2–4 mL/min/kg is normal for a cat. It is dependent on adequate blood flow to the kidneys, blood pressure, interstitial and intratubular pressures, as well as number of functioning nephrons. It is not easy to measure GFR directly, but it can be measured by studies that use substances which are freely filtered by the glomerulus, and that are neither secreted nor reabsorbed, such as inulin, iohexol, mannitol, *p*-amminohippuric acid, and exogenous creatinine. Glomerular filtration rate can also be estimated by endogenous creatinine clearance studies. The methodologies of these and other tests can be found in many other resources.

Because of the complexity of direct GFR measurement studies, it is seldom measured in veterinary medicine. Instead indirect evidence of decreased GFR is derived from serum [UN], [Ct], [phosphorus], and serum [calcium], and additional data is gleaned from the complete urinalysis, and / or urinary protein and creatinine clearance, as well as the fractional excretion of sodium, among other tests.

A decreasing GFR is the best indicator of renal insufficiency, and since UN and Ct are both freely filtered by the glomerulus they are the analytes most commonly used to estimate GFR (Fig. 23.1). As the GFR decreases plasma [UN] and [Ct] increase; however, GFR must be reduced by 75% before [UN] and [Ct] increase in blood plasma. Because azotemia is not evident until 75% of nephrons are no longer functioning adequately, and because the ability to concentrate urine is lost after 66% of nephrons are compromised, azotemia and dilute urine or polyuria caused by renal failure are not detected until a large portion of the total renal mass is compromised. Therefore, they are not early indicators of renal failure. These percentages indicate the tremendous reserve of renal function, since only 25% of total renal mass is needed to excrete sufficient nitrogenous waste to prevent azotemia, and only 33% is needed to concentrate urine and preserve the body's fluid volume.

It should be noted that no serum biochemical abnormalities indicate irreversibility of renal damage, for, while glomeruli have no regenerative capabilities, tubules have tremendous regenerative capacity if basement membranes are preserved. If glomeruli and tubules sustain severe damage the remaining nephrons compensate by hypertrophy. For example, if a dog with normal plasma [Ct] of 0.5 mg/dL developed renal disease such that plasma [Ct] increased to 1.0 mg/dL, then theoretically 50% of its nephrons are now not functioning properly. However, since that value of plasma [Ct] is still within the reference interval, that 50% loss of function would not be detected by measuring [Ct] or [UN] because the renal reserve, or compensation via hypertrophy of remaining nephrons, would work to maintain plasma [Ct] within the reference interval of a "normal" dog. However, and very importantly, once [Ct] is increased above the upper reference interval, then every doubling of plasma [Ct] indicates a loss of function of 50% of the remaining renal mass. Conversely, the recovery of

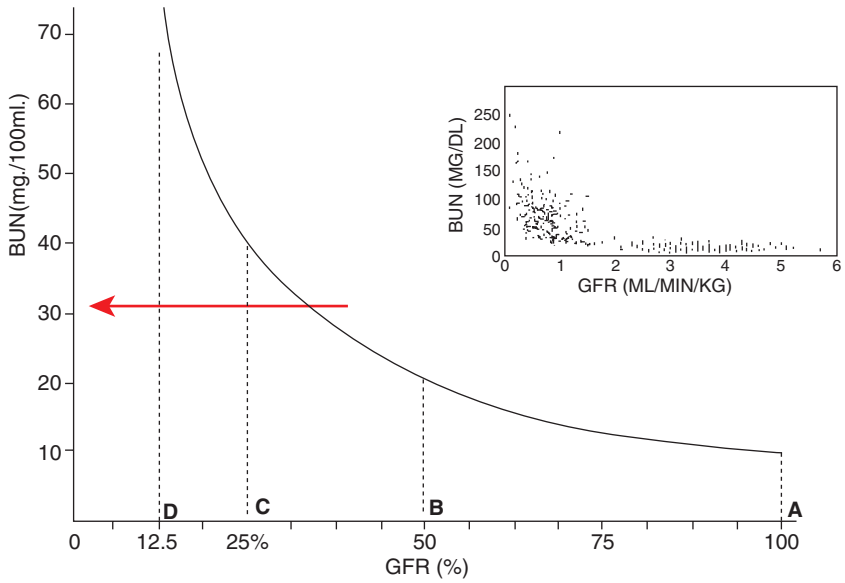


Figure 23.1 Glomerular filtration rate (GFR) as mL/min/kg body weight (inset) and as % functioning nephron units. A GFR of 3–6 mL/min/kg is normal for dogs and the BUN is less than 30 mg/dL and as GFR decreases the BUN increases (inset). In the example provided (A) correlates with 100% functional nephron units and a BUN of approximately 12 mg/dL. When 50% of the nephron units are compromised (GFR decreased 50%) the BUN has doubled to approximately 24 mg/dL but it is still in reference interval (occult renal issues). Not until approximately 75% of the nephron units are compromised (GFR is now 25% of normal) is the dog azotemic (C). Therefore BUN and serum creatinine (Ct) are poor indicators of early renal insufficiency. If the GFR is decreased by half again (D) by advancing renal disease, then a rule of thumb is the BUN and Ct will double. Likewise a decrease in BUN and Ct by half indicates the GFR has doubled, meaning there is improvement in renal function, e.g., more nephrons are functioning. Monitoring BUN or Ct to assess deterioration or improvement of renal function can be done only after they are increased, but GFR can be used to monitor patients with renal insufficiency at any time.

nephrons from an insult can be monitored, for every 50% reduction in plasma [Ct] or [UN] indicates that 50% of the nephrons have returned to function. This is because there is a logarithmic relationship of plasma [Ct] to GFR (Fig. 23.1). It is this principle that allows us to monitor progression of renal disease through measurement of plasma [Ct], once the upper limit of the reference interval has been surpassed. However, more sophisticated methods are required to determine if renal disease is present when plasma [Ct] is not increased because of the great amount of renal functional reserve, e.g., the tests of endogenous or exogenous creatinine clearance, inulin clearance, clearance of radioisotopes, and assessment of fractional excretion of electrolytes, etc.

It should be noted that plasma [Ct] is a better indicator of GFR than plasma [UN] because its rate of production and excretion are fairly constant, and it is neither metabolized by extrarenal nor renal processes. In contrast, plasma [UN] is influenced by more nonrenal factors than is [Ct] and a significant proportion of [UN] excreted into the glomerular filtrate is reabsorbed. Furthermore, the rate of reabsorption of [UN] varies with the hydration status of the animal, as well as the speed of flow of the glomerular filtrate within the tubules.

Plasma [Ct] within the reference interval does not mean the kidneys are normal, it means that 25% of the renal mass is functioning adequately enough to excrete creatinine and keep it within the reference interval. An endogenous creatinine clearance study is a better indicator of renal function than plasma [Ct] or [UN]. Endogenous [Ct] clearance can be used to estimate GFR because the production of [Ct] is relatively constant and essentially 100% is excreted via the kidneys (the small amount that is secreted by proximal tubules is negligible for this estimation). Creatinine clearance is useful when renal disease is suspected but the plasma concentrations of [Ct] and [UN] are not increased. Creatinine clearance can be calculated from the serum [Ct], urine [Ct], the volume of urine produced in a defined period, and the weight of the patient. The urinary bladder is completely emptied of urine at the start of the study and the volume produced in the duration of the study is measured.

$$\text{Creatinine clearance} = \frac{\text{Urine Ct} \times \text{volume urine/time/kg}}{\text{Serum Ct}}$$

Case. 40 kg dog has a serum Ct of 1.0 mg/dL and in 1 hour produces 30mL of urine that has a urine Ct of 200 mg/dL.

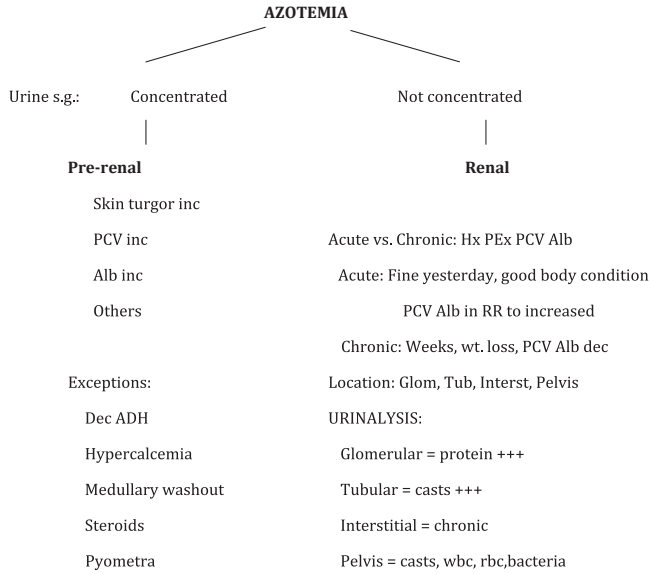
$$\text{Creatinine clearance} = \frac{200\ \text{mg/dL} \times 30\ \text{mL/60 min/40 kg}}{1.0\ \text{mg/dL}}$$

$$= \frac{200\text{mg/100 mL} \times 0.0125\ \text{mL/min/kg}}{1\text{mg/100 mL}}$$

$$= 2.5\ \text{mL/min/kg}$$

Reference range for dogs is 3–6 mL/min/kg

This dog has reduced Ct clearance not reflected by the serum [Ct], which is within the reference interval. This may be seen in posttreatment of renal failure, when the serum [Ct] has returned to reference interval: the patient feels better but there is still compromised renal function, e.g., some renal lesions may persist, such as interstitial fibrosis. These patients will benefit from dietary changes and *ad libitum* access to water. This might be predicted from clinical experience but it would not be known without assessment of urinary creatinine clearance. In a 24-hour study of endogenous creatinine clearance, the urinary bladder is emptied of urine at the start of the study, the volume of urine produced in 24 hours is measured, and a serum sample is collected, usually in the middle or at the end of the study.



Correlate azotemia with USG, PCV, albumin and hydration status to determine if azotemia is pre-renal or renal or a combination. Concentrated urine for a dog is > 1.030 and for a cat >1.040, the greater the USG over these low end ranges for adequate concentration the greater our confidence that the kidney can concentrate urine and the cause of the azotemia is pre-renal. Not concentrated means the USG is inappropriately dilute relative to azotemia and the other parameters being assessed. USG 1.007-1.013 (isosthenuria) with azotemia means there is renal failure, if the animal is dehydrated some of the azotemia may be due to hypovolemia. USG 1.014 to 1.029 in a dehydrated dog is probably inappropriate and suggests there may be concurrent renal issues and dehydration. Decreased ADH or increases in substances that interfere with ADH may prevent tubules from concentrating urine. This is confusing if the animal has azotemia from dehydration but the urine is dilute because of an interfering substance: hypercalcemia, steroids, pyometra etc.

Figure 23.2 General approaches to azotemia.

Endogenous creatinine clearance can also be used in the diagnostic evaluation of a patient in which occult renal disease is suspected. This patient is not azotemic but has polyuria, polydipsia, and dilute urine. This situation may be observed when functional renal mass is reduced to between 66% and 75%. If more accurate assessment of renal function was desired then clearance of exogenous creatinine, inulin or iohexol can be performed as well as injected radioisotopes or renal scintigraphy. These studies are usually only performed in specialty hospitals and when the clinician and owner want the patient monitored closely.

Urea nitrogen and creatinine

A major function of the urinary system is to excrete UN and Ct. When this function is lost, plasma concentrations of [UN] and [Ct] increase—a condition termed azotemia. Azotemia is the single best laboratory abnormality that indicates problems in the urinary system. Azotemia is caused by prerenal, renal or postrenal causes. Prerenal azotemia is due to hypovolemia (e.g., dehydration, shock). Renal azotemia is caused by lesions in one of five locations: glomeruli, tubules, intersititium, renal pelvis, or blood vessels, and postrenal azotemia is due to an obstruction anywhere after the nephron, or rupture within the urinary system. Any time azotemia is recognized the next step is to correlate it with the urine specific gravity (USG), and to determine if urine is exiting the body, and if so, in what quantities (i.e., is there anuria, oliguria, or polyuria?). Azotemia with concentrated urine is of prerenal origin (exception is some cats); azotemia with unconcentrated urine is of renal origin (Fig. 23.2). Azotemia due to postrenal causes has urine of various concentrations; and diagnosis of postrenal azotemia is dependent on determining that urine is not being excreted, i.e., there is an obstruction or rupture.

Mild azotemia is characterized by a serum [Ct] (in mg/dL) of: 1.5–2.0 in the dog, 1.6–3.0 in the cat. Moderate azotemia: 2.0–5.0 dogs, 3.0–5.0 cat, and severe azotemia is >5.0 in both the dog and cat. Extreme values >10.0 may be observed when hypovolemia (e.g., dehydration) is superimposed on renal or postrenal causes of azotemia. The magnitude of serum [UN] or [Ct] does not predict a pre-, renal or postrenal cause of azotemia.

Urea nitrogen and creatinine are byproducts of nitrogen metabolism and they, along with a complete urinalysis, are the most practical indicators of renal failure. Both UN and

Ct are excreted via glomerular filtration, and as the GFR decreases the plasma concentrations of [UN] and [Ct] increase, resulting in azotemia. Urea is produced in the liver from ammonium and bicarbonate. It has a molecular weight of 60 daltons and is the main means of nitrogen excretion in animals. The term "blood urea nitrogen" (BUN) is no longer a relevant term because of changes in measurement methodology. Originally, a urease method released nitrogen from urea and the amount of nitrogen was measured, i.e., BUN. Now urea is measured from the release of ammonium ions via urease. However, "urea" is not a substance defined by the International Clinical Chemistry Federation. They use "carbamide." It has been difficult to switch clinicians from BUN to UN, and impossible to force the acceptance of carbamide, so throughout this chapter, the term UN will be used.

Decreased plasma [UN] is uncommon. A decreased serum [UN] implies decreased production of urea, either due to hepatic failure, or portosystemic shunt, if additional abnormalities are present. Congenital hepatic shunts are probably the most common cause of decreased serum [UN], and acquired shunts will have the same end-result.

Urea nitrogen is freely filtered by glomeruli and approximately 50% is reabsorbed passively by the proximal tubules, with ~10% being actively reabsorbed by the collecting tubules. The amount reabsorbed varies with the rate of flow through the tubules. The slower the flow, the more UN is reabsorbed. Hence with dehydration more UN is reabsorbed and therefore serum [UN] may be disproportionately higher than serum [Ct] in a dehydrated animal ([UN]/[Ct] ratio is >30 rather than approximately 20 with normal hydration in dogs and cats). A portion of urea is also excreted via salivary glands into the gastrointestinal (GI) tract where it is degraded by bacteria into ammonium, absorbed and converted back into urea by the liver. Therefore, there is no net excretion of UN via the gastrointestinal system in most species. However, ruminants have a unique rumen microflora that can degrade and assimilate the urea into amino acids, and reabsorb these resulting in a protein gain and net urea excretion. This is the logic of feeding cattle nonprotein nitrogen sources such as liquid urea as a source of protein. And why it is especially critical in ruminants to correlate serum [UN] with [Ct] and urine specific gravity to predict renal disease. Increases in serum [UN] without concurrent increase in [Ct] have been used to suggest rumen stasis (and GI bleeding). Horses excrete [UN] via their GI, but not to the extent that cattle do, and serum [UN] is used in the assessment of renal function in this species along with [Ct] and urine specific gravity.

Creatinine is synthesized in muscles from the conversion of creatine and creatine phosphate into the ring structure that is creatinine. Failure to deliver blood to the kidneys (dehydration, cardiac insufficiency, or shock), or failure within the kidneys (variety of diseases), or failure to excrete urine (obstructed or ruptured urinary tract) all result in increased serum [UN] and [Ct]. If UN and Ct are not excreted in adequate amounts their concentration increases in plasma (azotemia), and this may lead to the clinical signs of urinary toxin accumulation in the patient, known as uremia. Although both substances are reported in the biochemical profile, an increased concentration of either implies the same thing: decreased GFR. Neither substance increases until approximately 75% of nephrons are nonfunctional. In fact, this percentage is probably closer to 80–90%. The remaining nephrons compensate by hypertrophy, especially in chronic renal failure. Therefore, when azotemia is detected the total nephron mass not functioning adequately may actually be greater than 75%. The remaining nephrons compensate and maintain the overall GFR such that serum [UN] and [Ct] stay within the reference interval, delaying azotemia until more nephrons are lost.

Since serum [UN] and [Ct] do not increase until 75% of the nephrons are compromised they are not useful in the detection of early renal failure, i.e., they are insensitive indicators of lower levels of renal dysfunction. However, they are fairly specific as relatively few nonrenal factors cause their increase. Table 23.2 lists nonrenal factors that increase these substances. The most common causes are dehydration (hypovolemia) and GI hemorrhage. Although dehydration eventually leads to increases in both substances, [UN] increases first. The ratio of serum [UN]:[Ct] in small animals is approximately 20:1 and in large animals 10:1. An increased ratio is associated with dehydration or intestinal bleeding whereas a decreased ratio is associated with fluid diuresis, the presence of noncreatinine chromogens, or the unique ability of cows and horses to metabolize and excrete UN through their gastrointestinal tracts.

GI hemorrhage will increase [UN] without increasing [Ct]. Blood in the GI tract is broken down, reabsorbed as amino acids and ammonia, delivered to the liver, and converted

Table 23.2 Nonrenal factors that increase serum [UN] and [Ct].

Both	UN	Ct
Dehydration Hypovolemia Shock	GI hemorrhage	Noncreatinine chromogens Oxyglobin Glucose Ketones Carotenes Uric acid Vitamins A and C

Protein-rich meals, fever, sepsis, and anorexia are listed as factors that will increase serum [UN]. While sepsis and developing cachexia can lead to increased release of creatine and increased creatinine production, these increases tend to be mild and do not interfere with clinical interpretation.

into UN for excretion by the kidneys. Hemorrhage does not have to be so severe as to cause anemia, and in fact, hemorrhage may be mild enough that it requires an occult blood test on feces to be certain it is present. Other "high protein meals" may increase UN production post prandially, but they do not cause azotemia in normal patients. This is the principle of "renal diets," i.e., the feeding of a diet low in nitrogenous substances (low protein, high carbohydrate) that should result in less UN production via the liver, and therefore reduce the work load on the failing kidneys. Excess muscle catabolism (e.g., starvation, or fever) could increase the production of UN but it rarely produces azotemia. If there is an increase it will be mild and will not interfere with clinical interpretation.

Case Example 23.1. 11-year-old Labrador retriever crosstreated with NSAIDs for chronic arthritis

UN 78 mg/dL Ct 1.2 mg/dL urine s.g. 1.034
UN : Ct ratio 65 : 1

First interpretation: A disproportionate increase in [UN] relative to [Ct], coupled with a concentrated urine is mostly likely due to GI hemorrhage secondary to gastric erosions and ulcerations, related to NSAIDs use.

Further tests: occult blood test on feces, and/or suspension of NSAIDs, and evaluation of hydration status.

Second interpretation: Dehydration resulting in increased renal retention of UN. However, since [UN] is increased and [Ct] is within reference interval GI hemorrhage is more likely than dehydration. Evaluate hydration status.

Dehydration can increase [UN] without a commensurate increase in [Ct] and the ratio of [UN]:[Ct] may be high, i.e., >20:1. One hundred percent of creatinine excreted in the glomerular filtrate passes out in the urine. However, approximately 50% of [UN] excreted in glomerular filtrate is reabsorbed via the tubules. The amount reabsorbed is a function of health of the tubules and the flow rate of filtrate through the tubules. The slower the flow rate (dehydration) the greater the reabsorption of UN; up to 70% of urea may be reabsorbed versus the expected 50%. The faster the flow rate (diuresis) the lower the amount of [UN] reabsorption, perhaps only 40% or less is reabsorbed. Therefore, with dehydration [UN] increases more than [Ct], and with diuresis [UN] decreases faster than [Ct], and both of these situations may be seen clinically. The faster decrease in [UN] during fluid therapy is due to increased production of glomerular filtrate, leading to a faster transit time of fluid in tubules, and therefore reduced time to reabsorb UN.

Case Example 23.2. 9-year-old dog, 6% dehydration noted clinically

[UN] 88 mg/dL, [Ct] 2.8 mg/dL, USG 1.058,
[UN] : [Ct] ratio 31 : 1

Interpretation: Prerenal azotemia, as evidenced by a disproportionate increase of [UN] compared to [Ct], with a highly concentrated urine, and clinical signs of dehydration. Urea reabsorption is increased secondary to decreased flow of glomerular filtrate caused by the hypovolemia of dehydration.

The packed cell volume and albumin may also be increased if dehydration is severe enough, and if neither PCV nor albumin were decreased below the lower reference interval prior to the onset of dehydration. If the dog was not anemic, then PCV may be increased; however, if the dog was anemic prior to the onset of dehydration, then the decrease in plasma volume may cause an increase in the PCV to within the reference interval thus masking the anemia.

Case Example 23.3. Mixed breed dog dehydrated 6% clinically

Initial results: [UN] 120 mg/dL, [Ct] 4.5 mg/dL,
USG 1.062, [UN] : [Ct] 26 : 1

Post IV fluids: [UN] 34 mg/dL, [Ct] 2.9 mg/dL,
USG 1.008, [UN] : [Ct] 12 : 1

Interpretation: Prerenal azotemia; disproportionate increase of [UN] to [Ct] with concentrated urine and clinical dehydration. Dog is still mildly azotemic post fluid therapy and the decrease in [UN] is of greater magnitude (70% less) than the decrease in [Ct] (33%). Serum [UN] is almost within the reference interval, and [Ct] is nearly twice the upper reference interval value. This is due to the increased flow of filtrate through the tubules caused by the fluid therapy which allowed less time for [UN] and water to be reabsorbed and, therefore, more [UN] and fluid remained in the plasma ultrafiltrate, allowing more to be excreted (polyuria). This led to a faster decrease in [UN] than [Ct]. During fluid diuresis the increased flow of fluid through the kidneys decreases the reabsorption of UN to <40% and therefore BUN decreases faster than creatinine during fluid therapy.

Approximately 40–60% of the UN excreted in the glomerular filtrate is reabsorbed via the tubules by passive (proximal tubules) and active mechanisms (via ADH in collecting ducts). There are urea transporters (UT1, UT2, UT3) that are active in different regions of the tubules to accomplish urea reabsorption. The amount reabsorbed is a function of the health of tubules and the rate of flow in the glomerular filtrate. A portion of the [UN] remains in the interstitium, along with sodium and chloride, and contributes to the hypertonicity of medulla that is part of the counter current multiplier system. The degree of saturation in the interstitium is proportional to the concentration of UN, sodium, and chloride in the medulla, which makes it hypertonic compared to the fluid in the tubules, and this gradient is needed to help concentrate the glomerular filtrate as it is processed into urine. Urea and sodium are the two substances primarily responsible for the passive reabsorption of water from tubules in the descending limb of the loop of Henle. If a patient has prolonged decreases in plasma [sodium] or [UN] it may result in decreased concentrations of either substance in the interstitium, leading to a decreased ability to reabsorb water passively and therefore, to a decreased ability to concentrate urine. Hence, a dilute urine results that is noted clinically as polyuria. This combination of events that leads to decreased medullary tonicity is referred to as medullary washout. It is seen with hypoadrenocorticism (prolonged hyponatremia) and with prolonged decreased production of UN due to hepatic shunts (congenital or acquired) as well as severe chronic liver failure. Urea is synthesized in the liver and chronic liver failure can result in decreased production and therefore decreased plasma [UN]. Single digit [UN] combined with hypoalbuminemia, and microcytosis may be subtle clues that indicate hepatic shunts.

Psychogenic polydipsia will wash out the medullary interstitium of UN and sodium via marked diuresis resulting in hyposthenuria and PU PD.

Case Example 23.4. 1-year-old small breed dog, poor growth, thin, and bizarre behavior (snapping at objects, not playful)

[UN] 5 mg/dL, [Ct] 1.1 mg/dL, USG 1.012, [UN] : [Ct] 4.5 : 1

Serum [albumin] 1.8 g/dL (Reference Interval (RI): 2.6–3.9); liver enzymes within RI.

Interpretation: Decreased [UN] and [albumin] in a young dog with possible CNS disturbances (snapping at objects) and dilute urine is likely caused by congenital hepatic shunt and decreased synthesis of [UN] and albumin by the liver. Inability to concentrate urine is due to a renal medullary interstitium that never developed a high [UN], resulting in an inability to produce concentrated urine (medullary washout). It would be a classical sign of congenital hepatic shunt if ammonium biurate crystals were observed in the urine. These crystals and ammonium biurate uroliths (green, green-brown (Figs. A23.18 and A23.19)) are caused by the markedly elevated ammonia concentration in plasma, which, since it is freely filtered at the glomerulus, contributes to an ultrafiltrate that is both supersaturated with ammonia and alkaline in pH and leads leads to ammonia crystallization. Most cases of congenital liver shunts have no to only mild increases in serum liver enzymes and are not bilirubinemic. This is in contrast to most cases of acquired liver shunts which have moderate to marked increases in liver enzymes and bilirubinemia because the severe liver lesion precedes the development of the shunt.

All species can excrete [UN] through their salivary glands. However, only ruminants and horses have unique microflora that convert the [UN] into amino acids, reabsorb these and have a net excretion of UN. Cattle are so efficient with this mechanism that it may take up to 1 week post bilateral nephrectomy before serum [UN] increases. Cattle can be fed liquid urea because of their unique rumenal microflora. Cattle on a nitrogen-deficient diet or those that are severely anorectic will excrete the majority of their UN via the GI tract. Horses are not as efficient but have microflora in their cecum and colon which accomplish some net excretion of UN (case examples are found in the creatinine section).

Normal dogs fed a high protein meal may have a slight increase in their serum [UN] peaking at 6 hours and lasting up to 18 hours. The protein is broken down in the GI tract and the ammonium produced is reabsorbed and converted into UN by the liver. This is the principle of feeding reduced protein diets to patients in renal failure. These patients may benefit from a low protein diet that results in less UN production, and therefore reduces some of the work performed by the kidneys. Other nonrenal factors that may increase serum [UN] include: intestinal hemorrhage (the globin portion of hemoglobin is a protein); sepsis and fasting, which increase protein catabolism; and decreased renal perfusion (favors increased tubular reabsorption). Conditions that may decrease UN production are: liver failure, intra- or extrahepatic shunts, malnutrition, hyperthyroidism (increased catabolism and increased GFR; hyperthyroidism may also decrease [Ct] due to cachexia), and diuresis.

Methodology considerations: Urea

Serum reagent strips that use urease to estimate serum [UN] are less accurate than strips that assay the release of ammonia, which is a semiquantitative determination. These methods are somewhat useful for broadly estimating "normal" or "increased" serum [UN] in after-hours situations, but are not accurate and should not be relied on to monitor a patient over time. They are good at identifying low or normal values and are not adequate to quantify abnormally high values. If the strip indicates that a patient is azotemic, then a blood sample should be collected prior to fluid therapy, and serum should be analyzed by a quantitative chemical methodology. If serum strips are used to assess uroabdomen, then the color difference between abdominal fluid and serum should be definitive, and it is recommended that results are confirmed with quantitation of [UN] or [Ct] in fluid and serum (refer to the uroabdomen section). Reagent strips are a benefit for after-hour estimations but should not replace chemical measurement of urea, urea nitrogen.

Creatinine is a waste product of creatine and creatine phosphate found in muscle. Creatine (from the Greek word for flesh *kreas*) is produced in the liver with a minor role from the pancreas, transported to skeletal muscle, where 95% of the total body creatine is located and enzymatically converted into creatine phosphate via the enzyme creatine kinase. Creatine phosphate serves as an energy store for production of ATP and along with creatine is spontaneously degraded in muscles to creatinine, which has no charge and freely passes out of muscle cells. Creatinine production is relatively constant (2%/day) and is roughly proportional to muscle mass. It is a ring structure, has molecular weight of 113 daltons, and is filtered freely through the glomeruli as the majority of circulating Ct is not protein bound and it has no charge. Although dietary meat protein (creatine) could increase the serum creatinine from gastrointestinal absorption this increase is offset by a concurrent increase in GFR stimulated by the meal. The increase in Ct from the meat is so mild that the postprandial increase in GFR actually decreases the serum concentration of Ct within 2 hours of the meal.

In a normal animal UN and Ct are in high concentrations in the urine, up to 300 mg/dL of Ct in urine and in low concentration in the serum, 1 mg/dL of Ct. If the kidney is functioning insufficiently there is less Ct excreted in the urine, 100 mg/dL or less and Ct is retained in the serum, 4 mg/dL or greater. The magnitude of increase in UN or Ct cannot be used to correctly identify if the azotemia is due to prerenal, renal or postrenal causes. Concurrent dehydration in an animal with renal or postrenal azotemia will amplify the increase in UN and Ct and can produce marked azotemia, UN >200 mg/dL and Ct >20 mg/dL. Fluid therapy will decrease the contribution from dehydration (prerenal) and where the UN or Ct stops its decline can be considered the reference point that is due to the renal or postrenal disease. Postrenal azotemia from urinary obstruction or a bladder rupture is generally considered to produce the greatest and most rapid increases in serum creatinine but is not diagnostic. Decreased serum Ct or at least decreased synthesis of Ct is seen rarely with chronic liver failure and is more frequently associated with conditions that decrease muscle mass such as chronic cachexia and is occasionally observed in hyperthyroidism of cats. In fact, the decrease synthesis of Ct with hyperthyroidism may mask the development of azotemia in geriatric cats with concurrent hyperthyroidism and chronic renal diseases. Interpretation of an increase in serum Ct is done by examining UN, USG, and the rest of the data. A small amount is secreted by the proximal tubules of male dogs but this is clinically inconsequential; cats and ponies do not secrete or reabsorb Ct. Factors that increase endogenous muscle catabolism such as sepsis, developing cachexia, can increase the release of creatine and, hence, the quantity of creatinine that is produced. However, these increases are mild and rarely interfere with clinical interpretation. An increased blood creatinine is most likely due to decreased excretion—prerenal, renal, or postrenal mechanisms.

Cattle and horses occasionally have disproportionate increases in serum [Ct] as compared to [UN], and this may be due to an increase in noncreatinine chromogens that falsely increase serum [Ct] but do not affect serum [UN]. Creatinine is measured spectrophotometrically, and any chromogenic substance (or noncreatinine chromogen) will also be measured, resulting in a false increase in the measured serum [Ct]. The Jaffé reagent is used most frequently in veterinary laboratories to measure creatinine and it reacts with many noncreatinine chromogens. At normal concentrations of Ct, noncreatinine chromogens can contribute up to 50% of the measured serum [Ct]. During the development of renal failure [Ct] increases but noncreatinine chromogens do not increase, and in nonruminants, there is a concurrent increase in [UN] so the interpretation will be azotemia. However, there are several situations where these noncreatinine chromogens contribute to the measured [Ct] such that they will interfere with clinical interpretation. In small animals, this is seen almost exclusively with artificial transfusates such as oxyglobin products. The serum [Ct] can be increased as high as 20 mg/dL, but serum [UN] will remain within the reference interval. In addition to other analytes, these animals will have falsely increased plasma hepatic enzymes, and the serum may be yellow-orange, depending on the dose of artificial transfusate administered as well as the cause of the anemia.

In large animals a disproportionate increase in serum [Ct] is seen most frequently in cases of equine colic and is attributed to an increase in chromogens other than creatinine. The most common noncreatinine chromogens are ketones, glucose, carotenes, and vitamin A—substances that tend to

be higher in herbivores—as well as pyruvate, ascorbic acid, and uric acid (Table 23.2).

When [Ct] is disproportionately higher than [UN], such that the serum [UN]:[Ct] ratio is 5 or less, then noncreatinine chromogens are a likely cause, especially in horses. This is particularly likely if [UN] is within or only mildly increased, above the RI, but [Ct] is clearly increased (3–6 mg/dL). The easiest way to determine if the increase in [Ct] is due to renal or nonrenal causes is to compare the USG and the serum [UN]. If the USG and the serum [Ct] are increased but [UN] is within the RI, then the horse has noncreatinine chromogens artifactually increasing the serum [Ct]. If the USG is increased and [UN] is increased but [Ct] is disproportionately higher than the increase in [UN], then the horse has prerenal azotemia and noncreatinine chromogens are contributing to the [Ct] measurement. If USG is isosthenuric and there is azotemia, then the animal has renal azotemia. This presents a clinical dilemma, which is compounded when the [UN] is not increased as much as the [Ct] due to the enteric excretion of UN in horses and cattle.

Case Example 23.5. 10-year-old horse with colic, clinical signs of dehydration are equivocal

Serum [UN] 35 mg/dL, [Ct] 5.1 mg/dL,
urine not obtained, [UN] : [Ct] ratio 7

Interpretation: Disproportionate increase of [Ct] to [UN] due to noncreatinine chromogens and/or possible excretion of [UN] in gastrointestinal tract. A [UN] of 35 mg/dL is mild to insignificant, however, a [Ct] of 5.1 mg/dL is a moderate increase and is of concern. This is especially true if banamine and/or phenylbutazone are to be administered to alleviate pain and aid in the prevention of laminitis. NSAIDs are contraindicated if the horse is dehydrated, not drinking, or azotemic as the propensity for NSAIDs to cause medullary crest necrosis in the kidneys is enhanced in these situations. This is a practical clinical dilemma. If the increase in [Ct] is due to noncreatinine chromogens, then the horse would benefit from NSAIDs. However, prerenal azotemia may be present in this horse, so obtaining the USG *before* fluid therapy is the best means to determine if there is a renal contribution to the azotemia. Fractional excretion of sodium <1% would be definitive evidence of no renal involvement, whereas fractional excretion of sodium >1% indicates renal disease. In the latter case NSAIDs are contraindicated. If they occur, the renal lesions induced by NSAIDs in horses are usually mild. From a practical view, colic is much more common than renal failure in horses and therefore odds are that the increase in [Ct] is due to noncreatinine chromogens.

A serum [Ct] of your shoe size or greater indicates azotemia is present, but it does not mean that the cause of the increase is irreversible. For example, prerenal azotemia is reversed by implementing fluid therapy, or, if a disease process leading to renal azotemia spares the basement membrane, then the tubules may regenerate. If a renal lesion is mild and remaining nephrons compensate, if a ruptured bladder causing uroabdomen is repaired, if an obstruction to urinary outflow tract is relieved, etc., then the cause of the azotemia may be corrected. However, it is equally true that a return of serum [Ct] to the reference interval does not mean the kidneys are normal or fully functional. In fact, it simply means that >25% of nephrons are functioning adequately to keep [Ct] within the reference interval. If serum [Ct] returns to the reference interval but renal concentrating ability does not return, this implies a reduction of functioning renal mass by two-thirds. If renal concentrating ability returns then it could mean the kidneys are entirely normal, or simply that the lesions in the kidneys involve less than two-thirds of the renal mass. That is to say that significant disease could still be present, and these patients should always have access to drinking water. In most clinical situations this apparent return to normal is cause for celebration. If you wanted to follow these patients over time, practical methods would be to monitor patient weight, water intake, urine volume, USG, and serum [UN] and [Ct]. If closer monitoring was desired, or if you wanted to more accurately determine the functional renal mass, then specialized studies such as exogenous or endogenous creatinine clearance, fractional excretion of sodium, microproteinuria, or ultrasonography could be offered or performed.

After azotemia is identified the next step is to decide if the cause is prerenal, renal, or postrenal. Determining the USG and urine volume are critical next steps (Fig. 23.2). Prerenal azotemia is due to hypovolemia, or inadequate blood flow to the kidneys, such that the kidneys cannot adequately filter and excrete waste substances from the plasma. The continued basal production of UN and Ct combined with the decrease in GFR will lead to increased plasma concentrations of both. Causes are hypovolemia due to dehydration, cardiac insufficiency, and shock. Dehydration is one of the most common causes of azotemia in veterinary medicine. Easily reversible with fluid therapy, but if persistent it can lead to ischemic renal damage and therefore, renal azotemia.

Urine [Ct]:serum [Ct] ratios can be used to distinguish renal and prerenal azotemias. Ratios >50:1 indicates prerenal azotemia and ratios <37:1 indicate renal azotemia.

Example: urine [Ct] 1000 mg/dL serum [Ct] 4
= 250 = prerenal azotemia
urine [Ct] 100 mg/dL serum [Ct] 4 = 25 = renal azotemia

Prerenal azotemia

Dehydration is the most common cause and characteristic results for this are increased serum [UN] and [Ct], concentrated USG, decreased urine volume, increased PCV and serum [albumin], and clinical signs of dehydration (Fig. 23.2).

Azotemia occurs because there is continued basal production of UN and Ct but neither is excreted because the decreased blood volume reduces GFR (inadequate blood flow to glomeruli). In cases of prerenal azotemia (dehydration, for example) urine production should decrease (oliguria, anuria) as the body attempts to conserve plasma volume (water), and USG should increase. In general, the magnitude of the azotemia cannot be used to prove that dehydration is the cause of the azotemia, but generally the increases in [UN] and [Ct] observed with dehydration are mild to moderate (e.g., serum [UN] 35–120 mg/dL and [Ct] 2–5 mg/dL). If dehydration is superimposed on a case of true renal failure, then the azotemia can be severe (e.g., [UN] >200 mg/dL, [Ct] >10 mg/dL). Fluid therapy will remove the prerenal contribution and decrease serum [UN] and [Ct]. The point at which these values plateau is the degree of azotemia that is due to the true renal lesions. If the patient does not have preexisting anemia or hypoalbuminemia then both of these analytes are expected to be increased in cases of dehydration. The only practical cause of hyperalbuminemia is dehydration. If the PCV was decreased prior to onset of dehydration then the PCV may shift upward to within the reference interval during dehydration, which would mask the anemia. In cases of prerenal azotemia, the fractional excretion of sodium is <1%.

Exceptions to the generalization that prerenal azotemia is associated with concentrated urine are concurrent conditions that prevent urine from being concentrated. These include primary and secondary diabetes insipidus, hypercalcemia, steroids, pyometra, and medullary washout. Despite clinical dehydration the kidneys cannot concentrate urine adequately because there is inadequate ADH, or a substance is interfering with ADH, or the renal medullary interstitium is no longer saturated with sodium and urea. Calcium interferes with the action of ADH and when hypercalcemia is present urine is often dilute, even if the animal is dehydrated and azotemic. Azotemia is present in 90% of dogs with hypoadrenocorticism (Addison's disease) due to dehydration, and a small percentage of these may have dilute urine because of chronic hyponatremia and resultant medullary washout. Because the constellation of laboratory and clinical signs associated with hypoadrenocorticism are similar to those caused by renal failure (namely, azotemia, inadequately concentrated urine (e.g., USG <1.020), anorexia, and vomiting) Addisonian dogs may be initially misdiagnosed. Fluid therapy will rapidly correct the azotemia in these dogs and when fluid therapy reverses azotemia overnight, or in hours then true renal failure was not present.

Renal azotemia

Any renal disease that causes damage to greater than 75% of the nephrons and reduces GFR below 25% will decrease the excretion of UN and Ct. If enough nephron units are compromised, and the remaining units cannot hypertrophy to compensate sufficiently, then the decrease in GFR will result in azotemia. The lesions do not have to directly involve glomeruli because of the concept that the nephron is a unit made of interdependent parts, and if one part of the nephron is injured then other part will be compromised. For example, if the tubular portion of the nephron is destroyed or damaged, then the glomerulus that freely filtered UN and Ct is rendered nonfunctional because the glomerular filtrate will have nowhere to drain.

Further, since 90% of the blood supply to the tubules passes through the glomerular capaillaries, if glomerular lesions are severe enough, blood flow to the tubules will be compromised, resulting in tubular defecits. Additionally, proteins that leak through diseased glomeruli may cause damage to tubules through various mechanisims including cytokine induction direct toxicity, and overload of lysosomal degradation mechanisms. If enough nephron units are compromised, and the remaining units cannot hypertrophy to compensate sufficiently, then azotemia will develop.

In cases of acute severe renal failure urine volume will be decreased (oliguria or anuria). In cases of chronic renal failure, urine production will be increased (polyuria). Patients with renal azotemia will have isosthenuric and some hyposthenuric urine and most will have several other abnormalities noted in the urinalysis, clinical chemistry panel, CBC, physical examination, history, etc.

Once renal failure is recognized, the next steps are to determine which region of the kidney is diseased, and to determine whether the renal failure is acute or chronic (Table 23.3). This distinction is critical, because acute renal failure may be reversible while chronic renal failure is not.

Characteristic features of acute renal failure are variable, but generally include a good body condition, sudden onset

Table 23.3 Expected results in acute versus chronic renal failure.

	Acute	Chronic
PCV	WRI	Decreased
Albumin	WRI	Decreased
K	Increased, variable	Decreased
Urine volume	Anuria, oliguria	Polyuria, polydipsia
Body condition	Good	Poor
History	Sudden onset	Gradual deterioration
Size of kidneys	Normal to enlarged	Small, irregular contours

These are generalizations and there is a range of actual results and species variations. (WRI = within the reference interval).

(the animal often being reported as "fine yesterday"), depression, lethargy, and decreased to absent urine output. Laboratory data will include PCV and albumin concentration within the reference interval (or increased if dehydration is present) and increased potassium concentration.

The most common cause of acute renal failure is nephosis, meaning tubular degeneration and necrosis, which is most commonly caused by a nephrotoxin. Acute nephrosis will be reflected in the urinalysis by isosthenuria, numerous casts, mild proteinuria, mild glucosuria, and variable cellular abnormalities in the sediment. If the kidneys can be imaged or palpated, they will be normal sized or enlarged and have regular contours.

Chronic renal failure can be the result of glomerular diseases as well as chronic interstitial nephritis, pyelonephritis, progressive familial nephropathy, and even bilateral staghorn calculi. In short, anything that can cause enough tissue damage to result in end-stage renal failure. Characteristic features of chronic renal failure are steady weight loss, mediocre to poor body condition, lethargy, polyuria and polydipsia, and laboratory data that include nonregenerative anemia, hypoalbuminemia, and hypocalcemia (the latter is uncommon in horses). Hypokalemia is often seen in cattle and cats. If the kidneys can be imaged they will be small and have irregular contours especially when the disease is fully developed.

Postrenal azotemia is due to obstruction of outflow or a rupture in the outflow tract. Azotemia is present due to an inability to excrete UN and Ct from the body, along with the continued basal production of both and the reabsorption of both from the abdomen or the subcutis if the urinary bladder or the urethra ruptures. The bladder will be enlarged on palpation if the obstruction is distal to the bladder and it will be small or undetectable if there is a rupture of the bladder. In this case, depending on duration and completeness of the obstruction, the azotemia may be mild to marked, UN > 200 and Ct > 10. If the animal becomes dehydrated, then part of the azotemia will be attributable to prerenal factors. In postrenal azotemia, USG is variable and does not help to identify the cause of the azotemia, however, the fact that urine production is decreased or absent (i.e., oliguria or anuria) is helpful in localizing the cause of the azotemia.

The dignosis of postrenal azotemia is made more from historical and physical examination findings than through laboratory evaluation. Most cases of postrenal azotemia are seen in males due to their narrow urethra. Obstruction of the outflow tract is uncommon in females due to their wide urethra. Urine obtained via cystocentesis in an obstructed animal is often red, and there are often a lot of red blood cells and inflammatory cells. Hyperkalemia can be severe (>8 mEq/L) and life-threatening, especially in male cats with a complete urethral obstruction. Blocked intact male cats can have rapid and marked increases of [UN] and [Ct] with [Ct] > 15mg/dL. The azotemia decreases rapidly following relief of the obstruction. Uroabdomen is confirmed by an abdominal fluid [Ct] > serum [Ct] and is discussed under diseases in this chapter.

Urine concentration

Correlate the urine specific gravity with serum [UN] and [Ct], as well as all the other case data. Measuring USG is a simple and effective test to assess renal function. Generalizations: azotemia and concentrated urine = prerenal cause; azotemia and unconcentrated urine = renal tubular disease/involvement.

Kidneys reabsorb more than 99% of the water that enters the tubules. Water is reabsorbed in the proximal tubules passively, in the descending loop of Henle passively due to the osmotic pull of the saturated medullary interstitium (countercurrent multiplier system), passively in the distal tubules, and actively in the collecting ducts through the actions of ADH. Failure in one or more of these locations may result in polyuria, and interference with of one or more of these mechanisms is used by different diuretics to stimulate water excretion.

The ability to produce concentrated urine is dependent on several factors. At least one-third of the renal mass must be functional, adequate amounts of ADH must be produced, the medullary intersitium must be saturated, the hydration status must be conducive, and there must be an absence of concurrent diseases. Therefore, animals with impaired urine concentration will have one or more of the following: lesions in two-thirds of the kidneys (tubules or interstitium), decreased ADH production (central diabetes insipidus), refractoriness to ADH (nephrogenic diabetes insipidus, hypercalcemia, excess glucocorticoids, pymometra, or hypokalemia), decreased medullary hypertonicity (medullary washout), over hydration, or solute overload (diabetes mellitus, diuretic administration).

Urine specific gravity must always be interpreted in light of hydration status. Dilute urine is "normal" in an overhydrated individual as the kidneys attempt to excrete the excess body water. For example, dilute urine is expected both in psychogenic polydipsia, and during fluid therapy. Isosthenuric urine in a dehydrated or azotemic patient is abnormal and suggests the renal lesion involves the tubules. Persistently dilute urine in a nonazotemic patient is abnormal and could involve renal or nonrenal mechanisms.

Kidneys concentrate and dilute the glomerular filtrate as it passes through tubules by removing solutes and water in different segments of the nephron. Glomerular filtrate is plasma minus albumin and it starts with a specific gravity approximately 1.010 (300 mOsm/kg), and in an animal with normal hydration, it finishes with a concentrated specific gravity.

If serial urinalyses reveal urine with a specific gravity of 1.007–1.013 it indicates that the kidney neither concentrated nor diluted the glomerular filtrate, i.e., the urine is

isosthenuric with respect to blood plasma, which has a specific gravity of approximately 1.007–1.014 and an osmolaltiy of 300 mOsmo. This is the least favorable range of urine specific gravities to detect over time. Urine specific gravities that are <1.007 or >1.013 imply there was some renal function. Dilute urine indicates that the kidney had enough function to remove more solutes than water, because dilution is an active process.

Any random USG can be "normal," hence the wide range of reference values, including 1.001. The most important task is to determine the pattern of concentrating ability, by correlating USG with serum [UN] and [Ct], urine volume, and the clinical data. If multiple urinalyses taken at different times of the day reveal hyposthenuria to isosthenuria, with or without azotemia, this is abnormal (i.e., USG: 1.004, 1.008, 1.005, etc.). A random urine sample with a USG of 1.008 taken from a nonazotemic animal with a history of PU PD is abnormal. Urine specific gravities that consistently stay below 1.007 are probably not due to renal lesions especially if the animal is not azotemic but are more likely due to lack of ADH (diabetes insipidus), or substances that inhibit the action of ADH or psychogenic polydipsia. Concentrated urine obviously indicates function, removal of more water than solutes. Although any urine specific gravity can be "normal" there are "expected" values for each species as well as values that indicate "adequate concentration" (Table 23.5). A morning urine sample is ideal to assess concentrating ability as this is the time of the day of maximum concentration.

Isosthenuria and hyposthenuria are associated with renal and nonrenal diseases (Table 23.4). When it is attributed to renal disease it implies that the lesion is primary within or involves the tubules. Glomerular diseases may be associated with dilute urine because 90% of the vascular supply to tubules passes through glomerular capillaries. Therefore if the glomerular lesion is severe enough it may eventually compromise tubular function. Interstitial diseases are often associated with dilute urine because tubules and interstitum are anatomically adjacent to each other and eventually lesions in one area involve the other. These examples illustrate the nephron concept: injury to one part of the nephron may eventually lead to injury of the rest of the nephron. This is why, in chronic renal failure, it is difficult to determine which part of the nephron was the site of the original or primary injury/disease. Following a patient's USG over time is a practical, affordable laboratory test to monitor the progression or improvement of renal function; however, it is not as discerning as fractional excretion studies or creatinine clearance studies.

Reagent strips should not be used to estimate USG, because false negatives and false positives are common, and they are therefore considered unreliable. Urine specific gravity is determined best via a refractometer and although this does not measure specific gravity directly, it estimates the specific gravity by the amount of light refracted, and this is adequate for clinical decision making. The USG is dependent on several factors, one of which is the concentration of small solutes in solution. Suspended particles, such as mucus, crystals, and cells do not affect USG because they are not dissolved; however, they can make the urine cloudy and may make reading the line on the refractometer difficult.

Adding soluble ions or molecules or removing fluid in excess of solutes will increase the osmolality/specific gravity and removing solutes and replacing them with water will lower the specific gravity resulting in dilute urine.

The scales in refractometers are calibrated to estimate specific gravity and/or proteins. The scales are different for each of dogs, cats, and horses. The differences are minor but significant such that the scale for one species should not be used for another. There is also a maximum reading, e.g., 1.060. Although the urine could be diluted to determine the exact reading this is not necessary, as the clinical decision is obvious; the patient can concentrate urine to a very high level.

Urine specific gravity is an estimate of osmolality and there is a good linear relationship between them. Measuring

Table 23.4 Causes of polyuria (polydipsia) and dilute urine.

Decreased ADH—Central diabetes insipidus (DI)
Pituitary (hypothalamic rare) tumor, abscess, idiopathic, congenital

Inadequate response of tubular cells to adequate ADH—Nephrogenic DI
Hypercalcemia, steroids, hypokalemia, pyometra *E. coli* endotoxin, congenital lack of response of tubular cells to ADH

Decreased renal mass = lesions in kidneys, loss of tubular cells
With azotemia = >75% involvement; especially if lesions in medulla and pelvis
Without azotemia = 66–75% involvement of total renal mass

Excess fluid intake
Psychogenic polydipsia
Fluid overload diuresis

Medullary washout—medullary interstitium not saturated with sodium and urea
Addison's—prolonged hyponatremia
Liver failure—decreased urea nitrogen (other laboratory data will also support); congenital and acquired shunts; end-stage liver disease
Psychogenic polydipsia
Fluid overload diuresis

Solute overload
Diabetes mellitus, acromegaly, Fanconi's syndrome, salt toxicity

Diuretics—many with actions at different regions of the tubules

Others/incompletely understood mechanisms
Hypoparathyroidism, hyperthyroidism, polycythemia, myeloma without hypercalcemia

Table 23.5 Urine specific gravity—expected, maximal, adequate, isosthenuric, and hyposthenuric concentration ranges.

	Dog	Cat*	Horse	Cow
Expected	1.020–1.045	1.020–1.050	1.020–1.045	1.020–1.045
Max. conc.	1.060 >	1.080>	1.050>	1.050>
Adequate	1.030 >	1.035>	1.025>	1.025>
Isosthenuria	1.007–1.013	across this table		
Hyposthenuria	<1.007	across this table		
With 5% Dehydration	1.040–1.075	1.045–1.088		

* Some cats with renal azotemia can concentrate urine.

osmolality via freezing point depression is the gold standard, but is rarely done. The measurement is dependent on the number and size of solutes in solution. As osmolality increases, the temperature at which the fluid freezes becomes lower. This is the principle of the protective effect of antifreeze on water in a radiator, and because ethylene glycol is fully miscible with water, it contributes to serum osmolality, and this is why the measured osmolality is so high in the serum of patients with ethylene glycol toxicity.

The measurement of osmolality is generally confined to reference laboratories, as the equipment is either too expensive, or used too infrequently to make it practical in private practice. Because of the linear relationship between specific gravity and osmolality, clinical decisions based on specific gravity (SG) are acceptable. It should also be noted that some solutes increase SG but have little effect on osmolality, which could lead to misinterpretations of the data. For example, in cases of marked proteinuria or glucosuria, every 1 g/dL of protein or glucose that is added to urine will increase the USG approximately 0.004, yet these solutes have little effect on the osmolality. The increased USG caused by proteinuria or glucosuria could lead to an overestimation of the USG and, therefore, an overestimation of the concentrating ability of the kidneys.

Additionally, adding the following substances to 1 mL of water will increase the specific gravity by 0.001 units: NaCl 1.5 mg; urea 3.6 mg; glucose 2.7 mg; albumin 4.0 mg. Each time the urine glucose increases by 1 g/dL (1+ on the dipstick) the USG increases by 0.004. A 4+ urine glucose would increase USG by approximately 0.010 units. These estimates and the principles are nice to know but they are rarely used to calculate or estimate a USG rather than simply measuring it.

An example of a potential clinical problem would be in a dog treated with an artifical colloid substance such as hetastarch (HES). This artificial volume expanding solution contains molecules of various sizes, some of which can pass through the glomerulus. In normal dogs 20 mL/kg of HES will increase the USG without a concurrent increase in the urine osmolality. If USG were increased by HES therapy, it could lead to an overestimation of renal concentrating ability, and mask an inadequate renal concentrating ability.

Mechanisms for the production of isosthenuric or dilute urine include: lesions in two-thirds of the kidneys, excess fluid intake, decreased ADH production, tubules refractory to ADH, decreased medullary hypertonicity, plasma solute overload, combinations of the foregoing, and unknown mechanisms (Table 23.4). Almost all of these disease states can be diagnosed without a water deprivation study. Psychogenic polydispsia, central diabetes insipidus (DI), and renal failure in patients that are not azotemic are the three diseases that may require a water deprivation study to differentiate them. The principle is to stimulate endogenous ADH production and release by withholding water and inducing mild dehydration of less than 5%. If an animal is able to concentrate urine with just water withdrawal, then psychogenic polydipsia is the diagnosis. If the patient does not concentrate until exogenous ADH is administered, then central DI is diagnosed, and if water withdrawal and exogenous ADH administration do not stimulate urine concentration, then renal disease is diagnosed. *Never* perform this test on an azotemic patient.

Water deprivation testing

Before the study commences the bladder is emptied, baseline data is gathered and additional values are recorded every 1–3 hours, depending on the severity of the PU PD until the study is stopped. The bladder is also emptied at each collection time point. Ideally, measurements should include body weight, skin turgor, PCV, plasma protein, serum [UN], USG, as well as plasma and urine osmolality. Osmolality does not have to be measured but it is ideal, and if it is measured the results will generally not be known for a day or more so USG and body weight are the more practical indicators to follow in private practice. A 3–5% weight loss is maximum stimulus to cause release of endogenous ADH. If this does

not cause urine concentration, or if USG has changed <10% over three consecutive measurements, then exogenous ADH should be administered. If plasma osomolality is known during the study, then ADH should be administered if plasma osomolality is >310 mOsm/kg, as this osmolality is considered adequate stimulus for the release of endogenous ADH. The study should be stopped when a USG from 1.025 to 1.035 is measured, as it indicates adequate urinary concentrating capacity. Because a common feature of these disease states is medullary washout, one should expect the USG to increase in a stepwise rather than rapid fashion. Medullary washout arises from prolonged polyuria that leaches solutes from the medullary interstitium, and until the interstitium is resaturated with urea, sodium, and chloride, the kidney will only concentrate accordingly. Depending on the severity of the medullary washout it may take up to 24 hours or longer of complete or partial water restriction for dogs with primary polydipsia to concentrate into the 1.030 range. Monitor serum [UN] and if azotemia develops stop the study, serum [UN] and / or [Ct] should not increase in a water deprivation study. See the section of Chapter 32 in this book that describes diabetes insipidus. Antidiuretic hormone (ADH, vasopressin) is produced by neurons in the hypothalamus, is transported to and stored in the pars nervosa of the pituitary, and is secreted in response to several stimuli including increased plasma osmolarity. ADH acts on the collecting tubules to stimulate water reabsorption.

An inherent risk of a water deprivation study is the possibility of exacerbating renal damage in the patient that has renal failure but is not azotemic. If urine remains unconcentrated after several hours then the disease present is either DI or renal failure. Monitor serum [UN], if azotemia develops, then stop the study. The renal failure patient has a reduced renal mass of between two-thirds and three-quarters and the closer the reduced mass is to the three-quarters mark, the easier it will be to induce azotemia via dehydration. USG in the isosthenuric range is more suggestive of occult renal disease than is USG <1.007.

Case Example 23.5 (reference intervals, mg/dL).

	A	B	C	D	E	F
Serum [UN] (10–30)	28	85	190	110	60	63
Serum [Ct] (<1.5)	1.1	4	9.2	3.2	3	3.1
Ratio serum [UN]/[Ct]	28	21	21	34	20	21
USG	1.034	1.006	1.010	1.058	1.014	1.044
Interpretation	Normal	Renal	Renal	Prerenal	Renal	Prerenal

A. "Normal": all results are within reference intervals, and USG indicates adequate concentrating capacity.

B. Renal azotemia: serum [UN] and [Ct] increased, and USG dilute.

C. Renal azotemia: [UN] and [Ct] increased and USG indicates isosthenuria, need repeat USG to see if concentration increases out of the 1.007–1.013 range.

D. Prerenal azotemia: [UN] and [Ct] increased and USG elevated (concentrated); [UN]/[Ct] ratio increased to 34 which also suggests prerenal; dehydration likely, evaluate hydration status.

E. Renal azotemia: [UN] and [Ct] increased and USG close to isosthenuric range, need repeat USG to see if patient can concentrate its urine.

F. Prerenal azotemia: [UN] and [Ct] increased and USG concentrated; nearly identical values to example "E" but different causes based on results of USG; there are no values for [UN] and [Ct] that are too high for prerenal azotemia; however often the greatest increases are seen when there is a combination of prerenal and renal azotemia.

Case Example 23.6 (reference intervals, mg/dL).

	A	B	C	D	E
Serum [UN] (10–30)	22	5	180	90	55
Serum [Ct] (<1.5)	1.5	1	9	2	10.2
Ratio [UN]/[Ct]	16	5	20	45	5.5
USG	1.024	1.005	1.010	1.055	Not performed
Interpretation	Normal	Shunt	Renal tubular	Prerenal	Horse or cow

A. "Normal": at least all results are within reference intervals, and USG indicates some concentrating capacity. Recall that any USG is possible on a random urine sample.

B. Hepatic shunt: [UN] is decreased and urine is hyposthenuric, implying renal ability to dilute urine but it may not be able to concentrate, need more USG to identify a pattern. The

decreased [UN] is attributable to decreased hepatic production; recommend evaluation of liver function.

C. Renal azotemia: [UN] and [Ct] are increased and USG indicates isosthenuria. The USG should be repeated to determine whether it will rise out of the 1.007–1.013 range. Dilute urine suggests tubular lesions, but we need to know the rapidity of onset, body condition of the animal, serum [albumin], and PCV to differentiate acute from chronic renal disease.

D. Prerenal azotemia: [UN] and [Ct] increased and high [UN]/[Ct] ratio suggestive of prerenal cause (e.g., dehydration leading to decreased GFR) with high USG (concentrated urine). One could observe similar values with GI hemorrhage, increased substrate delivery to liver for increased [UN] production (e.g., bleeding ulcer, hookworm infection, NSAIDs etc.).

E. Horse: Disproportionate increase in [Ct] relative to [UN] resulting in low [UN]/[Ct] ratio. Differential diagnoses include: an increase in noncreatinine chromogens combined with dehydration (e.g., noted with colic). Similar results are expected in cattle with renal failure coupled with GI excretion of UN, a urinalysis and especially USG would help differentiate these possibilities. If isosthenuric urine is observed, then the results are consistent with renal failure. If urine is adequately concentrated, then the results are consistent with prerenal azotemia, and the presence of noncreatinine chromogens.

Case Example 23.7 (reference intervals, mg/dL; PCV, %; albumin, g/dL).

	A	B	C	D	E-1	E-2 with fluid therapy
Serum [UN] (10–30)	20	98	121	220	225	68
Serum [Ct] (<1.5)	1.0	2	6	11	12	6.2
Ratio [UN]/[Ct]	20	50	20	20	20	10
PCV (30–50)	42	59	48	21	62	41
Albumin (2.8–4.0)	3.0	4.8	5.1	1.9	4.9	3.0
USG	1.022	1.044	1.059	1.009	1.006	1.010

Interpretations:

A. Normal

B. Azotemia with concentrated USG, increased [UN]/[Ct] ratio, polycythemia and increased serum [albumin] all attributable to dehydration causing a prerenal azotemia. GI hemorrhage could produce similar results, the PCV does not need to be decreased for GI hemorrhage to cause an increase in [UN] but an increased PCV fits better with dehydration, and an increase in [albumin] confirms dehydration.

C. Prerenal azotemia, there is not an increase in the [UN]/[Ct] ratio in this example; the *only* cause of increased serum [albumin] is dehydration (bisalbuminemia or a hepatocellular carcinoma causing increased albumin either through increased production by neoplastic hepatocytes, or due to reduced negative feedback on production; both conditions are incredibly rare).

D. Azotemia with isosthenuric urine indicates renal failure. The low PCV and low [albumin] indicate chronic renal failure. Correlate laboratory results with body condition of patient, physical exam results, and history. Ultrasonography of kidneys may reveal small, fibrotic kidneys.

E-1. Azotemia with unconcentrated urine indicates there is renal disease, but that the kidneys are functional to some degree as they are able to produce urine more dilute than plasma. The increases in albumin concentration and PCV indicate a prerenal component and indicate the renal failure is probably acute and therefore tubular lesions are likely, possibly a nephrosis; this is an example of renal and prerenal azotemia but it cannot be determined what portion of the azotemia is due to each of these factors. These data warrant performing serial urinalyses to monitor USG.

E-2. IV fluid therapy resulted in decreased azotemia supporting the interpretation that part of the azotemia was of prerenal azotemia. There is a greater decrease in [UN] relative to [Ct] which is typical of fluid diuresis and is caused by the increased rate of flow of filtrate leading to decreased reabsorption of UN in tubules. The decrease in PCV and [albumin] is due to fluid therapy; the second USG is isosthenuric and is expected following fluid therapy (i.e., it cannot be interpreted). A large portion of the azotemia before fluid therapy was due to dehydration. Fluid therapy should continue to determine if fluids and other treatments can lower the azotemia further. The point at which fluid therapy cannot decrease the azotemia further is the UN and Ct concentrations that are due to the true renal lesions. The patient can be further treated and monitored. Azotemia is not a death sentence. Many dogs and especially cats can be maintained with a low degree of azotemia and inability to concentrate urine for months or years with periodic treatments and monitoring the progression or improvement of their renal disease.

Case Example 23.8 (reference intervals, mg/dL; PCV %; albumin, g/dL).

	A	B	C
Serum UN (10–30)	200	221	120
Serum [Ct] (<1.5)	10	11	5
Ratio [UN]/[Ct]	20	20	22
PCV (30–50)	42	19	32
[Albumin] (2.8–4.0)	3.0	2.2	2.8
USG	1.012	1.009	1.006
Urine volume	Small amount	Increased	Increased
History	Fine yesterday	Week's weight loss	Week's weight loss
Body condition	Good	Poor	Poor, dehydrated 5%

Interpretations:

A. Azotemia, isosthenuric urine, history, and all other data point to acute tubular disease; therefore, suspect toxic nephrosis.

B. Azotemia, isosthenuric urine, history, and all other data point to chronic renal disease/failure.

C. Azotemia, lack of concentrated urine, history, urine volume, and body condition all point to chronic renal disease/failure but PCV and [albumin] do not support this interpretation; suspect concurrent dehydration detected on physical exam has raised these analytes into reference interval and after fluid therapy both will be decreased; seems likely given they are at low end of reference interval and dehydration is 5%.

Urinalysis

No other body system has an excretory product that is produced regularly, is easy to obtain, and that informs us so vividly on the health of the parent organ. A urinalysis (UA) is an essential component of the evaluation of the urinary system. Urinalyses have an excellent cost:benefit ratio as a screening test in all species. It is an absolute requirement for the diagnosis or to rule out urinary diseases. It is excellent as a follow-up test to determine the progression or improvement of urinary diseases. It is very useful in the diagnosis of some nonurinary diseases. It should be part of all geriatric exams, is performed in-practice, and it costs almost nothing. Some of the nonurinary diseases a UA helps diagnose are: hyperadrenocorticism (Cushing's disease), diabetes mellitus, hepatic diseases, hemolytic diseases, rhabdomyolysis, psychogenic polydipsia, and central diabetes insipidus. It is essential in distinguishing prerenal from renal azotemia.

Early detection of disease

- Glomerular diseases—proteinuria is the first abnormality, and precedes hypoalbuminemia, nephrotic syndrome, azotemia, etc. Persistent proteinuria found in a symptomatic or asymptomatic patient should prompt evaluation for glomerulonephritis, amyloidosis, and multiple myeloma.
- Renal disease, especially in geriatric patients—is typified by the inability to concentrate urine adequately, often before there is azotemia (exception some cats).
- Hematuria is a common clinical pathology abnormality and is detected with uroliths, transitional cell carcinoma, and several other diseases.

Monitoring of renal patients

- Urinalysis and especially USG are excellent inexpensive tests for following patients posttreatment to assess the progression or improvement of disease, especially if the patient is no longer azotemic. Persistent inability to concentrate urine in a previously azotemic patient suggests that the disease is still present, or that over 66% of the nephrons are still not functioning adequately. It is likely that they may have been destroyed by the disease process and fibrosis has replaced much of the renal mass. Return of concentrating ability indicates that the disease is gone or that less than 66% of the nephrons are still damaged. The presence of waxy casts in the urine sediment suggests persistence and chronicity of the disease.

Gross, microscopic, and chemical determinations are the key components of a UA. Table 23.6 summarizes expected results in normal animals. Table 23.7 provides guidelines for urinalysis findings to predict renal site of pathology that may be present. Urine composition is determined by the constituents and the quantity of plasma presented to the kidneys, renal function, and material added to the glomerular filtrate as it passes through the kidneys, bladder, urethra, and lower urinary tract. Urine examination should be done in-practice; there is no reason to send it out. Urinalyses should be performed on fresh urine (< ½ hour old), and if the macroscopic and reagent strip evaluations have no abnormalities, then performing the microscopic examination is optional because the sediment is rarely abnormal if the macroscopic exam and reagent strips are normal. This is especially true if the UA is being used to screen an apparently healthy patient.

Summary

- Excellent cost:benefit ratio, costs almost nothing to run
- Essential to distinguish prerenal from renal-origin azotemia
- Detects diseases in early stages

Table 23.6 "Normal"/expected findings in UA.

	Dog	Cat	Horse	Cow
Color	Yellow	Yellow	Yellow	Yellow
Clarity	Clear	Clear	Cloudy	Clear
SG	1.020–1.045	1.020–1.050	1.020–1.045	1.020–1.045
pH	5–7	5–7	7–8	7–8
Protein	Neg to trace	Neg	Neg	Neg
Bilirubin	Trace	Neg	Neg	Neg
Blood*	Neg	Neg	Neg	Neg
Glucose	Neg	Neg	Neg	Neg
Ketones	Neg	Neg	Neg	Neg
Urobilinogen	Do not use, same across this table			
WBCs	0–5	0–5	0–5	0–5
RBCs	0–5	0–5	0–5	0–5
Epithelial	0–5/few	0–5	0–5	0–5
Casts	Neg/none	Neg	Neg	Neg
Crystals	None	None	Ca carbonate/oxalate	None
Other	None	None	Mucus	None

* Small to trace amount with cystocentesis or catheterization collections.

Table 23.7 Renal azotemia and predicting location of lesion.*

Glomerular	Tubular (Interstitial)	Pelvis
Proteinuria	Casts	WBCs, bacteria urine sediment
Hypoalbuminemia	Isosthenuria	WBC casts, cellular casts
Ascites	Glucosuria, proteinuria/Active sediment, cells, bacteria	
Polyuria	Anuria, oliguria	Polyuria, dilute urine
Chronic, thin Weight loss	Acute Good body condition/Variable body condition	Chronic, acute exacerbations
Increased cholesterol		
Normal-to-small-sized kidneys	Normal-to-enlarged	Normal to irregular contours, dilated Irregularly shaped pelvis
Biopsy	Do not biopsy	Avoid biopsy
Amyloidosis versus glomerulonephritis	Toxins	Ascending infection

* This is assuming the diseases are behaving in a characteristic manner. Glomerular diseases usually present when chronic; tubular diseases are commonly acute but if they survive they can end up as chronic PU PD patients. Pyelonephritis is in an active phase when the infection is present. All these diseases will change over time, and depending on severity (mild, moderate, severe) and treatments.

- Detects nonrenal diseases
- Useful for monitoring patients
- If the macroscopic and reagent strips have no abnormalities then performing the microscopic examination is optional; UA should be performed in-house.

Collection

- *Voided, free catch*—morning sample preferred when urine is usually at maximal concentration; may add contamination of cells and bacteria from lower urogenital tract. Lower urethra has resident bacterial population, but upper urethra and bladder are sterile; bacteria, leukocytes commonly in prepuce; epithelial cells are added from genital tract and distal urethra.
- *Catheterization*—may induce a small amount of hemorrhage and introduces epithelial cells from urethra.
- *Cystocentesis*—preferred for culture, commonly introduces small amount of hemorrhage especially in cats.

Urinalyses are often not performed in large animals simply due to difficulty in collecting a sample. Horses often require catheterization but placing a horse in a stall and whistling is a commonly used technique for racehorses. Gentle rubbing below the ventral commissure of the vulva causes micturition in dairy cattle. Manually occluding the nares will cause sheep to urinate.

Timing

Perform on fresh urine, ideally less than ½ hour after collection, otherwise refrigerate (do not freeze) in an opaque airtight container to avoid deterioration of cellular components and metabolism or escape of analytes. Cells and casts will lyse if left at room temperature; glucose is metabolized; ketones and bilirubin decrease; pH increases as urea is converted into ammonia; CO_2 escapes; bacteria proliferate. Refrigeration can encourage crystal formation, so samples must be rewarmed for 20 minutes on the benchtop and swished gently to resuspend any settled particles.

Expected volume in healthy dogs and cats

Dogs = 20–40 mL/kg/d (1 mL/kg/h); cats = 10–20 mL/kg/d.

Color and clarity

Normal urine is yellow and clear, concentrated urine is deep amber, and dilute urine is clear to pale yellow. Red, brown, and various shades inbetween are seen commonly with hematuria, hemoglobinuria, and myoglobinuria. Uncommon differential diagnoses for red or brown urine include porphyria and administration of phenothiazine antehelmintics or aminopyrine (uncommon, given as urinary analgesic). See Table 23.8 for the differentiation of hematuria, hemoglobinuria, and myoglobinuria, and the specific diseases that cause them. Horse and rabbit urine may be cloudy in normal animals due to normal urinary tract mucus production and numerous calcium carbonate crystals. Guinea pigs may also have abundant calcium carbonate crystalluria. Cloudiness is caused by suspended solids (crystals, mucus, casts, cells, etc.) which do not change the specific gravity but may interfere with reading of the line. Cloudy urine in species other than those listed here is abnormal and should be assessed microscopically for the presence of cells, bacteria, casts, crystals, sperm, powder, contaminants, etc.

Table 23.8 Proteinuria.

Pre-glomerular	Glomerular	Post-glomerular
Myeloma	Glomerulonephritis	Cystitis
Bence Jones proteins	Amyloidosis	Tubular disease
Physiologic: fever, exercise	Chronic lesions	Fanconi syndrome
Hemoglobinemia		Hematuria
Myoglobinemia		

Hyperadrenocorticism and exogenous steroids cause proteinuria with and without cystitis. The mechanisms when the urine sediment is inactive are not as clear; however, glucocorticoid therapy given to healthy dogs resulted in a mild increase in UPC (slightly greater than 1) and glomerular lesions. Others have shown that hydrocortisone will increase the blood pressure of dogs and cause proteinuria, both of which are reversible within one month of stopping the steroid.
Microproteinuria, microalbuminuria: <30 mg/dL, and albuminuria is >30 mg/dL.

Specific gravity

See urine concentration and Tables 23.5 and 23.6 for the range of possible values.

Specific gravity is measured with a refractometer. Refractometers require only a drop of urine, results can be predictive of clinical problems, and it is a low-cost test. If USG is not concentrated the measurement should be repeated at different times of the day (especially in the first urine produced in the morning, the time of maximal concentration) to determine if urine can be concentrated and if there is a pattern.

Age of pup and UA

Approximately 4 weeks of age is when pups are able to concentrate urine comparable to other canine age groups. Urine specific gravity is significantly lower in pups younger than 4 weeks of age as compared to pups 4–24 weeks of age, but there are no differences in protein, blood, glucose, ketones, or bilirubin.

Chemical examination of urine

There are numerous products to help analyze urine, but it is beyond the scope of this writing to list them all and their associated false negative and false positive results.

Table 23.9 Expected results to differentitate hematuria, hemoglobinuria and myoglobinuria.

	Hematuria	Hemoglobinuria	Myoglobinuria
Signalment	Any	Any	Horse, exotics
History	Dysuria/obstructed	Variable	Exercise
PCV	Normal (RI)	Decreased	Normal to increased
Plasma	Clear	Pink to icteric	Clear
CPK	Normal (RI)	Normal, mild inc.	Markedly inc. 5+
AST	Normal (RI)	Normal, mild inc.	Markedly inc. 5+
Azotemia	No	Possible	Likely
	If obstructed yes	Can be high	May be lethal
Urine	Red	Red-brown	Red-brown-black
Blood	4+	4+	4+
RBCs	TNTC	None, few	None few
WBCs	+++	None, few	None few
Casts	None	Variable, many	Variable, many
Urine color post centrifugation	Yellow, pink	Red	Red-brown
Ammonium	Not performed	Red pellet	No pellet
sulfate ppt.		Supernatant clearer	Supernatant red-brown
Etiologies	Obstruction urolithiasis, trauma neoplasia, biopsy	Red maple, copper, zinc, IHA, postparturient hemoglobinuria water intoxication, *Babesia*	Exertional rhabdomyolysis capture myopathy, aortic thrombus

RI, reference interval.

Semiquantitative reagent strips

These are commonly used and provide a semiquantitative analysis of the concentration of most substances of interest in urine. The strips contain pads impregnated with various chemicals that, rather than detecting a specific compound, detect the product of a chemical reaction involving the specific analyte. Formation of the product leads to a color change, and the degree of color change is proportional to the concentration of the analyte (Fig. 23.3).

Product expiration dates should be adhered to as outdated strips can give false negative results. During storage, containers must be kept tightly closed as the reagent pads are sensitive to air and humidity. Additionally, reagent strips are designed for human urine and some of the reactions are not valid in animal urine; i.e., do not use strips to determine white blood cells, specific gravity, urobilinogen, or nitrite concentration.

Process

The urine sample is examined grossly for color and clarity. The reagent strip is immersed for the prescribed period (no longer or false increases will occur), and then removed with excess urine being drained while holding the stick laterally on a paper towel. Depending on the solute evaluated, the instructions will state how long after immersion that pad should be graded. Hold the reagent strip horizontally to prevent intermixing of reagents, and grade each reaction. The various reagents and their reactions produce different types of false negative and false positive results (e.g., see glucose below). Additionally, contaminants may be present that are able to participate in the chemical reaction of interest, producing a color change, and resulting in a false negative or false positive result. If results on a strip are equivocal, there are chemical tablets available that are generally more sensitive for each substance on the strip and that can be used to clarify results.

After immersion and blotting of the stick, the next step is to determine if a solute is present—a qualitative assessment. Then, proceeding by the time indicated on the container with the color grading scheme, the pads are read and graded, usually 1+ to 4+. Refractometer USG must be considered when assigning the grade, because the amount of solute detected is dependent on how much of the solute is excreted in a given volume of urine. Additionally, urine volume tends to vary inversely with urine specific gravity (i.e., with urine concentration decreases as volume increases). To illustrate this point, a 1+ grade in urine with a specific gravity of 1.060 is generally considered insignificant for most analytes, whereas a 1+ grade in dilute urine may be very significant. A 2+ grade in urine with a specific gravity of 1.020 would be 1+ if that urine sample was diluted to 1.010 (i.e., diluted by half). A dog with a urine protein of 50 mg/dL and a USG of 1.010 is losing as much protein per day as a dog with a urine protein of 100 mg/dL and a USG of 1.020. In dogs, a 1+

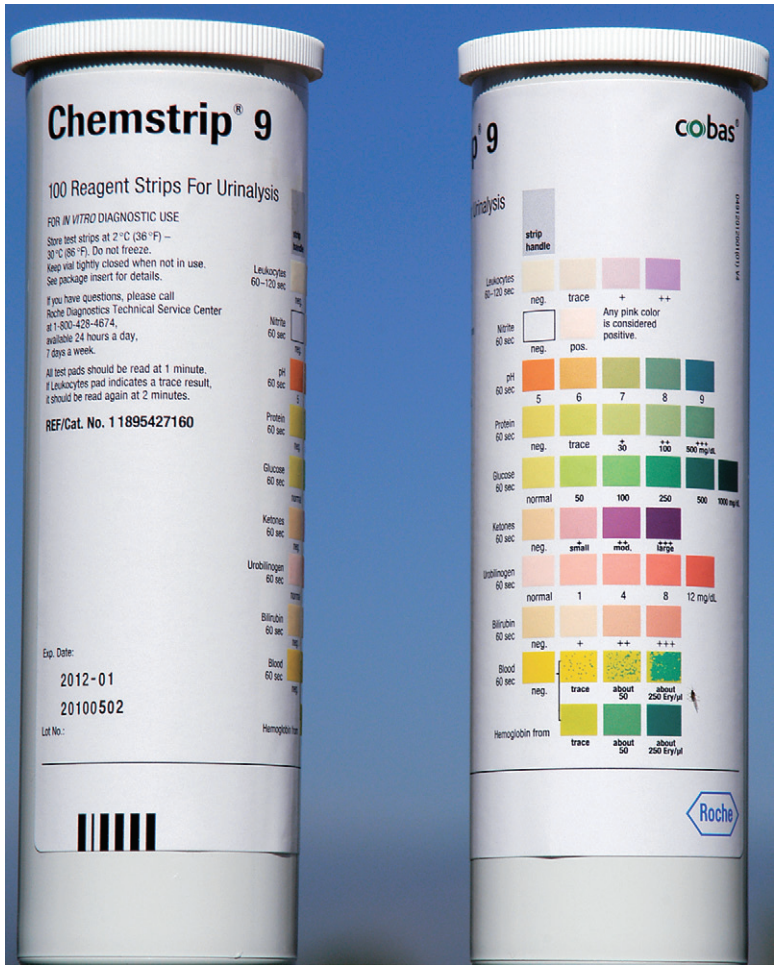


Figure 23.3 Two containers of representative urinalysis reagent strips. Container on left is oriented to show text labeling with storage and use directions and expiration date. Container on right is oriented to show a chart of reagent test pad color reactions. The patient urinalysis test strip is compared to the color chart for recording results. Alternatively, an optical color reflectance reader device is used to determine the urinalysis test strip results.

proteinuria in urine with an inactive sediment and a USG of 1.060 is less significant than trace or 1+ proteinuria in urine with a specific gravity of 1.008 and an inactive sediment.

pH

Dogs and cats have urine that is neutral to acidic and herbivores, neutral to alkaline. The pH reagent pads contain dyes that change color based on the pH: red in acidic, yellow in neutral, and blue-green in alkaline urine. The range detected is approximately 5.0–9.0. Because urine is produced from filtration of blood plasma, urine pH reflects plasma pH. Additionally, it is a crude index of blood gas status. Urine pH may be compared with TCO_2 (bicarbonate) to determine if they correlate, and usually they do. Paradoxical aciduria is a unique situation seen most frequently in dairy cattle with a left or right displaced abomasum. Affected cows commonly have a severe metabolic alkalosis characterized by profound hypochloremia, increased bicarbonate, coupled with an acidic urine (the paradox is that, logically, given the severe alkalemia, they should produce an alkaline urine rather than renally "wasting" acid that would restore a normal pH). Although there are multiple steps in the pathogenesis of this situation, the key component is severe hypochloremia. Until that is corrected the kidneys cannot excrete bicarbonate and the urine will remain acidic despite profound metabolic alkalosis. Alkaline urine in a dog with acidosis may be an indicator of "renal tubular acidosis," which indicates the inability to acidy urine in face of metabolic acidosis. Urease splitting bacteria can cause alkalinization of urine, as will prolonged storage at room temperature and the presence of certain disinfectants (contamination of urine collection container).

Normal dog and cat urine has a pH of approximately 5–7. If urine pH is below 5 or above 7.5 investigate the cause.

Struvite crystals form in alkaline urine. Alkaline urine with a pH > 8 may result in lysis of RBCs, WBCs, and dissolution of casts. It has been reported that a pH > 8 will falsely result in a 1+ protein on the reagent pad; however, recent studies suggest that this is no longer the case.

Protein

All chemical methods for protein determination are best at detecting albumin either because of its structure or overall high negative charge. Reagent pads on strips detect albumin up to 50 times better than globulins, but are able to detect other globulins, Bence Jones proteins (if present in sufficient concentration), hemoglobin, and myoglobin relatively well. The concentration of protein is assigned trace to 4+ and should always be correlated with USG, presence of blood in the urine, results of the serum biochemical profile, and results of the microscopic examination of urine sediment. In dogs a trace protein reading is ≤0.1 g/dL (10 mg/dL; 0.1 g/L) and a 1+ result indicates the renal loss of 0.03 g/dL (30 mg/dL; 0.3 g/L). Both are considered normal if urine is concentrated but abnormal if urine is dilute and/or if there is a positive blood reaction or urine sediment abnormalities.

Most proteins produce a positive color change on the pad. Hemorrhage with or without inflammation causes a positive pad result due to hemoglobin (a protein) being released from lysed RBCs, as well as any plasma proteins that may be exuded along with RBCs and inflammatory cells (WBCs). Pads are generally considered poorly reactive with proteins from WBCs and epithelial cells. If blood is positive on the strip and red blood cells are seen microscopically, then it is essential to obtain another urine sample before interpreting the proteinuria. A generalization is that visibly pink or red urine samples will be positive for protein from red blood cells and samples with only microscopic hematuria will not. Cystitis invariably produces proteinuria from inflammatory proteins and hemorrhage, but these, since they arise from the inflamed bladder, are of postglomerular origin. Proteinuria due to glomerular disease should be observed along with a "quiet" or "inactive" urine sediment (i.e., free of RBCs and WBCs).

Reagent strips do not easily recognize Bence Jones proteins associated with multiple myeloma as well as albumin because light chains have fewer free amino groups on light chain immunoglobulins to react with reagents. In general, approximately 25–50 mg/dL of Bence Jones proteins are required for a trace to 1+ reaction on the reagent pad. If concentrations are insufficient, a false negative result will ensue.

False positive dipstick protein results have been associated with excessive urine contact time, specific antibiotics, contamination of sample with disinfectants, and historically with highly alkaline urine.

Protein is also determined and scored via sulfosalicylic acid turbidity (SSA; 1+ to 4+). SSA also detects albumin the best, but does detect all proteins provided they are present in sufficient concentrations, and is considered better than the reagent pad on the dipstick. If urine is cloudy, it should be centrifuged prior to performing the SSA turbidity test, because suspended solids can cause a false positive result. False positive results are also possible with certain drugs (penicillin and sulfonamides), and radiology contrast media. False negative protein results are associated with the inability to read through very turbid urine and in markedly alkaline urine. If Bence Jones proteinuria and/or multiple myeloma are suspected, then consider sending urine to a reference laboratory to request SSA, and possibly urine and serum electrophoresis to determine if immunoglobulin light chains or a monoclonal gammopathy are present.

Protein can be quantified by a spectrophotometer in a spot urine sample or in a 24-hour collection. The latter is not recommended as it is too labor-intensive, and there is good correlation between 24-hour collections and urine [protein]/[Ct] ratios from spot urine samples. For further information, see section on protein abnormalities in this chapter.

Blood (occult blood, heme)

The reagent pad has a chromogen that changes to various shades of blue-green after oxidation of the enzyme. Anything that causes oxidation of the enzyme is detected and it implies blood is present only because that is how the pad is labeled and because iron in red blood cells (RBCs) is the most common substance that will stimulate this oxidation reaction. However, a positive blue color change is also seen with iron in free hemoglobin, iron in myoglobin, peroxidase in plants and bacteria, and cleaning agents. The reaction is sensitive and the color intensity varies with the amount of "heme" detected; it is more sensitive to free hemoglobin than intact RBCs.

Method

Immerse the stick in well-mixed noncentrifuged urine. A "speckled" pattern of green dots is due to individual RBCs that contact the pad and then lyse; this pattern is associated with microscopic hematuria. A uniform color change is due to a greater amount of hemorrhage, free hemoglobin, myoglobin, or contaminants. False negatives are uncommon but are associated with bacteria that produce nitrites and treatments with vitamin C. Ascorbic acid is a reducing substance that counteracts the oxidation reaction.

Correlate the results with microscopic enumeration of RBCs in urine, PCV, muscle enzymes, USG, and urine pH. Very dilute urine, less than 1.007, and alkaline urine may lyse red blood cells and mask hematuria. Distinction of hematuria, hemoglobinuria, and myoglobinuria is clinically important and is described in the section on diseases.

Degrees of sensitivity of various tests to detect blood

Hemastix, Labstix—hemolytic reagents in stick lyse the RBCs

positive = 1/8000 = 200 RBC/hpf

Occult test tablet

positive = 1/32,000 = 20 RBC/hpf

Microscope

positive = <10 RBC/hpf

Glucose

There should be no glucose in the urine of healthy animals. Reagent strips are sensitive, and detect glucose via an oxidation reaction that produces a color change. False negatives are due to vitamin C and outdated strips. False negatives can occur if the glucose in urine is in low concentrations and one of these substances is present: vitamin C, formaldehyde, ketones, bilirubin (large amount), salicylates, or tetracyclines. False positive results are observed when samples are contaminated with oxidizing cleaning agents and hydrogen peroxide. Cats with urethral obstruction reportedly have an unknown substance that reacts with the color indicator to produce false positive results. In an animal with a positive urine glucose, it is important to correlate the dipstick result with serum glucose as stress can cause a transient hyperglycemia that results in a transient glucosuria.

Tablets are available to detect glucose and do so through copper reduction. False negatives are due to outdated product and low glucose concentration in urine. False positives are fairly common as any reducing substance can cause the color change.

Always correlate results of urine [glucose] with serum [glucose]. The main clinical differential is transient (epinephrine, corticosteroids) versus persistent hyperglycemic disorders (diabetes mellitus, hyperadrenocorticism). When plasma glucose exceeds certain concentrations, the ability of the proximal tubules to reabsorb glucose is surpassed and glucose escapes into the urine. The renal threshold for glucose in dogs is approximately 200 mg/dL (range: 180–220 mg/dL), cats 300 mg/dL (range: 250–350 mg/dL), horses 150 mg/dL (range: 120–200 mg/dL), and cattle 100 mg/dL (range: 80–120 mg/dL). Positive urine glucose results coupled with serum glucose concentrations below the renal thresholds suggest a defect in tubular reabsorption mechanisms, and may be seen with nephrosis (toxic insults), tubular injury, primary or familial glucosuria, and Fanconi syndrome. Fanconi syndrome is a heritable or acquired disease characterized by defects in proximal tubular reabsorption of various substrates that include one or more of glucose, sodium, calcium, bicarbonate, amino acids, and phosphate. Primary glucosuria is seen in Scottish terriers, Norwegian elkhounds, and dogs of mixed breeding and is marked by glucosuria in the absence of hyperglycemia. Glucosuria without hyperglycemia may also be seen due to a time delay of urine collection relative to serum collection. The urine in the bladder may be several hours old relative to the serum and therefore a transient hyperglycemia may have occurred, while the serum [glucose] has meanwhile normalized. This can be seen with stress, postprandially, and after fluid therapy with glucose-enriched fluids. Glucosuria is common in cattle due to their low renal threshold for glucose and the ease of which cattle develop stress hyperglycemia. Cattle with CNS disease may have serum glucose values of 300 mg/dL or greater; pyloric obstruction can produce serum glucose values up to 500 mg/dL or greater.

Serum fructosamine is formed when sugar moieties become attached to plasma proteins, and its concentration can be used to distinguish transient from persistent hyperglycemia. The longer blood sugar has been elevated, the higher the serum fructosamine concentration. Serum fructosamine concentration reflects the average serum glucose concentration over the past 1–3 weeks (roughly the half life of albumin and globulins), and will not be increased by transient hyperglycemia.

Postprandial glucosuria lasts for approximately 1.5 hours; glucosuria beyond 2 hours is abnormal and should prompt examination of serum [glucose]. Persistent hyperglycemia and glucosuria is seen commonly with diabetes mellitus and hyperadrenocorticism (Cushing's disease), and less frequently with pheochromcytoma, pancreatitis, acromegaly, progesterone administration, and some cases of sepsis. A urinalysis used to screen for glucosuria is an acceptable screening test for diabetes mellitus and in monitoring the effect of treatment for diabetes. Hyperglycemia, glucosuria, and ketonuria are diagnostic for diabetes mellitus.

Glucose increases the USG by approximately 0.004 units for each 1 g/dL of glucose in the urine. A 4+ urine glucose would increase USG by approximately 0.010 units. Glucosuria promotes bacterial and fungal growth.

Ketones

There should be no ketones in the urine of healthy animals. Reagent strips detect ketones via a reaction with nitroprusside that produces a purple color change proportional to the concentration of ketones present. Ketones enter the circulation first and then spill into the glomerular filtrate. Ketonuria can be detected in the absence of ketonemia. Ketogenesis produces three kinds of ketone bodies, but only two have the form of a true ketone and are detected by the nitroprusside reaction. The proportions of ketones excreted in the urine are approximately 78% beta-hydroxybutyrate, 20%

acetoacetic acid and 2% acetone. The reaction mainly detects acetoacetic acid (approximately 90%), and reacts less efficiently with acetone (volatile). Beta-hydroxybutyrate, the most abundant product of ketogenesis, does not have the structure of a ketone and therefore, is not detected. The most sensitive test for ketones in cattle is Ketostix™. Tablets are available, but are less sensitive than Ketostix™. Tablets rely on the same nitroprusside reaction as the urine reagent pads. Place one drop of urine (or blood, serum, plasma, or milk) on the tablet and read the color change in thirty seconds. Lavender to deep purple is positive and the intensity is proportional to the concentration of ketones present in the urine or blood. Tablets are more sensitive than strips and can detect ketones in the urine at 5 mg/dL, as opposed to 10 mg/dL for strips, and 10mg/dL in blood.

If there is a trace ketone reaction on the dipstick consider confirming their presence with Ketostix™, or the tablet method. A positive test result can be due to: excessive fat catabolism, as with negative energy balance, inadequate carbohydrate content in the diet, cachexia, starvation, anorexia, hyperthyroidism, pregnancy toxemia, and diabetes mellitus. Ketonuria occurs most commonly in high production dairy cattle, which enter a state of negative energy balance that promotes formation of ketones. Diabetes mellitus is the most common cause of ketonuria in dogs and cats. Ketones are negatively charged and force the excretion of a cation (sodium or potassium) when they are filtered by the kidney. This may lead to hyponatremia and hypokalemia.

The chemicals used in the pads to detect ketones are light, humidity, heat and date sensitive therefore do not use expired strips and keep the lid secured tightly.

Blood glucose	Urine glucose	Urine ketones	Diagnosis
High	Positive	Positive	Diabetes mellitus
High	Positive	Negative	Diabetes mellitus, Cushings; uncommon causes
High	Negative	Negative	Epinephrine or corticosteroids
Low	Negative	Positive	Starvation, pregnancy toxemia, etc.
Normal	Positive	Negative	Transient hyperglycemia, time delay, nephrosis, other tubular diseases, Fanconia

Bilirubin

Use fresh noncentrifuged urine for analysis. Reagent strips detect only conjugated bilirubin using a diazo methodology similar to Ictotest tablets. Since the color reaction is beige to pink to red, any substance that imparts a red color to urine may interfere with interpretation. Large quantities of vitamin C may cause false negative results. As with all substances, correlate results with the USG. The sensitivity of strips is approximately 0.2–0.4 mg/dL, and tablets approximately 0.05–0.1 mg/dL. If interference is suspected (e.g., hemoglobinuria or myoglobinuria) use tablets.

The reaction does not detect biliverdin, and conjugated bilirubin will hydrolyze to biliverdin in urine exposed to light, so analyze fresh urine. Conjugated bilirubin is water soluble and readily enters the glomerular filtrate in most species (cats have a higher threshold). Unconjugated bilirubin is bound to albumin and therefore should not pass through glomeruli.

Bilirubinuria indicates possible liver disease or hemolysis rather than disease in the kidneys. It is most commonly associated with cholestasis. Any positive reaction in a cat is considered abnormal and warrants further investigation. Dogs have a low renal threshold for bilirubin, and additionally, it can be conjugated in small quantities in tubular epithelial cells of normal dogs. In dogs, up to 1+ bilirubin in concentrated urine is considered normal and is common, especially in male dogs but the finding must correlate with other clinical abnormalities. Approximately 20–25% of normal dogs will have positive reaction for bilirubin in urine via reagent strips, and up to 60% with tablets. Bilirubinuria precedes bilirubinemia and icterus, and may therefore be an early indicator of hepatic disease.

Urobilinogen

Urobilinogen is present on reagent strips used for human patients but it not present on reagent strips designed for animals as it has no value. The detection of urobilinogen indicates a patent bile duct and a fresh urine sample, and is ignored.

Nitrites

Detection of nitrites is indirect evidence of bacteriuria as some bacteria produce nitrite, however, the results are unreliable so do not use in animals. Determination of bacteriuria should be done by microscopic examination and culture of the urine.

Leukocytes

Reagent strips for white blood cells recognize a specific leukocyte esterase that is found in human neutrophils, eosinophils, basophils and monocytes but is not reliable for dogs

and cats. Determination of leukocytes in the urine of animals should be done by microscopic examination.

Urine specific gravity

The reagent strip tests for urine specific gravity do not correlate well with results from a refractometer therefore do not use. They estimate the specific gravity indirectly based on pKa which uses a shift in the pH to produce a color change. Maximum concentration detected is 1.030 and this is inadequate to detect the concentration ranges in cats and dogs.

Microscopic

It is standard to centrifuge (1500–2500 rpm for 5 min) 5–10 mL of well mixed fresh urine; however, the exact volume is not critical: just use a consistent amount or whatever is available. Pour off or aspirate and save the supernatant (for possible chemistry analysis) and resuspend the sediment in the urine that remains (0.5 mL is ideal and the amount left should be consistent) by flicking the tube several times with your finger until the pellet and its contents are well mixed. Place one unstained drop on a slide, add a coverslip and examine at low power field (10× objective) for casts and crystals and at a high power field (100× objective) for cells and bacteria. Reduce the light in the field by closing the iris diaphragm (preferable to lowering the condensor) until the material in the field is refractory. Scan the slide with the 10× objective and then record the results as number seen per low or high power field (#/lpf; #/hpf) by counting 10 fields and averaging the results. There also are manufactured systems that aid in these steps and results will be based on number seen per microliter. Change the focus during examination to better see the materials present as not all material is in the same plane of focus in wet mount preparations. If the sample of urine is excessively turbid dilute it with additional supernatant or physiologic saline (but results per magnification are now reduced) and if red blood cells are so numerous that it is difficult to see other structures then lyse the RBCs with 2% acetic acid (vinegar). All abnormalities can be seen in unstained preparations however if unusual structures are seen consider a wet-mount stain (follow directions) and or prepare an air dried film and stain it with a Diff-Quik type stain just like a cytology specimen; the latter is preferable. If neoplasia is suspected you may want a fresh sample of urine obtained with a wash as urine is harsh on cellular features and cells sitting in urine for several hours in the bladder will be visibly altered. Better yet is an ultrasound guided aspirate from the mass (see neoplasia). These slides should be air dried and stained with Diff-Quik type stains or Wright stains. Do not diagnose neoplasia on wet mount preparations, use air dried, monolayer preparations.

Always correlate results with mode of collection, USG, and clinical findings. Examples: 2 casts/lpf in urine with a USG of 1.004 in an azotemic animal is very significant, whereas 2 casts/lpf in urine with a USG of 1.044 in a wellness check is probably normal. Leukocytes in a voided sample may be from the genital tract. Normal findings or results expected in normal patients are in Table 23.6

Casts and heavier structures tend to aggregate along the edges of the coverslip so be sure to examine all regions of the cover slipped area. Count in a low power field but proceed to high power fields to better see their morphology and for more accurate identification.

Sources of technical error

Inexperience, open iris diaphragm, dirty objectives, old urine, poorly mixed urine, contamination, variable amounts of urine centrifuged, too much stain added, organisms growing in contaminated stain jars, random rather than systematic examination of 10 fields, and confusion of Brownian movement of small material with bacteria.

Refer to Appendix 23.1 for color imagery related to microscopic examination of urine; Appendix Figures A23.1–A23.37.

Red blood cells

Greater than 5 red blood cells (RBCs) per high power field (/hpf) is abnormal and is labeled hematuria; compare the number seen to occult blood on reagent pad. As with all cellular events in urine, correlate results with specific gravity, pH and mode of collection. Red blood cells lyse in urine with a USG of <1.008 and in alkaline urine. At USG >1.025 they tend to crenate (shrink and have irregular contours). At a USG in mid-ranges they are uniform, discoid, smoothly contoured, clear to light-yellow or rust-red, refractile, and have no internal detail. Results are recorded as number seen/hpf. Normal is considered <5/hpf, and the phrase too numerous to count (TNTC) is used when the field is crowded with RBCs. Cystocentesis and catheterization will induce minor hemorrhage.

There are too many causes of hematuria to produce a practical list. However, the common denominator is trauma: cystocentesis, catheterization, calculi, hit by car, obstruction, cancer, biopsy of kidney or bladder; and nonphysical traumatic events such as infection, estrus, nephrosis, feline lower urinary tract disease (formerly known as feline urologic syndrome), parasites, and bleeding disorders. Red blood cell casts are rare but localize the hemorrhage to the renal tubules. Red blood cell casts are very fragile and often disintegrate with handling of urine. Red blood cells may be confused with fat droplets, but fat droplets are not uniform

in size, are more refractile and some will be out of the plane of focus.

Leukocytes

Greater than 5 white blood cells (WBCs) per high power field (#/hpf)is abnormal and is termed pyuria. The magnitude of WBCs/hpf is the key and as always the results should be correlated with USG, the rest of the data in the UA, other data in the case (steroids will decrease the number of WBCs in urine), mode of collection, urine pH, etc. White blood cells lyse easily in alkaline urine and hypotonic urine (as do RBCs). Voided urine could have inflammation anywhere in the urogenital tract. Cystocentesis localizes the source of the inflammation to the kidneys or more likely the urinary bladder; however, reflux is possible, e.g., prostatitis.

White blood cells are slightly larger than RBCs (1.5–2× larger), are spherical, granular, have internal structures, a nucleus, and appear singular, in clumps, or in casts. White blood cell casts localize their source to renal tubules and indicate pyelonephritis. Although any WBC could be seen they are almost always neutrophils and differentiation of the types of WBCs is not necessary but if desired will require examination of a stained film. Neutrophils indicate inflammation in the urogenital tract, they are usually accompanied by RBCs and bacteria should be searched for microscopically and via culture. Inflammation is also seen with cancer, calculi, cystitis, prostatitis, and pyelonephritis. Eosinophils are rarely seen, can be associated with parasitic diseases and in dogs consider eosinophilic polypoid cystitis. The latter is a common histologic diagnosis that causes persistent hematuria and produces a mass in the urinary bladder that resembles a tumor. The mass is infiltrated with eosinophils and there is eosinophilpoiesis within the mass. It requires surgical removal.

Epithelial cells

These cells may be squamous (urethra, genital, or skin), transitional (bladder), renal (tubules), or neoplastic (usually transitional cell carcinoma). Squamous cells are huge (5× larger than WBCs, 10× larger than RBCs), have lined or sharp edges, with nuclei often not visible, and usually are in low numbers. If numerous squamous epithelial cells are seen in a free catch sample from a female dog in estrus they are genital in origin and if numerous squamous epithelial cells are seen in a male dog consider a sertoli cell neoplasm that is secreting estrogen and inducing squamous metaplasia of the prostate.

Transitional epithelial cells are from the bladder, ureter, or renal pelvis and most are from the bladder. Supposedly the depth of location in the urothelium changes their morphology enough to recognize the different sources but this is difficult to discern and does not aid a diagnosis. In normal urine there are only a few epithelial cells: <5/hpf. When in large numbers they are usually accompanied by RBCs and WBCs and are associated with cystitis. If numerous and in rafts they may be due to catheterization. Their appearance has a wide range; they are approximately 2× larger than WBCs, round to polygonal, have a nucleus, and are granular to homogenous. These are the most common epithelial cell seen in urine.

Renal tubular epithelial cells are the least common or the least commonly recognized. They are approximately the size of WBCs and look like WBCs. They are round, granular, and have a central round nucleus. Different shapes and sizes may be localized to different regions of the kidneys but too little is known in veterinary medicine for this to be clinically useful.

Neoplastic transitional epithelial cells may be found in this chapter, under "Diagnoses."

Bacteria

The kidneys, ureters, bladder, and proximal urethra are sterile therefore urine should be sterile, at least until it passes through the distal urethra where normal flora can be added as a contaminant. Centrifugation at speeds used for urinalysis will not take bacteria out of suspension so the supernatant or the pellet is equally satisfactory for microscopic detection. Use the pellet as that is what is used for everything else in the microscopic examination and it will permit visualization of cells and therefore the possibility of seeing bacteria inside neutrophils. This will be seen best if a film is prepared and stained, rather than a wet mount. The key to seeing bacteria is the number present, the type of bacteria, and the observer's ability to see them. *Reduce the light considerably* to help outline them (decrease the iris diaphragm or lower the condensor). The greater the numbers the more likely they will be seen. They are usually reported as few, moderate or many and with no reference of number/field.

A bacterial infection should be confirmed with culture and preferably sensitivity. Bacteria can be seen in wet mounts with the 100× objective; however, Brownian movement of particulate matter is easily confused with bacteria and single cocci are easy to confuse with debris. If bacteria are seen but culture results are negative consider misinterpretation of particulate material and Brownian movement as a possible explanation. These "false positive" observations are relatively common in clinical practice. Additional explanations for visualization of bacteria but culture negative are inhibition of growth by cold or frozen storage, or patient is on antibiotics. It is not due to decreased sensitivity of culture technique, as culture is better to identify bacteria than is visualization. It takes about 10,000 rods/mL urine to see

them and up to 100,000 cocci/mL to see them as opposed to only 1–10 bacteria/mL to produce a positive culture.

Localization of the infection is critical as they could be from kidney, bladder, urethra, prostate, uterus, prepuce, external genitalia, or the environment. If bacteria are detected in urine that was collected aseptically via cystocentesis an infection in the kidneys or bladder is the diagnosis. Correlate results with the rest of the urinalysis and if white blood cell casts are present (tubules involved), especially with concurrent azotemia, then pyelonephritis is the source. These patients may also have concurrent cystitis as an ascending infection from the bladder is the most common cause of pyelonephritis. Catheterized and voided samples that are positive for bacteria because of contamination will be culture positive but bacteria are usually in too low a number to visualize. If cystocentesis is not possible then use a sterile catheter and take as many precautions as possible to reduce contamination from the catheter. If a voided sample is used for culture clean the genitalia and catch a midstream sample. Voided samples are to be avoided to rule in an infection but they can be used to rule out an infection. Most contaminants and normal flora are Gram positive. Bacteria can replicate in urine readily therefore examine and culture soon after collection. Refrigeration and especially freezing will inhibit growth and or kill bacteria and therefore may result in false negatives.

Bacterial infections should be accompanied by pyuria, hematuria, proteinuria and various clinical signs including dysuria, stranguria, etc., so correlate all the data available. Intracellular bacteria in neutrophils is good evidence for in vivo infection but can happen in vitro if samples are allowed to sit at room temperature. Results of urinalysis may look similar for cystitis and pyleonephritis. Cystitis will not have azotemia or casts in the urine and pyelonephritis will be associated with azotemia and casts if the disease is active and widely disseminated (great than 75% involvement or kidneys). Infectious cystitis is more common in females due to their wider urethra and it is due to an ascending infection. It should be treated aggressively as it may lead to an ascending pyelonephritis. Sterile cystitis can occur with uroliths and neoplasia but all cases of cystitis/pyuria should be cultured before they are determined to have a nonbacterial etiology. Bacterial infection may also be a secondary event, regardless it requires therapy. Bacteriuria without neutrophils is a paradox but is associated with contamination; Cushing's disease or exogenous steroids which inhibit the influx of neutrophils; dilute urine or alkaline urine which may lyse the neutrophils; and antibiotic therapy which inhibit growth but bacteria are still seen. Cushing's disease has an increased prevalence of urinary tract infections, due to dilute urine and decreased urinary bladder immunity.

Microscopic absence of bacteria does not rule out an infection this requires negative cultures to be certain. Aerobic Gram negative rods account for most infections in the urinary tract and *E. coli* is the most common pathogen cultured. Commercial products are available to culture urine and or enumerate colonies in practice but they will require an incubator. Results obtained can be correlated with results of UA and this is a rapid way to identify a urinary tract infection and/or differentiate the discrepancy of brownian movement versus true bacteria. Urine should be placed in the system as soon after collection as possible for best results. Some systems use select media that turn different colors around different bacterial colonies. If numerous different colors are identified consider contamination. In these systems the colonies are enumerated or the entire culturette can be sent to a referral lab for enumeration, culture, and identification. Positive results determined in practice should be confirmed, bacteria classified, and a sensitivity recommended. In samples obtained by cystocentesis and enumerated with these products >1000 cfu/mL is significant, 100–1000 is suspect and <100 is probably contamination. For samples obtained by catheritization these guidelines are increased by tenfold.

Quantitative bacterial culture results report the number of colonies as colony forming units per milliliter of urine: cfu/mL. Approximately 20% of normal female dogs will have >100,000 cfu/mL urine via catheterization but normal male dogs rarely have >1000 cfu/mL via catheterization. The following numbers can be used as guidelines to diagnose true infection via collection technique when correlated with all the other data.

Catheterization female dog >100,000 cfu/mL
Catheterization male dog >1000 cfu/mL
Catheterization female and male cat >1000 cfu/mL
Cystoscentesis cat or dog either sex >100 cfu/mL
Voided urine is not reliable to rule in but can be used to rule out an infection

Yeast and fungi

Fungal hyphae and yeast forms in urine sediment are most commonly due to overgrowth of contaminants in old samples, or from the skin, litter box, etc.; however, if they are seen in a fresh sample/cystocentesis collected, then a fungal infection of the kidneys and/or bladder should be suspected. They are colorless, often plentiful when present; appear as long hyphae or budding ovals and spheres when in yeast forms. They tend to occur in immune-suppressed patients or patients on long-term antibiotic therapy. If noted in a feline, the feline immunodeficiency virus and feline leukemia virus status should be determined.

Parasites

Capillaria plica or *felis, Dioctophyma renale*, and *D. immitis* are seen rarely in urine of dogs and cats; *Klosiella* spp. in horse

urine has been seen but is exceedingly rare. Capillaria ova look like whipworm ova, oval-shaped, bipolar plugs, rough surface, and colorless to light yellow-tan. Check a fecal float for *Trichuris* and differentiate fecal contamination from dual infection. Microfilaria of heartworm may be seen due to hematuria, and a heartworm check should be performed if the status is not already known. Adult *Dioctophyma renale*, or the kidney worm, is sometimes seen during a laparotomy and the ova are rarely seen in urine as large oval-shaped structures with an internal structure and a pitted tan to light brown surface.

Debris

Spermatozoa, talc crystals from gloves, pollen, fibers, and hair are some of the more common large "debris" seen with the 10 or 20× objectives. Brownian movement that can be confused with bacteria is seen with 40, 50, or 100× objectives.

Lipid

Lipid droplets are clear, variable in size, refractile, round, in different planes of focus, and common in cat urine. They may appear gray to black at low magnification and rarely can be seen grossly in urine from cats. Cat kidneys have considerable amounts of lipid in tubular epithelium and presumably this is the source of the lipid. There is no correlation between lipiduria and lipemia in these individuals.

Casts

Casts are molds of tubular lumens. Their primary component is the Tamm-Horsfall mucoprotein that is secreted by tubular epithelial cells. Casts may contain variable amounts of cells, lipid, and debris. When present in significant numbers they reflect active disease in renal tubules, nephrosis, which usually has an acute toxic etiology. Casts are not a reliable marker of onset, severity, or reversibility of the tubular disease but they do imply that the disease is still active or ongoing and is in the kidneys. Tubules have excellent regenerative capabilities if the basement membrane remains intact. Casts form in the loops of Henle, distal tubules and the collecting ducts. Absence of casts does not rule out tubular involvement. If they are not reported but tubular disease is suspected then repeat the UA as they may be shed intermittently or in showers. If casts continue to be shed post treatment they indicate the disease is still ongoing. Waxy casts are often seen in chronic progressive tubulointersititial renal disease.

Casts are classified based on their microscopic appearance and an attempt is made to correlate their appearance with a pathologic process, which has variable success. They are reported as the number seen/lpf (10× objective) and they are seen more easily if contrast is increased by closing the iris diaphragm. Normal urine should have no casts or only a few casts, and 1–2 hyaline or fine granular casts /lpf in concentrated urine is considered acceptable. Correlate number of casts with the USG as 1–2 casts in dilute urine, especially if other abnormalities are present, is abnormal. Increased number of casts is termed cylindruria.

Hyaline casts

These are the most difficult to see, are clear, have rounded or blunt ends, tend to dissolve in dilute or alkaline urine, and are composed almost entirely of mucoprotein and albumin with no cells or granularity. In elevated numbers, they imply glomerular disease or, less likely, a preglomerular proteinuria. The albumin that leaks through glomeruli apparently stimulates excess secretion and precipitation of tubular Tamm-Horsfall mucoprotein leading to formation of these casts. A few hyaline casts in concentrated urine are normal and a few in dilute urine are abnormal; as are many in urine of any specific gravity. When they are observed, the possibility of glomerular disease or, less likely, multiple myeloma (Bence Jones proteinuria) should be pursued.

Granular casts

These are clear to tan-brown, easy to see, and consist of cells and mucoprotein. They are from epithelial cells that recently sloughed, became entrapped in the mucoprotein, and formed a cast. They indicate possible nephrosis, pyelonephritis, or infarction. The longer the casts are in the tubules before they are released, the more their granular appearance changes from rough and coarse to fine and then waxy which is the final stage of degeneration of granular casts. Differentiation of coarse and fine granular casts is not necessary but can be attempted. One to two fine granular casts per lpf in concentrated urine are considered normal, but the presence of coarse granular and elevated numbers of fine granular casts is abnormal.

Waxy casts

Waxy casts are clear, with no internal structure, and they have sharply defined margins and squared-off ends as they are brittle and break. To the novice, they may resemble hyaline casts which are smoother and the ends are more rounded. Waxy casts imply chronicity and particularly when found in large numbers indicate a pathologic process.

Cellular casts

These can be composed of epithelial, red, or white blood cells, and all can have a granular appearance (granular casts). If red blood cells are recognized in the cast it indicates

hemorrhage in the tubules and if white blood cells are recognized in the cast it indicates pyelonephritis. It can be difficult to recognize the type of cells in casts as the cells are degenerating and they are not stained well; red blood cell casts tend to be fragile.

Lipid casts

These are composed of lipid and are seen in cats; one may see refractile droplets in the casts. These casts are associated with tubular disease (degeneration) and diabetes mellitus. Confirmation of lipid content may require use of fat stains such as Sudan black B or Oil-red-O.

Hemoglobin and myoglobin casts

These are seen rarely and indicate intravascular hemolysis or myoglobinuria, respectively. They are yellow to pink-red to brown, homogeneous and smooth or may be granular if tubular cells are dying and sloughing into the lumens of the tubules. Correlate their presence with the rest of the case data.

Crystals in Urine = Crystalluria (see Appendix 23.1 for visual examples)

Urolith = stone in bladder; nephrolith = stone in kidney

Crystals in the urine can be found in normal patients (incidental finding, no treatment needed) or sick patients wherein the crystals may correlate with a known disease or they may indicate a primary disease that was formerly unidentified. For example, calcium oxalate dihydrate may indicate a hypercalcemic disorder; calcium oxalate monohydrate may indicate ethylene glycol toxicity; and ammonium biurate may indicate liver failure. Crystals in urine are often incidental and of no diagnostic significance; struvites, carbonates, and oxalates can be present in urine from normal patients. The observation of crystalluria should be correlated with clinical and other clinical pathology data. Crystals are only one of several risk factors for urolithiasis. For crystals to form, the urine must be supersaturated and therefore the potential for nephrolith or urolith formation is present, but not guaranteed. Stones must be analyzed to identify them properly as urinary crystals may or may not be the same as the urolith. Analyses of uroliths always require a send out test.

Urethral obstruction in most species is due to a urolith and is almost exclusive to males. However, obstructions in male cats are usually due to a plug of mucoid material and phosphate crystals rather than a stone.

Crystals should be identified in fresh urine, as storage, refrigeration, and preservatives may influence their formation or dissolution. Delaying examination for 6–24 hours can induce crystal formation especially in refrigerated samples. The formation of crystals depends on multiple factors including, species, breed, pH, hydration, diet, and underlying diseases. Urine pH influences precipitation of some crystals and changing urinary pH through diet modification can lead to crystal dissolution.

There are expected pH ranges for different crystals but these are generalizations and urinary pH does not establish identification of the crystal. Identification is done visually by matching photos with what is seen in the urine; shape, color, size of crystal are all used. Rarely, chemical analyses or x-ray diffraction are used for crystal identification.

Ammonium biurate

Brown, tan, yellow, greenish; spiky spheres; protrusions give "mite-like" or "thorn-apple" appearance. These can be seen in normal animals, especially in Dalmatians and English bulldogs. They may also be suggestive of liver failure, congenital or acquired shunts, in which there is decreased conversion of ammonia to urea. In these cases the serum urea nitrogen may be decreased while serum [ammonia] is increased and excreted in urine where it forms urate crystals, and may lead to nephrolith or urolith formation (tan to green in color). Their formation is generally favored in lower pH urine, but can form in any pH. Patients in liver failure may have concurrent hypoalbuminemia, microcytosis, decreased serum cholesterol, and variable liver-derived serum enzyme activity.

Bilirubin

Yellow, yellow-red, red; needle-like or granules; associated with bilirubinemia and bilirubinuria but these abnormalities do not have to be present to form bilirubin crystals; common in canine urine, especially in concentrated specimens, abnormal in urine of other species; pH<7; may resemble tyrosine crystals.

Calcium carbonate

Colorless, tan, brown; large with or without radial striations; spheres, dumbbell-shaped. Commonly seen in herbivores, such as horses, rabbits, and guinea pigs. Not normal in dog or cat. Dumbbell-shaped crystals in a dog or cat are more likely to be calcium oxalate monohydrate than calcium carbonate.

Calcium oxalate dihydrate

Colorless; "x" pattern through the crystal imparts "Maltese cross" or "envelope" pattern; square or rectangular. These are common in horses and cows, associated with ingestion of oxalate-rich plants. May be seen in normal dogs and cats, but also suggests hypercalcemia and hypercalciuria. Especially if numerous, their presence should prompt investigation of a potential hypercalcemic disorder, such as hyperparathyroidism or idiopathic hypercalcemia. These crystals are seen in conjunction with calcium oxalate monohydrate crystals in ethylene glycol toxicity.

Calcium oxalate monohydrate
Colorless; shapes include "Washington monument," "double-ended picket fence" (may look like double-ended hippuric acid crystals), dumbbell (may look like calcium carbonate), spindle, sheaths, hemp seed-like; can be normal in herbivores or in normal dogs and cats. However, their presence suggests ethylene glycol toxicity (correlate with signs and laboratory data); also should consider hypercalcemic diseases in dogs and cats, especially if dihydrate forms are also present; associated with plants that contain oxalate; any pH possible, associated with pH <7. When observed in dilute urine in any number from a dog or cat with acute renal failure they indicate ethylene glycol toxicity.

Calcium phosphate
Colorless to tan; amorphous aggregates or spheres and elongated prisms, needles that may aggregate into sheaths or rosettes; can be in normal dogs; can form uroliths; usually in alkaline urine.

Cholesterol
Colorless transparent; large flat plates, rectangles often with a notch in one corner, may stack on top of each other; with polarized light are birefringent and colorful; present in normal dogs; may suggest hypercholesterolemic syndromes and protein losing nephropathy.

Cystine
Colorless; hexagonal; may stack on top of each other; not always abnormal; seen with rare inherited metabolic disorder cystinuria; occurs in both male and female dogs, but clinical signs are observed almost exclusively in male dogs. Most common in Newfoundland, Australian cattle dogs, mastiffs, Scottish deerhounds, English bulldogs, and dachshunds.

Drug-associated
Dark to light brown, commonly form needles arranged in sheaves, bundles, fans, radiating spokes. Seen most commonly with sulfa family drugs, ampicillin, allopurinol, xanthine crystals, radiographic contrast media, and others. Correlate this finding with history of drug administration.

Hippuric acid
Distinguish from calcium oxalate monohydrate by noting single-ended point but distinction is difficult.

Leucine
Yellow, brown; spheres with concentrically radiating circles; rarely to never identified in veterinary species; suggestive of liver disease.

Magnesium ammonium phosphate—struvite—triple phosphate (a misnomer)
Colorless; "coffin shaped" pattern to surface, prisms, plates; three to six sides, oblique ends; very common crystal in dogs and cats; usually accompany alkaline urine; urease splitting bacteria that produce free ammonia and alkaline urine enhance their formation; can aggregate; common to form uroliths; can be numerous in normal dogs and cats.

Struvite
See *magnesium ammonium phosphate* (above).

Tyrosine
Colorless or yellow; needle-like; resemble bilirubin crystals; rare; suggest liver disease.

Urate ammonium
See *ammonium biurate* (above).

Uric acid
Yellow, yellow-brown; diamonds, rhomboid, rosettes; imply the same as urates. During purine degredation uric acid is produced and Dalmatian dogs have a genetic defect in the gene for a uric acid transporter therefore uric acid is not metabolized resulting in increased plasma and urine concentrations of uric acid. Significant disease can be present or they may be incidental; correlate with clinical signs. These dogs have adequate uricase in hepatocytes as compared to an absence of this enzyme in people but Dalmatians cannot transport uric acid into hepatocytes for uricase to convert uric acid to allantoin therefore they excrete uric acid in their urine. These crystals may also be seen in dogs with primary hepatic disease that fail to convert uric acid to allantoin and ammonia to urea.

Xanthine
Brown, tan; look identical to ammonium biurate crystals but xanthine crystals usually form after treatment with allopurinol, a xanthine oxidase inhibitor to prevent formation of uric acid in dogs with urate calculi; may be familial in Cavalier King Charles spaniels and dachshunds.

Electrolyte abnormalities

There is a tremendous amount of information on how and where in the tubules electrolytes, ions, and other substances are reabsorbed and excreted. This section focuses on the abnormalities seen with renal failure and related diseases more than the physiology. Electrolyte abnormalities are common in renal failure and generalizations are predictable; however, the severity of the renal failure and the stage of compensation make accurate predictions difficult and therefore serum electrolytes must be measured. Hyperphosphatemia is expected anytime GFR is reduced. If chronic renal failure is in a compensatory state then sodium, potassium, and chloride will likely be within reference intervals. If chronic or acute renal failure is not compensated then there

will be disturbances of these electrolytes that run the gamut of increased to decreased.

Calcium and phosphorus in renal failure

Evaluate these two electrolytes together. Most dogs and cats with renal failure will have normocalcemia and hyperphosphatemia, next most frequent is hypocalcemia and hyperphosphatemia; horses tend to have hypercalcemia and hypophosphatemia; hyperphosphatemia is a nearly constant association with chronic renal failure in all species except the horse. As renal failure progresses from stage 1 to stage 4 the serum concentrations of urea nitrogen, creatinine and phosphorus increase accordingly as well as the Ca × P product. At stage 1 (mild renal disease) hyperphosphatemia is observed in approximately 20% of dogs and the magnitude is mild, e.g., 6–8 mg/dL. Hyperphosphatemia progresses to 100% of dogs in stage 4 (severe end stage) and increases are severe 17–25 mg/dL. Similarly PTH increases overtime, 33% of dogs will have increased values at stage 1 and 100% at stage 4.

Approximately 80% of phosphorus entering the glomerular filtrate is reabsorbed in the proximal tubules and 20% is excreted. The most common cause of hyperphosphatemia in veterinary medicine is decreased GFR, pre-, renal, and post renal causes, acute and chronic renal failure all do this. In dogs with chronic renal failure increases in serum phosphorus are roughly parallel to increases in UN. In a ruptured urinary bladder phosphorus increases in the serum because it is reabsorbed along its concentration gradient from a high concentration in the urine/abdominal fluid across the peritoneum and into the blood. Serum phosphorus may increase before azotemia in some patients with ethylene glycol toxicity if the antifreeze product ingested also contains a phosphate rust inhibitor.

Parathyroid hormone inhibits P reabsorption in the proximal tubules and therefore promotes phosphaturia. Increased concentrations of parathyroid hormone help prevent hyperphosphatemia in renal failure for sometime but when the GFR is decreased below 20% of normal this compensatory adaptation is overwhelmed and hyperphosphatemia develops. Hyperphosphatemia and hypocalcemia are the major stimulatory factors for renal secondary hyperparathyroidism.

Hyperphosphatemia greater than 10 mg/dL is common and it can be severe, >15 mg/dL. At these concentrations phosphorus may amplify renal failure via mineralization of tubular cells and cellular organelles, direct nephrotoxicity, and vasoconstriction. When the serum Ca × P product is >70, soft tissue mineralization is possible and if it is >100 soft tissue mineralization is occurring. Soft tissue minerlaization is enhanced in renal failure due to underlying vasculits which damages tissues. Phosphorus is more important in mineralization than is calcium; therefore mineralization of soft tissues will be occurring even if there is hypocalcemia as long as there is hyperphosphatemia. Example: serum total calcium is 7.8 mg/dL and serum phosphorus is 16 mg/dL, the product is 125 and soft tissue mineralization is occurring even though there is hypocalcemia. Mineralization occurs outside the kidneys as well and predisposed sites are blood vessels throughout the body, midzonal gastric mucosa, lungs, and heart. On rare occasions the mineralization in blood vessels is severe enough to be seen in radiographs. This metastatic calcification is very harmful and contributes to mortality in animals with renal failure. Treatment of renal failure includes dietary changes and medicinal products to bind phosphorus and decrease GI absorption in an attempt to lower serum [P].

Normophosphatemia in an azotemic patient is unusual and should prompt consideration that there is another disease lowering the serum P such as primary hyperparathyroidism or, more likely, hypercalcemia of malignancy. Hypophosphatemia occurs in some horses with renal failure but does not occur in small animals unless it is caused by treatment. It is estimated that approximately 66% of horses in renal failure will be hypercalcemic and 50% will have hypophosphatemia. The mechanism is not clear. It is relatively easy to hypothesize on the hypercalcemia but hypophosphatemia is problematic; there may be increased excretion in the intestines. Some horses may adapt to the high calcium diet in alfalfa by excreting calcium and reabsorbing phosphorus in their kidneys. These horses may then retain calcium and excrete phosphorus during renal failure. This is the opposite of normal renal physiology. Hypercalcemia and hypophosphatemia in a horse is most likely due to renal failure but in a dog hypercalcemia of malignancy is the most likely diagnosis.

Normocalcemia is seen most frequently in animals with renal failure (50–75%); hypocalcemia is relatively common (up to 40%); and hypercalcemia is recognized, depending on the species, stage of compensation of the renal failure, and methodology for calcium measurement. Hypocalcemia can be explained via six mechanisms: decreased tubular cells to reabsorb the Ca, decreased concentrations of vitamin D, decreased albumin, soft tissue mineralization, reciprocal decrease in serum due to increased P, and if renal failure is due to ethylene glycol toxicity, the chelating effect of oxalate on calcium. The hypocalcemia seen with acute renal failure casued by ethylene glycol can be severe, <6 mg/dL. Hypocalcemia is more common with chronic than acute renal failure and usually is mild to moderate, 7–8 mg/dL, and asymptomatic. The rate limiting step in the synthesis of vitamin D is in the kidney hence chronic renal failure is associated with decreased production of vitamin D. Prolonged hypocalcemia stimulates parathyroid hyperplasia which may lead to metabolic bone disease, osteopenia, or renal fibrous osteodystrophy, or "rubber jaw." Although the bone lesions are generalized they are best seen radigoraphically in the mandible and maxillae.

The changes seen in total serum calcium are usually the same for ionized calcium but some cases of renal failure in dogs may have decreased ionized calcium while total serum calcium is normal or increased. Rarely are there clinical signs in these patients referable to this change in calcium. If ionized calcium is available it is the best fraction to measure to predict biologic action of calcium. If fluid therapy corrects the metabolic acidosis rapidly then these patients may develop tremors, tetany, and neuromuscular signs that could be due to the shift of calcium from ionized (acidosis) to protein or complexed compartments (alkalosis). Use of calcium products to correct this possible effect is probably contraindicated as the administered calcium would combine with the existing hyperphosphatemia to speed soft tissue mineralization. Hypocalcemia is expected in cows with renal failure due to the mechanisms listed above as well as the tendency for cattle to develop alkalosis with renal failure and the observations that many sick cattle with a variety of diseases will have mild hypocalcemia.

Hypercalcemia is seen in cases of canine and feline renal failure (10–20%) and equine renal failure (66%) and the explanation is not clear. A likely mechanism is an acquired defect in the calcium sensing protein receptor. This protein is critical for the parathyroid gland to recognize the concentration of calcium and adjust synthesis and secretion of parathyroid hormone appropriately to normalize serum calcium. If the molecule is abnormal as in congenital and acquired disorders in humans the parathyroid cells do not decrease their secretion of parathyroid hormone at a concentration of serum calcium that would normally signal the parathyroid gland to stop production and release of parathyroid hormone. Continued secretion of parathyroid hormone stimulates calcium reabsorption in the proximal convoluted tubules and osteolclastic osteolysis, further contributing to the hypercalcemia. Hypercalcemia with canine renal failure is seen most frequently in young dogs with progressive familial renal nephropathy. It is seen with other types of renal failure as well. Dogs and cats with renal failure–associated hypercalcemia will have hyperphosphatemia and the threat of soft tissue mineralization is high.

Renal failure is the second to third most common cause of hypercalcemia in dogs whether total serum calcium or ionized calcium is used to assess the calcium status. Several studies have highlighted that total serum calcium does not correlate with the ionized calcium in up to one-third of the cases of chronic renal failure in dogs. Approximately 4–10% of dogs with renal failure will have increased ionized calcium and 5–15% will have increased total serum calcium. Total serum calcium also may not reflect the ionized calcium in cats with chronic renal failure: ionized calcium increased in 6% versus 20% via total calcium and ionized calcium decreased in 25% versus 8% with total calcium. If the ionized calcium is desired then it must be measured and the measurement of ionized calcium is preferred to accurately assess calcium status. Correction formulae for calcium and albumin used in dogs were not designed to predict ionized calcium. When deciding which calcium to use for predicting the Ca × P product use the total serum calcium.

Deciding if renal failure is the cause or the result of the hypercalcemia is problematic. The easiest way to decide is to look at all the data and see if a primary diagnosis is evident. For example if a dog has lymphoma, azotemia, and hypercalcemia then hypercalcemia is due to lymphoma and its associated paraneoplastic syndrome and the azotemia is secondary to possible dehydration, soft tissue mineralization and or lymphoma in the kidneys. The serum [P] in these dogs will not be markedly increased despite azotemia due to the phosphaturic effect of PTH-rp. Parathyroid hormone (PTH), and parathyroid hormone related (PTH-rp) protein (cancer associated hypercalcemia) stimulate urinary phosphorus excretion and calcium reabsorption. If chronic renal failure can be established because of laboratory data, breed disposition, biopsy, etc., then that is the most likely cause of hypercalcemia. Inability to concentrate urine can be due to hypercalcemia or renal failure so USG is not a distinguishing feature.

If a primary diagnosis is not evident, then the rules of thumb are: the greater the serum [Ca] the more likely there is a primary calcium disease, and the greater the serum [P] the more likely it is primary renal failure. The lower the serum [P], the more likely there is a primary disease causing the hypercalcemia such as primary hyperparathyroidism or hypercalcemia of malignancy, both of which stimulate phosphaturia and a decrease in serum phosphorus. However, only 5% of dogs with primary hyperparathyroidism have azotemia so it is much more likely they have hypercalcemia of malignancy as azotemia is fairly common in these dogs. If serum phosphorus is within the reference interval in an azotemic patient with hypercalcemia, then there is a primary calcium disease and a hormone that is stimulating phosphaturia. Additionally if the total serum calcium is increased but the ionized calcium is normal or decreased, then renal failure is the more likely cause of the hypercalcemia.

Hypercalcemia is associated with dilute urine and PU PD. There are multiple mechanisms for this including the interference with the action of ADH, decreasing the movement of AQP2 to the apical membrane and effectively preventing water reabsorption, blocking receptors on renal epithelial cells, and mineralization of cells. Biochemical steps may be reversed, but structural lesions induced by mineralization may not. Basement membranes and cellular organelles will become mineralized and lead to death of cells further contributing to both concentrating defects and renal azotemia.

Hypercalcemia, steroids (hyperadrenocorticism), and pyometra (*E. coli* endotoxin) are examples of diseases or substances that interfere with the action of ADH and frequently result in dilute urine and PU PD. If these patients are also azotemic it can be difficult to differentiate prerenal from

renal azotemia. This is because they will have dilute urine due to the inhibitory substance but the azotemia may actually be due to concurrent dehydration and the kidneys are fine.

Calcium-oxalate urolithiasis or crystalluria are clues to look for hypercalcemia in small animals. Usually they are dihydrate oxalate crystals but both dihydrate and monohydrate forms have been seen in dogs and cats with hypercalcemia, e.g., primary hyperparathyroidism and idiopathic hypercalcemia of cats. Hyperparathyroidism is often asymptomatic and it is the presence of hypercalcemia found on a routine chemistry panel or calcium crystalluria that is the first clue that this disease is present. Calcium-oxalate monohydrate and dihydrate crystals can also be normal findings and they also suggest ethylene glycol toxicity in dogs and cats, therefore their presence must be correlated with all the clinical and lab data. Calcium-oxalate monohydrate and dihydrate crystals are seen in normal horses, rabbits, and guinea pigs. Horses and rabbits have cloudy urine and excessive mucus in their urine and have reference interval serum calcium values greater than others species, up to 13 mg/dL depending on the lab and methodology.

Sodium and chloride

Essentially 100% of sodium in the glomerular filtrate is reabsorbed: 65% in proximal convoluted tubules, 25% in the ascending loop of Henle, 5% in each distal tubules and collecting ducts. If the fractional excretion of sodium exceeds 1% it indicates renal tubular failure. Most cases of renal failure have normal concentrations of serum sodium and chloride; however, chronic renal failure is associated with hyponatremia and hypochloremia, especially in horses and cattle. Hyponatremia and hypochloremia can be seen in dogs and cats with chronic renal failure, and additionally, are characteristic electrolyte abnormalities of uroabdomen in all species.

Postassium and magnesium

Most potassium entering the filtrate is reabsorbed in the proximal tubules and potassium is excreted in collecting tubules via aldosterone stimulation of cellular channels. Primary hypoadrenocorticism is associated with hyperkalemia because of a lack of stimulation of these ion transporters. Potassium increases in the serum with postrenal azotemia and in some cases of acute renal failure, especially if oliguria and inorganic acidemia are present. Potassium may increase to life-threatening concentrations (>8 mEq/L) in obstructed male cats. Hyperkalemia with renal failure or postrenal diseases is due to decreased excretion of potassium and concurrent acidosis which shifts potassium from its intracellular location to an extracellular one, in an exchange for hydrogen ions to maintain intracellular electroneutrality. Potassium decreases with chronic renal failure especially if polyuria is present and it may decrease during the diuretic phase of acute renal failure if dietary intake does not match reanl loss. Cattle with renal failure have hypokalemia from renal loss, salivary loss, anorexia, and metabloic alkalosis. Alkalosis shifts potassium into cells in exchange for a hydrogen ion to buffer excess plasma bicarbonate. Alkalosis is due to ileus and forestomach atony secondary to uremia.

Historically, and with commercial food formulations with low potassium, approximately 30% of cats with chronic renal failure developed hypokalemia with an increased fractional excretion of potassium. The mechanisms were not clear. When severe, the hypokalemia caused myopathy, generalized muscle weakness, and cervical ventroflexion. Hypokalemia may also contribute to renal failure by causing degeneration of tubular cells and it also interferes with ability to concentrate urine by decreasing the responsiveness of tubular epithelial cells to ADH. The condition was known as feline kaliopenic polymyopathy-nephropathy syndrome.

Magnesium entering the glomerular filtrate is reabsorbed via active and passive routes in the proximal tubule and in the thick ascending loop of Henle. The primary route of magnesium excretion is via the kidney and therefore serum magnesium concentrations increase with renal failure in most species, although it is seldom measured. Horses with blister beetle intoxication tend to have severe hypocalcemia and hypomagnesemia. These horses have hemorrhages in numerous tissues including the urinary bladder which results in hematuria.

Fractional excretion of electrolytes

All the electrolytes can be tested but sodium, potassium, and phosphorus are most clinically useful. Fractional excretion of electrolytes is performed to determine whether there is renal tubular failure; in an animal that is hyponatremic or hypokalemic, they are used to determine the contribution of the kidney to the electrolyte's decrease. Fractional excretion of phosphorus is examined to determine the likelihood of increased parathyroid hormone in a nonazotemic animal. Single-spot urine and serum samples are adequate and are preferred over 24-hour urine collections for clinical assessment. Collect a serum sample close to the time of the urine sample and submit both for measurement of the electrolyte(s) desired and creatinine in serum and urine. The reference laboratory should calculate the result or use this formula:

$$\frac{\text{Serum Ct}}{\text{Urine Ct}} \times \frac{\text{Urine electrolyte}}{\text{Serum electrolyte}} \times 100 = \text{Fx Exc of electrolyte}$$

99% of the sodium entering the filtrate is normally reabsorbed.

Increased FE_{Na} > 1% is consistent with tubular failure or decreased aldosterone activity; correlate with rest of data.

FE_{Na} in an azotemic patient <1% is consistent with prerenal azotemia, and if hyponatremia is observed, is consistent with sodium loss via the GI.

FE_P > reference interval indicates increased serum parathyroid hormone or PTH-rp.

Reference intervals

Fx excretion, %	Dog	Cat
Sodium	<1	<1
Potassium	<6–20	<6–20
Chloride	<1	<1.5
Phosphorus	<20	<73

Fractional excretion of sodium in normal horses is 0.01–0.70; 0.80–10.10 in horses with renal failure, and 0.02–0.50 in horses with prerenal azotemia. It can also be used to determine if renal failure is present in nonazotemic patients and to follow recovery post treatment of an azotemic patient.

Parathyroid hormone and PTH-rp stimulate phosphorus excretion. Animals with hypercalcemia and primary hyperparathyroidism or humoral hypercalcemia of malignancy (if mediated by PTH-rp) will both have increased fractional excretion of phosphorus and, therefore, measuring the fractional excretion of phosphorus does not help distinguish these two diseases. Physical examination to locate the cancer is the best means to distinguish these differential diagnoses. If that is inconclusive then concurrent measurement of PTH, PTH-rp and serum calcium usually is definitive with or without imaging techniques of the parathyroid-thyroid region of the neck.

Blood gas

Metabolic acidosis is expected with renal failure in all species; however, it will depend on the severity of the renal failure, the presence of concurrent diseases, and compensatory mechanisms. Some cattle develop metabolic alkalosis due to ileus and forestomach atony secondary to uremia. The result is sequestration of acid-rich secretions in the abomasum and rumen. These cows will also have hypochloremia, hyponatremia, and hypokalemia, and an increased anion gap due to retained uremic acids.

Compensated renal failure patients have normal blood gas values and uncompensated renal failure patients may have severe acidosis. Hydrogen ion excretion is decreased in the tubules, as is potassium excretion therefore hyperkalemia may be present, especially with oliguria or anuria. Serum potassium will also increase due to shifting of hydrogen ions into cells and potassium ions out of cells to maintain intracellular electroneutrality. Total CO_2 concentration <15 mEq/L and an increased anion gap due to retention of uremic acids indicate metabolic acidosis; these patients will have acidic urine as well.

Paradoxical aciduria is an event that happens most commonly in cattle with displaced abomasum, especially right-sided. These cows have severe metabolic alkalosis and hypochloremia due to trapping of chloride-rich fluid in the displaced abomasum. They are also dehydrated, hypokalemic, and hyponatremic. Sodium reabsorption is stimulated by dehydration via activation of the renin-angiotensin-aldosterone system, which obligates potassium excretion. Additionally, in health, potassium excretion is elevated in cattle and it takes time for the kidneys to reach a new potassium excretion equilibrium. Aldosterone also promotes hydrogen ion excretion, and this potentiates tubular formation of bicarbonate, which is reabsorbed. Normally, chloride would be reabsorbed; however, it is not available because of the profound hypochloridemia. The reabsorption of bicarbonate ion further exacerbates the alkalosis, and the hydrogen ion excretion results in acidic urine. Until the displaced abomasum is replaced, and the severe hypochloremia is corrected the paradoxical aciduria with metabolic alkalosis will persist.

Protein abnormalities

Proteinuria

Although proteinuria has preglomerular, glomerular, and postglomerular causes (Table 23.8) it is used clinically to recognize renal disease, especially diseases affecting glomeruli. Preglomerular and postglomerular causes do not produce hypoalbuminemia, only glomerular causes result in hypoalbuminemia. The two main glomerular diseases are glomerulonephritis and amyloidosis. Chronic renal disease that involves glomeruli will also produce proteinuria and hypoalbuminemia. Significant proteinuria can precede azotemia and occur in animals that can still concentrate their urine. Quantification of renal proteinuria is a practical means to identify renal disease, predict glomerular disease, monitor renal disease, and formulate treatment plans. Excess protein in the urine is detected by colorimetric dipsticks, SSA turbidity testing or other chemical assays.

Albumin

An increased concentration of serum albumin is seen only with dehydration. There is no specific correlation of hyperalbuminemia with renal diseases, but dehydration is often present in patients with renal failure and the distinction of prerenal versus renal azotemia is made by measuring the urine specific gravity while assessing skin turgidity, PCV, and serum albumin to assess hydration. Decreased serum albumin is seen with glomerular diseases and end-stage renal disease and is also seen with diseases in the gastrointestinal, liver, and cardiovascular systems.

Albumin is conserved at the level of the glomerulus and hypoalbuminemia suggests glomerular lesions and chronicity. Glomeruli retain proteins larger than approximately

68,000 Da and that are negatively charged. With glomerular disease, albumin leaks because of its small molecular size (66,000) and negative charge. Glomeruli lose their negative charge because immunoglobulins (positively charged) are deposited in glomerular basement membranes as part of the pathogenesis of glomerulonephritis and amyloidosis. In health, the small amounts of albumin that leak through are reabsorbed by tubular epithelium and degraded by cellular lysosomes. Although albuminuria may be due to tubular lesions the amount of protein in the urine is small and this mechanism never results in hypoalbuminemia. If glomerulonephritis and amyloidosis are severe enough there will be proteinuria, hypoalbuminemia, edema, ascites, thrombi, and hypercholesterolemia with or without azotemia—nephrotic syndrome.

Preglomerular diseases that result in marked hyperproteinemia may damage the filtration barrier, overload the proximal tubular capacity for reabsorption, and result in persistent proteinuria, referred to as "overload proteinuria." Hemoglobinemia, myoglobinemia, and paraproteins of small molecular weight (<45,000 Da) as seen in multiple myeloma will do this. Critical to the diagnosis of preglomerular proteinuria is to recognize one of those three problems. Serum proteins in patients with multiple myeloma may exceed 9 g/dL and this will induce preglomerular, overload proteinuria that is persistent. If hemoglobinemia is so severe it overwhelms carrying proteins such as haptoglobin then excess hemoglobin will enter the glomerular filtrate. Myoglobin does not have carrier proteins and it readily passes into the glomerular filtrate producing dipstick results that are positive for protein and blood. Mild, clinically insignificant preglomerular proteinuria is also associated with excess exercise, seizures, fever, stress, etc., and is referred to as functional proteinuria. This type of proteinuria is transient and is not significant. Historical and physical exam data and UA will identify these causes. One study demonstrated that excercise did not increase the urinary excretion of albumin in dogs.

Postglomerular proteinuria is very common, is probably the number one cause of proteinuria and is due to inflammation in the urogenital tract: cystitis, pyelonephritis, trauma, neoplasia, urolithiasis, prostatitis, etc. Hypoalbuminemia is not expected from these diseases and the results of urinalysis indicate active sediment. If inflammatory cells are present in the urine then the proteinuria is most likely due to inflammatory proteins and concurrent hemorrhage. The hemoglobin in the red blood cells will produce a positive result for protein and blood. Cystitis is the most common cause of postglomerular proteinuria. Attention is focused on the diagnosis and treatment of cystitis not proteinuria. If proteinuria persists after the inflammation is gone then consideration is given to causes of persistent proteinuria. Postglomerular proteinuria can also be seen in tubular resorptive defects as seen in familial or acquired Fanconi syndrome.

The key to differentiating postglomerular from glomerular proteinuria is by examining the urinalysis. Glomerular proteinuria requires persistent proteinuria with inactive urine sediment (free of RBCs, WBCs, etc.). Urinalyses that reveal blood, RBCs, leukocytes, or other indicators of inflammation have active urine sediment and are classified as postglomerular proteinuria; the underlying cause should be sought.

Detection starts with positive protein reaction on the urinalysis via qualitative dipsticks or SSA. The amount of protein should *always be correlated with urine specific gravity*. In the dog, 1+ proteinuria coupled with concentrated urine is considered insignificant, whereas 1+ protein in dilute urine is considered significant, if the urine sediment is inactive (no cells, blood, etc.). The next critical step is to determine proteinuria is not just transient, but that it persists over time. If there is persistent proteinuria of any magnitude then the amount may be quantified via urine protein to creatinine ratio (UPC) to help estimate the severity and the disease present. A recent study correlated dipstick urine protein with urine specific gravity in an attempt to predict when quantification of the urine protein creatinine ratio (UPC) was indicated. These results were then compared to UPC to determine false negative and false positive as well as positive predictive values. Only cases with inactive urine sediments were included. An interpretative summary of their results is:

0 + (neg) at any USG = nonproteinuric, UPC not needed

1 + (30 mg/dl) at > 1.012 = nonproteinuric, UPC not needed

1 + (30 mg/dl) at < 1.012
= proteinuric * correlate with clinical presentation

2 + (100 mg/dl) at any USG
= proteinuric, UPC recommended

*gray zone results = 1 + (30 mg/dL) at <1.012; requires integration of clinical presentation with results; if there are clinical signs or suspicion of problems recommend UPC; if there are no clinical signs or suspicions then UPC is not indicated, consider serial UA.

These recommendations would miss cases of "microproteinuria." Veterinarians could use the above to screen and still consider testing for microproteinuria in geriatric patients and patients with historical features that suggest occult renal disease.

Determination of UPC is accomplished by collecting urine and serum simultaneously and requesting the laboratory measure protein and creatinine chemically in both. The amount of protein in the urine is divided by the amount of creatinine in the urine and a ratio is derived, Prot:Ct. Usually the lab reports the ratio and provides interpretative ranges. Sex of dog or time of day of collection does not influence results. Creatinine excretion is relatively constant and reduces the variability due to concentration of urine (water)

therefore an increased ratio is attributed to an increased concentration of protein. Any cause of increased protein will increase this ratio; therefore, preglomerular and postglomerular causes must be ruled out before an increased UPC is assigned to a glomerular cause or renal disease.

Results for dogs

UPC <0.2 = nonproteinuric, "normal"; UPC 0.2–0.5 gray zone, borderline proteinuia; UPC >0.5 = proteinuric, abnormal, any renal disease.

Generalizations

Prot:Ct > 5 = glomerular; Prot:Ct > 10 = glomerulonephritis; Prot:Ct > 20 = amyloidosis. Given the wide overlap of ranges and the severity and chronicity of the diseases, the best means to distinguish glomerulonephritis and amyloidosis is renal biopsy.

The distinction of glomerulonephritis and amyloidosis could have different treatment and prognostic implications and therefore the differentiation of these two diseases is sometimes desired. The correlation of renal biopsy results with histologic sections taken at necropsy is 98%. This is because the lesions of glomerulonephritis and amyloidosis are diffuse. Essentially 100% of glomeruli have lesions, at least to some degree so biopsy, even if only 1–3 small pieces are obtained will adequately sample the disease process as long as the cortex is sampled. The most common complication is minor hemorrhage, resulting in hematuria; but rarely, the complications are severe and life-threatening. Results of biopsy that indicate severe end stage renal disease may influence the decision to treat.

The majority of dogs 9 years of age and older will have some form of microscopic glomerulonephritis. The majority of these cases are subclinical. The recognition of these animals with newer screening tests that detect microproteinuria (urine albumin <30 mg/dL, correlating to 1+ on the urine dipstick) may prove beneficial for long-term care. It is reported that 2% of clinically healthy dogs have proteinuria and 20% have microalbuminuria. Proteins that are leaking through diseased glomeruli may cause damage to tubules via cytokines, direct toxicity, and overload of lysosomal degradation mechanisms. Therefore recognizing persistent proteinuria and preventing its progression may have benefical effects for the health of the kidney and therefore the animal.

Determining UPC ratios on several samples taken 24 hours or more apart is recommended for even more reliable results as there can be random variation in the amounts of albumin loss in any one sample. However, measuring serial UPC may be cost-prohibitive. In these situations, pooling one mL aliquots taken from three urine samples collected 24–48 hours apart yields results that are clinically useful and more cost effective. To determine the disease is progressing or improving, a change of at least 40% in UPC should be observed.

Mean 24-hour urine protein excretion, 24-hour Prot:Ct ratio, and 4-hour Prot:Ct ratios have been determined for horses and ponies and the ranges are similar to those established for other species. Reference interval for Prot:Ct is 0 to 0.40 and there is minimal day-to-day variation in these species.

Microproteinuria, microalbuminuria is an extension of proteinuria and refers to situations where the amount of protein in the urine is small or below the limit of detectability of most qualitative and some routine quantitative assays. A trace or 1+ protein in concentrated urine is considered insignificant; however, it can imply early or occult renal disease (minimal change nephropathy). The primary protein in the urine of these patients is albumin and assays are now available to measure microalbuminuria. Microalbuminuria is albumin <30 mg/dL and albuminuria ("overt albuminuria") is urine albumin >30 mg/dL in urine normalized to specific gravity of 1.010. The limit of detectability of dipstick colorimetric pads is approximately 6–10 mg/dL and SSA 5 mg/dL. If these trace amounts are detected in urine with a specific gravity of 1.020 then the concentrations would double if the USG was 1.040. Similarly, trace amounts in urine with a specific gravity of 1.020 would be undetectable with these qualitative methods in urine with a specific gravity of 1.010 or less, yet this may be significant. The detection of microproteinuria may be of value in geriatric patients, patients with suspected occult renal disease, monitoring chronic renal patients, breeds with known familial nephropathies or sick patients with an unknown diagnosis. Although its use is implied to recognize occult glomerular or chronic renal disease, any cause of proteinuria, pre-, glomerular and postglomerular, will be positive with these microalbumin assays. Therefore these other causes must be ruled out before steps are taken to recognize occult renal disease.

Microalbuminuria may be a marker of early renal disease and there is some evidence to suggest it is a mediator of disease; therefore, there could be benefits to reducing the amount of protein entering the urine. Recognizing occult renal disease opens avenues for patient management such as restricted diets and angiotensin-converting enzyme (ACE) inhibitors. It is not known if these treatments can change the progress of the disease or is beneficial to these patients but it may prove helpful, and the first step is to recognize the problem exists.

There are qualitative (reagent pad) and semiquantitative immunologic test strips (ELISA) available to estimate Prot:Ct ratios but their clinical utility has not been proven.

The other methodologies, protein electrophoresis and immunoturbiditmetric tests are quantitative and are performed in reference labs. Semiquantitative immunologic test strips and protein electrophoresis are less accurate than automated immunoturbiditmetric assays. Semiquantitative immunologic test strips are easy to use. Urine is diluted to

a specific gravity of 1.010 by adding distilled water and the strip is immersed for 3 minutes and the color intenisty compared to various categories. The sensitivity and specificity of the test strips are 91% and 92%, respectively. They have a false positive rate of 8%, false negative rate of 9%, and at the high and very high positive categories are detecting overt proteinuria that can be assessed with routine dipsticks.

Antithrombin

Antithrombin (also known as antithrombin III) is a small alpha globulin synthesized in the liver and lost in the urine of patients with glomerular diseases. Antithrombin (AT) has a molecular weight just lower than albumin; hence both proteins are lost in the filtrate of patients with glomerular diseases. Antithrombin is the most potent inhibitor of the coagulation cascade. When AT is decreased a prothrombotic state exists. The most common location of thrombi is in the pulmonary artery but thrombi are also located in the aortic quadrification and in many other vessels if they are examined. The hypoalbuminemia also stimulates platelet hypersensitivity which further contributes to the formation of thrombi. In contrast, many cases of severe renal failure tend to bleed due to several mechanisms: concurrent DIC, as uremia alters platelet function leading to prolonged bleeding times, and increased clotting associated with uremia induced vasculitis.

Fibrinogen

Cattle in renal failure tend to have marked increases in fibrinogen, 1000–2000 mg/dL.

Other abnormalities

Generally as the renal disease progresses from stage 1 to stage 4 (mild to severe) the laboratory abnormalities worsen in their magnitude and or the percentage of patients with each abnormality increases.

Packed cell volume; erythroid

Chronic renal failure is characterized by nonregenerative anemia in all species. Horses will have an anemia due to chronic renal failure but distinction of regenerative versus nonregenerative is not practical in horses. Decreased production of erythropoietin in the kidneys is the main causes; however, other factors that contribute to anemia are decreased life span of erythrocytes, blood loss due to tendency of uremic patients to bleed, anemia of chronic inflammatory disease, bone marrow suppression, hyperphosphatemia, and increased serum parathyroid hormone concentration. The anemia of chronic renal failure is usually mild to moderate; a PCV in high teens to twenties is typical. If the anemia is severe, such as low teens or single digits then search for an additional cause and or gastrointestinal bleeding.

Rarely a renal tumor produces erythropoietin and increases the PCV. Any tumor in the kidney can produce this paraneoplastic syndrome. The result is an absolute polycythemia and it has been reported with nonneoplastic renal masses as well.

Cholesterol

Increased cholesterol is seen with the nephrotic syndrome. The mechanism is not known but there are numerous publications that make the association and attempt to explain hypercholesterolemia: increased hepatic production of lipoproteins, defective lipolysis of lipoproteins, and decreased conversion of cholesterol into bile acids are some of the hypotheses.

Parathyroid hormone

Chronic renal failure patients will have parathyroid hyperplasia and hypertrophy secondary to hypocalcemia and hyperphosphatemia. This may result in clinically detectable fibrous osteodystrophy and osteopenia due to increased bone resorption triggered by increased serum concentrations of parathyroid hormone. Parathyroid hormone (PTH) will be increased due to decreased renal clearance and concurrent production and release in the hyperplastic parathyroid glands. These mechanisms result in increased parathyroid hormone no matter what assay is used. As renal failure progresses from stage 1 to stage 4 PTH concentrations increase (along with increases in serum urea nitrogen, creatinine, and phosphorus): 33% of dogs will have increased serum PTH at stage 1 and 100% at stage 4. There is ample evidence that increased serum PTH concentrations are one of the uremic toxins that contribute to the vasculitis and suppress bone marrow function.

Vitamin D

Vitamin D concentrations will eventually decrease in patients with renal failure as the rate limiting step in the synthesis of vitamin D is in the kidneys. The decreased serum concentration of vitamin D contributes to hypocalcemia and hyperparathyroidism. It is not necessary to measure serum vitamin D concentration in patients with renal failure but knowledge of the possible consequences of reduced vitamin D may be useful.

Lipase and amylase in serum

These enzymes are inactivated or excreted through the urinary system and any cause of azotemia (decreased GFR) may result in increased serum concentrations of one or both. The magnitude of increase is usually one to threefold. If the increase in lipase is greater than threefold then a purely renal contribution is unlikely and pancreatitis should be evaluated. Increases of fivefold or greater are usually due to pancreatitis. Prerenal azotemia can produce as great an increase in lipase or amylase as renal azotemia. Expect

amylase or lipase or both to increase in 70% of patients with spontaneous renal failure. Approximately 33% of azotemic patients will have an increase in both; 33% will have increased lipase only and 33% increased amylase only.

Enzymes in urine

Gamma-glutamyltransferase (GGT) and N-acetyl-glucosaminidase (NAG).

Enzymes found in urine have two sources, filtered at glomeruli or released from tubular epithelium. Enzymes that are too large to be filtered at glomeruli but that are released from damaged tubular epithelial cells can be a useful adjunct to determine if there is an acute tubular lesion before azotemia develops. Two such enzymes are gamma-glutamyltransferase (GGT) and N-acetyl-beta-glucosaminidase (NAG). GGT is a membrane-bound enzyme and NAG is a lysosomal enzyme. Although both enzymes are produced in other tissues neither is filtered at the glomerulus, and therefore, any amount in the urine reflects a tubular source. Proximal tubular epithelium contains more of these enzymes than other tubular cells, and most toxins affect the proximal convoluted tubules preferentially due to their high metabolic rate. These enzymes are measured in a random urine sample along with urine [Ct], and the ratio of enzyme:Ct is reported. Samples should be refrigerated but not frozen as freeze thawing destroys the activity of enzymes. Enzymes cannot accumulate in the urine because they are voided from the bladder on urination. Therefore the amount measured indicates the amount released since the last urination, and an increased amount implies that the lesion is active or ongoing. These enzymes are not absorbed into the circulation. GGT and NAG have proven useful in dogs, cats, horses, sheep, and cattle, particularly in cases of drug-induced tubular injury (e.g., gentamicin, neomycin, NSAIDs). Urinary NAG varies with sex, being twofold greater in male dogs, circadian rhythm, and species.

Reference values should be obtained from the laboratory and not the literature due to analytical variation in how the enzymes are measured. Despite the early detection of renal tubular disease afforded by urinary enzymes, they are seldom measured. This may be because the diagnosis of acute renal failure is easily made by the time animals with spontaneous renal diseases present,and use of urinary enzymes is unnecessary. A potential use would be to monitor an animal placed on a nephrotoxic drug such as an NSAID or gentamicin, where an increase in these enzymes would warrant discontinuing drug therapy. In experimental gentamicin nephrotoxicity, increased tubular enzymes occurs before increased fractional excretion of electrolytes, and precedes azotemia by 7–8 days and decreased creatinine clearance by 4–6 days (Fig. 23.4). Increases in C-reactive protein and retinol binding protein also occur with tubular disease and may be useful disease markers.

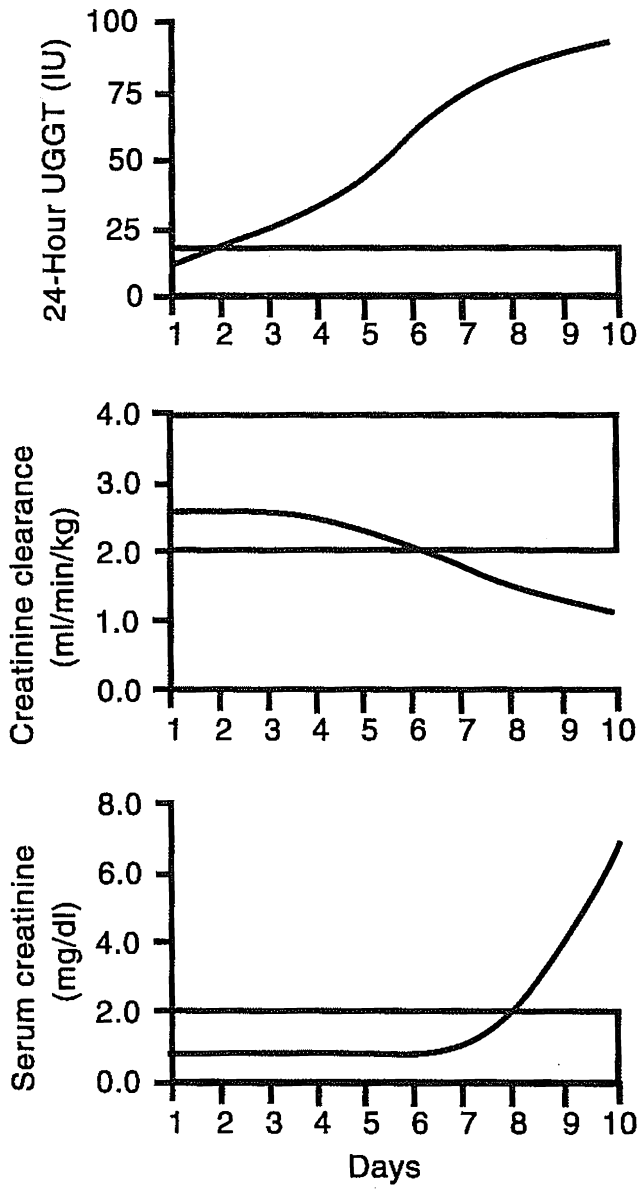


Figure 23.4 Serum creatinine, 24 hr endogenous creatinine clearance, and 24 hr urine GGT activity in experimental gentamicin nephrotoxicosis.

Dogs mean + SD GGT : Ct 0.39 + 0.18 IU/mg;
24-hour 1.42 + 0.82 IU/kg/24 hr
NAG : Ct 0.06 + 0.04 IU/mg; 24-hour 0.19
+ 0.14 IU/kg/24 hr

Diseases/Syndromes

Ethylene glycol intoxication

Ethylene glycol is sweet tasting and is one of the most common lethal toxins in veterinary medicine. First it causes vomiting, ataxia, and CNS depression such that owners

might think their pet is "drunk." At this stage a UV light may reveal fluorescence in the oral cavity, in vomitus, and in urine as fluorescent dye is commonly present in commercial antifreeze preparations (the dye helps detect leaks in a radiator). Negative fluorescence does not rule out ethylene glycol (EG) ingestion as not all antifreeze products add this dye. EG itself is not nephrotoxic but alcohol dehydrogenase converts EG to toxic metabolites, principally oxalic acid. Competitive inhibition of alcohol dehydrogenase by ethanol is the treatment of choice for cats as 4-methylpyrazole (fomepizole) does not work well in cats. 4-Methylpyrazole directly inhibits alcohol dehydrogenase in dogs and is therefore recommended to treat dogs exposed to antifreeze.

Key to successful treatment is to confirm the diagnosis before the patient becomes azotemic. Azotemia is usually not present until about 24 hours post exposure, and therefore the goal is to diagnose acute renal failure in a patient that is not azotemic. Five ways to accomplish this are: UV fluorescence, visualize calcium oxalate monohydrate crystals in urine (present in 3 hours post exposure in cats and 6 hours post exposure in dogs), increased serum osmolality and increased serum osmole gap (occurs in first hour post ingestion), increased anion gap (first 3–6 hours), and perhaps the easiest way is a test kit that can be run in-house. Additionally, the FE_{Na} will be increased in the first 3 hours post exposure prior to the onset of azotemia.

The commercial kit detects serum EG via a color change and takes 15 minutes to perform. The test is designed for serum and it is useful only in the first 12 hours post exposure as it recognizes the parent compound but not metabolites. At North Carolina State University, the kit has been used on aqueous humor, serum, and urine up to 2 days post exposure and positive results achieved and the diagnosis confirmed with histology; however, this is too late for effective therapy to be implemented. Commercial kits: Ethylene glycol test kits (Allelic Biosystems, Kearneysville WV, and PRN Pharmacal, Pensacola, FL).

Serum phosphorus may increase before azotemia if the antifreeze product ingested also contains a phosphate rust inhibitor. Calcium oxalate monohydrate crystals are more plentiful in EG toxicity than are dihydrate crystals. Both types can be seen in urinalyses but they require careful searching with increased contrast as there may only be low numbers.

Osmole gap

Markedly increased osmole gap is diagnostic for EG. The increased osmolality and the gap are due to the parent compound ethylene glycol and both are increased within one hour of ingestion. Osmolality is measured in serum by freezing-point depression. Serum osmolarity is calculated using the following formula, and osmole gap is calculated by subtracting the calculated osmolarity from the measured osmolality:

$$\text{Calculated osmolality} = 1.86\,([\text{Na} + \text{K}] + \frac{\text{Glucose}}{18} + \frac{[\text{UN}]}{2.8}$$

Osmole gap = Measured osmolality (–) Calculated osmolarity

Osmole gap reference range is < 5; > 20 suggestive;
> 30 diagnostic

However, serum osmolality is rarely measured in private practices and the blood sample must be kept cool in transit to the referral laboratory. The technique to measure serum osmolality is based on the freezing point depression of a fluid. Ethylene glycol is a small molecular weight molecule that lowers the freezing point of serum and therefore increases the measured osmolality and does so within an hour of ingestion.

The increased anion gap (AG) is due to the metabolites of EG and therefore an increased AG is seen after the increased osmole gap, usually around 3–6 hours post ingestion. Most laboratories now report the AG in a chemistry panel, but it can be calculated via the following equation:

$$(\text{Na} + \text{K}) - (\text{Cl} + \text{HCO}_3)$$

Reference range is approximately < 9

AG >35 is suggestive of EG toxicity; >45 very suggestive; combine results with all the other data in the case, do not diagnose just on the AG.

Hypocalcemia often develops because the toxic metabolite, oxalate, will chelate calcium from the serum. This may produce marked hypocalcemia, e.g., 4–5 mg/dL. Many of the other laboratory and clinical abnormalities present in patients with antifreeze toxicity are not unique to this toxicity but are part of acute renal failure: oliguria to anuria, dilute urine, casts in UA, hyperphosphatemia, etc. Azotemia does not develop for almost 24 hours post exposure.

Diethylene glycol (DEG)

This is related to ethylene glycol; however, it is less nephrotoxic. It is found in a variety of industrial products: brake fluid, dyes, oils, ink, glue, lubricants, and heating/cooking fuel, as well as personal care products such as skin creams, deodorants, and toothpastes, and as an adulterant to create sweet wine or sweeten cough syrups. It is metabolized in the liver into various aldehydes and the acid 2-hydroxyethoxyacetic acid (HEAA) which is believed to be the nephrotoxic metabolite. Much like EG toxicity the first phases of DEG toxicity affect the GI and central nervous systems producing inebriation-like state that is followed by renal failure. Similar to EG toxicity both ethanol and fomepizole are used in the treatment (to prevent the formation of HEAA) but unlike EG there are no calcium oxalate crystals

in the kidneys. Scattered cases in pets have been observed. The LD 50 for small mammals is between 2 and 25 g/kg.

Uroabdomen

Abdominal fluid Ct > serum Ct in concurrent samples; hyponatremia, hyperkalemia, hypochloremia, hyperphosphatemia; hole is usually located dorsally in the bladder; some animals can urinate and retain contrast dye; more common in males.

The best method to confirm uroabdomen is to measure creatinine in the abdominal fluid and serum concurrently. Abdominal fluid creatinine greater than serum creatinine is diagnostic. The slower diffusion of creatinine as opposed to UN (4 hr vs. 90 min) is why creatinine is measured preferential to UN in the diagnosis of uroabdomen but either can be used. This difference in diffusion rates may be due to the difference in sizes of these molecules (urea nitrogen is 60 Da and creatinine 113 Da) or their shape (urea is a simple chain and creatinine a ring structure). If the serum creatinine is increased in a patient with uroabdomen then the abdominal fluid creatinine will always be greater than the serum Ct concentration. The greater the difference between the serum and abdominal Ct values the greater the level of confidence of the diagnosis. The abdominal fluid creatinine does not have to be twice as high as the serum creatinine to confirm the diagnosis. Do not wait for a threefold or greater difference as the patient will benefit from surgical correction as early as possible. The hole in the bladder is almost always located dorsally and therefore the patient may still be able to urinate and retain radiopaque dye in the bladder. Cytologic examination of the abdominal fluid is not useful to establish the diagnosis. On rare occasions urinary crystals and or sperm may be seen in the abdominal fluid which does confirm the diagnosis of uroabdomen. Classically uroabdomen is a disease of males due to their narrow urethra which is more easily obstructed than the wider urethra of females. In horses uroabdomen occurs most frequently in male foals less than 7 days old that were fine for several days post birth and now are lethargic and anorectic. The urinary bladder ruptured during birth and there are no calculi. Uroabdomen in sheep, goats, cattle, and cats occur in males that have a calculus or mucus plug (cats) lodged in the most narrow portion of the urethra: sheep and goats, urethral appendage; cattle, sigmoid flexure. In steers the rupture may also occur in the urethra; the bladder is intact and these individuals tend to have less severe clinical pathology abnormalities and a better surgical prognosis. In dogs uroabdomen is most typically seen post trauma, e.g., hit by car. The following serum electrolyte abnormalities are characteristic: hyponatremia, hyperkalemia, and hypochloremia, along with hyperphosphatemia. In the experimental induction of uroabdomen in dogs these electrolyte abnormalities develop slowly over a 48-hour period.

Substances that are excreted into the urine in high concentration are now greater in the abdominal fluid than the blood and diffuse from the abdominal fluid into the blood, following their concentration gradient and gradually increasing the serum concentration of these substances: UN, Ct, K, P. Substances that are excreted into the urine in low concentration are lower in the abdominal fluid than the blood and therefore diffuse from the blood into the abdominal fluid; gradually decreasing the serum concentration of these substances: Na, Cl. Additionally fluid will flow into the abdominal cavity due to the irritation and increased osmolality from the urine mixing with the peritoneal fluid producing a third space and dilution effect on multiple analytes.

Differential diagnoses for hyponatremia, hyperkalemia, and hypochloremia are: uroabdomen, Addison's, renal failure, GI disease (whipworms, salmonella, colibacillosis), Akita and other dog and sheep breeds with potassium-rich red blood cells, chylothorax with drainage, and others (see Addison's disease in this book).

Patients in renal failure and with an intact urinary bladder will have increased concentrations of Ct and UN in all body fluids due to diffusion of Ct and UN from the blood. Therefore Ct is increased in comparable amounts in abdominal fluid, thoracic fluid, cerebrospinal fluid, aqueous and blood; however, the increase in these fluids will be less or nearly the same as the increased serum Ct concentration. The diffusion of Ct into other fluids is the basis of peritoneal dialysis in patients with renal failure.

Examples:

Serum Ct mg/dL	8.1	4.5	5	6.3	11	1.2
Abdominal Ct mg/dL	13.4	6.2	15	6.1	10	0.9

The first three examples are all characteristic of uroabdomen; the next two examples are azotemia with an intact bladder; and the last a "normal" or at least nonazotemic patient. The patients with an intact bladder and azotemia may benefit from peritoneal dialysis: remove the abdominal fluid, inject warm saline into the abdomen, and allow time for Ct to diffuse from the blood into the saline and repeat until serum creatinine is reduced to an acceptable concentration. In acute renal failure, peritoneal dialysis may, along with other treatments, permit the patient to survive long enough for tubules to regenerate.

Azostick determination of abdominal versus blood UN has been used to diagnose uroabdomen and may be beneficial if after-hour chemical measurements are not available. However, the color differences should be obvious and the qualitative results confirmed with chemical measurements and or all the data should correlate superbly: e.g., male animal that cannot urinate or does so in small volumes, bladder is small or collapsed, excess fluid in abdomen, azotemia, and characteristic electrolyte abnormalities.

Medullary washout

Primary diseases: Psychogenic polydipsia, liver failure, and hypoadrenocorticism.

Urea and sodium chloride are the main solutes that saturate the renal medullary interstitium. This hypertonic medullary intersitium combined with the vasa recta form the *counter current multiplier system*. This system is responsible for the passive absorption of water from the proximal convoluted tubules and is the first step in the process of concentrating the glomerular filtrate. If either or both urea and sodium are decreased in the interstitium of the medulla then passive absorption of water from the tubules is compromised and the filtrate cannot be concentrated. Two syndromes that do this are chronic liver failure, such as acquired and congenital shunts (decreased urea) and hypoadrenocorticism (chronic hyponatremia). Liver failure caused by congenital shunts will have decreased serum concentrations of other substances produced by the liver such as albumin and cholesterol, and may have microcytosis as clues that this disease is present. Addison's patients will have azotemia, Na:K ratio <23 and basal cortisol <2 µg/dL.

Psychogenic polydipsia will "wash out" the medullary interstitium via marked diuresis which provides insufficient time for urea and sodium reabsorption.

Fanconi syndrome

This is a heritable or acquired disease characterized by defects in proximal tubular reabsorption of various substrates that include one or more of glucose, sodium, calcium, bicarbonate, amino acids, and phosphate. Abnormal lab data includes: dilute urine, glucosuria, and proteinuria, increased fractional excretion of electrolytes, cystinuria, and aminoaciduria. Clinical signs range from asymptomatic to marked clinical alterations and death from renal failure. The disease is heritable and is present in 10–33% of Basenjis and is seen in Norwegian elkhounds, Shetland sheepdogs, and schnauzers. It is recognized by breed susceptibility, clinical signs, and UA.

Nephrotic syndrome

Proteinuria, hypoproteinemia, hypoalbuminemia, ascites, and hypercholesterolemia, with or without azotemia, define this syndrome.

The combination of proteinuria, hypoalbuminemia, edema, ascites, and hypercholesterolemia are the classical features of the nephrotic syndrome but not all are present in every case, or are not detected. Ascites and edema may not always be present but if the rest of the features are present that is adequate to use this term (see section on "Protein Abnormalities" and tables and figures in this chapter). The nephrotic syndrome implies a lesion is present in glomeruli, either amyloidosis or glomerulonephritis. End-stage kidneys from any cause could present with similar features. If the lesion is severe and chronic the dogs usually have peripheral edema and some develop thrombi due to a decrease in antithrombin III (AT III). This is a common syndrome in veterinary medicine because glomerulonephritis is so common in older dogs.

Proteinuria will precede azotemia in most cases. The more severe the lesions the more likely azotemia will be present; approximately half of the dogs with glomerulonephritis will be azotemic and 75% of the dogs with amyloidosis will be azotemic. Dogs that are azotemic at initial diagnosis have the shortest survivals. Dilute urine will be seen in 50–60% of the cases; casts are often present and they can be hyaline (protein-rich) or of other types. Amyloidosis may account for more cases simply because the glomerular lesions are more severe and therefore there is greater proteinuria. There is no effective treatment for amyloidosis. Glomeruli cannot regenerate but if the lesions are mild and or the underlying cause of inflammation predisposing to glomerulonephritis can be removed there are cases that have survived for several years.

Progressive familial renal nephropathy/dysplasia

Chronic progressive familial nephropathy (renal dysplasia) is one of the most common causes of renal failure in young dogs, some presenting as early as 8 weeks of age. This is not hypoplasia as the kidneys start with a normal number of nephrons and then progressively lose nephrons over time. In severe cases the kidneys are shrunken and fibrotic but there often are regions of embryonic glomeruli, tubules, and interstitium. There also are other familial glomerulopathies that look similar clinically and histologically. Regardless of nomenclature there are several diseases of high prevalence in many purebred animals that present at a young age. Amyolidosis has breed predlictions as well. The lesions vary in severity as do the laboratory findings. Clinical pathology results look like chronic renal failure, the kidneys appear shrunken on imaging but the patient is a young or middle aged purebred dog. Of all the causes of renal failure these diseases probably have the highest incidence of concurrent hypercalcemia. Hyperphosphatemia will accompany the azotemia and therefore soft tissue mineralization is prominent.

- Progressive familial renal nephropathy is observed in the Lhasa Apso, Shih Tzu, soft-coated Wheaten terrier, standard poodle, miniature schnauzer, Alaskan malamute, golden retriever, Norwegian elkhound, and Doberman breeds.
- Inherited glomerulopathies have been documented in the Samoyed, Bernese mountain dog, bull terrier, chow chow, English cocker spaniel, and Rottweiler breeds.
- Amyloidosis is noted in cats: Abyssinian, Siamese, and Oriental shorthair, and in dog breeds: Shar Pei, beagle, English foxhound.
- Polycystic kidney disease is observed in the Persian cat, West Highland white and Cairn terrier, and bull terrier.

Hematuria hemoglobinuria myoglobinuria—red, red-brown urine

The main differential diagnoses for urine discolored red to red-brown are hematuria, hemoglobinuria, and myoglobinuria. Uncommon causes are aminopyrine (urinary analgesic), porphyria, and phenothiazine antehelmintics. When all the data are considered distinguishing the common causes of red urine is straightforward (Table 23.9). One of the easiest procedures is to simply look at the urine before and after centrifugation. If the color is cleared or greatly reduced by centrifugation then the diagnosis is hematuria; if the color persists after centrifugation then hemoglobinuria or myoglobinuria is present. If the color remains post centrifugation look at all the data to distinguish these two differentials and if it is still not obvious then ask a lab to perform an ammonium sulfate precipitation test on the supernatant. This will precipitate hemoglobin such that after centrifugation a red pellet forms and all or the majority of the supernatant is cleared and is now yellow or semiclear to light pink. An 80% saturated solution of ammonium sulfate will not precipitate myoglobin and the supernatant remains red-brown. If there is still doubt there are additional tests that can be requested: electrophoresis, spectroscopic, immunoprecipitation, and ultrafiltration. Hematuria, hemoglobinuria, and myoglobinuria will all produce positive reactions to blood and protein on urine strips.

Hematuria will have numerous red blood cells in the urine (TNTC) and will likely have numerous white blood cells due to inflammation triggered by the cause of the hematuria: infection, cystitis, urolithiasis, trauma, neoplasia, etc. Depending on the amount of hemorrhage the PCV will be normal or decreased; a normal or reference interval PCV is most likely. If the urine has a pink hue post centrifugation it is due to hemolysis in vitro which can be due to alkaline urine, USG <1.008, prolonged storage, and or rough handling.

Hemoglobinuria is due to intravascular hemolysis (not extravascular) of such magnitude that the buffering mechanisms are overloaded and free hemoglobin spills into the glomerular filtrate. Plasma during the hemolytic event will be pink, the MCHC will be increased (artifact of free hemoglobin), and gradually the plasma and the patient will become icteric. Heinz bodies should be searched for in all species with this problem. The PCV is decreased to variable degrees depending on the severity of the hemolysis. Etiologies to consider are those that cause intravascular hemolysis: most cases of IHA are actually extravascular but intravascular hemolysis can occur; parasites that are in the red blood cells (*Babesia*) as opposed to on the surface (*Mycoplasma*); metals such as zinc or copper; water intoxication in cattle (osmotic lysis); *Clostridium hemolyticum*; post parturient hemoglobinuria in cattle; Heinz body anemias; and acetaminophen, onions, garlic, baby food, red maple in horses, etc.

Myoglobinuria is rarely seen in dogs (racing, crush injuries), cats (aortic thrombus, crush injury), or cattle but it is relatively common in horses and captured wild animals. Of the many types of equine myopathies the most common form associated with myoglobinuria is exertional rhabdomyolysis, which has various common names: azoturia, Monday morning disease, tying up syndrome, and capture myopathy. In these situations there is massive muscle necrosis and release of myoglobin. Myoglobin is of a small molecular weight compared to hemoglobin (18,000 vs. 68,000 daltons) such that it readily overflows into the glomerular filtrate discoloring the urine but the plasma remains clear. In horses the plasma and the patient may turn icteric due to concurrent anorexia. The PCV will be in reference interval or increased due to concurrent dehydration and or splenic contraction secondary to pain. Muscle enzymes will be markedly increased. The serum CPK can be as high as 1,000,000 IU/mL in horses with this disease. An increased CPK means the muscle necrosis is still active. CPK increases first, it is cleared rapidly once the muscle necrosis stops and is followed by increases in AST within hours to days of the onset of the disease. This disease can be lethal and requires immediate treatment. It is typically seen in horses that are over exercised following periods of rest and full feed ("couch potatoes"), large breed horses undergoing prolonged anesthetic procedures (pressure necrosis and ischemia of muscles), and wild animals that are chased for prolonged periods, equidae, ruminants, etc. It is rarely seen in domestic ruminants even with severe vitamin E Se responsive disease but can be seen in young ruminants chased excessively, similar to capture myopathy of exotics.

Hematuria is not associated with azotemia unless the cause of the hematuria is obstruction of urine outflow. Azotemia is expected with myoglobinuria and often occurs with severe cases of hemoglobinuria. Neither hemoglobin nor myoglobin is the nephrotoxin; apparently small molecular weight substances released concurrently are the toxic substances. Regardless azotemia is possible and intravenous fluid therapy to help prevent "hemoglobinuric and myoglobinuric nephrosis" is warranted. Interestingly, some cases of severe myoglobinuria do not develop azotemia even when the muscle enzymes are markedly increased (>500,000 IU/mL) and the urine is brown-black. These cases have a better prognosis and the absence of azotemia may be due to the absence of the nephrotoxic substance in the muscles of these horses. Casts are anticipated with hemoglobinuria and myoglobinuria and should not be present with hematuria. The casts may be of any type due to concurrent nephrosis or they may be characteristic for the diseases: myoglobin or hemoglobin casts.

Neoplasms

Lymphoma is the most common tumor in the kidneys; transitional cell carcinoma (TCC) is the most common tumor in

the urinary tract from the renal pelvis to the distal urethra and it is highly malignant; 30% of TCC can be diagnosed via urine cytology, 75% via washes, and 90% via aspiration cytology.

Rarely is a renal tumor diagnosed from examination of cells in the urine; most of these will be diagnosed from ultrasound guided fine needle aspiration into the renal mass. Primary renal tumors are tubular adenomas and carcinomas, nephroblastomas in young animals, fibromas, and hemangiosarcomas.

Renal lymphoma

Neoplastic lymphoid cells appear as they do in lymph nodes and other organs infiltrated with lymphomatous cells. Sometimes tubules are aspirated concurrently (Fig. A23.7). If lymphoma is in the kidneys it will also be located elsewhere. The only unusual feature of lymphoma in the kidneys is the occasional tumor that stimulates erythropoietin production resulting in polycythemia. This is not unique to lymphoma and has been seen with other neoplasms and nonneoplastic renal masses. More typical is a nonregenenerative anemia due to decreased erythropoietin production combined with anemia of chronic inflammatory disease.

Transitional cell carcinoma is by far the most common tumor of the urinary bladder and urinary excretory system of animals, and it can be diagnosed by finding neoplastic cells in the urine (Figs. A23.4–23.6). Most cases of bladder cancer are recognized when the tumor is advanced, and therefore the prognosis is uniformly poor. Approximately 20% of dogs have clinically detectable metastases at the time of clinical diagnosis, 50% actually have metastases and 90% are expected to develop metastases if the tumor is allowed to progress. Clinical signs include weight loss, weakness, dysuria (85%), pollakiuria (40%), and incontinence (10%). Approximately 90% of dogs with epithelial or mesenchymal tumors of the urinary bladder or urethra have one or more abnormalities detected on urinalysis: hematuria (76%), pyuria (50%), and bacteriuria (28 %). Hematuria is due to physical disruption of blood vessels, either in the tumor or from contact and/or invasion of the tumor into adjacent parenchyma.

Hypercalcemia has been reported with a few tumors of the lower urinary tract. Azotemia is present in only 15% of dogs with bladder or urethral tumors and is most likely due to obstruction of the outflow of urine (postrenal azotemia). Invasion through the wall of the bladder by the tumor or rupture of the bladder and production of uroabdomen is extremely rare.

Cytological diagnosis of neoplastic cells in the urine seems a logical diagnostic aid but must be interpreted cautiously as inflammation of the urinary tract stimulates hyperplasia and dysplasia of transitional epithelium, making the distinction of hyperplasia from dysplasia or neoplasia difficult. In dogs, approximately 30% of TCC can be diagnosed from urine cytologic examination, 75% from urethral or prostatic washes, and 90% from ultrasound guided guided fine needle aspirational cytology.

The best method to diagnose TCC in urine is to collect a fresh sample, prepare a concentrated preparation, make a film of the sediment, and stain with a Romanowsky stain (do not diagnose from a wet mount, sedi-stain preparation). Additionally a diagnostic laboratory can prepare a cellblock of the sediment and prepare a histologic section. Tumor cells will be in clusters or individual, neoplastic cells will be extremely large (>40 microns diameter), and will have marked cytologic and nuclear atypia (various sizes and shapes of cells, nuclei, and nucleoli) and some cells will contain large cytoplasmic vacuoles (Figs. A23.5 and A23.6). The more numerous these abnormalities and the less inflammation present, the more likely the cells are neoplastic. If only a few of these cytologic abnormalities are identified and there is inflammation, then the cellular atypia is more likely due to dysplasia or hyperplasia than to neoplasia. Correlate results with other data, such as a mass in the trigone region of the bladder, unresponsive hematuria and age of patient. TCC can seed and grow in the abdominal incision site used for surgical removal of the tumor. A few reports exist of seeding from fine needle aspiration cytology, but this should not prevent attempts to diagnose the tumor via this means.

Basic fibroblast growth factor (bFGF) is a proangiogenic peptide used as a marker for urologic and nonurologic tumors in humans and has been detected in high concentrations in the urine of dogs with bladder cancer. Although the numbers of dogs were small, one study demonstrated significantly higher concentrations of bFGF in dogs with bladder cancer than in normal dogs or dogs with urinary tract infection (UTI). Results are expressed as ng/g creatinine, and the median concentration of bFGF was 2.23 in normal dogs, 2.45 in dogs with UTI, and 9.86 in dogs with bladder cancer. Of dogs with cancer 86% could be correctly identified by increased concentrations of bFGF, and 90% of dogs with UTI did not have increased concentrations. The commercially available ELISA test kit uses a monoclonal antibody to recognize natural and recombinant human bFGF.

Another commercially available test is the bladder tumor associated antigen (BTA). The assay detects a glycoprotein antigen complex that is of host basement origin and partly of tumor origin. The dipstick test was used on 65 dogs, 20 with TCC, 19 healthy controls, and 26 controls. Test sensitivity (dogs with cancer have positive results) was reported to be 78% and the specificity (dogs without cancer have negative results) 90%; results are not quantified: they are either positive or negative. False positive results can be seen with pyuria, hematuria, proteinuria, and glucosuria. When these abnormalities are present the utility of the dipstick test is greatly limited, and if used, the test should be performed in conjunction with cytology and other ancillary tests. The dipstick test may be more appropriately applied as a screen-

ing test in older dogs for bladder cancer; however, cost and index of suspicion may limit utility.

Second-generation BTA statistical tests use a monoclonal antibody to recognize a human complement complex that is secreted into the urine of humans with bladder cancer. When applied to dogs with TCC the results have been negative, and they were attributed to the lack of crossreactivity of the monoclonal antibody to canine TCC generated antigens.

Definitions, glossary, and principles

Definitions

Urea nitrogen (UN)

This is produced in the liver from ammonium and bicarbonate, and is excreted from the body via glomerular filtration through the kidneys. Approximately half of the UN excreted into the tubules is reabsorbed passively in the proximal tubule and actively by the cells of the collecting ducts. Urea is held in the medullary interstitium. The units of serum or plasma [UN] are reported in mg/dL, and as µmol/L, internationally. The conversion factor between these units is: 1 mg/dL × 0.7140 = µmol/L (e.g., 10 mg/dL UN is 7.1 µmol/L).

Creatinine (Ct)

This is a breakdown product of muscle creatine and creatine phosphate that is excreted via glomerular filtration in the kidneys. Serum or plasma Ct concentration ([Ct]) is not increased until approximately 75% of nephons are not filtering adequately. Creatinine concentration is reported in mg/dL and as µmol/L internationally. The conversion factor between these units is: 1 mg/dL × 88.4 = µmol/L (e.g., 1 mg/dL Ct is 88.4 µmol/L). Noncreatinine chromogens, such as glucose, ketones, vitamins A and C, carotenes, oxyglobin, pyruvate, and uric acid, may falsely increase measured creatinine. This occurs most frequently in cows and horses.

Azotemia

This is the most commonly used laboratory indicator of renal dysfunction and occurs when the serum concentration of urea nitrogen and/or creatinine concentrations are increased. Serum or plasma UN or Ct concentration are not increased until approximately 75% of nephrons are not filtering adequately. Azotemia can be caused by prerenal, renal (glomerular, tubular, interstitial, pelvis), or postrenal problems. Patietns may be azotemic but are not yet uremic.

Uremia

This is the term used when clinical signs are attributed to azotemia. With progression of renal disease, a clinical state known as "uremia" can develop. Among other things, uremia results in anorexia, weight loss, depression, stupor, vomiting, electrolyte and fluid imbalances, and hormone deficits and or increases. This is caused by accumulation of nitrogenous waste products and uremic toxins in blood.

Prerenal azotemia

This is recognized when azotemia is noted in blood and urine specific gravity (USG) is concentrated. To be considered concentrated, USG should be greater than 1.030 in dogs, 1.035 in cats, and 1.025 in horses and cattle. Causes of prerenal azotemia include any state that results in a decreased renal plasma flow: hypovolemia due to dehydration, shock, and cardiac insufficiency. Prerenal conditions are a common cause of azotemia, and if these conditions persist, they can lead to kidney damage and renal azotemia.

Renal azotemia

This is recognized when azotemia is coupled with inability to concentrate urine, especially isosthenuric urine, as indicated by a USG between 1.007 and 1.013. Isosthenuria implies that the kidneys are damaged to such an extent that they are no longer able to concentrate nor dilute urine. Renal azotemia can be due to acute or chronic renal failure. The defect in renal function may arise from one of several diseases of the glomeruli, tubules, interstitium, renal pelvis, and, least likely, from within renal blood vessels.

Postrenal azotemia

This is associated with any obstruction to the outflow of urine or rupture of urinary bladder. Oliguria or anuria will be observed, and any urine specific gravity is possible.

Nephrons

These are the smallest individual anatomic units in the kidney, and are composed of a glomerulus, tubule, and collecting tubule. There are approximately one million glomeruli per kidney.

Renal disease

This is classified as a structural or biochemical lesion in kidney. If the lesion is focal, it may never produce clinical problems (such as with the interstitial nephritis caused by ascarid migration). Renal disease is a continuum that begins with renal insufficiency and finishes with end-stage renal disease.

Renal insufficiency

This is a state in which nephrons are functionally impaired, but not yet sufficiently damaged as to result in clinically apparent disease. Unaffected individual nephrons compensate for these losses by hypertrophy, but with progression of disease, enough nephrons become impaired so that they are unable to maintain the health of the animal.

Renal failure

This exists when roughly two-thirds of functional renal mass is impaired; urine cannot be concentrated adequately and polyuria, oliguria, or anuria results. When three-quarters of nephrons are dysfunctional, remaining nephrons cannot compensate and azotemia is detected.

Clinical signs associated with renal failure are attributable to a loss of functional renal tissue, and accumulation of nitrogenous and other waste products in blood. Signs may include anemia, vomiting, lethargy, anorexia, weight loss, vasculitis, glossal and oral ulcers, gastric erosions or ulcers, bleeding diatheses, petechiae, thrombosis, gastrointestinal tract bleeding, parathyroid hyperplasia, mineralization of soft tissues, and fibrous osteodystrophy.

Glossary

Anuria: Refers to a state wherein there is no urine output.

Dysuria: Describes painful or difficult urination.

Glomerular filtration rate (GFR): The volume of plasma filtered by the glomerular capillaries into Bowmans space per unit of time. A GFR of 3–6 mL/min/kg is normal for dogs and 2–4 mL/min/kg is considered normal in cats. A decrease in GFR can occur due to prerenal, renal or postrenal causes.

Microalbuminuria: Describes a state in which small quantities of protein are lost in urine, but are below the limit of detection of reagent sticks. Microalbuminuria is urinary albumin 1–30 mg/dL and albuminuria ("overt albuminuria") is urine albumin >30 mg/dL in urine normalized to a USG of 1.010. Persistent microalbuminuria may indicate early or mild renal disease.

Oliguria: Indicates reduced urine output.

Pollakiuria: A term indicating an increased frequency of urination; however, the total volume of urine produced may not be increased. Among other conditions, pollakiuria may be seen with cystitis.

Polydipsia (PD): A term denoting an increased volume of water is consumed within a 24-hour period; associated with renal failure; multiple other causes. This usually occurs secondary to polyuria caused by the loss of urinary concentrating ability. In dogs, polydypsia is considered as drinking >90 mL/kg/day, and in cats >45 mL/kg/day.

Polyuria (PU): A term denoting an increased total volume of urine produced wthin 24 hours. The normal range in dogs is 20–40 mL/kg/day (1 mL/kg/hr), and in cats, 10–20 mL/kg/day.

Proteinuria: Refers to protein in the urine as detected by reagent sticks or sulfosalicylic acid (SSA) protein precipitation methods. It is caused by preglomerular, glomerular, or postglomerular casues. Persistent proteinuria coupled with a quiescent urine analysis suggests a glomerular lesion, such as amyloidosis or glomerulonephritis.

Stranguria: Refers to straining to urinate.

Principles

The ability of the kidneys to concentrate urine is a good indicator of renal function, loss of this capability is one of the earliest signs of renal failure, preceding azotemia in all species except cats.

Normal urine specific gravity: 1.001–1.080 and is dependent on the hydration status of animal.

The expected USG of a random urine sample from a healthy animal is: dog 1.020–1.045; cat 1.025–1.050; horse and bovine 1.020–1.045. The USG must always be considered in conjunction with the hydration status.

On any random urine sample, an adequate USG is considered to be >1.030 in the dog; >1.035 in cat; and >1.025 in horse and bovine.

Dogs are not born with an adult's level of concentrating capacity, and dilute urine is expected up to 4 weeks of age. There are no differences in protein, blood, glucose, ketones, or bilirubin based on age.

Hyposthenuria occurs when the kidneys actively produce urine with a USG <1.007, or with an osmolality less than that of plasma, e.g., <300 mOsm/kg. Hyposthenuria has several renal and nonrenal causes, and indicates that the kidneys are healthy enough to actively dilute the plasma ultrafiltrate.

Isosthenuria indicates a USG similar to the specific gravity of plasma, i.e., 1.008–1.012 and 1.007–1.013 are reported in different sources. This USG implies that the nephrons were unable to either concentrate or dilute the plasma ultrafiltrate. Isosthenuric urine and azotemia are indicative of renal failure and the lesion may be in or involve the tubules or the medulla.

Hypersthenuria, or baruria, are two rarely used terms that describe urine with a specific gravity greater than 1.013. These terms imply that urine has been concentrated to greater than the isosthenuric range.

Differential diagnoses for polyuria and polydipsia (PU/PD) include renal failure (with or without azotemia), diabetes mellitus, primary or secondary diabetes insipidus, hyperadrenocorticism, hypercalcemia, pyometra, psychogenic polydipsia, medullary washout (associated with hypoadrenocorticism or liver failure). A complete list is in Table 23.4.

Water reabsorption occurs passively/osmotically in the proximal convoluted tubules and descending loop of Henle, but is actively reabsorbed in the collecting tubules through the actions of antidiuretic hormone (ADH).

Medullary washout—this occurs when the solutes urea and sodium chloride, located in the interstitium of the renal medulla, are decreased. This loss of hypertonicity in the

medulla results in production of a dilute urine, and the clinical signs of PU PD.

Creatinine clearance is the volume of plasma that is cleared of creatinine per unit time and can be used to estimate GFR. In dogs essentially 100% of creatinine entering the filtrate is excreted (male dogs also secrete a small amount of Ct via the proximal tubules). However, 40–60% of UN is reabsorbed from the filtrate and the amount reabsorbed varies with hydration status. Therefore creatinine clearance is an acceptable means to estimate GFR and UN clearance is not.

Acute renal failure (ARF) is generally accompanied by anuria or oliguria. The onset of acute renal failure is swift, commonly due to a nephrotoxin that causes necrosis of tubules (nephrosis). Acute renal failure is typified by azotemia and inability to concentrate urine coupled with hyperphosphatemia, variable changes in potassium, a normal to increased PCV, and good body condition. Potassium increased >8 mEq/L can be life-threatening.

Chronic renal failure (CRF) can be due to chronic glomerulonephritis, amyloidoss, chronic interstitial nephritis, chronic pyelonephritis, progressive familial renal dysplasia, etc. Lab data include azotemia, inability to concentrate urine, and mild nonregenerative anemia, and if the disease process involves the glomeruli, there may be hypoalbuminemia. The onset of CRF is chronic, progressing over months to years, and the patient has poor to thin body condition. The etiology is often unknown as the primary event was months to years ago.

Formed elements in urine

Casts may be observed during microscopic examination of urine, and suggest tubular disease. They are formed in the loop of Henle and in the distal and convoluted tubules. Rare hyaline and fine granular casts (1–2/low power field) may be observed in concentrated urine and are considered normal; however, casts found in dilute urine are considered abnormal.

Crystalluria refers to crystals in urine. Nephroliths are stones found in the kidney, while the term urolith indicates the presence of stones in the bladder.

Staging renal disease

The severity of renal disease is graded as 1, 2, 3, or 4 based on severity of clinical signs, physical examination results, and laboratory abnormalities. As the stage of renal disease progresses so does the severity of the laboratory abnormalities and the percentage of animals that have an abnormality. For example, 20% of azotemic dogs also have mild hyperphosphatemia (~6 mg/dL) in stage 1. This percentage increases to 100% of dogs in stage 4 renal failure, where the serum phosphorus will be markedly increased (>20 mg/dL).

The **nephrotic syndrome** is characterized by proteinuria, hypoproteinemia, hypercholesterolemia, ascites, and edema. There may or may not be an azotemia. With nephrotic syndrome, the lesion is in glomeruli, e.g., amyloidosis or glomerulonephritis.

Other terms, disease conditions, and methods of analyzing renal function

Chronic progressive familial nephropathy (renal dysplasia) is one of the most common causes of renal failure in young dogs, and it has a high prevalence in many purebred breeds. In severe cases, kidneys are shrunken and fibrotic and look like any end-stage kidney disease but they retain regions of embryonic glomeruli, tubules, and interstitium.

Uroabdomen or uroperitoneum is typified by hyponatremia, hypochloremia, and hyperkalemia. Serum [Ct] and [UN] are variable, but abdominal fluid [Ct]:serum [Ct] is ≥ 1.5–2:1. This commonly occurs in males due to their narrow urethra that becomes obstructed, or in male foals in which the dorsal bladder wall ruptures during birth or from being stepped on.

Paradoxical aciduria is a unique situation seen in dairy cattle with displaced abomasums, or other animals with proximal duodenal blockage, and is typified by profound hypochloremia, severe metabolic alkalosis, and acidic urine.

Transitional cell carcinoma (TCC) is a highly malignant tumor of transitional epithelium and is the most common tumor of the urinary bladder and the urinary excretory system.

Monitoring renal disease patients over time is advisable for purposes of prognostication and for monitoring response to therapy. Practical methods include accurate weight of the patient, water intake and urine volume measurement, USG, and periodic serum [UN] and [Ct]. If closer monitoring is desired, then specialized studies such as ultrasonography, endogenous or exogenous creatinine clearance, fractional excretion of sodium, monitoring of microproteinuria, clearance studies of inulin, iohexol, radioisotopes, and renal scintigraphy can be undertaken.

Essentially 100% of sodium is reabsorbed from the glomerular ultrafiltrate and therefore less than 1% is excreted in the urine of animals with normal renal function. If there is increased renal loss of sodium as measured by an increase in the urinary fractional excretion of sodium (>1%) it indicates renal insufficiency or failure. If the fractional excretion of sodium is <1% then prerenal azotemia is indicated.

Urinary [Protein]:[Ct] Ratio (UPC)

The concentration of protein and creatinine are measured in a random urine sample, and urine [protein] is divided by urine [Ct]. This is used to quantify the degree of proteinuria, and to identify what the most likely disease process affecting the kidneys is.

In dogs, a UPC <0.5 is considered normal, 0.5–1 is inconclusive, and >1 is considered abnormal.

Urine [Cortisol]:[Ct] Ratio (UCCR)

Urinary [cortisol] is a good estimate of cortisol production over the preceding 24 hours, and the UCCR is used to rule out hyperadrenocorticism (Cushing's disease). Of dogs with normal UCCR 90% do not have hyperadrenocorticism; 95% of dogs with hyperadrenocorticism have increased UCCR; but 80% of dogs with nonadrenal disease have increased UCCR. The latter animals are generally sick and stressed, and the finding of an increased UCCR in these individuals is considered a false positive result. Creatinine and cortisol are measured in a sample of urine collected at home first thing in the morning.

Urine [Bile Acid]:[Ct] Ratio

With increased production, bile acids are excreted in the urine. Increased urine [bile acid]:[Ct] ratios have the same diagnostic value as measuring serum [bile acid]. Creatinine and total bile acids, or bile acid components, are measured in a random urine sample from a nonfasted dog or cat, a ratio is calculated and compared to published data or the reference laboratory's reference interval.

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Suggested Reading

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Appendix 23.1

Urinalysis and urinary system imagery

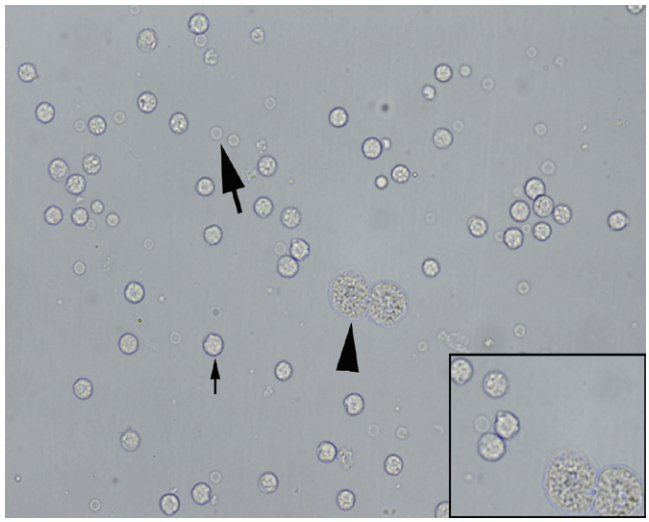

Figure A23.1 Urine sediment, unstained. There are numerous leukocytes (small arrow), fewer erythrocytes (large arrow), and two epithelial cells (arrowhead). Despite the number of white cells, there are no bacteria visible. A culture of the urine is required to verify there are no bacteria. ×400.

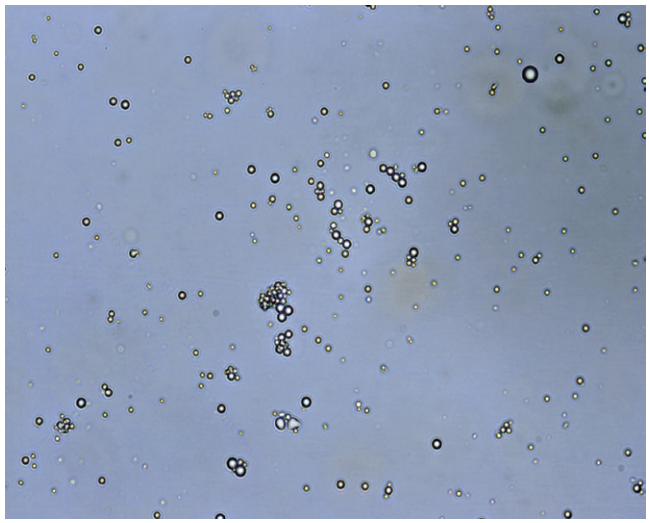

Figure A23.2 The numerous lipid droplets present are frequently noted in feline urine and are suspected to come from renal tubular epithelium but have unknown significance. They may be mistaken for red blood cells, but lipid droplets are variably sized and are in a different plane of focus from cellular elements in the urine due to their lower density. ×100.

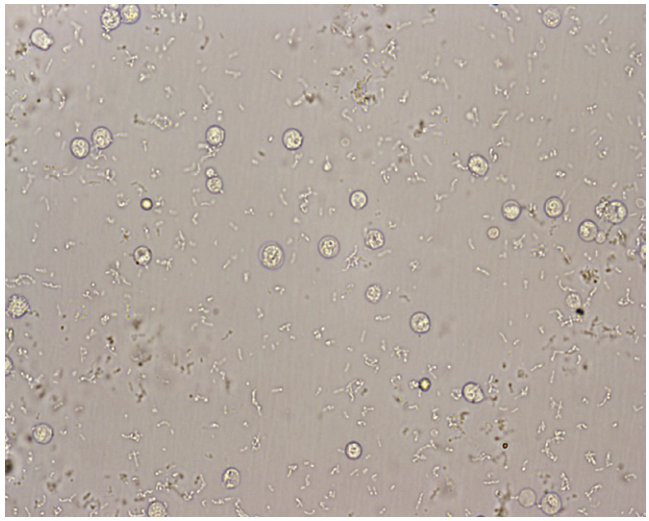

Figure A23.3 Urine sediment, unstained. There are numerous leukocytes and rod-shaped bacteria present, indicating bacterial urinary tract infection. ×400.

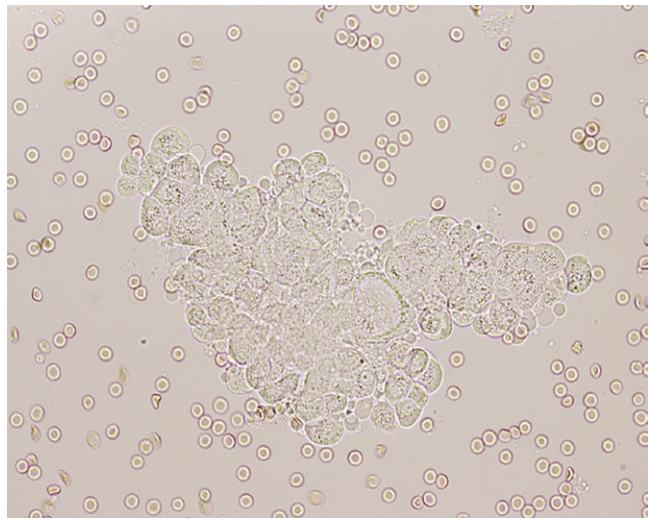

Figure A23.4 Urine sediment, unstained. Note the large cluster of many pleomorphic epithelial cells on a background of erythrocytes. This dog had a lesion in the trigone of the urinary bladder confirmed as transitional cell carcinoma. ×400.

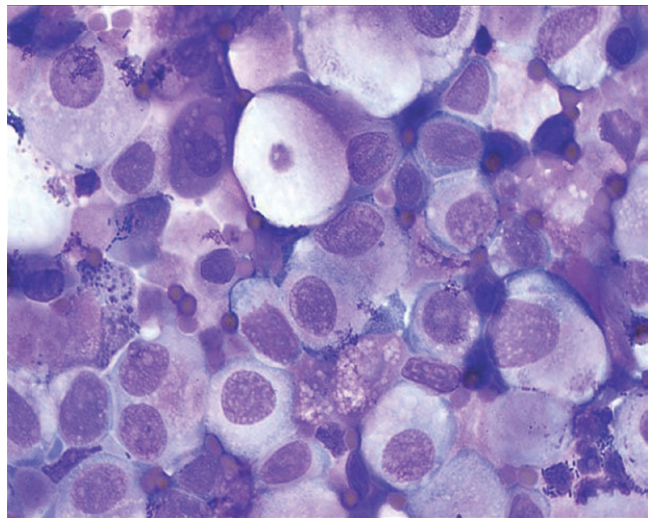

Figure A23.5 Urine sediment, Wright-Giemsa stain. There are many pleomorphic epithelial cells without any inflammation consistent with transitional cell carcinoma. Key to the diagnosis is the overall number of epithelial cells, the variability in sizes and shapes of these cells, and the absence of inflammation. ×1000.

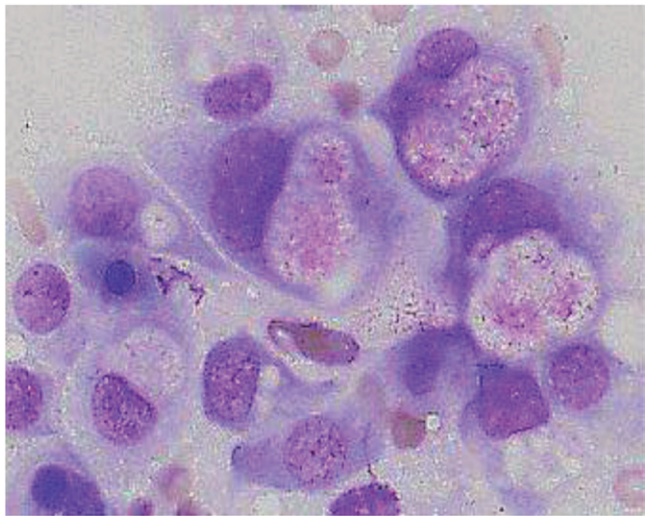

Figure A23.6 Urine sediment, Wright-Giemsa stain. These pleomorphic epithelial cells, seen in the absence of inflammation, occasionally have large cytoplasmic vacuoles containing pink material characteristic of transitional cell carcinomas. ×1000.

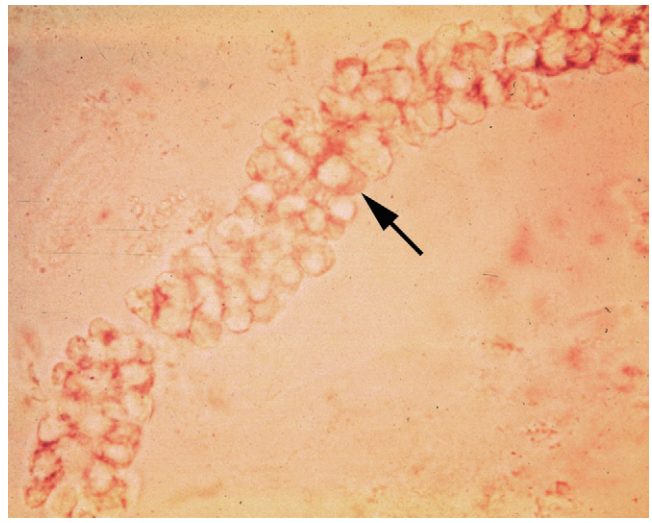

Figure A23.8 Urine sediment, unstained. Leukocyte casts (arrow) are quite fragile, seen infrequently, indicate inflammation is in renal tubules and therefore suggests pyelonephritis. ×400.

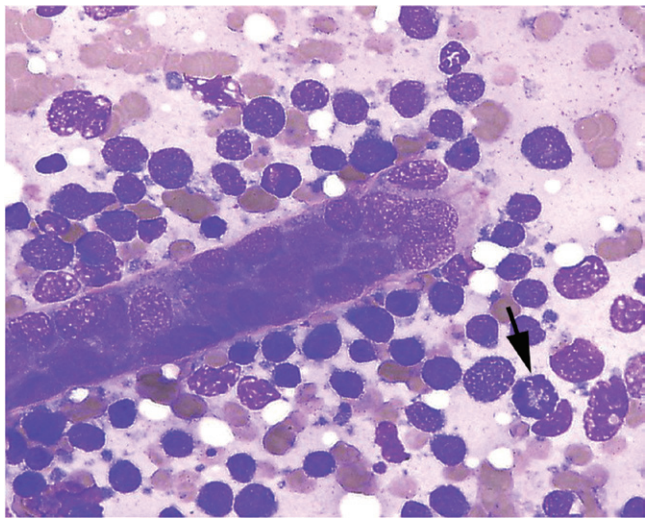

Figure A23.7 Kidney aspirate, Wright-Giemsa stain. A renal tubule is surrounded by numerous intermediate to large lymphoid cells with one mitotic figure (arrow); diagnosis is renal lymphoma. ×500.

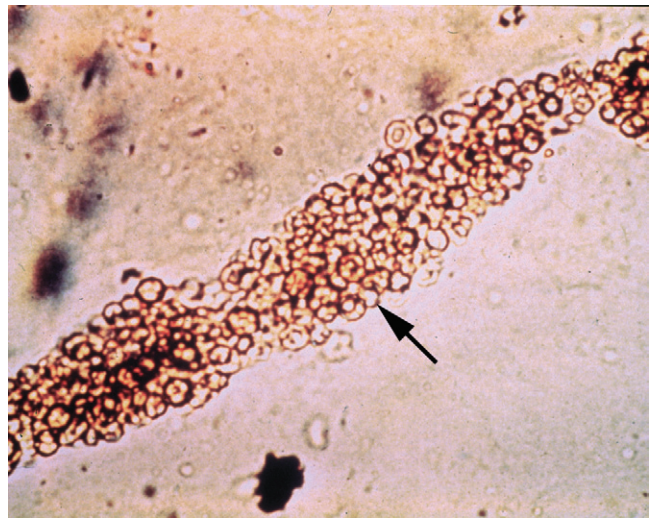

Figure A23.9 Urine sediment, unstained. Erythrocyte casts (arrow) are fragile and infrequently seen. When present, they indicate hemorrhage within the renal tubules. ×400.

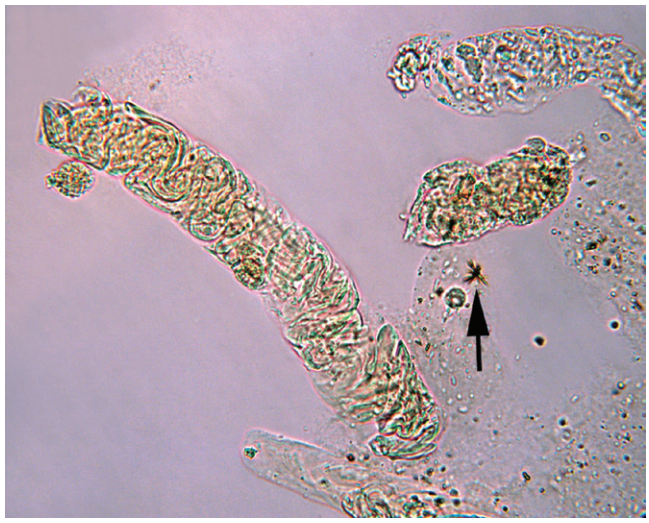

Figure A23.10 Urine sediment, unstained. Note the cellular casts and bilirubin crystal (arrow) present. ×400.

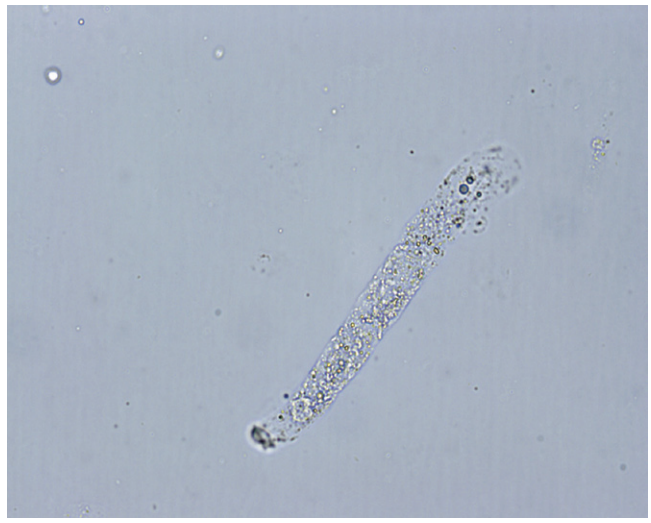

Figure A23.12 Urine sediment, unstained. The granular cast is progressing from coarse to finely granular; the distinction is not important, the critical factor is that these type of casts indicate an active tubular lesion when they are numerous and if urine is not concentrated. ×400.

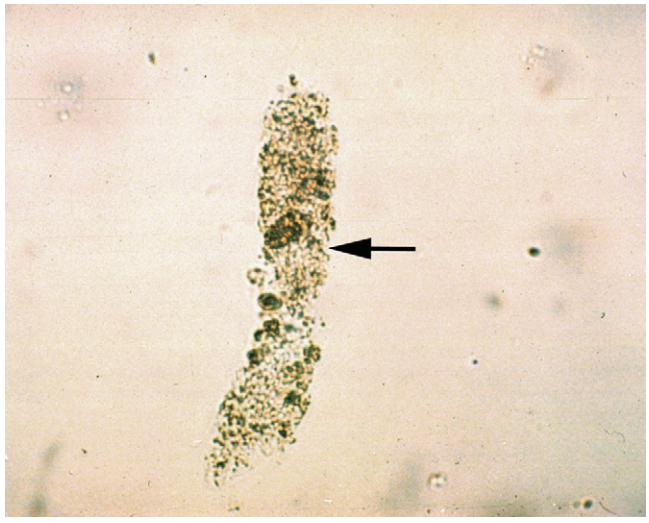

Figure A23.11 Urine sediment, unstained. The coarse granular cast (arrow) shown here is seen most frequently with a toxic insult (nephrosis) and can be caused by renal ischemia. ×400.

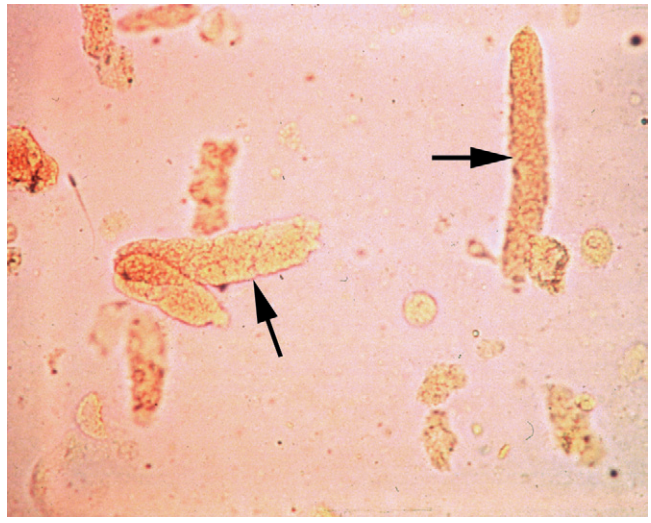

Figure A23.13 Urine sediment, unstained. These fine granular casts (arrows) occur under the same circumstances as coarse granular casts. ×100.

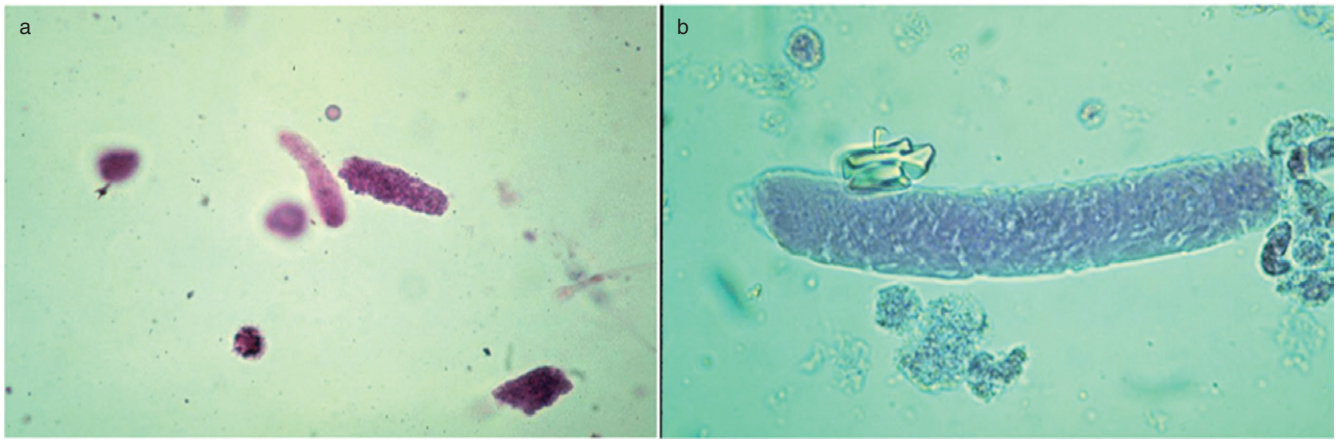

Figure A23.14 Urine sediment, sedi-stain. The multiple granular casts present at (a) low power (×100) and (b) at high power (×400) were found in urine with a specific gravity of 1.008, indicating hyposthenuria and active tubular disease (nephrosis).

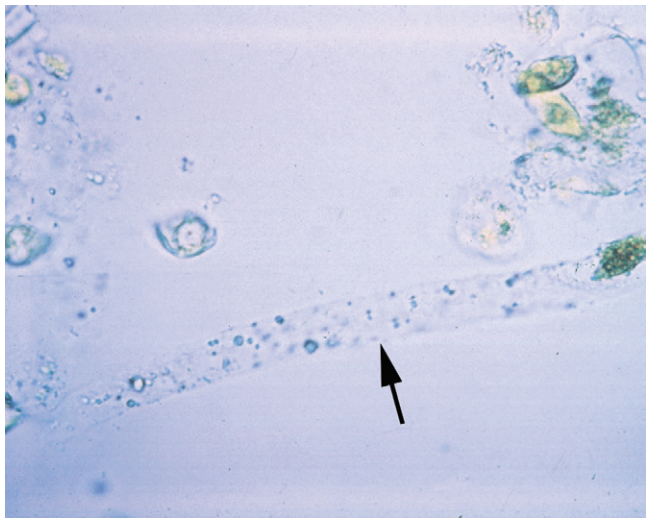

Figure A23.15 Unstained urine sediment with a hyaline cast (arrow). These casts can be seen in low numbers in healthy patients that have concentrated urine; they also are associated with proteinuria (particularly the nephrotic syndrome). ×400.

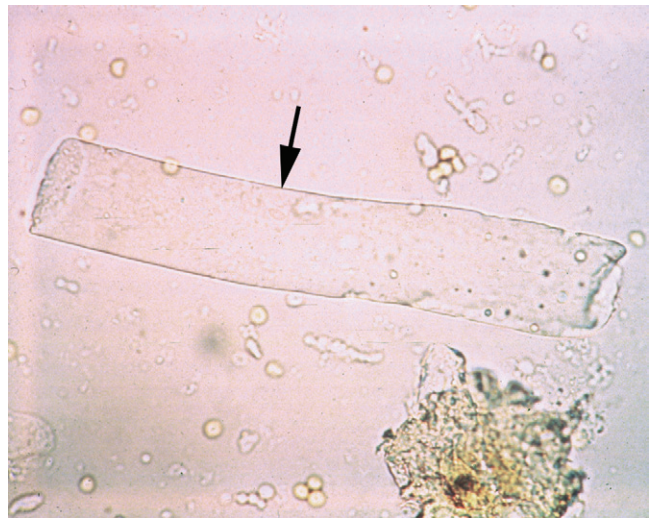

Figure A23.17 Urine sediment, unstained. The waxy cast (arrow) depicted here has sharp, linear edges with blunt ends and has a brittle appearance. When seen, waxy casts indicate prolonged periods of decreased tubular flow, most likely due to chronic renal lesions. ×400.

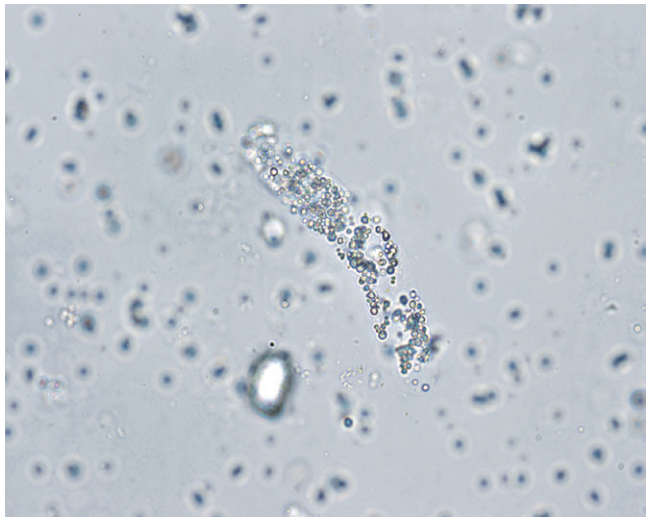

Figure A23.16 Urine sediment, unstained. Note the lipid (fatty) cast. ×400.

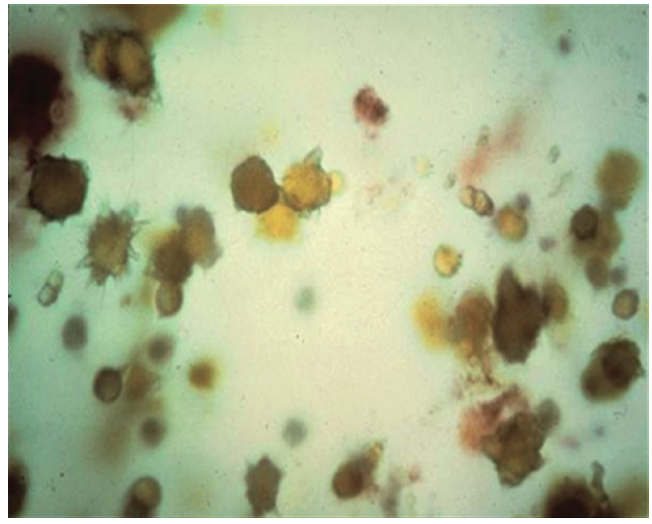

Figure A23.18 Note the many crystals with a thorn-apple appearance typical of ammonium biurate, which can be seen in health in English bulldogs and Dalmations and are also associated with portosystemic shunts and severe liver failure. Clinical chemistry should be performed to identify low BUN, glucose, cholesterol, and albumin confirming decreased synthesis of these substances by the liver.

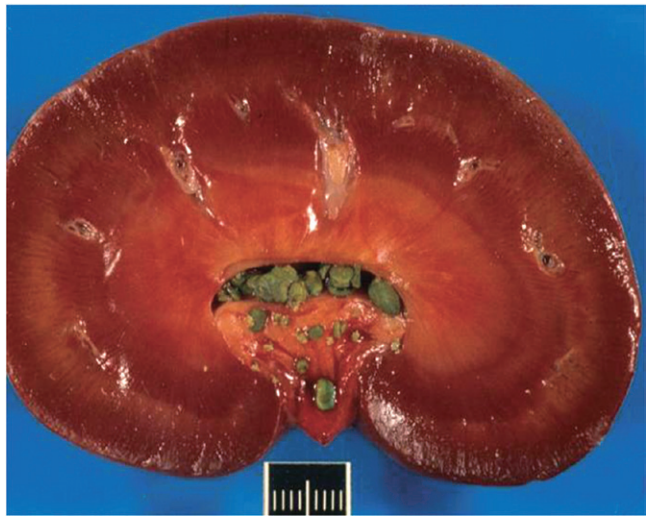

Figure A23.19 These variably sized green calculi located in the renal pelvis are nephroliths. This dog had a portosystemic shunt as well as the ammonium biurate crystals seen on sediment analysis in Figure A23.18.

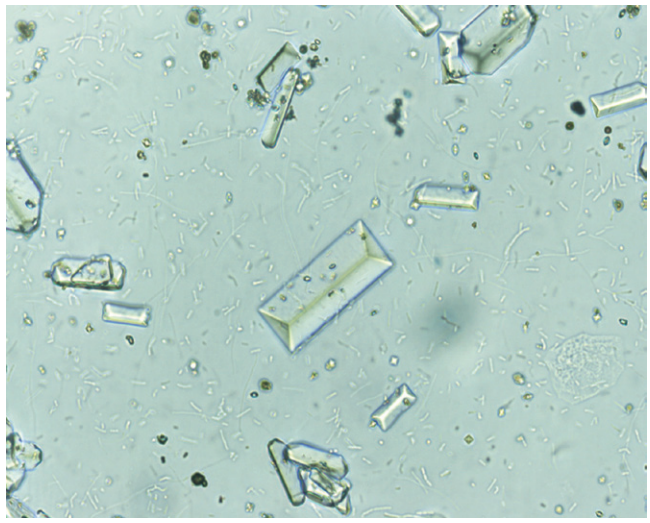

Figure A23.21 These multiple struvite crystals present on a background of many rod bacteria indicate bacterial overgrowth, as evidenced by the lack of leukocytes. ×400.

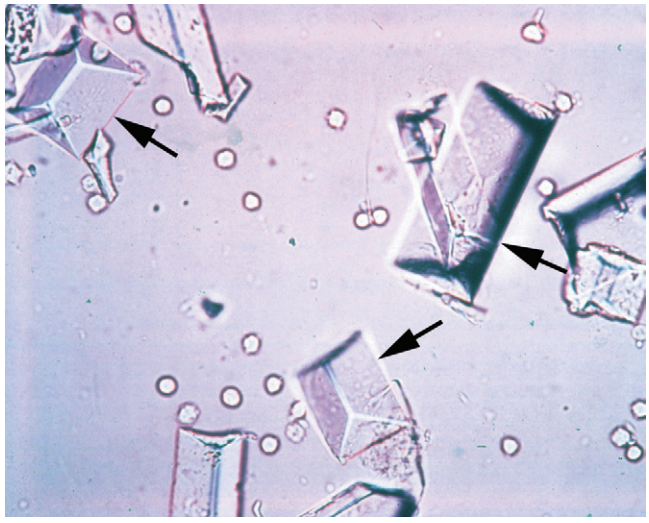

Figure A23.20 Urine sediment, unstained. Note the magnesium ammonium phosphate crystals (arrows) and the prism-like appearance. These are the most common crystals seen in cats and dogs. In dogs, they are associated with bacterial urinary tract infection. ×400.

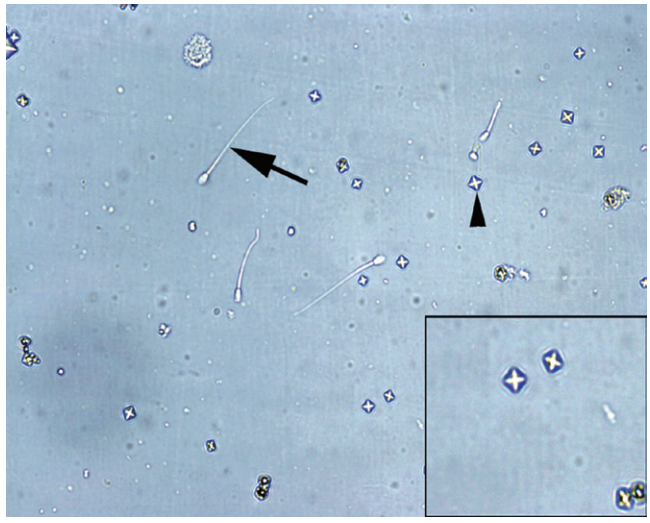

Figure A23.22 Urine sediment, unstained. The many crystals (arrowhead) depicted here have a Maltese cross appearance typical of calcium oxalate dihydrate. These crystals are seen in neutral to acidic urine. They can occasionally be seen in normal urine although when seen persistently are a clue to investigate hypercalcemic disorders. These crystals can be seen alone or with calcium oxalate monohydrate in ethylene glycol toxicity. There are also occasional sperm (arrow) present. ×100.

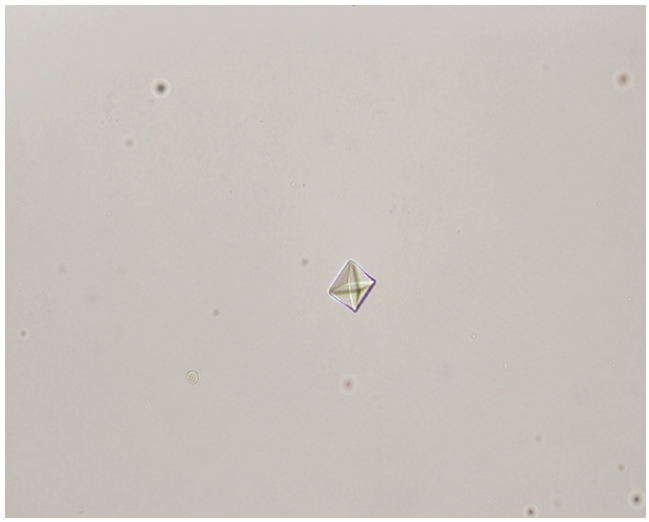

Figure A23.23 Urine sediment, unstained. Note the calcium oxalate dihydrate crystal at high power. ×400.

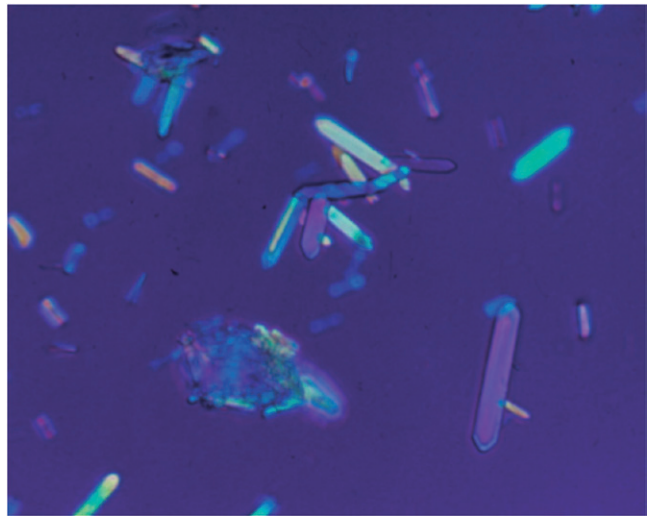

Figure A23.25 Urine sediment, unstained under polarized light. Note the many "picket-fence" calcium oxalate monohydrate crystals seen with ethylene glycol toxicity.

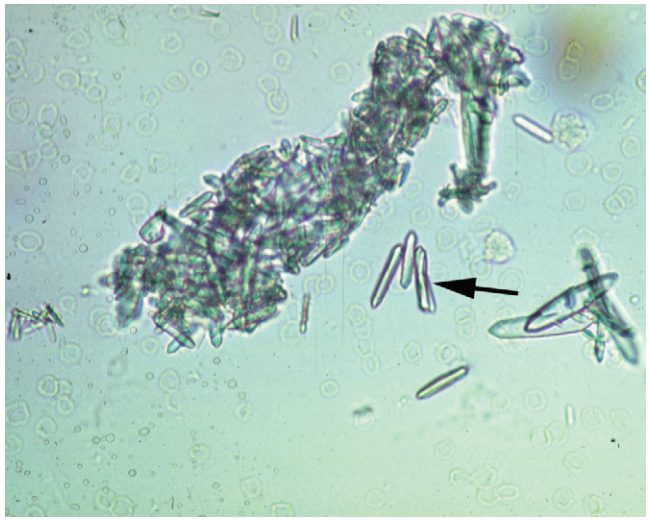

Figure A23.24 Urine sediment, unstained. These calcium oxalate monohydrate crystals shown here are present individually and in aggregate. If these crystals are found in a patient with acute renal failure, they are diagnostic for ethylene glycol intoxication. Intoxication can be confirmed with the ethylene glycol test kit, or a marked osmolar and anion gap. ×400.

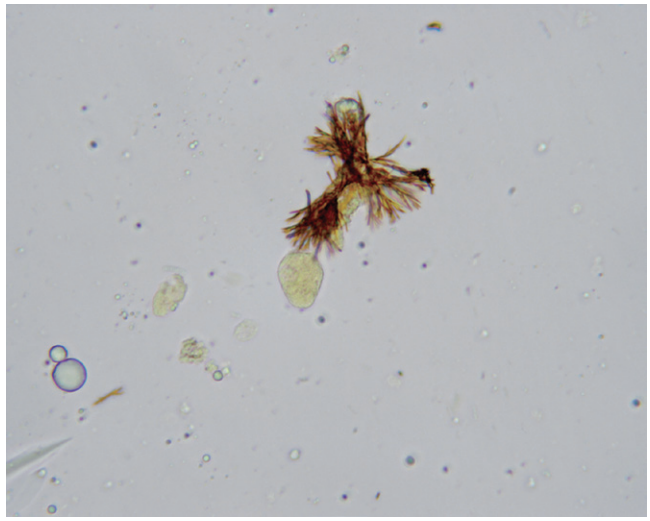

Figure A23.26 Urine sediment, unstained. This cluster of crystals is bilirubin, which can be seen with hemolysis, hepatocellular disease, or intra or extra-hepatic cholestasis. The cellular elements present are also stained with bilirubin. ×400.

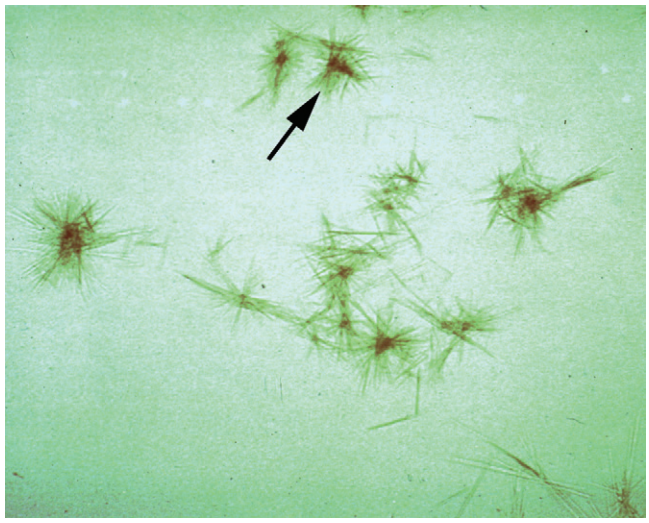

Figure A23.27 Urine sediment, unstained. Numerous tyrosine crystals (arrow) are present. These crystals are associated with hepatic disease, resemble bilirubin crystals, and occur under similar circumstances. ×400.

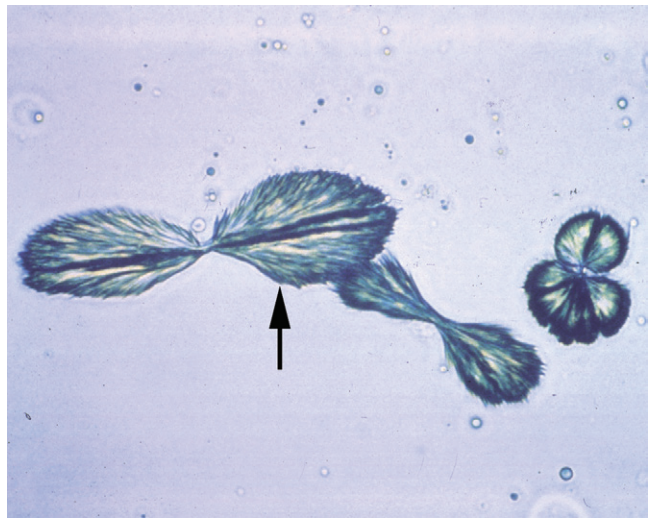

Figure A23.29 Urine sediment, unstained. Note the sulfonamide crystals (arrow). Nephrotoxic drugs may lead to the appearance of bizarre crystals in urine. ×400.

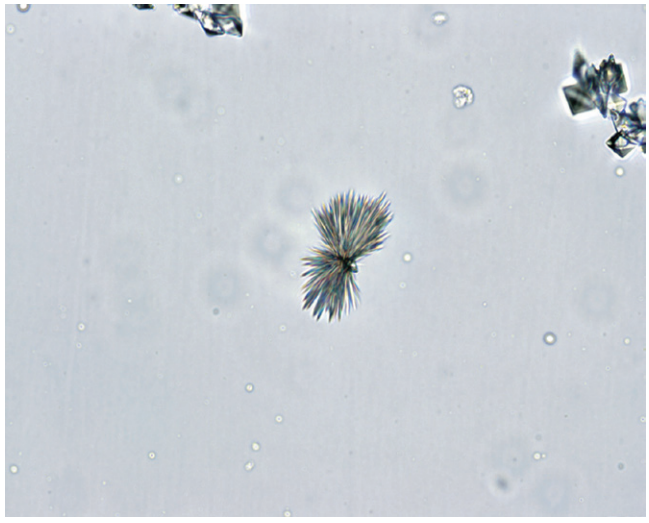

Figure A23.28 Urine sediment, unstained. Note the radiographic contrast crystal. These can be seen after intravenous contrast studies are performed. ×400.

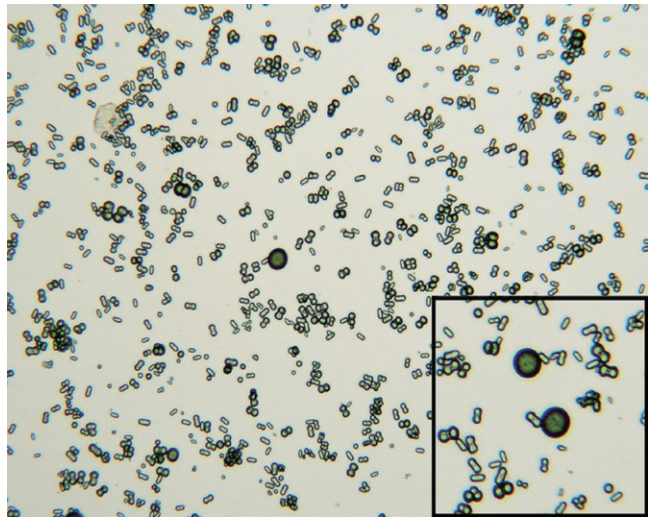

Figure A23.30 Urine sediment, unstained. Note the calcium carbonate crystals seen commonly in horses, rabbits and guinea pigs, predominantly in the spherical forms. ×400.

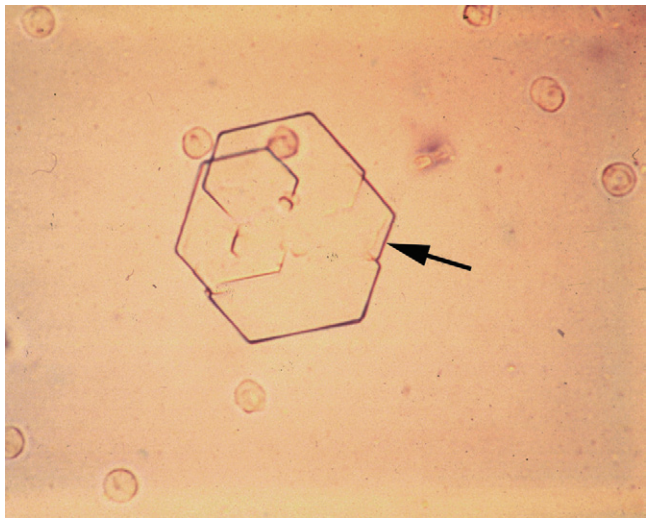

Figure A23.31 Urine sediment, unstained. The cystine crystals (arrow) seen here are always an abnormal finding and indicate cystinuria, which is due to a tubular defect heritable in English bulldogs, Mastiffs, chihuahuas, dachshunds, Newfoundlands, Australian cattle dogs, and American Staffordshire terriers. ×400.

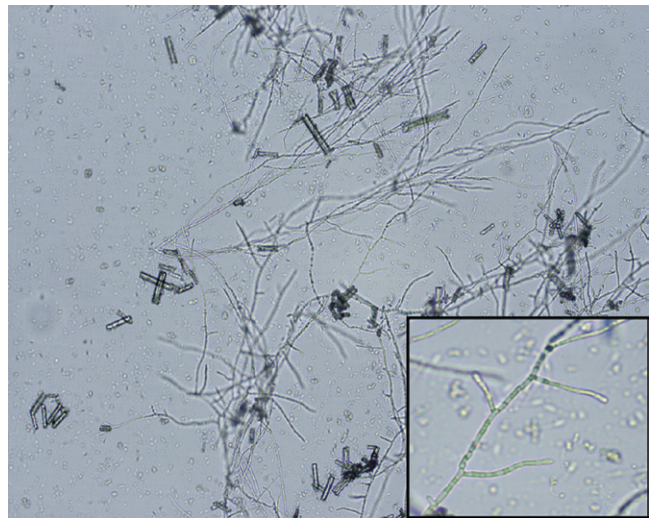

Figure A23.33 Urine sediment, unstained. Note the pseudohyphae and budding yeast forms in a pet with confirmed *Candida albicans* cystitis. These can also be contaminants; therefore, correlate presence of organisms with presence or absence of inflammation and clinical findings. ×500.

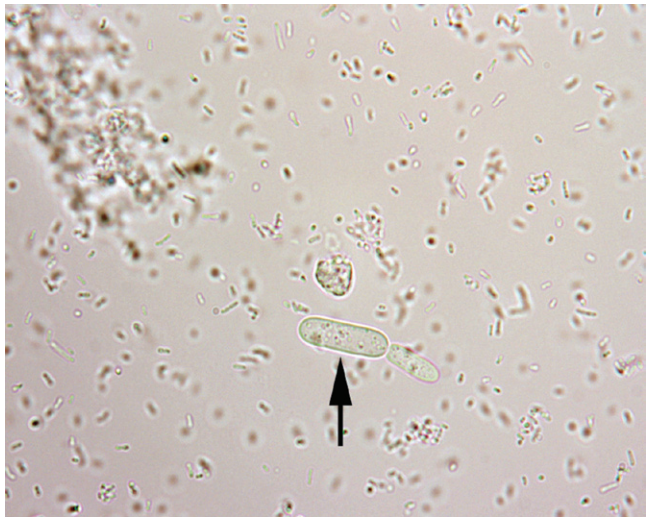

Figure A23.32 Urine sediment, unstained. The large, cylindrical shaped budding yeast structure here is typical of *Cyniclomyces guttulatus*. The *Cyniclomyces* yeast can be seen in urine due to fecal contamination. This yeast is seen uncommonly, is regarded as non-pathogenic, and can be seen due to ingestion of rabbit feces. Many rod bacteria are also present within the background. ×1000.

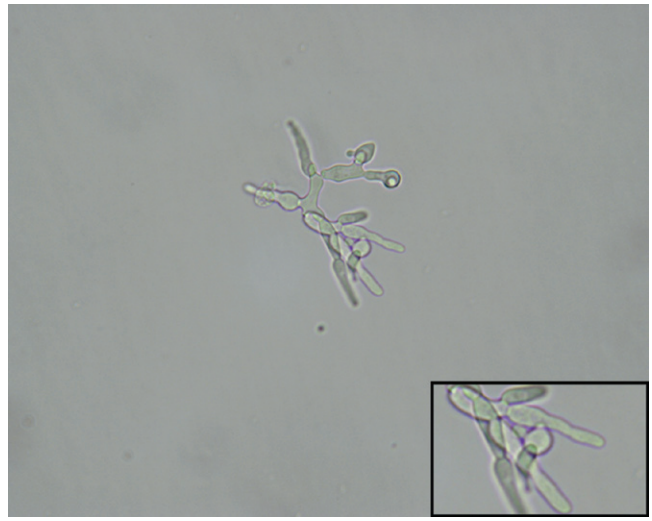

Figure A23.34 Urine sediment, unstained. Note the pseudohyphae and budding yeast forms in a young golden retriever with an incompetent urethral sphincter treated chronically with antibiotics and subsequently diagnosed with *Candida albicans* cystitis. ×500.

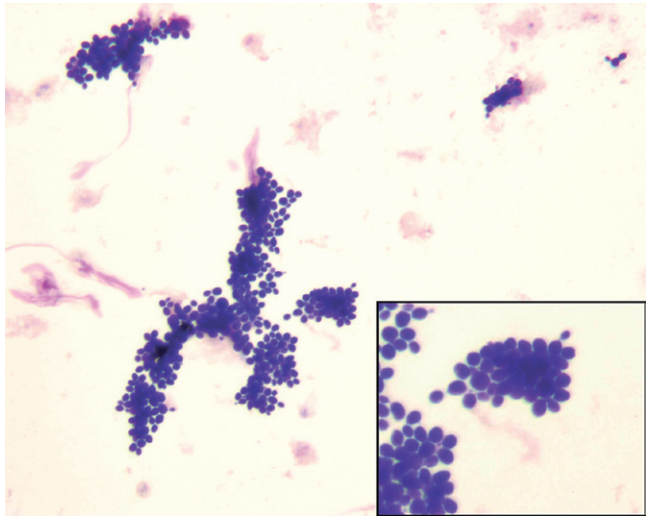

Figure A23.35 Urine sediment, Wright-Giemsa stain. There are numerous budding yeast present confirmed on culture as *Candida albicans*. These organisms are larger than bacteria and smaller than red blood cells, despite the lack of cells in this field for size comparison. This feline patient was a newly diagnosed diabetic with a history of multiple urinary tract infections and was chronically treated with antibiotics. ×500.

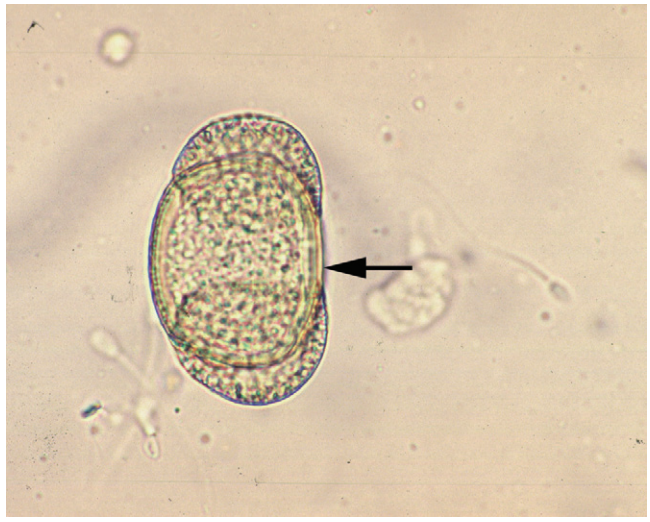

Figure A23.37 Urine sediment, unstained. The pine pollen (arrow) shown here can be commonly seen in free-catch urine specimens. ×400.

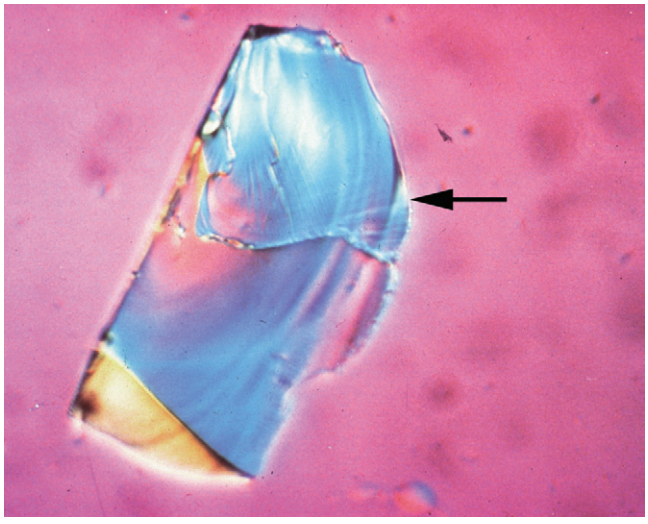

Figure A23.36 Urine sediment, unstained. The glass fragment (arrow) seen here may be from urine specimen containers or environmental contamination. It is important that this and other debris not be mistaken for crystals of pathologic significance. ×400.

24 Laboratory Evaluation of Electrolytes

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Electrolytes are present in all intracellular and extracellular body fluids, but we typically measure their concentration in blood, plasma, or serum. The serum electrolyte concentration may not accurately reflect the balance of that particular electrolyte within the whole body, especially for electrolytes that are predominantly intracellular. Sodium and chloride are the electrolytes whose concentrations are greatest in extracellular fluid (ECF). The concentration of potassium, calcium, phosphorus, and magnesium are highest in intracellular fluid (ICF). Maintaining the intra- and extracellular concentration of each electrolyte within narrow limits is essential to life.

Intake of all of the electrolytes is via the oral route. The common organs that are important in maintaining all serum electrolyte levels are the gastrointestinal (GI) system and the kidneys. Additional regulatory mechanisms as well as the consequences and causes of imbalances for each individual electrolyte will be covered when each electrolyte is discussed in more detail.

Sodium

Sodium has many important functions, including maintaining normal blood pressure and volume and maintaining normal function of muscles and nerves. These functions are dependent on keeping plasma sodium concentrations within a narrow range. The concentration of sodium in the blood is predominantly a balance between what is consumed in food and drink and what is excreted in urine. Only a small amount is normally lost through stool and sweat, but these routes can become more important in certain disease or physiological states, depending on species.

The regulation of sodium cannot be discussed without also discussing water balance since these substances are intricately tied together. Water comprises approximately 60% of body weight, with about two-thirds in ICF and one-third in ECF. Approximately one-quarter of ECF is within the vasculature while three-quarters is present in the interstitium. Water balance between different compartments is dependent on osmotic pressures. As the most abundant cation of plasma, sodium, along with its associated anions, is the major determinant of extracellular osmolality. (For further information on osmolality, see Box 24.1.) Sodium pumps maintain concentration differences across cell membranes, but sodium can freely cross vascular walls, equilibrating between interstitial and vascular spaces. Serum concentration of sodium does not necessarily reflect whole body sodium content.

Water and sodium regulation is associated with maintaining normal blood volume and osmolality. Sensors of osmolality and vascular pressure result in changes of sodium and/or water handling by the kidney. As little as a 1–2% increase in plasma osmolality will be detected by osmoreceptors in the hypothalamus, resulting in vasopressin (antidiuretic hormone) secretion from the posterior pituitary. Alternatively, a perceived deficit in blood volume of 10% will result in vasopressin release regardless of osmolality. Vasopressin enhances water reabsorption in the renal collecting duct to replenish vascular water. Osmoreceptor cells are also involved in the sensation of thirst.

If arterial and atrial baroreceptors sense elevated blood pressure or blood volume, impulses are sent to the hypothalamus to inhibit vasopressin release. They also act to decrease sodium reabsorption in the distal nephron. The juxtaglomerular cells of the kidney are baroreceptors that detect low blood pressure. These cells activate the renin-angiotensin-aldosterone system (RAAS) by secreting renin. Renin cleaves angiotensinogen to angiotensin I which is then converted to angiotensinogen II by angiotenin converting enzyme. Angiotensinogen II causes the release of aldosterone from the adrenal glands, increases secretion of

Veterinary Hematology and Clinical Chemistry, Second Edition. Edited by Mary Anna Thrall, Glade Weiser, Robin W. Allison, and Terry W. Campbell.

Box 24.1 Explanation of osmolality.

Osmolality

Solutes = substances that are dissolved in plasma (electrolytes, proteins, etc.)

Osmolality = the concentration of solutes in plasma (only the number matters, not size or weight)

– osmolality = concentration of osmotically active particles per kilogram solvent
– osmolarity = concentration of osmotically active particles in one liter of solution

In plasma, osmolality and osmolarity are nearly equal and can be used interchangeably.

Water will flow from low to high osmolar solutions

Osmolality is measured by determining either the freezing point depression or vapor-point elevation of a solution compared to water.

Osmolality can be estimated by a calculation:

$$2[Na] + [\text{glucose mg/dl}]/18 + [\text{urea mg/dl}]/2.8 = \text{osm}$$

Calculated osmolality is typically about 300–310 mOsm/liter, approximately 10 mOsm less than the measured value in a healthy state.

$$\text{Osmolar Gap} = \text{Measured Osm} - \text{Calculated Osm};$$
$$\text{normally} \sim 10 \text{ mOsm}$$

An increase in the osmolar gap indicates the presence of osmolar substances not included in the calculation (mannitol, ethylene glycol, etc.)

Box 24.2 Physical and biochemical parameters used in the assessment of hydration status.

Assessment of body water content

Physical exam:
Skin turgor = interstitial tissue consistency
Capillary Refill Time = peripheral vascular blood flow
Change in body weight

Biochemically:
PCV and plasma protein concentration
Serum urea concentration
Serum sodium concentration

Box 24.3 Assessment of dehydration.

Isontonic dehydration: Proportional loss of NaCl and water—some diarrheas and renal diseases
[Na] and [Cl] do not change
PCV and [Plasma protein] increase
No change in osmolality; water does not shift between ICF and ECF, therefore ECF volume decreases

Hypertonic dehydration: (ECF becomes hypertonic)
Water loss > NaCl loss
Diabetes insipidus
Water deprivation/hypodypsia
Respiratory loss with high temperature/panting
Osmotic diuresis
Diarrhea
[Na] and [Cl] increase
PCV and [Plasma protein] increase
Osmolality increases; water shifts from ICF to ECF to maintain ECF volume

Hypotonic dehydration: (ECF is hypotonic)
NaCl loss > water loss
Secretory diarrhea
Vomiting
Third-space loss
Equine sweat
[Na] and [Cl] decrease
PCV and [Plasma protein] increase
Osmolality decreases; water shifts from ECF to ICF leading to volume depletion

Protein and/or PCV may not appear increased if there is concurrent protein loss and/or anemia.

vasopressin, and stimulates thirst centers. Aldosterone acts on the renal cortical collecting tubules to reabsorb sodium. The reabsorption of sodium is coupled with either the secretion of potassium (another very important function of aldosterone) or the absorption of chloride to maintain electroneutrality.

When evaluating serum sodium concentration, the animal's total body water must be taken into consideration. Is there clinical or biochemical evidence of low body water (dehydration, Boxes 24.2 and 24.3) or does it appear normal or, possibly, increased? An increase in serum sodium concentration can be due to more sodium, less water, or a combination of causes. A decrease in serum sodium concentration can be due to less sodium, more water, or a combination of causes.

Hypernatremia

Hypernatremia (Fig. 24.1) is most commonly associated with an imbalance in body water. This can be due to decreased intake or loss of water that exceeds the loss of electrolytes.

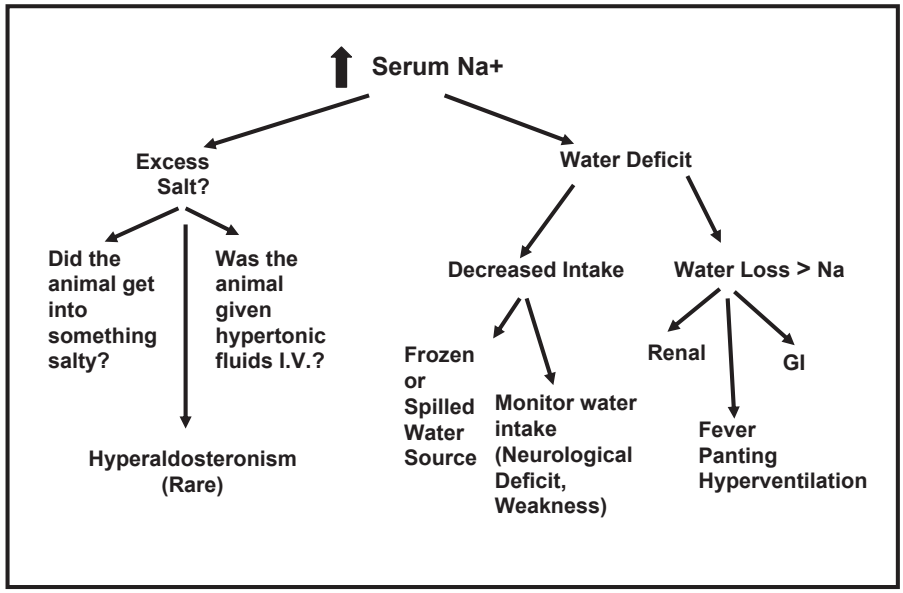

Figure 24.1 Diagnostic pathways for determining the cause of hypernatremia.

Decreased intake can be due to water deprivation, defective thirst response, or a physical inability to drink. Loss of water can occur through insensible losses (respiratory or skin losses) or loss from the kidney or gastrointestinal systems.

For hypernatremia to develop, more water needs to be lost than electrolytes. Pure water loss occurs when there is an increase in insensible fluid losses, heat stroke as an example, or when the kidney cannot conserve water, as with central or nephrogenic diabetes insipidus. In many instances of water loss, electrolytes are also lost, as with vomiting, diarrhea (osmotic diarrhea, ruminal acidosis), or diuresis. Animals that lose electrolytes with water will become hypovolemic. With pure water loss or inadequate intake of water, the total body sodium content is normal, and intracellular water is drawn into the extracellular spaces, maintaining plasma volume (isovolemic hypernatremia).

Sodium excess is an uncommon cause of hypernatremia and concurrent water restriction or lack of urine concentration is usually present. Hypernatremia can occur either by ingesting excess salt or iatrogenically with the administration of hypertonic fluids. Decreased excretion of sodium can also lead to sodium excess; this can occur with the rare condition of hyperaldosteronism. These animals will become hypervolemic. If excess sodium is present in ECF, intracellular water will shift to the ECF and cells will become dehydrated.

Hyponatremia

Hyponatremia (Fig. 24.2) may be either due to sodium loss that exceeds water loss or an increase in body water. Hyponatremia is associated with hypoosmolality except in cases of pseudohyponatremia or in translocational hyponatremia in which high numbers of alternative osmoles are present.

Pseudohyponatremia can occur when sodium concentration is measured in whole plasma and not just plasma water because sodium is only dissolved in the water component of plasma. Marked hyperlipidemia or hyperproteinemia cause volume displacement and decrease the percent of serum or plasma that is water. If the method used to measure sodium uses whole plasma, an artifactually low sodium concentration may be obtained, although measured plasma osmolality is normal. Ion-selective electrodes measure sodium concentration in just the water component of plasma and pseudohyponatremia will not occur using this method of measurement.

Translocational hyponatremia is due to the presence of other substances in the plasma causing hyperosmolality. Substances that readily cross cell membranes, like urea, will not cause translocational hyponatremia. Tonic substances, osmolar substances that cannot easily cross cell membranes, will draw water from the ICF to the higher osmolality of the ECF, diluting out the sodium that is present. If glucose cannot enter cells due to the lack of insulin or its actions, hyperglycemia can result in translocational hyponatremia. In this case, measured and calculated osmolality will be similar. Exogenous substances (mannitol, ethylene glycol) that cause translocational hyponatremia will result in an increase in osmolar gap.

Hypoosmolar hyponatremia occurs because of either increased water content or decreased sodium content. Increased water content occurs if there is impaired renal excretion of free water or dilute urine or if water intake exceeds the maximal renal excretory capacity. Excess water intake is rare, but can occur with psychogenic polydipsia. With excess intake, urine osmolality as well as plasma osmolality will be low.

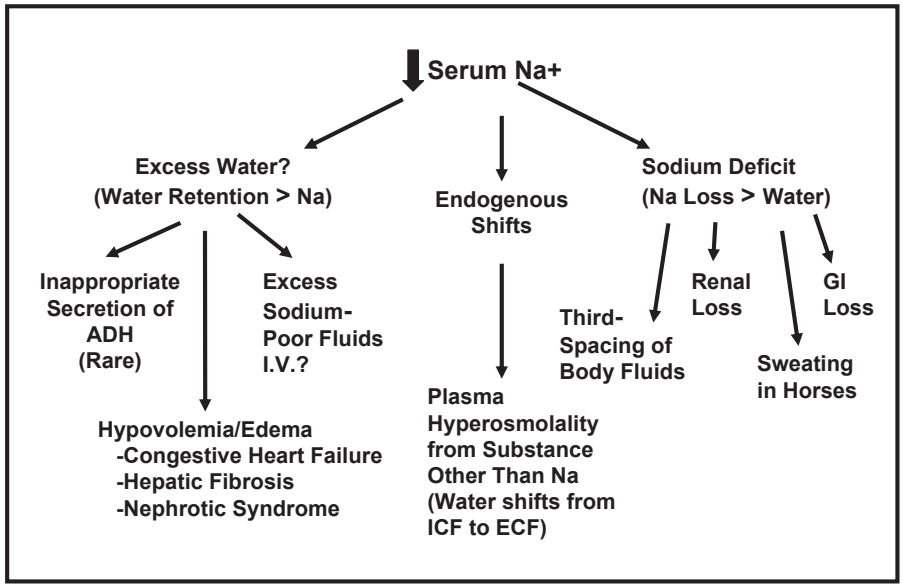

Figure 24.2 Diagnostic pathways for determining the cause of hyponatremia.

Decreased renal excretion of free water in response to perceived hypovolemia can lead to an increase in total body water. This occurs due to third-spacing (accumulation of fluid within body cavities) of fluid associated with congestive heart failure, cirrhosis of the liver, or nephrotic syndrome. Vassopressin is released in response to perceived hypovolemia, resulting in increased reabsorption of water. Impaired renal excretion of water due to renal failure can also lead to hyponatremia with hypervolemia.

Hypovolemia typically accompanies loss of sodium from the body. Sodium is rarely lost from the body without some water, but to become hyponatremic there either has to be loss of hypertonic fluid (more sodium lost than water) or isotonic or hypotonic fluid loss resulting in volume depletion which stimulates drinking and renal water retention, diluting the remaining body fluids. Hypovolemic, hypoosmolar, hyponatremia can result from gastrointestinal loss (vomiting, diarrhea), renal loss (hypoadrenocorticism, prolonged diuresis), third-space loss (body cavity effusions), or sweating in horses. Primary hypoadrenocorticism (Addison's disease) is associated with aldosterone deficiency, resulting in decreased renal reabsorption of sodium and increased retention of potassium in cortical collecting tubules (Box 24.4).

It is important to keep in mind that an animal may be normonatremic in many of the conditions listed above despite being dehydrated or hypervolemic if there is a net loss or gain of isotonic fluids or if an equilibrium has been reached. Hypovolemia not only stimulates vasopressin release, but triggers the RAAS which leads to sodium retention.

Box 24.4 The sodium potassium ratio. A decrease in the ratio is associated with hypoadrenocorticism, as well as several other diseases.

Sodium Potassium Ratio (Reference: 27:1 to 40:1)

The sodium potassium ratio is often reported and has been used in the diagnosis of hypoadrenocorticism, but is not specific for any disease process. Decreases in the ratio <27 : 1 can be due to either an absolute or relative increase in potassium, decrease in sodium, or a combination of these changes; elevated potassium is the most common reason.

Hypoadrenocorticism should always be a differential with a decreased Na : K ratio BUT not the only one.

Other diseases/conditions commonly associated with low Na : K ratios include:

- Renal/urinary tract disease.
- Gastrointestinal disease; parasitism in dogs.
- Body cavity effusions.

Low Na : K ratios have also been reported with:
Diabetes, pancreatitis, cardiorespiratory disease, pyometra, disseminated neoplasia, grade III patellar luxation, mushroom poisoning, behavior problem, ocular disease, skin disease.

Na : K ratios <15 are more commonly associated with hypoadrenocorticism in dogs.

A small percentage of dogs with primary hypoadrenocorticism have a normal ratio.

The ratio is often normal in dogs with secondary hypoadrenocorticism (low ACTH).

Sources: 12–18.

Chloride

Chloride is the major anion in the ECF and, similar to sodium, chloride is important in the transport of electrolytes and water. Chloride also serves as a conjugate anion in acid base metabolism. To maintain electroneutrality chloride either moves in the same direction of the positively charged sodium or exchanges with the negatively charged bicarbonate ions. When evaluating an abnormality in serum chloride concentration, it is important to compare chloride levels with sodium levels and to the animal's acid base status. If abnormalities in chloride concentration appear to be in proportion to abnormalities in sodium concentration (Box 24.5), differentials to consider are similar to those given for hyponatremia or hypernatremia above. If the change in chloride concentration appears greater than a change in sodium concentration, bicarbonate concentration should be evaluated and a blood gas analysis may be indicated (Chapter 23).

Box 24.5 Using sodium to correct chloride concentration in the analysis of chloride abnormalities.

Correction of chloride for water imbalance

Can the abnormality in chloride be attributed to the water imbalance that is affecting sodium concentration?

This can be estimated by correcting chloride proportionally with the sodium concentration. The middle of the reference interval can be used as the "normal" sodium value. Divide this value by the measured sodium concentration to arrive at the factor with which to multiply the measured chloride concentration. The corrected chloride concentration can then be compared to the reference interval for chloride.

Normal sodium/Measured sodium × Measured chloride = Corrected chloride

Examples:	Na = 164 mEq/L	(reference 134–144 mEq/L; middle = 139)
	Cl = 136 mEq/L	(reference 105–125 mEq/L)
139/164 × 136 = 115	Therefore, Cl shifts are due to same process as Na shifts.	
	Na = 124 mEq/L	(reference 134–144 mEq/L; middle = 139)
	Cl = 75 mEq/L	(reference 105–125 mEq/L)
139/124 × 75 = 84	The corrected chloride concentration is still markedly outside of the reference interval. Bicarbonate is expected to be increased.	

Hyperchloremia

If the degree of hyperchloremia is proportional to concurrent hypernatremia, consider the same differentials as hypernatremia. Hyperchloremia is usually associated with a water deficit.

Alternatively, hyperchloremia can be related to hypobicarbonatemia. Loss of bicarbonate can occur from the GI tract with diarrhea, loss of saliva in cattle which contains a high bicarbonate concentration, or vomiting intestinal contents as can occur with intestinal obstruction. Renal loss of bicarbonate occurs with proximal or distal tubular acidosis. In response to a respiratory alkalosis, there is decreased renal conservation of bicarbonate, resulting in retention of chloride.

Artifactual increases in serum chloride concentration can occur when other halides are present in high concentrations. Ion selective electrodes are not specific for chloride and, if bromide or iodide is present, they will be measured as chloride, causing a false elevation in chloride concentration.

Hypochloremia

If the degree of hypochloremia is proportional to the degree of hyponatremia (Box 24.5), the same differentials listed for hyponatremia apply.

If chloride is decreased to a greater degree than sodium, differentials related to metabolic alkalosis must be considered. In the process of secreting HCl into the stomach, serum chloride is decreased and serum bicarbonate is increased. These changes are normally reversed when hydrogen and chloride ions and water are reabsorbed in the intestines. If gastric fluid is lost due to vomiting or sequestered due to a displaced abomasum, pyloric obstruction, or functional obstruction, serum chloride will remain low and bicarbonate will remain elevated.

Serum chloride levels decrease when bicarbonate concentrations increase in the compensatory response to chronic respiratory acidosis.

Potassium

Potassium is a major intracellular cation that plays an important role in resting cell membrane potential. Clinical signs associated with abnormal serum potassium concentrations manifest as cardiac and skeletal muscle dysfunction and hyperkalemia can have life-threatening effects on cardiac conduction. Therefore, it is important to maintain serum potassium concentrations within narrow limits. Total body potassium is a balance between what is ingested (100%) and what is excreted from the kidneys (normally ~90–95%) and

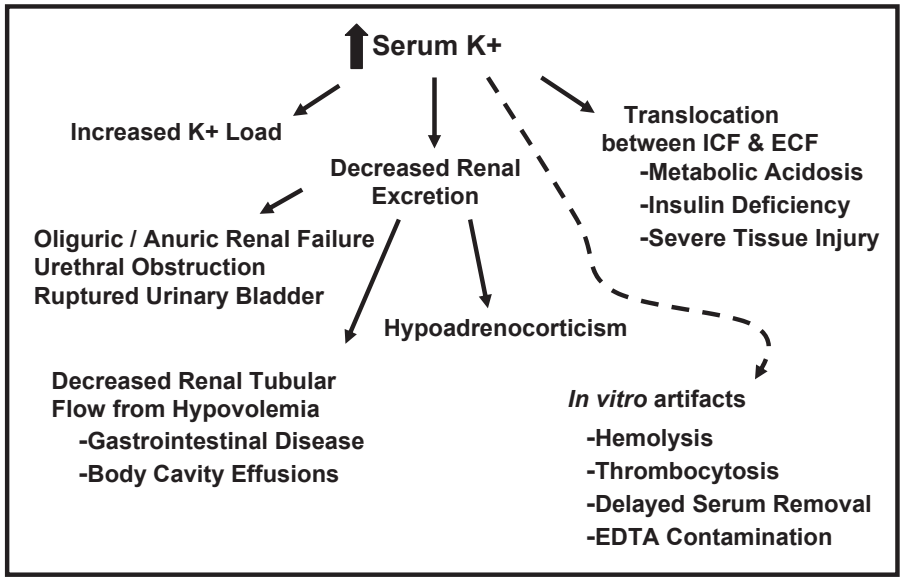

Figure 24.3 Diagnostic pathways for determining the cause of hyperkalemia.

colon (normally ~5–10%). The concentration of ECF (serum) potassium is also reliant on the translocation of potassium between the ECF and ICF. Less than 5% of total body potassium is present in the ECF; therefore serum potassium concentration is an unpredictable representation of total body potassium content.

Hyperkalemia

Hyperkalemia (Fig. 24.3) occurs if there is an increased potassium load, a decrease in potassium excretion, or a shift of potassium from ICF to ECF. Increased ingestion of potassium is unlikely to result in hyperkalemia unless there is a concurrent decrease in renal excretion. An increase in potassium load can occur iatrogenically and can result in death when fluids containing high concentrations of potassium are mistakenly given.

Decreased renal excretion of potassium is a common cause of hyperkalemia and can result from renal or postrenal diseases of the urinary tract. In anuric or oliguric renal failure the kidney itself does not have the capacity to remove excess potassium from the body. Postrenal processes that result in decreased removal of urine from the body such as urethral obstruction or ruptured urinary bladder can also result in hyperkalemia.

Aldosterone acts to increase serum sodium and decrease serum potassium concentrations by reabsorbing sodium and excreting potassium in the renal cortical collecting tubules. Aldosterone deficiency results in decreased renal excretion of potassium and hypoadrenocorticism is commonly associated with hyperkalemia and hyponatremia (Box 24.4).

Potassium renal excretion is decreased with a decreased tubular flow rate which can occur with hypovolemia. This is thought to be the reason for elevated serum potassium concentrations associated with body cavity effusions and gastrointestinal disease. Hypovolemia and hyponatremia become more severe with repeated drainage of body cavity effusions, and hyperkalemia is more commonly seen with effusions that have been repeatedly drained. The gastrointestinal disease that is most commonly associated with hyperkalemia is severe whipworm infestation.

Translocation between ECF and ICF plays a large role in maintaining serum potassium concentrations. A condition that moves potassium from the ICF to ECF causing hyperkalemia is the movement of acid into cells with metabolic acidosis. Experimentally, this has been reproduced only with nonanion gap metabolic acidosis. Since insulin is important in normal movement of potassium from the ECF to ICF, insulin deficiency can result in hyperkalemia. Given the high concentration of intracellular potassium, potassium released from injured cells can increase its concentration in the ECF, especially if there is also decreased renal excretion. A large degree of tissue injury is typically necessary to result in hyperkalemia, which can occur with tumor lysis syndrome, rhabdomyolysis, or severe trauma.

Pseudohyperkalemia occurs if large amounts of potassium leak out of cells during or after blood is drawn. Platelets contain abundant intracellular potassium that is released upon activation. Blood clotting, therefore, can result in an elevation in serum potassium concentration, especially if a thrombocytosis is present. Reference intervals for serum potassium are typically about 0.5 meq/L higher than for plasma. Hemolysis causes potassium to leak from erythrocytes. The amount of potassium in erythrocytes varies with species and even breed. Horses, pigs, and cattle have high

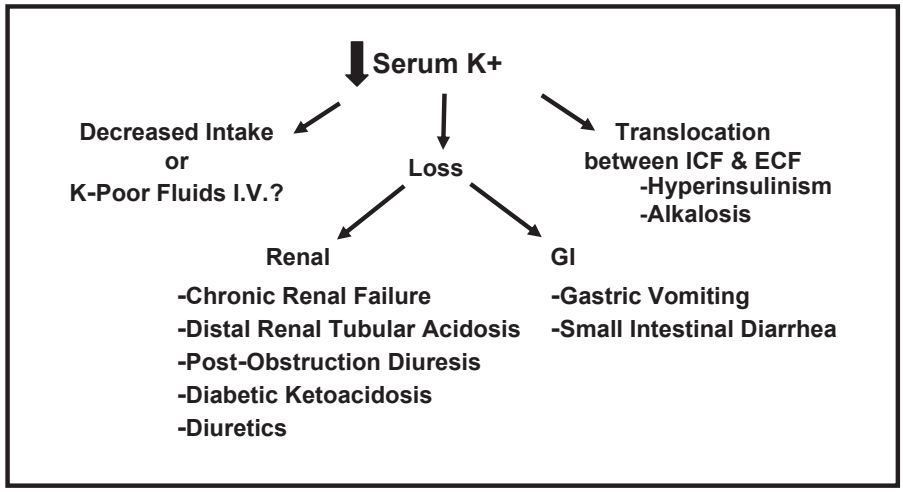

Figure 24.4 Diagnostic pathways for determining the cause of hypokalemia.

erythrocyte potassium concentrations. Cats and dogs have lower erythrocyte potassium except for the Akita and other Japanese dog breeds.

False elevations in potassium also occur if a sample has been contaminated with potassium EDTA. In this case, calcium and magnesium should also be very low.

Hypokalemia

Hypokalemia (Fig. 24.4) is one of the more common electrolyte disturbances in critically ill veterinary patients, although a definitive cause cannot always be identified. Hypokalemia may be due to decreased intake, increased excretion or loss, shifts between the ECF and ICF, or (often) a combination of these. Decreased intake from diet can contribute to hypokalemia, but is usually not a cause on its own. Hypokalemia can be caused iatrogenically with potassium-poor fluids.

Loss of potassium occurs from the gastrointestinal or renal systems. Vomiting and small intestinal diarrhea can result in hypokalemia. Renal losses can occur for a variety of reasons. Hypokalemia associated with chronic renal failure occurs more commonly in cats. Distal renal tubular acidosis, postobstructive diuresis, diabetic ketoacidosis, and diuretic administration all can lead to increased potassium excretion and hypokalemia. Hyperaldosteronism is a rare cause of hypokalemia.

Increased movement of potassium from the ECF to ICF can cause hypokalemia. This can be due to an excess of insulin or a glucose infusion or because of alkalosis. Catecholamines can also cause a shift of potassium from ECF to ICF with pain, sepsis, or trauma.

Anion gap

We measure several anions and cations in the blood, but there are many others that are not routinely measured. The predominant cations of ECF are sodium, potassium, calcium, and magnesium and the predominant anions are chloride, bicarbonate, plasma proteins, organic acid ions, phosphate, and sulfate. The number of unmeasured anions is greater than the number of unmeasured cations, and the difference between these is called the anion gap. The greatest change in the anion gap is when an elevation occurs due to an increase of organic acids in the circulation. The anion gap, therefore, is important in determination of the acid-base status of an animal (Chapter 25). The anion gap is essentially used to determine the cause of decreased blood bicarbonate concentrations (metabolic acidosis) or to detect metabolic acidosis during a mixed acid-base disorder in which bicarbonate may be normal or increased.

An indirect method is used to calculate the anion gap (Box 24.6). The calculation is based on the law of electroneutrality (The number of positive charges need to equal the number of negative charges in the body). The cations and anions that are considered "measured" are (a) sodium and potassium and (b) chloride and bicarbonate, respectively. The anion gap is the difference between these anions and cations as illustrated in Figure 24.5.

Since cations rarely change enough to affect the anion gap, a decrease in bicarbonate has to be accompanied by either an increase in unmeasured anions or a decrease in chloride to keep the equation equal and to maintain electroneutrality (Figs. 24.6 and 24.7). Unmeasured anions that have the most affect on anion gap are the endogenous products lactate, ketones, and uremic acids, as well as the exogenous substances salicylate and the metabolites of ethylene glycol toxicity. Lactic acidosis is produced during hypoxia and anaerobic metabolism. Keto acids are produced when there is a negative energy balance and metabolism switches from primarily glycolysis to lipolysis. Uremic acids are phosphates, sulfates, and organic acids that are no longer ade-

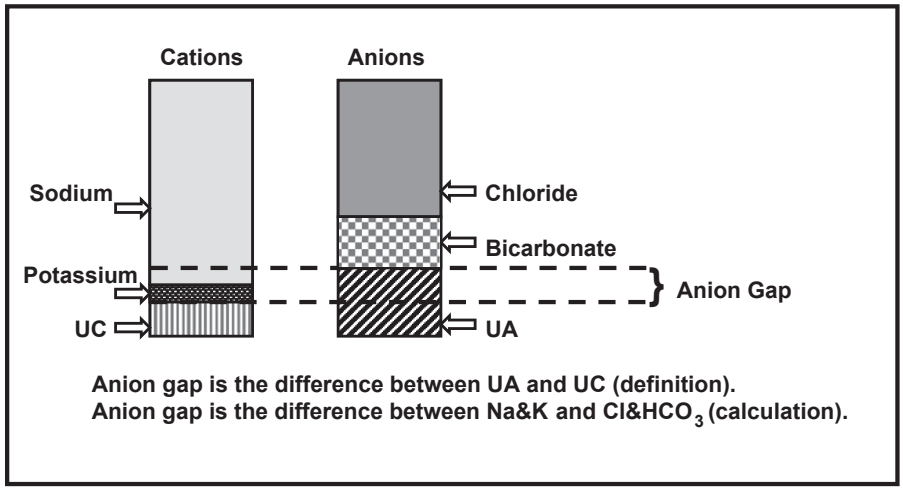

Figure 24.5 Illustration of why the difference between measured cations and measured anions is equal to the difference between unmeasured anions (UA) and unmeasured cations (UC), or anion gap.

Box 24.6 Calculation of the anion gap.

Anion gap calculation

Given the law of electroneutrality (where UC is unmeasured cations and UA is unmeasured anions):

$$Na^+ + K^+ + UC^+ = Cl^- + HCO_3^- + UA^-$$

Subtracting UC^+ and the anions Cl^- & HCO^-_3 from both sides:

$$(Na^+ + K^+) - (Cl^- + HCO_3^-) = UA^- - UC^+$$

Rewriting the equation:

$$\text{Anion Gap} = UA^- - UC^+ = Na^+ + K^+ - Cl^- - HCO_3^-$$

quately filtered because of decreased glomerular filtration rate (GFR). For further utilization of the anion gap, see Chapter 25.

Calcium, phosphorus, and magnesium

Calcium, phosphorus, and magnesium are required for vital extra- and intracellular functions. Like potassium and in contrast to sodium and chloride, intracellular concentrations of calcium, phosphorus, and magnesium are higher than extracellular concentrations. As with the other electrolytes, their intake is by ingestion and the regulation of their blood concentration involves the kidneys and gastrointestinal tract. Bone is another essential player in regulation, as the majority of total body calcium, phosphorus, and magnesium are stored in bone. Maintaining appropriate circulating concentrations of these electrolytes is largely dependent on hormonal control. Regulatory hormones are shared by these electrolytes, with magnesium regulation being less well understood. The main hormones that function to maintain normal calcium and phosphorus levels are calcitonin, parathyroid hormone (PTH), and 1,25-dihydroxyvitamin D (calcitriol or vitamin D).

Calcitonin is produced by thyroid parafollicular cells (C-cells). It is released in response to hypercalcemia and its release is inhibited by hypocalcemia. The main function of calcitonin is to limit postprandial hypercalcemia by inhibiting osteoclastic bone resorption and decreasing reabsorption of calcium and phosphorus by the kidney tubules. Its overall effect is to decrease serum calcium and phosphorus concentrations (Fig. 24.8).

Parathyroid hormone is produced by chief cells in the parathyroid gland and is the principle hormone in fine, minute-to-minute blood calcium regulation. It is released in response to hypocalcemia and its release is inhibited by elevated calcium levels and vitamin D. Parathyroid hormone acts to increase the activity of vitamin D, increase calcium and phosphorus reabsorption from bone, and increase calcium while decreasing phosphorus reabsorption by the kidneys. Because of its potent phosphaturic action, the overall effect of PTH is to increase serum calcium and decrease serum phosphorus concentrations (Fig. 24.8).

Vitamin D results from cholecalciferol production in the liver, where it is metabolized to calcidiol. Calcidiol, under the influence of PTH, becomes activated to calcitriol in the kidney. The activation of calcitriol is influenced by serum calcium, phosphorus, PTH, and calcitriol concentrations and the effects of calcium and calcitriol concentrations on PTH release. Vitamin D predominantly acts to increase the

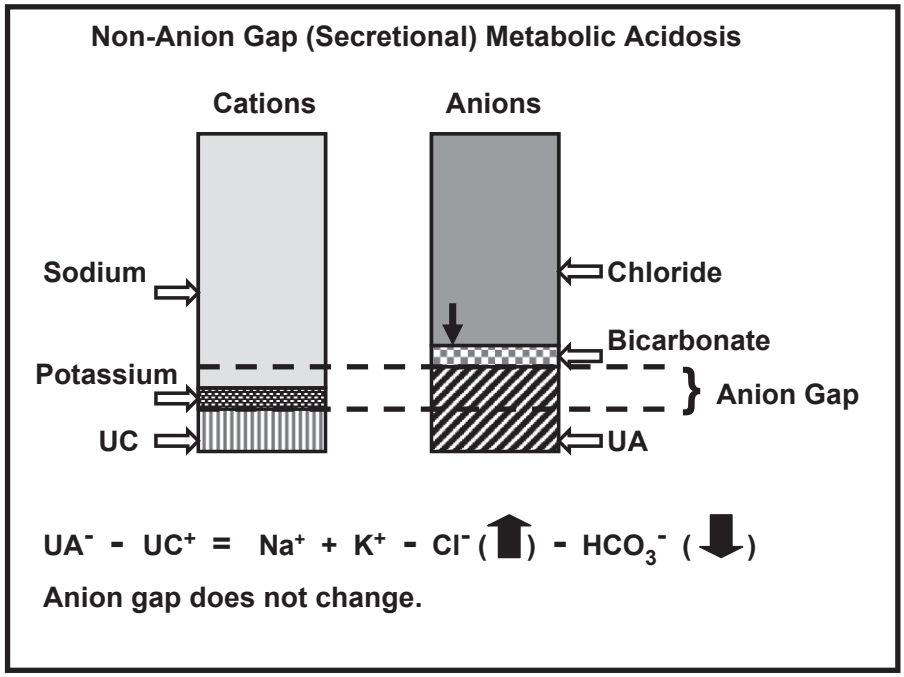

Figure 24.6 Illustration of why hyperchloremia and the resultant hypobicarbonatemia do not result in a change in anion gap during secretional metabolic acidosis (compare to Figure 24.5).

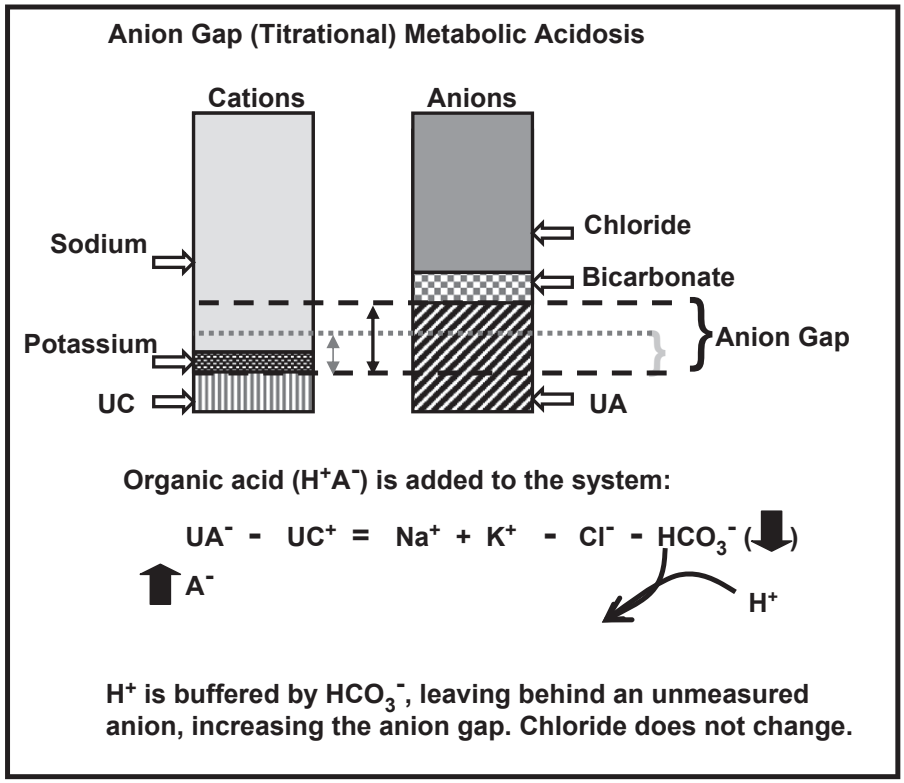

Figure 24.7 Illustration of how addition of organic acid increases the anion gap while bicarbonate is decreased during titrational metabolic acidosis (compare to Figure 24.5).

absorption of calcium and phosphorus from the gastrointestinal tract. Its overall action is to increase both serum calcium and phosphorus concentrations (Fig. 24.8).

Calcium

Why is calcium measured? One reason is that alterations in blood calcium concentrations can result in severe clinical problems, including death. Another reason is that recognizing and pursuing the cause of calcium abnormalities often aids in diagnosing the underlying disease process.

When measuring serum concentrations of calcium, it is important to understand the difference between the measurement of total calcium and free, ionized calcium. Free (unbound) ionized calcium (iCa) is the biologically active, hormonally regulated fraction that comprises approximately 50% of total calcium. Measuring the concentration of iCa is necessary to confirm if abnormalities in total calcium concentrations are significant or if calcitonin, PTH, and vitamin D concentrations are appropriate. Ionized calcium is not routinely included in serum chemistry panels because it is measured by a methodology using an ion-selective electrode which is not a technique available in the large chemistry analyzers. Total calcium is what is routinely reported on a serum biochemical panel; it is measured via a colorimetric technique. The total calcium measurement includes all calcium, whether bound or unbound. The bound fractions of total calcium are those that are bound to protein (~40–45% of total calcium) and complexed with nonprotein ions such as phosphates, citrate, lactate, etc. (5–10% of total calcium). Changes in the amount of calcium bound to proteins or other ions will affect total calcium but will not affect the concentration of iCa. Bound calcium is essentially removed from the biologically active pool of calcium. Therefore, as long as regulatory mechanisms are functioning properly, the iCa concentration will remain within a narrow range, even if total calcium concentrations decrease or become elevated due to changes in the amount of substances that bind calcium in the blood (Fig. 24.9).

Ionized calcium is required for vital intracellular and extracellular functions, including muscle tone and contraction, nerve conduction, hormone secretion, enzymatic reactions, blood coagulation, and cell growth, division, and function. It is also required for skeletal support. Some of the more common sequelae to marked hypercalcemia include

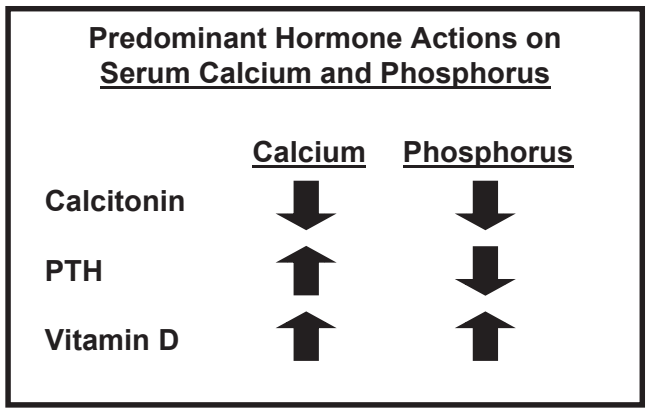

Figure 24.8 The effect of hormones on blood calcium and phosphorus concentrations.

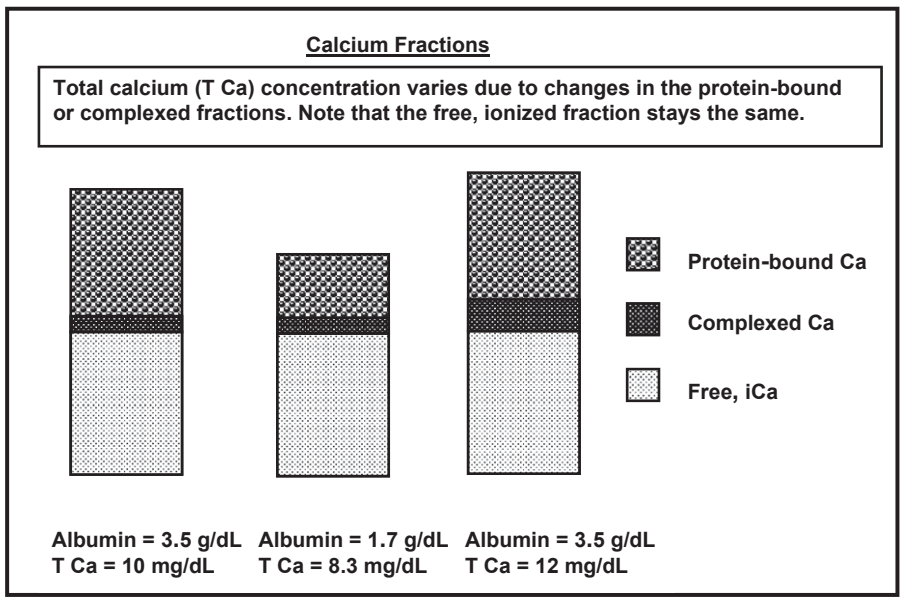

Figure 24.9 Ionized calcium, the biologically active fraction, normally stays within a very narrow range while total calcium concentration is affected by calcium that is bound or complexed and, therefore, inactive.

Box 24.7 An acronym to help remember the differentials for hypercalcemia.

Acronym for most hypercalcemia differentials (total calcium)

- G ranulomatous inflammation
- O steolytic lesions
- S purious results
- H yperparathyroidism (primary)
- D vitamin toxicity
- A ddison's disease
- R enal disease (chronic)
- N eoplasia
- I diopathic
- T ransient

polyuria, constipation, acute renal failure, and cardiac arrhythmias. The majority of signs related to hypocalcemia are due to the importance of calcium in muscle function. Signs may include muscle fasciculations, tetany, seizures, paresis, tachycardia, hypotension, and respiratory arrest. Abnormalities in blood calcium concentration result from an imbalance in hormonal regulation, altered absorption from the gastrointestinal tract, pathologic excretion from the kidneys, or altered distribution involving bone or other tissues.

Hypercalcemia

Hypercalcemia (Box 24.7), if ignored, can lead to serious consequences such as acute renal injury and failure. Therefore, depending on the situation, it is a good idea to recheck a high total calcium concentration or, ideally, to measure the iCa concentration when total calcium is elevated. If the iCa concentration is normal, this suggests that abnormalities in total calcium are due to changes in the amount of bound calcium and, therefore, an increase in the concentration of complexing or binding substances. If the iCa concentration is elevated, this indicates that there is a problem with calcium regulation.

Hypercalcemia can occur due to an increase in PTH or PTH-like substances. High concentrations of calcium normally feed back to decrease PTH secretion. If PTH concentrations are higher than they should be in the face of hypercalcemia, the cells producing PTH are not responding to feedback signals, as occurs with parathyroid tumors. The disease associated most commonly with parathyroid adenoma is called primary hyperparathyroidism, in which the thyroid gland itself is inappropriately over producing PTH. Along with elevated iCa and PTH levels, hypophosphatemia is typically associated with primary hyperparathyroidism, unless there is a concurrent decrease in GFR, with which normal or elevated phosphorus levels may be seen.

A substance that has similar actions to PTH is PTH-related protein (PTHrP). While this substance has normal functions, it becomes a problem when produced by neoplastic cells. One of the most common causes of hypercalcemia is neoplasia, and PTHrP is associated with many of these cases of humoral hypercalcemia of malignancy (also called pseudohyperparathyroidism). The more common neoplasms associated with humoral hypercalcemia of malignancy are lymphoid neoplasms and apocrine gland adenocarcinoma of the anal sac, although many different tumors have been associated with hypercalcemia, including thymoma and various carcinomas. An assay to measure the concentration of PTHrP is available for dogs and cats. In cases of humoral hypercalcemia of malignancy, PTHrP is often elevated, iCa concentrations are increased, and PTH is appropriately low. Phosphorus levels are typically decreased, unless there is a concurrent decrease in GFR.

Vitamin D toxicity results in increased absorption of calcium from the gastrointestinal tract. Toxicity can occur with cholecalciferol rodenticide poisoning, ingestion of plants containing vitamin D glycosides (*Cestrum diurnum*, *Solanum malacoxylon*, and *Trisetum flavescens*), ingestion of calcipotriene, an analog of calcitriol found in a topical preparation used to treat psoriasis in people, or over supplementation. Some granulomatous diseases as well as some neoplasms activate vitamin D precursors in an unregulated manner, resulting in hypercalcemia. Hypervitaminosis D results in increased iCa and phosphorus and low PTH concentrations.

Renal disease can be associated with hypercalcemia, but should only be attributed as the cause after other differentials are ruled out since hypercalcemia can result in renal failure. Hypercalcemia is a common finding in chronic renal failure in horses, as the horse kidney plays a more important role in excreting excess calcium than in other species. In equine chronic renal failure, iCa is often high, phosphorus low, and PTH appropriately low. Only approximately 10% of dogs with chronic renal failure are hypercalcemic; within this population, dogs with hereditary disease are more common. Hypercalcemia is also a common finding in dogs with grape- or raisin-induced renal failure.[1] Hypercalcemia is more common in cats with chronic renal failure than in dogs. In cats and dogs, although total calcium may be elevated in chronic renal failure, iCa is usually normal or low, consistent with increased complexing of calcium, and PTH may be increased.

Hypoadrenocorticism (Addison's disease) is a common cause of hypercalcemia in dogs. Approximately one-third of dogs with hypoadrenocorticism are hypercalcemic. The mechanism of hypercalcemia is unclear and the concentration of iCa is variable.[2,3]

Because bone contains high amounts of calcium, osteolytic lesions occurring from inflammatory conditions or from metastatic neoplasia can result in hypercalcemia.

Idiopathic hypercalcemia is diagnosed if all other potential causes have been ruled out. This is an acknowledged entity and is becoming more commonly recognized in cats. As the name implies, the underlying mechanism is unknown. Hypercalcemia is typically mild to moderate, with increased iCa, low to normal PTH, and normal vitamin D concentrations.[4]

There can be nonpathological reasons for hypercalcemia. Mild, transient increases may occur postprandially. Dehydration concentrates proteins within the blood which can result in mild elevations in total calcium concentration. Young, growing animals normally have higher calcium concentrations; consequently, if adult reference intervals are used, they will appear hypercalcemic when, in fact, their calcium concentrations are normal for their age. Depending on method of analysis, lipemia or marked hemolysis may interfere with the colorimetric measurement of total calcium. If there is significant lipemia or hemolysis present in the sample, a new sample should be collected and measured or iCa determined.

Diagnostic tools for further working up the cause of a persistent hypercalcemia include laboratory techniques as well as other diagnostic modalities. If hypercalcemia was detected by measuring total calcium, determining the iCa concentration is important in order to interpret the importance of the abnormality as well as helping differentiate between causes. A CBC, serum chemistry panel, and urinalysis are critical for the assessment of underlying disease processes. A thorough physical exam, including careful palpation of lymph nodes and the perianal area as well as radiographs and/or ultrasonography are valuable in the detection of masses or osteolyic lesions. Cytologic or histologic examination of mass lesions provides additional diagnostic information. It is possible to measure PTH and vitamin D concentrations as well as, in dogs and cats, PTHrP for assessment of hormonal status.

Hypocalcemia

It is uncommon for hypocalcemia (Box 24.8) to be severe enough to cause clinical signs, but mild hypocalcemia is often detected on a serum biochemistry panel when total calcium is measured. The most common reason for hypocalcemia is a decrease in the protein-bound fraction when hypoalbuminemia is present. When this is the cause, the iCa concentration is normal. A simple correction factor is frequently used to correct for the decreased protein-bound fraction of calcium (3.5 – patient albumin + patient calcium = corrected calcium), but it must be kept in mind that this equation is only for use in dogs and is only a rough estimate that tends to underestimate the incidence of deficient iCa concentrations. Ideally, instead of the correction factor, iCa concentrations should be measured to determine the significance of decreased total calcium concentrations.

Box 24.8 An acronym to help remember the differentials for hypocalcemia.

Acronym for most hypocalcemia differentials (total calcium)

- M agnesium deficiency
- I njury to tissues (severe)
- L actation / pregnancy
- D vitamin deficiency
- P ancreatitis
- R enal disease
- A lbumin deficiency
- I ntake from GI decreased
- S epsis
- E thylene glycol

Primary hypoparathyroidism is an uncommon cause of hypocalcemia. It should be considered after other causes are ruled out. Iatrogenic hypoparathyroidism is more common and occurs if the parathyroid glands are mistakenly removed during thyroidectomy. With hypoparathyroidism, PTH is inappropriately low in the face of a low iCa concentration. Phosphorus concentration is typically elevated.

Dietary deficiency of calcium seldom leads to hypocalcemia because of the regulatory mechanisms in place to maintain normal blood calcium, but a severe decrease in absorption from the gastrointestinal tract due to a malabsorption or maldigestion disease process or due to cantharidiasis (blister beetle toxicity in horses) can result in hypocalcemia. Decreased absorption associated with vitamin D deficiency can result in hypocalcemia as well as hypophosphatemia.

Imbalances of other electrolytes can lead to hypocalcemia. Hypomagnesemia decreases PTH secretion and action, resulting in hypocalcemia. This is a common occurrence in bovine grass tetany, but also occurs in other species. Hyperphosphatemia can also lead to hypocalcemia as high levels of phosphorus decrease the activation of vitamin D and decrease the action of PTH on bone. This pattern of events is most commonly associated with renal disease (increased phosphorus with decreased GFR) or nutritional imbalances (excess phosphorus or low calcium:phosphorus ratio) and is termed secondary hyperparathyroidism because PTH levels become elevated secondarily to the persistent hypocalcemia.

During late pregnancy or lactation, the demand for calcium may be greater than the bearer's body can maintain. Puerperal hypocalcemia most commonly occurs a few weeks

post whelping in dogs and is rare in cats. Parturient paresis most commonly occurs within 3 days on either side of calving in cattle, but can occur several weeks before or after parturition in sheep and goats. In horses, hypocalcemic tetany usually occurs 1 to 2 weeks after foaling (lactation tetany). Hypocalcemic tetany can also occur with increased calcium loss with excessive sweating in horses.

Transport tetany has occurred in cattle, small ruminants, and horses. Pregnancy or lactation can be a contributing factor, but the primary cause of the hypocalcemia is thought to be due to stress and decreased intake.

Altered distribution of calcium results in hypocalcemia when there is deposition of calcium in tissues as can occur with soponification of fat with pancreatitis or in massive tissue injury, including acute tumor lysis. Precipitation of calcium with oxalates occurs with ethylene glycol toxicity.

Although the mechanism is not completely understood, inflammatory mediators appear to influence calcium regulation. Hypocalcemia associated with sepsis and critical illness is well recognized in human medicine and has been reported in dogs with sepsis[5,6] and with equine colic.[7–10] Total calcium concentrations and calcium concentrations corrected for albumin are not reliable indicators of iCa in these cases. Free, iCa should be measured in critically ill patients, especially if signs of hypocalcemia are evident.

Phosphorus

Phosphorus is required for energy metabolism, nucleic acid synthesis, and cell signaling. It is an important buffer in blood and urine and an important component in structural plasma membrane phospholipids and phosphoproteins and in bone. Abnormalities in serum phosphorus concentrations can be due to abnormalities in hormonal balance, intestinal absorption, renal excretion, or tissue or cell distribution. Serum concentrations of phosphorus may not reflect total body levels.

If there is a concurrent abnormality in serum calcium, pursuing and determining the cause of the calcium abnormality will often provide explanation for an abnormality in phosphorus. Examining the pattern of change between calcium and phosphorus can provide important clues.

Hyperphosphatemia

Hyperphosphatemia (Box 24.9) typically occurs when the phosphorus load (from GI absorption, cellular release, or exogenous administration) exceeds excretion and tissue uptake. In most species, the primary route of phosphorus excretion in via the kidneys, but in ruminants, it is via the gastrointestinal tract. The most common cause of hyperphosphatemia is decreased renal excretion associated with a decrease in glomerular filtration rate. Chronic renal failure is the most common cause of hyperphosphatemia in adult dogs and cats. In ruminants, upper GI obstruction can lead to hyperphosphatemia because of decreased GI excretion.

An excess phosphorus load via increased intestinal absorption can occur with a high phosphate diet or ingestion of nondietary substances containing high concentrations of phosphate such as ethylene glycol that contains phosphate

Box 24.9 An outline for working through the differentials for hypophosphatemia and hyperphosphatemia.

Working through phosphorus abnormalities

1. What is the serum calcium concentration?
   – Is there a pattern consistent with hormonal imbalance?

Hypophosphatemia	Hyperphosphatemia
Hypercalcemia – Primary hyperparathyroidism? – Hypercalcemia of malignancy? Hypocalcemia – Vitamin D deficiency?	Hypercalcemia – Vitamin D toxicosis? Hypocalcemia – Hypoparathyroidism?

2. What do renal parameters look like?
   – Is there evidence for decreased excretion or resorption?

Hypophosphatemia	Hyperphosphatemia
Prolonged diuresis? – Treatments? – Hyperglycemia? – Polyuria? – Dilute urine? Possible tubular defects? – Glucosuria? Horse with hypercalcemia and azotemia?	Is there evidence for decreased GFR?? – Elevated serum BUN and creatinine? **Most common cause of hyperphosphatemia**

3. Is there any evidence of dietary or gastrointestinal problems?

Hypophosphatemia	Hyperphosphatemia
Prolonged anorexia? Diarrhea? Vomiting? Low phosphorus diet?	High phosphorous diet? Phosphate enema use? Ingestion of ethylene glycol with phosphate rust inhibitors? Ruminant with upper GI obstruction?

4. Could there be redistribution, a shift between the ICF and ECF?

Hypophosphatemia	Hyperphosphatemia
Insulin therapy? Carbohydrate loading? Alkalosis?	Evidence of extensive tissue damage? Presence of osteolytic bone lesion? Acidosis?

rust inhibitors. Hypervitaminosis D increases the amount of phosphorus absorbed from the GI tract, leading to hyperphosphatemia and hypercalcemia. Phosphate enemas can also lead to severe hyperphosphatemia.

Given that the majority of total body phosphorus is stored in bone and that the concentration of intracellular phosphorus is more than ten times that of ECF, the redistribution of phosphorus from bone or from the intracellular space can result in hyperphosphatemia. Release from bone can occur with osteolytic lesions. Release from cells occurs with injury, which must be extensive in order to significantly affect the serum concentration of phosphorus; this can occur with acute tumor lysis or acute myopathies. Acidosis decreases the cellular uptake of phosphorus and can contribute to hyperphosphatemia.

Improper sample handling may cause falsely increased serum phosphorus concentrations. This includes hemolysis of the sample or a delay in the removal of serum from erythrocytes after collection. Mild, transient increases may occur postprandially. Young, growing animals have higher concentrations of serum phosphorus; consequently, if adult reference intervals are used, they will appear hyperphosphatemic when, in fact, their phosphorus concentrations are normal for their age.

Hypophosphatemia

Hypophosphatemia (Box 24.9) occurs from hormonal imbalances, decreased renal reabsorption, decreased intestinal absorption, or redistribution from ECF to ICF. Hormonal imbalances typically include concurrent calcium abnormalities, the pattern of which can aid diagnosis. Low phosphorus with elevated calcium concentration is the pattern seen with primary or pseudo-hyperparathyroidism. A decrease in both phosphorus and calcium is the pattern seen with hypovitaminosis D. Hypophosphatemia can also occur due to elevated PTH in response to pariparturient hypocalcemia (physiologic hyperparathyroidism).

Decreased renal phosphorus reabsorption (increased phosphate excretion) leads to hypophosphatemia. This can occur due to congenital or acquired defects in the proximal tubules where the majority of reabsorption normally occurs, often called Fanconi's syndrome. Diuresis results in decreased renal phosphate reabsorption which, when prolonged, can lead to hypophosphatemia. Diabetic ketoacidosis leads to hypophosphatemia because of osmotic diuresis, as well as phosphate's role as a buffer for excreted acid. Although the mechanism is poorly understood, hypophosphatemia is often seen with the hypercalcemia associated with chronic renal failure in horses. Increased phosphate excretion is also the mechanism of hypophosphatemia associated with hyperparathyroidism.

Decreased intestinal absorption of phosphorus is an uncommon cause of hypophosphatemia as the body typically can maintain normal blood levels even with decreased intake, although anorexia or a low phosphate diet, if prolonged, may lead to hypophosphatemia. Impaired absorption because of vomiting, diarrhea, or an intestinal malabsorption disease can also lead to hypophosphatemia. Decreased intestinal absorption is the mechanism of hypophosphatemia with hypovitaminosis D.

Redistribution of phosphate from ECF to ICF can result in hypophosphatemia. Insulin causes phosphate to move into cells. Hypophosphatemia can occur with administration of insulin or with insulin-producing tumors. It can also occur with carbohydrate loading or intravenously administered glucose which induces the secretion of insulin. Respiratory alkalosis has been associated with hypophosphatemia because phosphate shifts to the intracellular space when CO_2 moves out of the cell. Because phosphate is required for energy metabolism, accelerated metabolism, in general, will result in intracellular shifts of phosphate, decreasing extracellular concentrations.

Magnesium

Magnesium is primarily an intracellular ion and is a cofactor of many enzymatic reactions, including all reactions involving the formation and utilization of ATP and many mitochondrial reactions. It is also required for protein and nucleic acid synthesis. Vitamin D and PTH influence, but do not regulate magnesium metabolism.[11] Homeostasis is primarily a balance between intestinal absorption and renal excretion. Magnesium has a similar charge as calcium and, as does calcium, exists in free ionized, protein-bound (approximately 30%), and complexed forms in serum. Serum magnesium contains only approximately 1% of total body magnesium and therefore is not necessarily an accurate representation of total body magnesium.

Hypomagnesemia is more commonly associated with morbidity than hypermagnesemia. Neuromuscular signs occur with hypomagnesemia, including hyperexcitability, muscle tremors, spasms, and fasciculations, and ataxia. Other complications associated with hypomagnesemia include the development of hypokalemia or hypocalcemia. These deficiencies may not be able to be corrected unless hypomagnesemia is corrected first.

Hypomagnesemia is typically associated with either increased loss or decreased intake. Losses, the most common cause of hypomagnesemia in small animals, are through the renal or gastrointestinal systems. Renal loss occurs with diuresis and renal disease. Renal reabsorption can also be inhibited by hypercalcemia. Malabsorption and diarrhea are causes of gastrointestinal magnesium loss.

Decreased intake is a common cause of hypomagnesemia in ruminants. Grass tetany is a disease that is associated with ruminants eating lush green pastures that are high in potassium and low in magnesium content. Elevated potassium

ingestion blocks normal magnesium absorption in the rumen. Milk tetany is a disease that is associated with older calves being fed milk-only diets. Prolonged anorexia or poor diet can lead to hypomagnesemia, especially if an animal is lactating. Prolonged intravenous fluids or parenteral nutrition can also lead to hypomagnesemia if magnesium supplementation is not included.

Other causes of hypomagnesemia include redistribution and hypoalbuminemia (if total magnesium is measured instead of free, ionized magnesium). Redistribution of magnesium is not well understood, but may be influenced by administration of insulin or glucose, sepsis, trauma, or pancreatitis.

Hypermagnesemia

Hypermagnesemia is typically a less significant clinical problem, unless it develops acutely. It can result in cardiac or neurological problems and cause nausea and vomiting. Hypermagnesemia can occur iatrogenically or due to decreased renal excretion, primarily associated with acute renal failure or urethral obstruction.

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25 Laboratory Evaluation of Acid-Base Disorders

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Introduction

Acid-base analysis, also known as blood gas analysis, is a growing point-of-care diagnostic application established for complicated medical cases and in critical care settings. Assessment of acid-base status is typically done in conjunction with electrolyte evaluation to determine the presence and severity of fluid and electrolyte derangement attributable to the animal's underlying disease process. Advances in technology have made acid-base evaluation possible and even routine within the in-clinic laboratory. These determinations allow for correction of such clinical abnormalities, which in turn aids recovery time and may improve mortality outcome.

Blood gas analysis has historically been regarded as complex and intimidating for many clinicians. This is likely because measurement and interpretation of partial pressures of gases in blood solution are less conceptually intuitive than concentration measurements dealt with in hematology and conventional clinical chemistry. The subject also is complicated by many derivative specialty calculations that may appear on laboratory reports. Sample transportation to a central laboratory, perceived complex sample handling, and a perception that blood gas analysis necessitates arterial blood collection are factors that have contributed to very infrequent use. However, the clinician is encouraged to embrace this capability made possible by the availability of simplified point-of-care analysis systems that eliminate most of these barriers.

Recommendations to facilitate use include the following. It may help to think of blood gas analysis more simply as analysis of acid-base balance. This approach may be more intuitive. Today's point of care electrochemical analyzers makes capability to evaluate acid-base status routine. Incorporation of this capability into the practice routine provides the use frequency needed to build and maintain interpretive skills. Users should focus on a few key values and not be overwhelmed by the various possible derivative calculations that may appear on laboratory reports. Most of the calculations may be ignored while initial interpretive skill is acquired. Users may then adopt a few selected calculations as skill is accumulated.

The purposes of this chapter are to present a background of the technical aspects of acid-base analysis and an introductory approach to interpretation of basic acid-base laboratory data. The introductory approach is designed to help the reader recognize and interpret basic, common acid-base abnormalities; there is no intention to discuss advanced concepts used by various specialties. The latter is left to literature and advanced training that exists for specialty applications.

Technical considerations

Measurement of acid-base parameters

Acid-base laboratory data are generated on electrochemical analyzers (Chapter 1). These typically use the same sample to simultaneously determine both electrolyte and acid-base determinations. Venous blood collection is adequate for evaluation of the metabolic complications of disease that result in acid-base and electrolyte disturbances. Arterial blood is typically only required when it is necessary to critically evaluate blood oxygenation.

While a number of parameters may appear on an acid-base report, there are very few direct measurements and calculations that are important for interpretation of acid-base balance. A systematic focus on this small number of parameters will simplify interpretation. These include the following.

Veterinary Hematology and Clinical Chemistry, Second Edition. Edited by Mary Anna Thrall, Glade Weiser, Robin W. Allison, and Terry W. Campbell.

pH

The primary measurement of blood acidity. It is directly measured by an ion specific electrode (see Chapter 1). pH is the negative log of hydrogen ion concentration. Therefore a decrease in pH value indicates an increase in free hydrogen ion concentration (relatively acidic) and an increase in pH indicates a decrease in free hydrogen ion concentration (relatively alkaline). pH is very tightly regulated in the body by a number of buffering systems.

PCO_2

The partial pressure of carbon dioxide (CO_2) gas dissolved in blood, measured in mm of mercury (mmHg). This may be measured in either venous or arterial blood.

HCO_3 or bicarbonate concentration

Using the pH and pCO_2 values, bicarbonate concentration is calculated by the instrumentation software. It is expressed in mmol/L. The pH and bicarbonate values are the most useful for interpretation of acid-base disturbances when venous blood is the sample.

PO_2

The partial pressure of oxygen (O_2) gas dissolved in blood, measured in mm of mercury (mmHg). This measurement is typically only useful for analysis of blood oxygenation and therefore is only of interpretive value when arterial blood is sampled specifically to evaluate the patient for oxygenation pathology.

Other calculated values

There are a number of possible calculated indices. The presentation of these on reports varies by the manufacturer. Their use is somewhat optional and most are typically adopted by critical care specialists. Some relate to arterial human applications that are rarely used in animals.

Sample handling requirements

Proper sample handling instructions are available and are simplified by the capability provided by point-of-care analysis. pH may be affected by changes in pCO_2. Both CO_2 and O_2 may move toward ambient equilibrium in blood removed from the body if not properly handled. The following are proper sample handling guidelines for electrochemical analyses that should be supplemented by instructions accompanying point-of-care analyzers.

- Avoid manual heparinization of syringes. This can cause gross over heparinization of the sample leading to errors in any of the acid-base and electrolyte results.
- It is recommended to use a balanced heparin-containing syringe produced specifically for electrochemical analysis samples (see Chapter 2).
- Any gas bubbles in the collection syringe should be expelled.
- Unless the sample is introduced for analysis immediately after collection, the syringe should be capped to prevent sample contact with air. Recommended collection devices have a cap for this purpose.

Physiologic considerations

Regulation of blood pH

It is critical for most physiologic functions that acid-base balance be maintained within a narrow range of pH, typically 7.35 to 7.45, with some minor species variations indicated in reference interval tables. A number of pathologic conditions may add or subtract acid in blood and body fluids. The blood contains considerable buffering capacity to aid regulation of blood pH. Major buffers include hemoglobin and the bicarbonate buffer systems. Minor contributory buffers include inorganic phosphate and plasma proteins. Through equilibrium reactions, these molecules may incorporate or release hydrogen ions in the effort to maintain pH. The bicarbonate buffer system is important because of its rapid buffering capacity and because its components are readily measured for assessment blood pH and associated therapeutic monitoring. In addition, the bicarbonate system interacts with the mass quantity of hemoglobin in both pH regulation and gas exchange between tissues and respiration. The equilibria within the bicarbonate buffer system are shown in Figure 25.1.

The relationship of the bicarbonate buffer system to pH is described as:

$$pH = pK + \log \frac{[HCO_3^-]}{[H_2CO_3]}$$

where pK is the pH at which 50% of an acid is dissociated; this is ~6.1 for carbonic acid.

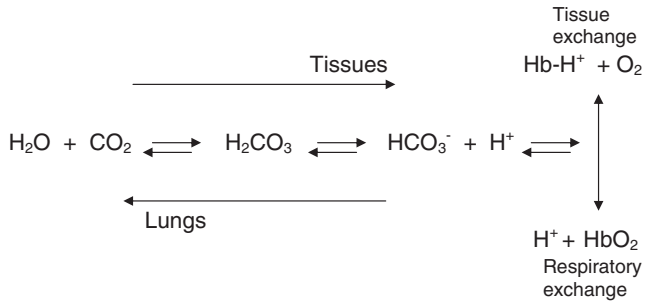

Figure 25.1 Bicarbonate buffer system interactions. At the lung level, hemoglobin binds oxygen; this creates a molecular change that favors dissociation of H^+ from hemoglobin. This will push the equilibrium to the left, yielding CO_2 and water that is expired. At the tissue level, metabolism yields CO_2 and considerable acid. Hemoglobin releases O_2 to tissues and deoxygenated hemoglobin then binds H^+. Thus, equilibrium is driven to the right.

Table 25.1 Acid-base disturbances resulting in abnormal blood pH. For each disturbance category, the major abnormality in the bicarbonate buffer system is indicated. Also, common causes or processes that may cause a given disturbance are listed.

Acid-Base Disturbance	Metabolic Acidosis Decreased pH	Metabolic Alkalosis Increased pH	Respiratory Acidosis Decreased pH	Respiratory Alkalosis Increased pH
Primary bicarbonate buffer alteration	Decreased bicarbonate	Increased bicarbonate	Increased CO_2	Decreased CO_2
Causes	Any cause of generalized poor perfusion leading to lactate production	Loss of acid due to vomiting or functional upper GI obstruction	Any cause of respiratory failure, e.g.:	Pulmonary pathology resulting in impaired O_2 alveolar diffusion
	Diabetic ketoacidosis Lactic acidosis	Excess bicarbonate therapy	Severe pneumonia Pneumothorax Severe pleural fluid	Excess positive pressure ventilation during anesthesia
	Renal failure	Excess diuretic therapy	Airway obstruction	
	Ethylene glycol toxicity		Hypoventilation due to anesthetic overdose	
	Diarrhea			

Because pCO_2 can be measured and the dissolved CO_2 in blood is proportional to the carbonic acid concentration, this relationship may be simplified to:

$$\text{Blood pH} = 6.1 + \log \frac{[HCO_3^-]}{\alpha pCO_2}$$

where α pCO_2 is pCO_2 multiplied by its solubility constant to yield the amount of CO_2 gas dissolved in blood. Using the solubility constant, the above formula may be rearranged to:

$$\text{Blood pH} = 6.1 + \log \frac{[HCO_3^-]}{0.03 \times pCO_2}$$

Normally, the ratio of bicarbonate to CO_2 is 20:1. At this ratio, the log of 20 plus 6.1 yields the desired blood pH of 7.4. For interpretation purposes it is helpful to think of bicarbonate as being the metabolic component of blood pH regulation and pCO_2 as being the respiratory component of blood pH regulation. For example, if bicarbonate is utilized to buffer an increase in metabolic acid (H^+), a decrease in bicarbonate in the above equation results in a decrease in pH, or acidosis. In response, respiration may increase CO_2 expiration to partially normalize the ratio, known as compensation. The compensation response attempts to normalize the ratio, which in turn helps normalize or regulate pH. This framework of metabolic and respiratory components in the above equation will be very helpful for identifying and interpreting acid-base abnormalities.

Acid-base balance pathology

General processes that may result in life-threatening abnormalities in acid-base balance are classified in the following four primary categories. Common disease entities that may result in these categories are outlined in Table 25.1. Metabolic acid-base disturbances develop relatively slowly, usually over a period of days, whereas respiratory acid-base disturbances may develop very acutely.

Bicarbonate (HCO_3^-) depletion—metabolic acidosis

This is due to pathologic metabolic production of acid in the form of hydrogen ions. The increased hydrogen ions are buffered by combining with bicarbonate to form carbonic acid that then dissociates to CO_2 gas and water. The CO_2 is then rapidly eliminated from the system via respiration. Common examples of pathologic metabolism resulting in metabolic acidosis include lactic acidosis, ketoacidosis, renal failure, and acid toxicities (e.g., ethylene glycol toxicity). Alternatively, bicarbonate may be lost from the system such as may occur with severe diarrhea. By any of these mechanisms, depletion of bicarbonate establishes metabolic acidosis.

Metabolic acidosis is the most common acid-base disturbance. This is attributed to the fact that dehydration and poor tissue perfusion leading to lactic acid production is a process common to many primary internal medical disorders. Renal failure and diabetes mellitus, relatively common disorders in veterinary patients, also contribute to the incidence of metabolic acidosis.

CO_2 retention due to hypoventilation—respiratory acidosis

This is due to acute respiratory failure with accumulation of CO_2. Causes include hypoventilation during anesthesia or any pathologic cause of acute spontaneous hypoventilation

or severe impairment of gas exchange at the blood—lung interface.

Bicarbonate excess—metabolic alkalosis

This is due to metabolic production and accumulation of excess bicarbonate. Gastric parietal cells produce hydrogen ions by combining CO_2 and water to form carbonic acid, which then dissociates to H^+ and bicarbonate. Bicarbonate is exchanged to the plasma for chloride ions. H^+ and Cl^- are then excreted into the gastric lumen as part of the gastric acid (H^+Cl^-) digestive response. The most common cause of metabolic alkalosis is upper gastrointestinal obstruction. The hydrogen ions are secreted into the stomach and are lost in the obstructive process, while bicarbonate is retained.

CO_2 loss due to hyperventilation—respiratory alkalosis

This may occur when there is hypoxemia that stimulates hyperventilation, as seen with some forms of pneumonia. With inflammatory or fluid pneumonia, the gas diffusion barrier is increased. Oxygen diffuses more slowly than CO_2 across such barriers. Increased ventilation due to poor oxygenation can result in loss of CO_2 since it diffuses more freely across the barrier. More severe pneumonia that impairs even CO_2 diffusion is more likely to lead to respiratory acidosis as a result of CO_2 retention. Excess positive pressure ventilation during anesthesia may also cause respiratory alkalosis.

Relationship of acid-base pathology to anion gap

The anion gap is discussed in Chapter 24. The anion gap is useful in classifying metabolic acidosis into one of two categories, which may help determine the cause of the acid-base disturbance. The most common pathway to metabolic acidosis is accumulation of acid due to either production of organic acid or the presence of an acid toxicity. The accumulation of a dissociable acid yields H^+ and the respective anion. As the H^+ is buffered by bicarbonate, the bicarbonate concentration decreases. The anion is unmeasured in calculation of anion gap and its accumulation results in the increased anion gap. Representative unmeasured anions associated with metabolic acidosis disorders are listed in Table 25.2.

Table 25.2 Causes of metabolic acidosis with increased anion gap, with associated pathologic acids.

Disease with unmeasured anions	Pathologic acid(s)
Diabetes, unregulated	Ketoacids: acetoacetate, beta-hydroxybuterate
Renal failure	Sulfates, phosphates, and lactate when dehydrated
Hypoxemia and/or poor perfusion	Lactic acid
Acid toxicity, e.g., ethylene glycol toxicity	Metabolism to form oxalic and glycolic acids

The alternative cause of metabolic acidosis is due to a primary process of bicarbonate loss. This may be due to severe diarrhea or, less commonly, renal tubular acidosis in which there is pathologic renal loss of bicarbonate. In this situation, the anion gap will be normal because there is no accumulation of unmeasured anions. Increased serum chloride may develop with this type of acidosis. The kidneys will continue to reabsorb the vast majority of filtered sodium. Because active renal sodium reabsorption requires either bicarbonate or chloride as a counterion, more chloride may be retrieved when there is relatively severe deficit of bicarbonate. As a result, hyperchloremia may develop.

Approach to interpretation of acid-base data

Evaluation of acid-base status begins with analysis of blood pH, bicarbonate concentration, and the partial pressure of carbon dioxide. It is desirable to develop a stepwise approach to interpretation of the acid-base data. A recommended stepwise approach is described below and diagrammed in Figure 25.2.

Step 1—Evaluate pH. The pH is abnormal if either decreased or increased beyond the reference interval limits. If the pH is decreased, the animal by definition has acidemia. This is due to some process that is causing acidosis or accumulation of acid in the system. If the pH is increased, the animal by definition has alkalemia. This is due to some process that is causing alkalosis or accumulation of base in the system.

If the pH is within the reference interval, a major acid-base disturbance is unlikely. However, there is still merit in checking the bicarbonate buffer pair for abnormality. If the pH is near the lower or upper limit it should still be evaluated as a possible indicator of acidosis or alkalosis. Borderline pH values may be an indication that a process causing acidosis or alkalosis is present, but there has been some compensation in an attempt to normalize the pH. Alternatively, a mixed acid-base disturbance may be due to severe disease, but result in a relatively normal pH value. A mixed acid-base disturbance is due to two or more processes affecting acid and/or base addition to the system.

Because of the relationship between pH and HCO_3^- and pCO_2 described above, the bicarbonate buffer system should next be evaluated to determine the cause of the acid-base disturbance defined by the pH. This involves a determination of whether the metabolic component (HCO_3^-) or respiratory component (pCO_2) of the buffer system is responsible for the primary acid-base disturbance.

Step 2—Evaluate bicarbonate (HCO_3^-) concentration. Interpret if the HCO_3^- value is below the reference interval limit, indicating increased metabolic acid (acidosis), or above

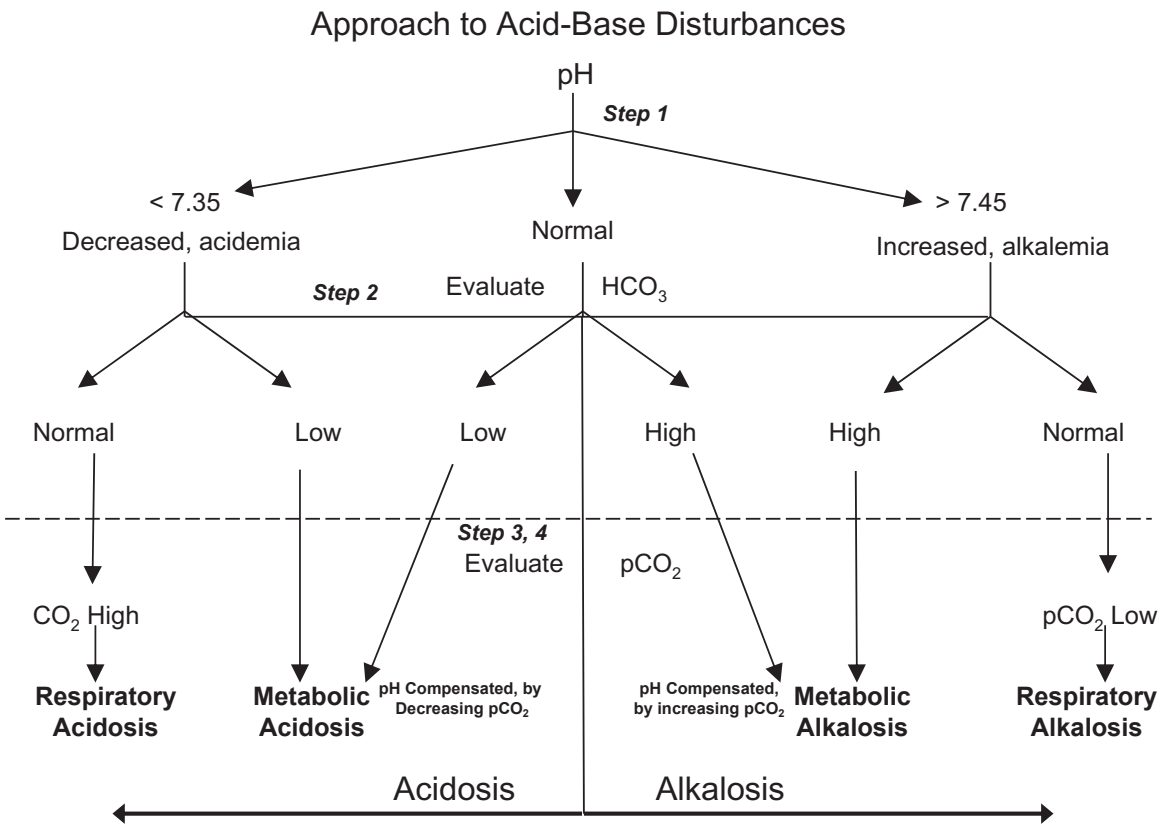

Figure 25.2 Stepwise approach to interpretation of acid-base disturbances. See text for narrative discussion.

the reference interval limit, indicating increased metabolic base (alkalosis).

Note that some references may suggest evaluation of pCO_2 first. However, because most acid-base disturbances identified in animals are due to metabolic disorders, evaluation of HCO_3^- first usually will readily define the acid-base disturbance.

Step 3—Evaluate the pCO_2. If the pCO_2 value is normal, the acid-base disturbance is defined by the bicarbonate concentration abnormality. Interpret if the pCO_2 value is above the reference interval limit, indicating increased respiratory acid (acidosis), or below the reference interval limit, indicating increased respiratory base (alkalosis).

Step 4—If needed, evaluate any combined abnormalities between HCO_3^- and pCO_2. If both the HCO_3^- and pCO_2 values are abnormal, the primary acid-base base disturbance is almost always due to the most abnormal value in the pair. The change in the least abnormal value is almost always due to a compensation response in an attempt to normalize the pH. In addition, the compensating value is either abnormal or moving toward abnormal in a direction *opposite* what would be required to cause the pH abnormality. This step may also aid in identification of acid-base disturbances due to more than one process, known as a mixed acid-base disturbance.

This approach may be illustrated in the following example of a common patient acid-base data, with comparison reference intervals (RI):

pH 7.21 RI = 7.35–7.45
decreased—indicates a primary acid disorder (acidemia)

HCO_3^- 12 mmol/L RI = 15–23 mmol/L
decreased—indicates acid response,
metabolic component of buffer system

pCO_2 30 mmHg RI = 35–40 mm Hg
decreased—indicates base response,
respiratory component of buffer system

Both determinants of the bicarbonate buffer system are abnormally decreased. The determinant abnormality that adds acid or base consistent with the pH change is the primary cause of the acid base disturbance. In this example, a decrease in HCO_3^- is interpreted as an addition of acid. The decrease in pCO_2 cannot explain the pH because this change would add base to the system, increasing the pH. Therefore, the concluding interpretation is metabolic acidosis with some respiratory compensation. The decrease in pCO_2 is a compensation response. Increased respiratory elimination of

CO_2 tends to compensate to normalize the pH to some degree. Some will find it useful to utilize the formula of this relationship when making these interpretations:

$$\text{Blood pH} = 6.1 + \log \frac{[HCO_3^-]}{0.03 \times pCO_2}$$

Looking at this equation, it is apparent that either a decrease in HCO_3^- or an increase in pCO_2 would be required to decrease blood pH.

Compensation is a normal, active physiologic action to attempt pH correction in response to the primary acid-base disturbance. Unless the acid-base disturbance is very acute, a compensation response is expected. Typically respiratory compensation for metabolic disorders occurs faster than metabolic compensation for respiratory disorders. Table 25.3 shows abnormalities in the bicarbonate buffer system for various acid-base disturbances and the expected respective compensation responses. It is important to note that compensation will move the pH toward normal, but may at best only partially normalize the pH. For certain, mechanisms involved in compensation will not overcompensate pH normalization. This perspective is helpful for recognizing the presence of mixed acid-base disturbances.

Table 25.3 Expected values for pH, HCO_3, and pCO_2 in various acid-base disturbances, including expected compensation responses. Note that acute respiratory acidosis may not effectively compensate.

Disturbance	pH	HCO_3	pCO_2
Metabolic acidosis	Dec	Dec	N
Metabolic acidosis, compensating	Dec or low N	Dec	Dec or low N
Metabolic alkalosis	Inc	Inc	N
Metabolic alkalosis, compensating	Inc or high N	Inc	Inc or high N
Respiratory acidosis	Dec	N	Inc
Respiratory acidosis, compensating	Dec	Inc or high N	Inc
Respiratory alkalosis	Inc	N	Dec
Respiratory alkalosis, compensating	Inc or high N	Dec or high N	Dec

N = normal value, within reference interval; Dec = decreased; Inc = increased.

More advanced considerations

Mixed acid-base disturbances

As mentioned above, a mixed acid-base disturbance is composed of two or more pathologic processes that affect pH. The pH value will reflect a balance of the contributing processes. An example of a mixed acid-base disturbance might be recognized in a dog with severe pancreatitis. In this situation, there may be dehydration with poor tissue perfusion and prerenal azotemia, processes leading to metabolic acidosis. If there is protracted or disproportionate vomiting, there is a superimposed process of metabolic alkalosis. The latter process may be suspected if there is a disproportionately decreased serum chloride concentration.

Recognition of a mixed acid-base disturbance should start with suspecting it based on diagnosis of the existing clinical problem or problems. Some example clinical scenarios that may result in mixed acid-base disorders include:

- Hypoadrenocorticism with disproportionate vomiting—metabolic acidosis and alkalosis
- Protracted vomiting and aspiration pneumonia—metabolic alkalosis and respiratory acidosis
- Heart failure with severe pulmonary edema—metabolic and respiratory acidosis
- Renal failure with disproportionate vomiting—metabolic acidosis and alkalosis
- Gastric dilatation/volvulus—variable, depending on manifestations
- Anesthesia in excess, with preexisting disorder—variable, depending on disorder.

The acid-base data may aid in confirming the presence of a mixed disturbance. Using the clinical findings and the most likely primary acid-base data abnormality, identify the primary acid-base disturbance. Then, predict the expected compensation response to the primary disturbance. Guidelines for magnitude of compensation changes in Table 25.4 may aid this determination. If the expected compensation response is either not present, excessive, or in the opposite direction of what is expected, then a second primary disturbance should be suspected, indicating a mixed acid-base disturbance. These guidelines should be used with considerable latitude and only large deviation from the expectation should be interpreted as inappropriate. By way of example, consider the following case data.

pH 6.99 RI = 7.35–7.45
severely decreased—indicates
a primary acid disorder (acidemia)

HCO_3^- 12 mmol/L RI = 15–23 mmol/L
decreased—indicates acid response,
metabolic component of buffer system

Table 25.4 Guidelines for expected magnitude of compensation of primary acid-base disturbances. For each acid-base disturbance, the table shows the associated primary alteration, action for compensation, and expected magnitude of change in the compensation response.

Acid-Base Disturbance:	Metabolic Acidosis Decreased pH	Metabolic Alkalosis Increased pH	Respiratory Acidosis Decreased pH	Respiratory Alkalosis Increased pH
Primary bicarbonate buffer alteration	Decreased bicarbonate	Increased bicarbonate	Increased CO_2	Decreased CO_2
Expected compensation:	Decrease pCO_2 by respiration change	Increase pCO_2 by respiration change	Increase HCO_3^- by metabolism	Decrease HCO_3^- by metabolism
Expected magnitude of compensation:	0.7 mm Hg per 1.0 mmol/L decrease in HCO_3^-	0.7 mm Hg per 1.0 mmol/L increase in HCO_3^-	Acute: 1.5 mmol/L HCO_3^- per 10 mmHg increase in pCO_2 Chronic: may approximately double	Acute: 2.5 mmol/L HCO_3^- per 10 mmHg decrease in pCO_2 Chronic: may approximately double

pCO_2 50 mm Hg RI = 35–40 mm Hg
increased—indicates acid response,
respiratory component of buffer system

At first glance, this may appear confusing if one is suspecting a single primary disturbance. If it is initially assumed that metabolic acidosis is the primary abnormality, one would expect a compensatory decrease in pCO_2 of about 2 mm Hg below the lower RI limit, or ~33 mm Hg (Table 25.4). However, the pCO_2 result is grossly different from the compensation expectation. If it is initially assumed that respiratory acidosis is the primary abnormality, one would expect an increase in HCO_3^- of about 1.5–3.0 mmol/L, or ~ 25 mmol/L. Again the result is grossly different than the compensation expectation. This should prompt consideration of a mixed acid-base disturbance. In this case there are two acid responses consisting of both respiratory acidosis and metabolic acidosis. The two primary acid-base disturbances are recognized as a mixed disturbance. Therefore, both the pulmonary function and potential causes of metabolic acidosis should be investigated (Table 25.1).

Arterial blood oxygenation

A conventional blood gas analyzer measures pO_2 along with other values discussed above. It then calculates or predicts the percent hemoglobin saturation with oxygen (SO_2) based on the expected behavior of hemoglobin affinity at a given pH, temperature, and pO_2. The relationship between percentage saturation and these variables is characterized by the hemoglobin-oxygen dissociation curve. These values are present on all blood gas analyzer reports, but are typically only useful for arterial samples performed specifically to evaluate oxygenation. As an initial alternative to arterial sampling, a pulse oximeter may be used as a hemoglobin percentage saturation screening measurement to rule out severe oxygenation defects.

When breathing ambient air and blood oxygenation is normal, the animal will have an arterial pO_2 in the range of 85–100 mm Hg. At this level, the SO_2 is typically 95% or higher. Generally, large pathologic change in pO_2, e.g., <60–70 mm Hg, is required to result in clinically important changes in SO_2. The calculation of percentage saturation is reasonably accurate when hemoglobin is normal. However, it is not accurate in the presence of the toxicities such as methemoglobinemia and carboxyhemoglobinemia (below) and in some hemoglobin abnormalities present only in humans.

Base excess calculation

The base excess (BE) value is typically included on the acid-base data report. This value is calculated to account for the combined bicarbonate and hemoglobin buffering capacity of blood. This is a complex calculation that utilizes information from a nomogram built on the relationship between pH, pCO_2, and HCO_3^- in blood. This relationship is similar for human and dog, but may vary with other species. Instrument software typically uses the human calculation to derive BE. The normal range of BE is slight positive and negative deviation around zero. A positive abnormal value indicates an excess of base, or alkalosis. A negative value indicates the magnitude of HCO_3^- deficit in mmol/L in metabolic acidosis. The amount of deficit may be useful in planning fluid and bicarbonate therapy.

The BE utility is for calculation of the amount of bicarbonate replacement in fluid therapy formulae. These calculations result in the target amount of bicarbonate that would be administered to normalize the bicarbonate concentration and pH. The calculation is based on body weight and the goal to deliver bicarbonate to the extracellular fluid space,

which is approximately 30% of body weight. A representative formula using the absolute value of BE is:

$$\text{Bicarbonate dosage (mmol)} = 0.3 \times \text{Body weight (kg)} \times \text{BE (mmol/L)}$$

One may encounter variations of the above formula, but the general principle is unchanged. The user must realize that bicarbonate replacement in acidosis is a moving target. The administration of bicarbonate is initially distributed to the extracellular fluid space, but there is movement intracellularly over time. Furthermore, the process(es) causing acid-base disturbance may change or continue to affect bicarbonate balance. As a result, the acid-base status should be monitored during replacement therapy and ongoing bicarbonate administration should be adjusted accordingly.

Cooximetry

In contrast to measurement of pO_2, co-oximetry is the measurement of hemoglobin (HGB) spectrophotometrically using multiple wavelengths of light. HGB will absorb light maximally at different wavelengths depending on its configuration. The 4 common absorption maximum wavelengths used in these devices are to provide measurement of:

- oxygenated hemoglobin (oxy-HGB or O_2-HGB), which is expressed as true, measured oxygen saturation percent
- unoxygenated hemoglobin (deoxy-HGB)
- carboxyhemoglobin (CO-HGB)—used to detect the presence and severity of carbon monoxide poisoning
- methemoglobin (Met-HGB)—used to detect the presence and severity of methemoglobinemia.

Because cooximetry is used to characterize HGB oxygenation, it is typically useful only for arterial blood analysis.

Recommended Reading

For case management of medical problems that may have acid-base disturbances, e.g., diabetes mellitus with ketoacidosis, lactic acidosis, renal failure, etc.

DiBartola SP (ed.) (2012) *Fluid, Electrolyte, and Acid Base Disorders*, 4th ed. St. Louis: Saunders-Elsevier. Recommendations: Section III "Acid Base Disorders," pp. 231–329. Other sections may be useful, e.g., Section IV "Fluid Therapy," as it relates to treatment of acid-base disorders.

Silverstein DC, Hopper K (eds.) (2009) *Small Animal Critical Care Medicine*. St. Louis: Saunders-Elsevier. Recommendation: Section V, "Electrolyte and Acid-Base Disturbances," pp. 224–59.

Tilley LP, Smith FWK (eds.) (2011) *Blackwell's Five-Minute Veterinary Consult: Canine and Feline*, 5th ed. West Sussex, UK: John Wiley & Sons, Ltd.

26 Laboratory Evaluation of the Liver

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The liver functions in an amazing variety of biologic processes that are essential to life. These functions include metabolism of carbohydrates, lipids, proteins, hormones, and vitamins; detoxification and excretion of waste products and other toxic substances, digestion (especially of fats), and production of most clotting factors. The liver is highly vascular and uniquely situated to receive not only arterial blood via the hepatic artery, but also venous blood via the portal vein. In fact, the majority (70–75%) of blood flow to the liver arrives from the portal circulation, and the liver's capacity to remove various solutes from portal blood is central to many of its functions.[77] Because of the liver's remarkable diversity, liver dysfunction may result in a variety of laboratory abnormalities.

Liver disease vs liver failure

Liver disease includes any process that results in hepatocyte injury, cholestasis, or both. These include hypoxia, metabolic diseases, toxicoses, inflammation, neoplasia, mechanical trauma, and intrahepatic or extrahepatic bile duct blockage. It is important to realize that the liver is frequently secondarily affected by primary disease processes occurring in other tissues, such as inflammatory bowel disease and pancreatitis. Liver failure may result from liver disease, and is recognized both by failure to clear the blood of those substances normally eliminated by the liver and by failure to synthesize those substances normally produced by the liver. Liver disease, however, does not always result in liver failure. The liver has a marked reserve capacity, and 70–80% of the functional hepatic mass must be lost before liver failure occurs. Tests for liver disease or failure fall into three main categories:

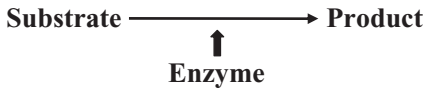
- Serum enzyme assays that detect hepatocyte injury.
- Serum enzyme assays that detect cholestasis.
- Tests that evaluate liver function.

Introduction to enzymology

A basic understanding of diagnostic enzymology is necessary to interpret the results of serum enzyme assays used to detect liver disease. Principles of diagnostic enzymology include:

- Different organs, tissues, or cells contain different enzymes. In some cases, only a few organs or tissues contain a given enzyme; these "tissue-specific" enzymes tend to be the most diagnostically useful.
- Increased serum enzyme activity results when an increased quantity of the enzyme passes into the blood, either because of leakage from injured cells or because of increased production.
- Detection of increased serum enzyme activity, therefore, suggests either injury to the cells of origin or stimulation of the cells to produce increased quantities of the enzyme.
- Diagnostic enzymology is a means of locating where tissue injury or stimulation of increased enzyme production has occurred.
- Results of diagnostic enzymology, in combination with other clinical and laboratory data, are helpful in understanding the disease process and in making a diagnosis.
- Diagnostic enzymology does *not* provide information about tissue *function*.

In the body, enzymes catalyze biochemical reactions by converting a substrate into a product. For example,



To measure enzyme activity, a standard quantity of serum containing the enzyme to be measured is mixed with a solution containing the substrate for that enzyme. The reaction is then allowed to occur, and the enzyme's activity is

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measured by the rate of either substrate disappearance or product formation. The more rapidly that either one occurs, the greater the patient's serum enzyme activity. Frequently, the product is not measured directly; rather, it is incorporated into a second reaction, which often involves the conversion of NAD^+ (or $NADP^+$) to NADH (or NADPH), or vice versa. This second reaction results in a change in the sample's light absorbance, which can be measured using a spectrophotometer[101] (see Chapter 1 for additional measurement principles).

Enzyme concentrations are not measured directly, but the serum activity of an enzyme is considered to be directly proportional to its concentration. Currently, enzyme activities are reported in terms of units per liter (U/L), with a unit defined as the quantity of enzyme that catalyzes the reaction of 1 µmol of substrate per minute.[101] Although this unit was historically referred to as an international unit (IU), the actual SI unit (Système International, for international uniformity) is the katal, which describes enzyme activity in moles per second; using the SI system enzyme activity is reported in terms of katals per liter (kat/L).[101] For conversion of units, IU/L × 0.01667 = µkat/L.[156]

Basic concepts and information that must be considered to properly interpret the results of serum enzyme assays include:

- the difference between leakage enzymes and induced enzymes;
- the duration of enzyme activity after passage into the blood (i.e., the enzyme's biologic half-life in the blood);
- the tissue specificity of enzymes;
- the proper handling and storage of serum for enzyme assays.

Leakage vs induced enzymes

Increased serum enzyme activities can result from either leakage or induction. This distinction is most applicable to the liver because of the array of enzymes used in evaluation of liver disease. Enzymes can be released when cell injury alters cell membranes; enzymes that pass into the extracellular space and then into the serum by this mechanism are termed *leakage enzymes* (Fig. 26.1). The term is somewhat misleading, however. Although membranes of fatally injured cells can certainly leak enzymes as they degrade, sublethally injured cells may release membrane blebs that later rupture, resulting in increased serum enzyme activity.[60] Nevertheless, it is useful to think of the process in terms of leakage. By contrast, induction involves the increased production of an enzyme by cells that normally produce the enzyme in smaller quantities. This increased production is induced by some type of stimulus, and it results in increased release of the enzyme from the cells and increased activity of the enzyme in serum. Enzymes that pass into the serum by this mechanism are termed *induced enzymes* (Fig. 26.2).

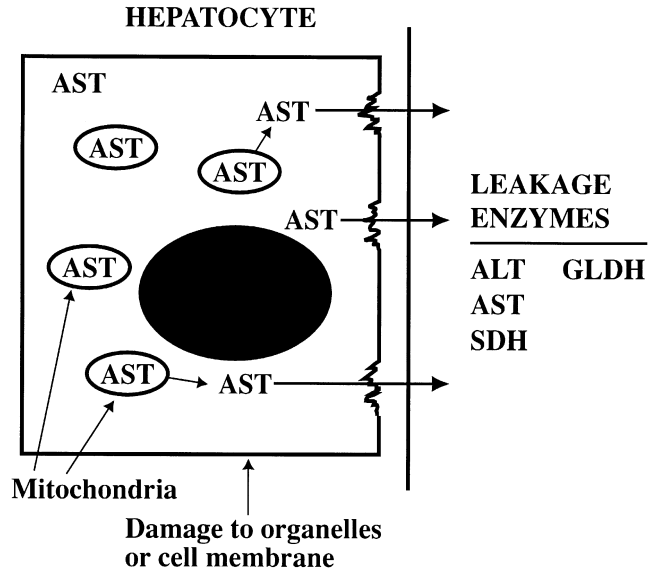

Figure 26.1 Leakage enzymes escape from the cell because of altered plasma membranes. Some leakage enzymes, such as AST, are also present in the organelles. More severe damage is required to cause leakage from these organelles.

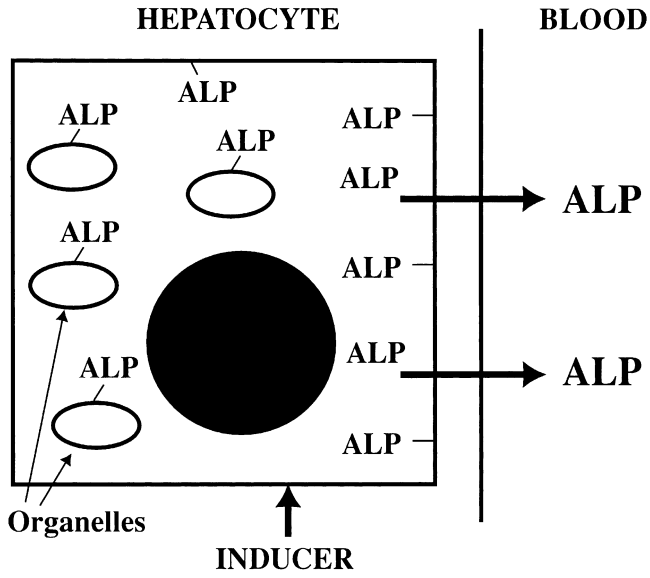

Figure 26.2 Increased serum activities of induced enzymes result, in part, from increased production of these enzymes, with a subsequent increase in secretion. This increased production is caused by some type of inducer.

Leakage enzymes are present in the cytosol, organelles, or both, and escape following sublethal or lethal (i.e., necrosis) cell injury. Increased serum enzyme activities can be detected within hours of the injury.[24] By contrast, induced enzymes are attached to cell membranes. Increased serum activity of these enzymes depends primarily on increased production and develops more slowly (i.e., days rather than hours).[24]

The concept of leakage versus induced enzymes is important, but the difference is not entirely clear-cut in clinical situations. For example, acute hepatocyte injury can result in loss of leakage enzymes, but enzyme production may be up-regulated in the subsequent reparative process of hepatic regeneration, resulting in a slower decline of serum enzyme activity than expected based on the enzyme half-life.[108] In addition, the release of membrane blebs containing membrane-bound enzymes may cause rapid but generally mild increases in serum activity of those "inducible" enzymes.[24] Release of induced enzymes can also occur secondary to less acute membrane alterations. The increased serum activities of alkaline phosphatase (ALP) and γ glutamyltransferase (GGT) that occur as a result of cholestasis are examples. A portion of the increase in the serum activities of these enzymes probably results from increased enzyme production, but the bile acids sequestered in bile canaliculi and ducts can solubilize membranes of hepatocytes and bile duct epithelial cells, resulting in increased release of these enzymes.[124,129]

Although activities of both leakage and induced enzymes often increase during most types of liver disease, the relative magnitudes of the increases can provide a hint about the underlying liver lesions. In diseases characterized primarily by hepatocyte injury, the activities of leakage enzymes tend to be increased to a greater degree than are the activities of induced enzymes. Similarly, in diseases characterized primarily by cholestasis, the activities of induced enzymes tend to be increased to a greater degree than are the activities of leakage enzymes. However, many liver diseases (particularly chronic diseases) result in both hepatocyte injury and cholestasis, so these distinctions are not always useful.

Enzyme half-life

After leakage or secretion from cells, the enzymes eventually are degraded and/or excreted from the body. Some enzyme molecules also lose their activity in the serum over time. The rate at which the loss of activity, degradation, or excretion occurs determines the length of time during which the enzyme activity is detectable in the serum after leakage or secretion. The disappearance rate of enzyme activity typically is measured as the biologic half-life of the enzyme, which is the time required for one-half of that enzyme's activity to disappear from the serum. Knowledge of the average biologic half-life of an enzyme is helpful when assessing how recently leakage or increased production has occurred and whether these processes are continuing. The biologic half-lives of various diagnostic enzymes and the use of enzyme half-lives in assessing tissue injury are discussed later.

Tissue specificity

It is important to know from which tissue the enzyme most likely originated. Knowledge of tissue specificity allows the diagnostician to narrow the list of possible tissues that are involved in a disease process. Tissue specificity is a function of:

- The presence or absence of the enzyme in the tissue. When increased serum activity of an enzyme is detected, only the tissues in which that enzyme is normally present are considered potential sites of injury.
- The concentration of the enzyme in tissues. An enzyme can be present in many tissues, but have high concentration in only one or a few. When increased serum activity of an enzyme is detected, the tissues in which that enzyme is found at the highest concentrations are the most likely sites of injury.
- Where the enzyme goes after leakage or secretion. Enzymes that are detected in serum have either leaked or been secreted into the extracellular spaces and then passed into the serum. Some tissues may have high enzyme concentration, but leaked or secreted enzymes are not readily accessible to blood. For example, injury to renal epithelial cells results in leakage of the enzyme GGT.[11] This enzyme leaks from the brush border of the cell into the lumens of the renal tubules, rather than into the extracellular space. Thus, increased GGT activity can be detected in the urine, but the serum activity does not increase.
- The half-life of different isoenzymes. Enzymes with the same catalytic activity might be produced in several different tissues, but these enzymes can vary regarding other properties. These different forms of enzymes are termed isoenzymes or isoforms (see later discussion of ALP isoenzymes versus isoforms), and they may have different half-lives in serum. If an isoenzyme has a very short half-life (e.g., minutes to a few hours) it is less likely to accumulate in the serum after leakage or secretion and, therefore, is less likely to be detected. If an enzyme originates from two different tissues, but the half-life of the isoenzyme from one tissue is minutes and that of the isoenzyme from the second tissue is days, then the increased serum enzyme activity is more likely to have originated from the second tissue. For example, the placenta of dogs contains large quantities of the enzyme ALP, but the half-life of the placental isoenzyme is minutes. Therefore, the placenta is not considered to be a likely source when an increased serum ALP activity is detected.[24]

The ideal diagnostic enzyme would be specific for only one tissue. Increased serum activities of such an enzyme would direct the diagnostician to that tissue as the site of a disease process. Almost no diagnostic enzymes are found in only one tissue; however, some are found in only a few tissues.

Diagnosticians commonly attempt to relate the magnitude of increased serum enzyme activities with the type or degree of injury in a tissue. It is tempting to assume that higher enzyme activities are indicative of more severe tissue injury (especially in the case of leakage enzymes), but this assumption is not always true (Fig. 26.3). Dead cells release all of

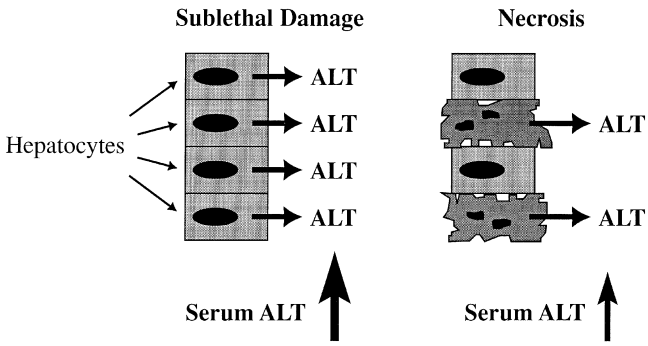

Figure 26.3 The magnitude of serum enzyme activity is not necessarily related to the severity of tissue injury. Serum ALT leaks from hepatocytes when their plasma membranes are injured. The resultant serum ALT activity can be greater after sublethal injury to many hepatocytes than after necrosis of a few hepatocytes.

their enzymes, and they produce no additional enzymes. Sublethally injured cells, however, lose only a portion of their enzyme content and continue to produce enzymes (possibly at an increased rate). Such cells can ultimately release more enzyme than can dead cells. In other words, necrosis can result in increased enzyme activity, but diffuse, sublethal injury to the same tissue can result in even greater serum enzyme activity. Thus, the magnitude of the serum enzyme activity is not a reliable indicator of the type or degree of tissue injury. The relative magnitude of increased serum enzyme activity is often referred to in terms of the fold increase above the upper reference limit (URL); for example, 3× URL signifies a 3-fold increase above the upper reference limit.

Sample handling

Unlike substances in serum that are directly measured (e.g., urea, creatinine, electrolytes), serum enzyme concentration is determined by measuring serum activity. It is then assumed that the activity is proportional to the serum concentration. If serum samples are not properly handled the enzyme activity can be altered leading to erroneous results. It is important to realize that serum enzymes are proteins that are subject to degradation or denaturation by heat, changes in pH, variable inherent stability, and exposure to various chemicals, any of which can result in loss of enzyme activity.[101]

Regardless of whether serum enzyme activities are assayed in an in-clinic laboratory or a reference laboratory, some delay usually occurs between sample acquisition and testing, making proper sample handling essential. While serum to be used in these assays should be harvested and assayed as soon as possible, most enzymes are stable in refrigerated, separated serum for 24 hours. The degree of degradation that occurs after 24 hours varies considerably depending upon the particular enzyme.[136] Hemolysis and lipemia should be avoided because of the potential to interfere with spectrophotometric assays (increased serum bilirubin may also interfere, but is not preventable). Some enzymes are present in erythrocytes, and hemolysis can also directly contribute to the increased enzyme activity. (See Chapter 3.)

Hepatocyte injury

Hepatocyte injury is detected by measuring the serum activities of hepatocellular leakage enzymes. Three serum enzymes (discussed below) are routinely measured to provide information about hepatocyte injury. An additional enzyme, GLDH, is available, but is primarily utilized in countries other than the United States. However, numerous other enzymes are utilized in laboratory animals as biomarkers of hepatic toxicity.[107]

Alanine aminotransferase

Alanine aminotransferase (ALT), previously referred to as serum glutamic pyruvic transaminase (SGPT), is a leakage enzyme that is free in the cytoplasm. In dogs and cats, the highest concentrations of ALT occur in hepatocytes (especially those in periportal regions), and the ALT assay is commonly included in the serum biochemical profiles of these species.[9] ALT activity is sometimes the only test used to detect hepatocyte injury in dogs and cats because ALT is much more liver specific than AST (discussed below). However, ALT is not totally liver specific; severe muscle damage or disease can cause increases in serum ALT activity.[138] ALT activity of muscle is less than that of the liver (activities in skeletal and cardiac muscle are approximately 5% and 25% that of liver activity, respectively).[9] Because the total mass of muscle is much greater than that of the liver, muscle can be a significant source of ALT leakage. While increased serum ALT activity in dogs and cats is usually indicative of either hepatocyte death or sublethal hepatocyte injury, necrosis or sublethal damage to muscle cells must be considered as well. When ALT activity is increased, measurement of a serum enzyme activity that is more muscle specific (e.g., creatine kinase [CK]) is helpful in order to determine if muscle damage is a possible source of the increased ALT.

Horses and ruminants have low ALT concentration in hepatocytes; consequently, serum ALT activity is not useful for detecting liver disease in these species.[9] Moderate amounts of ALT are present in the muscle of horses and ruminants, and moderate increases in the serum ALT activity occur with muscle injury in these species;[9] however, ALT is not included in large animal biochemical profiles. Other muscle-specific enzymes (e.g., CK) are more commonly used for detecting muscle injury in these species.

In dogs and cats, a wide variety of liver diseases can produce increased serum ALT activity. Hypoxia, metabolic alterations resulting in hepatocyte lipid accumulation, bacte-

rial toxins, inflammation, hepatic neoplasia, and a multitude of toxic chemicals and drugs can cause hepatocyte injury, thereby resulting in ALT leakage. Acutely, the serum activity of ALT is proportional to the number of cells that are injured, but as illustrated in Figure 26.3, the magnitude of ALT activity is not indicative of the *cause* of the injury or of the *type* of damage to the hepatocytes (sublethal damage versus necrosis). After acute severe injury, such as from a toxin, serum ALT activity can increase markedly within a day or two.[24] If the injury is not ongoing, ALT activity slowly decreases over several weeks. Serum ALT activity can also be increased during recovery from liver injury when active hepatocyte regeneration is occurring; this may explain why ALT activity does not always return to normal as quickly as expected based on its serum half-life, which has been estimated at 17 to 60 hours in dogs and 3.5 hours in cats.[108,10] More chronic inflammatory liver diseases can result in periodic "flares" of increased ALT activity. Thus, repeated measurement of serum enzyme activity may give insight about the underlying disease process. However, it is important to recognize that in certain situations significant liver disease can occur with normal or only slightly increased serum ALT activity. For example, if liver disease is severe and the hepatic mass is markedly decreased there may be too few remaining hepatocytes to result in markedly increased serum enzyme activity, even if the remaining cells are injured and leaking ALT. Some chronic diseases may have little active hepatocyte injury taking place, resulting in little enzyme leakage. Also, a few toxins (aflatoxin, microcystin) seem to interfere with transaminase production; massive acute hepatic necrosis may occur with minimal increases in ALT (or AST).[24,43]

Increases in serum ALT activity can also be observed in dogs with hyperadrenocorticism or that have been administered corticosteroids.[120] These increases are generally mild (two- to fivefold), but ALT activity increases can vary widely among dogs receiving corticosteroid therapy, depending on the dose and duration of treatment.[6,45,99] It is not entirely clear whether these increases in ALT activity are due to induction of enzyme production by steroids (as is well-documented for the induction enzymes ALP and GGT) or due to actual hepatocyte membrane injury; morphologic changes in hepatocytes develop within days after steroid therapy begins and resolve slowly after therapy ceases.[6,45,61]

Anticonvulsant drugs (e.g., phenobarbital, primidone, phenytoin) also cause mildly increased serum ALT activity in dogs.[17,150] Because most dogs remain clinically healthy and liver biopsies have not shown morphologic evidence of liver damage these increases have been attributed to induction (up-regulation of enzyme production); however, in vitro studies have not supported that hypothesis.[56,102] In addition, some dogs receiving anticonvulsants will develop a toxic hepatopathy, in which case ALT activity may be markedly increased due to hepatocyte injury.[41]

Aspartate aminotransferase

Aspartate aminotransferase (AST), previously termed serum glutamic oxaloacetic transaminase (SGOT), is present at highest concentrations in hepatocytes and muscle cells (both skeletal and cardiac) of all species.[9] Therefore, AST is not a liver-specific enzyme. AST is a leakage enzyme that is found predominantly in the cytoplasm, with about 20% located within mitochondria.[80] By contrast to ALT, hepatocyte AST is in highest concentration in cells of the periacinar region, surrounding central veins (zone 3).[24] Increased serum AST activity can result from lethal or sublethal injury to either hepatocytes or muscle cells.

In dogs and cats serum AST activity will increase as a result of the same liver diseases previously listed for ALT and generally parallels ALT activity, but the magnitude of the increase may be less than that of ALT.[24] The serum AST activity may return to baseline faster than ALT following acute liver injury in some animals, making repeated measurements useful for monitoring disease resolution. Although AST is less liver specific than ALT, it may be more sensitive than ALT for detecting some liver diseases in dogs and cats.[24] For example, one study reported 89% of cats with hepatic lipidosis had increased AST activity compared to 72% for increased ALT activity.[23]

Similar to ALT, mild increases in AST activity may be seen in dogs as a result of enzyme induction due to corticosteroids and possibly phenobarbital, although there is some controversy in the literature.[44,102,150] Because muscle is a possible source of serum AST activity, measurement of an enzyme specific for muscle injury (i.e., CK) is useful to determine if the increase in AST activity is due to muscle injury.

In horses and ruminants, AST is often used for the routine detection of hepatocyte injury since it is included in most large animal biochemical profiles and because of low hepatocyte ALT concentration (discussed earlier). In these species, an increased serum AST activity can result from the same spectrum of liver diseases (both sublethal and necrotic) as listed for ALT. The major problem with AST in detecting hepatocyte injury is its lack of liver specificity. As in dogs and cats, increased serum AST activity in horses and ruminants can result not only from hepatocyte injury, but also from muscle injury. This problem can be mitigated to a certain extent by assaying a muscle-specific enzyme such as CK along with AST. Increased AST activity with normal CK activity may be seen if the source of the AST is the liver, suggesting hepatocyte injury has occurred. Uncertainty remains in such a case, however, because the half-life of CK is shorter than that of AST (Fig. 26.4). Serum activities of both enzymes may increase as a result of muscle injury, but the CK activity may return to normal earlier than the AST activity.[20] These problems with use of AST in detecting hepatocyte injury in horses and ruminants have led to use of more liver-specific enzymes (such as sorbitol dehyrogenase [SDH]) in these species.

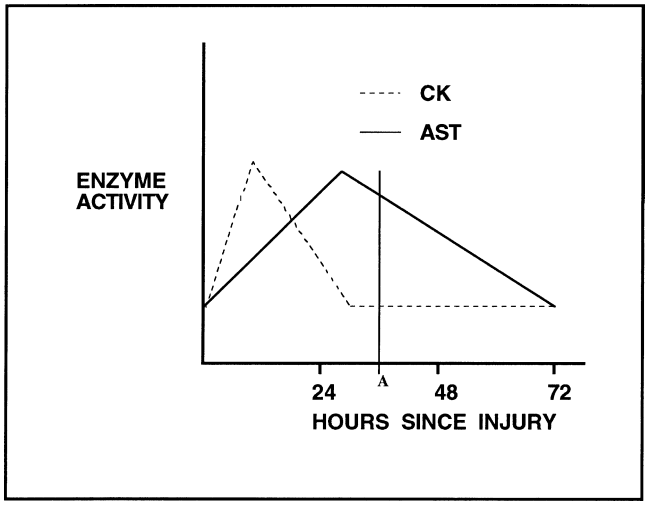

Figure 26.4 Serum activities of both AST and CK increase because of muscle injury. As illustrated here, these activities increase and decrease at different rates. Depending on when a blood sample is analyzed after muscle injury, it is possible to detect increased serum AST activity and normal serum CK activity (note time A) and to erroneously interpret this as being an indication of hepatic injury.

As discussed for ALT, the serum activity of AST may be normal or only slightly increased with significant liver diseases that are chronic and low-grade, that have resulted in markedly decreased hepatic mass, or that are due to toxins that inhibit transaminase activity. In dogs and cats the half-life of AST is shorter than that of ALT, and has been estimated between 4 and 12 hours in dogs, and 77 minutes in cats.[9,148] In horses, the half-life of AST has been estimated at 7 to 8 days.[20]

Sorbitol dehydrogenase (iditol dehydrogenase)

Sorbitol dehydrogenase (SDH), also called iditol dehydrogenase (ID), is a leakage enzyme that is free in the cytoplasm. It is present at high concentration in the hepatocytes of dogs, cats, horses, and ruminants, but its concentration in other tissues in these species is low.[9] Therefore, SDH is a liver-specific enzyme. Increased serum SDH activity is suggestive of either hepatocyte death or sublethal hepatocyte injury. SDH is not superior to ALT for detecting hepatocyte injury in dogs and cats, and it is not commonly used in these species. In horses and ruminants, however, SDH is much more specific than AST for detecting hepatocyte injury. The half-life of SDH is very short (<2 days); serum activities may return to normal within 4–5 days after acute hepatocyte injury.[8] The main disadvantage to SDH is that it is less stable in vitro than most other diagnostic enzymes, and the stability varies by species. In both horses and cattle, however, SDH is stable in room temperature or refrigerated serum for as long as 5 hours (24 hours refrigerated in cattle), and for as long as 48 hours (72 hours in cattle) when frozen.[71] In llamas, SDH is stable in serum for 8 hours, and in refrigerated or frozen serum for up to one week.[143] In dogs, SDH is stable in serum for 4 hours at room temperature, 48 hours refrigerated, 1 week frozen at –20°C, and 1 month frozen at –70°C.[42] In most cases, these time periods should be sufficient to allow the delivery of serum to a laboratory for an SDH assay. Because SDH is preferable to AST for detecting hepatocyte injury in horses and ruminants, one should identify a laboratory that can perform this assay within the appropriate time frame. This assay is not available in a point of care format.

Glutamate dehydrogenase

Glutamate dehydrogenase (GLDH) is a leakage enzyme present in highest concentration within mitochondria of hepatocytes, predominantly in periportal regions.[9] The same types of reversible and irreversible hepatocyte injury that cause increased serum activity of ALT will cause increased serum activity of GLDH. Increased serum concentration of this enzyme is reported to have excellent sensitivity for the detection of canine hepatic disease.[114,136] The sensitivity for equine hepatic disease is reported to be good, but slightly less than that of GGT.[89] GLDH activity is stable in canine serum for 2 days at room temperature, 7 days refrigerated at 4°C, and 6 months frozen at –20°C.[114] Serum activity of GLDH may increase in dogs with hyperadrenocorticism; increases have also been documented in dogs receiving anticonvulsants.[1,114] Assays for this enzyme have not been routinely available in the United States, but are used more commonly in other countries.

Cholestasis

Cholestasis (impaired bile flow) can be detected by measuring the activities of serum enzymes whose increased production is induced by cholestasis or by measuring the serum concentrations of substances (either endogenous or exogenous) that normally are considered tests of liver function and are discussed later. The two serum enzymes used to detect cholestasis are alkaline phosphatase (ALP) and γ-glutamyltransferase (GGT).

Alkaline phosphatase

Alkaline phosphatase (ALP) is an induced enzyme that is attached to cell membranes and synthesized by many tissues such as liver, bone, kidney, intestine, pancreas, and placenta.[9,50,80] In domestic animals two ALP isoenzymes are produced from two different genes; these are referred to as the intestinal isoenzyme and the tissue-nonspecific isoenzyme.[50] The tissue-nonspecific isoenzyme undergoes further posttranslational modification in different tissues resulting in different isoforms from liver (LALP), bone (BALP), kidney, and placenta. The intestinal isoenzyme can also undergo

further modification to produce the unique corticosteroid induced isoform (CALP) in dogs. These different isoforms are often mistakenly referred to as different isoenzymes, but isoenzymes must be produced from different genes. Most of the normal serum ALP activity originates from the liver. The half-life of intestinal, renal, and placental ALP in dogs is approximately 6 minutes, and the half-life of intestinal ALP in cats is approximately 2 minutes, thus they are unlikely to cause increased serum ALP activity.[50] Increased ALP production and increased serum ALP activity commonly occur with cholestasis, increased osteoblastic activity, induction by certain drugs (primarily in dogs), and a variety of chronic diseases.

Increased serum alkaline phosphatase activity

Hepatobiliary disease

ALP in the liver is associated with biliary epithelial cells and canalicular membranes of hepatocytes.[24] A variety of hepatobiliary diseases can result in increased serum ALP activity due to increased enzyme production, solubilization of membranes by the action of bile salts, and release of membrane blebs after cell injury.[50,60,124,129] Cholestatic diseases can result in marked increases in serum ALP activity in dogs (greater than 10 fold URL), but increases are more variable in other species.[24,35,50,68] Impaired bile flow induces increased ALP production, and sequestration of bile salts in the biliary system causes solubilization of ALP molecules attached to cell membranes, which are then released into the blood.[65,78,124,129] The half-life of the cholestasis-induced LALP is approximately 3 days in dogs but only 6 hours in cats.[24] In cats, this short half-life, in addition to lower liver ALP concentration per gram of tissue, contributes to the relatively smaller magnitude of serum ALP activity increases seen with liver disease in cats compared to dogs.[24] However, ALP is still a useful enzyme for evaluation of feline cholestatic liver disease if one keeps in mind that even mild increases (2–3× URL) can be significant.[24] The utility of ALP for detection of cholestasis in horses and ruminants is generally considered inferior to that of GGT (discussed later).[50,68] Wide reference intervals for ALP in horses and ruminants contribute to the reduced sensitivity of the serum ALP assay for the detection of liver disease in these species.

When cholestasis is the cause of increased serum ALP activity, serum total bilirubin and bile acid concentrations may be increased concurrently. In dogs with cholestasis, serum ALP activity often increases prior to increases in serum bilirubin concentration; thus ALP is a more sensitive indicator of cholestasis in dogs.[133,136] However, even if the serum bilirubin concentration is normal, bilirubinuria may accompany cholestasis-induced increases in ALP. Whereas lesions primarily involving the intra- or extrahepatic biliary system are common causes of cholestasis, hepatic diseases resulting in significant hepatocyte swelling (e.g., lipidosis or inflammation of the hepatic parenchyma) can obstruct small bile canaliculi and induce increased ALP production and release.[22] It is also important to realize that pancreatic and intestinal lesions can sometimes be the primary cause of cholestasis due to extrahepatic bile duct obstruction.

Osteoblastic activity

Increased serum ALP activity associated with increased osteoblastic activity occurs in all species. These increases are most often detected in young, growing animals when the results of ALP assays are compared with adult reference intervals for ALP. For instance, the mean serum activity of alkaline phosphatase of bone origin (BALP) in immature cats in one study was more than ten times that of adult cats, resulting in a mean total ALP activity more than twice that of the adults.[70] Another study found the reference interval for total ALP activity in four-week old kittens was 97–274 U/L compared to 10–80 U/L for adult cats.[86] Since age-specific reference intervals are seldom provided, one must remember that young animals commonly have serum ALP activities greater than adult reference intervals. In puppies, kittens, and calves, ALP activity increases attributed to bone growth are generally mild (<4–5× URL), but foals may have increases up to 20× URL in the first three weeks of life.[50,64]

A few causes of increased osteoblastic activity in mature animals may result in mildly increased serum ALP activity due to production of BALP. Osteosarcoma and other bone neoplasms (both primary and secondary) inconsistently result in increased serum ALP activity because of osteoblast proliferation in these processes, and dogs with osteosarcoma and increased serum ALP activity appear to have a worse prognosis than those with normal ALP activity.[47,50,54] Fracture healing usually results in localized increases in osteoblastic activity and mild increases in serum ALP that may be useful for monitoring the progression of healing. In one study dogs with uncomplicated fracture healing had mild increases in serum ALP activity that returned to normal with bone union, while dogs with failure of bone union had no changes in serum ALP activity.[82] Canine hyperparathyroidism (primary or secondary) and feline hyperthyroidism may result in increased bone turnover and increased osteoblastic activity; mild increases in serum ALP may be detected in patients with these diseases.[50]

Induction by drugs (dogs)

Serum ALP activity can be markedly increased when enzyme production is induced by certain drugs. Drug-induced ALP production is well documented in dogs, but not in other species, thus the following discussion pertains to dogs only. Corticosteroids (exogenous or endogenous) and anticonvulsants (e.g., phenobarbitol, phenytoin, primidone) induce increased ALP production by canine hepatocytes. Exogenous corticosteroids in any form (oral, parenteral, topical, ophthalmic, and otic) have been implicated.[72,92] Increased serum ALP activity induced by corticosteroids

varies depending on dose and duration of exposure, but can be marked (>20× URL).[6,24] Anticonvulsants generally cause somewhat milder increases (<10× URL).[24,41,55]

Corticosteroids induce production of a unique isoform (CALP) that is distinct from that produced by hepatocytes in response to cholestasis (LALP). Although it is possible to distinguish LALP from CALP with special laboratory tests, the clinical utility of such distinction is uncertain. In dogs given corticosteroids the initial rise in serum ALP activity is due to LALP, while CALP increases after a 10-day lag period, and there appears to be considerable individual variation in the degree of ALP induction caused by corticosteroids.[45,99,130,131,154] Although not specific, increased CALP activity is common in dogs with naturally occurring hyperadrenocorticism, and a *lack* of increased CALP argues against hyperadrenocorticism in suspect cases.[75,131] Although many dogs with increased blood corticosteroid concentration will have increased serum ALP activity due to induction, some will develop a steroid hepatopathy with swelling of hepatocytes due to glycogen deposition. Evidence of decreased hepatic function (increased total bilirubin or bile acids) is uncommon in these dogs, but can occur in severe cases.[24] Chronic stress resulting in increased blood concentration of endogenous steroids may also cause increases in CALP activity.[24,50]

To help distinguish cholestasis-induced from corticosteroid-induced increases in serum ALP in dogs, other tests can be performed. These tests include serum and urinary bilirubin concentration, serum bile acid concentration, and tests to detect hyperadrenocorticism. A suggested approach is presented in Figure 26.5. The concurrent presence of increased ALP activity and hyperbilirubinemia is strongly suggestive of cholestasis, but the serum bilirubin concentration may be normal in some cases of cholestatic disease (e.g., early in the disease process or if only a portion of the biliary tree is obstructed). In the latter situation, the unobstructed portion of the biliary system excretes enough bilirubin that serum concentration remains within the reference interval.

Dogs receiving anticonvulsant drugs (e.g., phenobarbitol, phenytoin, primidone) often have increased serum ALP

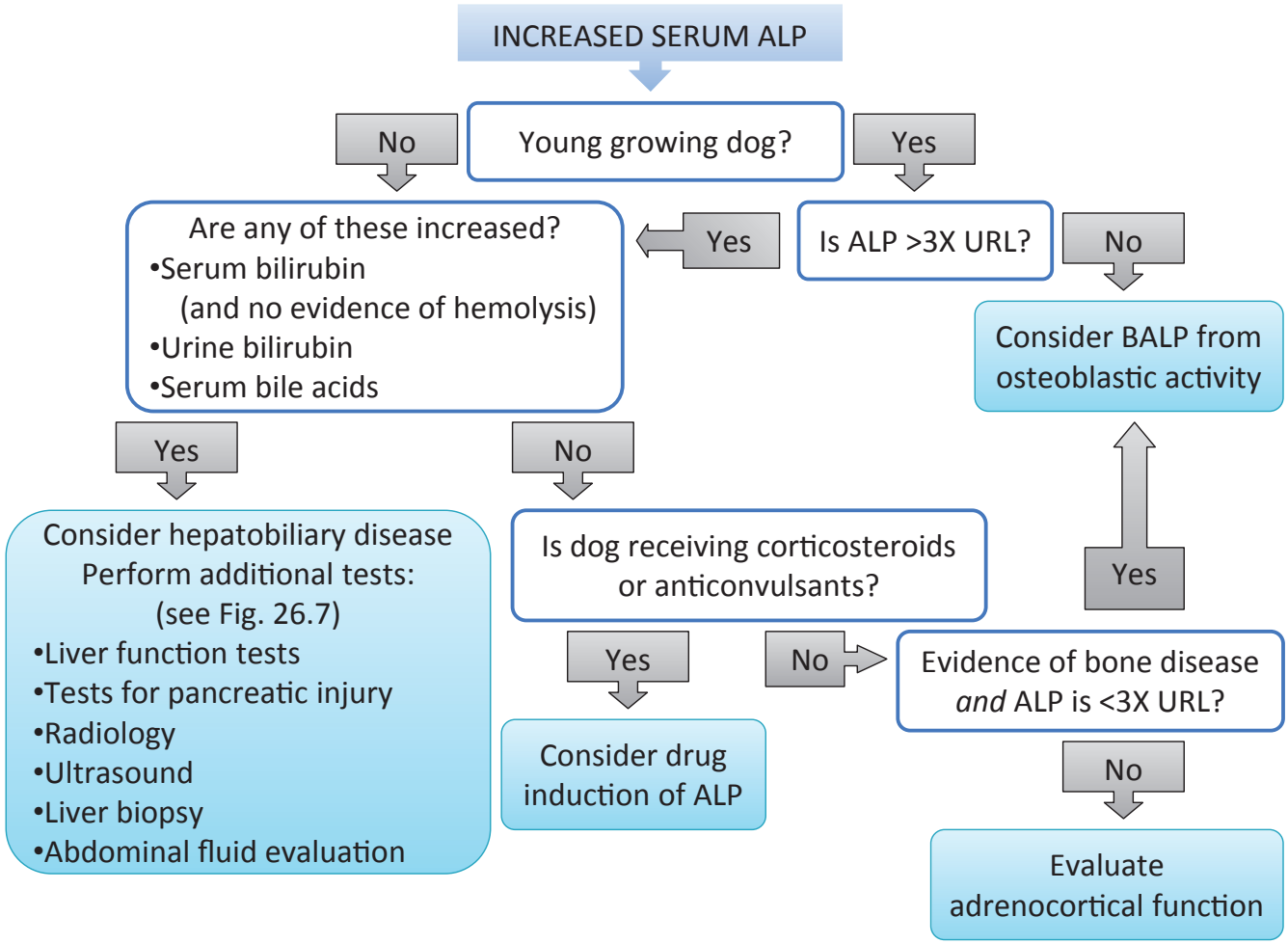

Figure 26.5 Flow chart for evaluating the possible causes of increased serum ALP activity in dogs.

activity, which may be due to LALP, CALP, or both.[24,55] Most of these dogs remain clinically healthy, but anticonvulsants are also known to cause a toxic hepatopathy in dogs. In healthy dogs the serum ALP increases have been attributed to induction, but this has not been confirmed by in vitro studies and remains controversial.[56,102] Animals that develop a toxic hepatopathy will usually have other indications of decreased hepatic function (increased total bilirubin or serum bile acids), as well as histologic abnormalities.[41]

Miscellaneous other causes

Neonates of several species have high serum ALP activity following ingestion of colostrum. During the first few days of life puppies, kittens, and lambs have transient marked increases in serum ALP activity (up to or >30× URL for adult animals).[14,34,86] Foals and calves do not have such marked increases following colostrum ingestion, although serum ALP activity is increased compared to adults due to BALP, as discussed earlier.[10,64,157]

A variety of endocrine diseases have been associated with increased serum ALP activity. Hyperadrenocorticism has already been discussed as a cause of often marked corticosteroid-induced ALP activity increases in dogs. The precise mechanisms for the generally mild increases seen with other endocrine diseases are not clearly defined, but are likely multifactorial; remember that stress associated with any chronic disease may increase endogenous corticosteroids and result in induction of CALP in dogs. Such diseases include diabetes mellitus, canine hypothyroidism and hyperparathyroidism, and feline hyperthyroidism; as many as 80% of hyperthyroid cats are reported to have increased serum ALP activity (generally <4× URL, due to both BALP and LALP).[15,50,52,69,115]

Neoplasia may be associated with increased serum ALP activity. Hepatic neoplasia may directly cause cholestasis, and bone neoplasia may be associated with increased osteoblastic activity (both discussed earlier). In addition, mammary gland neoplasia, without metastases to bone or liver, has been identified as a cause of increased serum ALP activity in dogs. Serum ALP activity increases may be seen with benign or malignant mammary gland tumors, are generally mild (<8× URL), and do not appear to have prognostic value.[63,79]

In cattle there are mild increases in ALP activity associated with pregnancy (mid to late gestation) and early lactation.[123,155] In dogs serum ALP activity increases slightly during pregnancy, but remains within reference intervals.[50]

Breed-related increases in serum ALP activity have been identified in Siberian husky and Scottish terrier dogs. ALP activity in some Siberian huskies was >5× that of siblings in eight related litters; no underlying cause was identified.[84] Similarly, increased serum ALP activity in comparison to other breeds has been reported in Scottish terriers, with ALP activity as high as 15× URL.[53] These observations await further characterization.

γ–Glutamyltransferase

γ–Glutamyltransferase (GGT) is considered an induced enzyme. Acute hepatic injury, however, can produce rapid increases in serum GGT activity, likely due to release of membrane fragments to which GGT is attached.[60] Most body tissues synthesize GGT, with the highest concentrations occurring in the pancreas and kidney.[9,12,80,118] It also is present at lower concentrations in hepatocytes, bile duct epithelium, and intestinal mucosa and at high concentrations in the mammary glands of cattle, sheep, and dogs. Most of the serum GGT activity originates in the liver (except for neonates of some species, discussed later). Release from renal epithelial cells results in increased urinary GGT activity, but not increased serum GGT activity (see Chapter 23). Similarly, pancreatic cells release GGT into pancreatic ducts rather than into the blood.

Increased GGT production, release, and subsequent increased serum GGT activity occur with cholestasis and biliary hyperplasia.[16,85] The increased serum GGT activity that occurs with cholestasis may result from both increased production and solubilization of GGT attached to cell membranes.[24] In dogs, increased GGT activity also occurs as a result of drug induction, similar to that described for ALP.[24]

Experimentally, extrahepatic bile duct obstruction in dogs results in increases in GGT activity up to 50 fold within two weeks; similar studies in cats found increases up to 16 fold.[24,105,132] For the detection of liver disease in dogs, GGT is more specific, but less sensitive, than ALP.[35] For the detection of liver disease in cats, GGT is more sensitive, but less specific, than ALP (the exception is hepatic lipidosis, discussed below).[27] In both dogs and cats, results of serum ALP and GGT assays performed in combination to detect hepatobiliary disease are more diagnostically valid than those of either enzyme assay used alone.[27,35] Cats with hepatic lipidosis usually have greater relative increases in serum ALP activity compared to GGT, which may be within the reference interval or only minimally increased.[24,30] However, if there is an underlying necroinflammatory disease present that is the primary cause of the hepatic lipidosis, GGT activity may be increased to a greater degree than is ALP activity.[23,27]

Similar to ALP, increases in serum GGT activity are seen in dogs receiving corticosteroids, but it is not clear whether these increases are due to increased GGT production or are secondary to steroid hepatopathy.[61,130] When the increase in GGT activity is induced by corticosteroids, GGT activity increases more slowly and to a lesser degree than does ALP activity.[6] Drug induction of GGT activity has also been reported in dogs receiving anticonvulsant medication, but resulting increases are minimal (2–3× URL) and may not even exceed reference intervals.[24,93,102] If increases of greater magnitude are seen in dogs receiving such medications, they are more likely the result of cholestasis. Marked increases in the serum GGT activity of a dog being treated

with anticonvulsant medication may be indicative of a drug-associated toxic hepatopathy with life-threatening implications.[18,24]

In horses and cattle, GGT is generally considered more sensitive than ALP for detection of cholestasis. In horses with experimental cholestasis induced by bile duct ligation, serum GGT activity increased to a greater degree than did ALP activity.[68] Cattle and horses with pyrrolizidine alkaloid toxicity, which causes marked biliary hyperplasia and eventual liver failure, consistently have early increases in serum GGT activity.[38,39] However, ALP activity may be increased to a greater degree than GGT activity in more chronic cases.[39] Cattle with moderate to severe hepatic lipidosis have only mild increases in serum GGT activity (2–3× URL).[21]

High serum GGT activity in the colostrum of dogs, cattle and sheep can result in extremely high activities in the serum of young puppies, calves, and lambs that have consumed colostrum.[34,111,140] In calves, the GGT activity can be >50× URL for adult animals during the first few days after birth.[10,140,157] Typically, the GGT activity declines over a period of weeks to reach normal adult levels by about 5 weeks of age. Lambs also have markedly increased serum GGT activity after colostrum consumption, with this activity falling to within the adult reference intervals by approximately 30 days of age.[111] In puppies, marked increases in GGT activity (up to 100× URL for adults) following colostrum ingestion return to normal adult levels more quickly, by about 10 days of age.[34] Increased serum GGT activity also occurs in foals and is typically 1.5–3× URL during the first month of life, but this enzyme activity is apparently not of colostral origin.[13,110]

Liver function

Tests of liver function include measurement of the serum concentrations of substances that normally are removed from the blood by the liver and then metabolized or excreted via the biliary system (e.g., bilirubin, bile acids, ammonia, cholesterol), and substances that normally are synthesized by the liver (e.g., albumin, globulins, urea, cholesterol, coagulation factors). Although abnormal blood concentrations of these substances can result from nonhepatic factors, the detection of abnormal concentrations *in addition to evidence of liver injury* (as detected by changes in leakage or induced enzyme activities) can supply further evidence of significant liver disease or liver failure. Often, however, liver biopsy is required for a definitive diagnosis.

Bilirubin

Normal bilirubin metabolism

Bilirubin is formed primarily from the degradation of hemoglobin (Fig. 26.6), with a small contribution from other

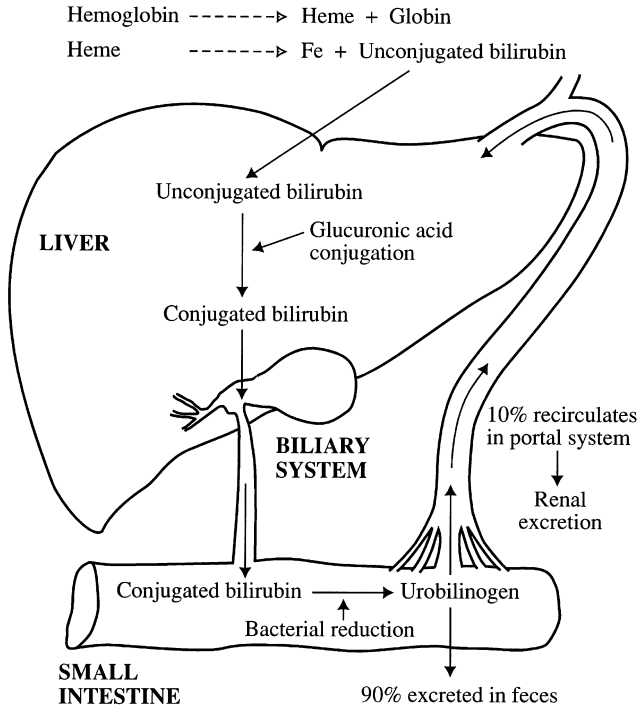

Figure 26.6 Normal bilirubin metabolism.

hemoproteins (e.g., myoglobin, cytochromes, peroxidase, catalase).[119] Erythrocytes normally are destroyed at a constant rate because of aging, but they also can be destroyed at an increased rate because of hemolytic processes (discussed later). Senescent erythrocytes, which have reached the end of their normal life span, are phagocytized by mononuclear phagocytes primarily in the spleen, but also in the liver and the bone marrow. The hemoglobin of phagocytized erythrocytes is catabolized. The globin portion is converted to amino acids, and the heme portion is split into iron and protoporphyrin. The iron is recycled, but the protoporphyrin is converted first to biliverdin and then to bilirubin. The newly formed unconjugated bilirubin (U-bilirubin, also called indirect bilirubin) is released from macrophages, noncovalently bound to albumin, and transported in blood to the liver sinusoids, where it is released from albumin and enters hepatocytes. Passage through the hepatocyte membrane is facilitated by a carrier, the capacity of which can be saturated if too much bilirubin is presented to the liver (as occurs with increased erythrocyte destruction).

Once inside the hepatocyte U-bilirubin is bound by proteins (Y protein or ligandin, and Z protein), which limit efflux of U-bilirubin back into the plasma.[36] In the hepatocyte, U-bilirubin is conjugated to sugar groups forming conjugated bilirubin (C-bilirubin, also called direct bilirubin). In many mammals, the major sugar group to which bilirubin is conjugated is glucuronic acid, resulting in the formation of bilirubin glucuronide. This reaction is cata-

lyzed by membrane-associated enzymes of the UDP-glucuronosyltransferase superfamily.[74] Both monoglucuronides and diglucuronides are formed in mammals, with the latter being the predominant form of conjugated bilirubin in bile. In addition to glucuronides, alternate conjugates (e.g., glucosides, glucoside-glucuronide mixed conjugates, xylosides) are produced in some species, with glucosides predominating in horses.[37,51] Conjugated bilirubin is not tightly protein bound, and is more water soluble than the protein-bound, unconjugated bilirubin. Most C-bilirubin is actively transported against the concentration gradient into bile canaliculi and then excreted in the bile. A small amount of C-bilirubin normally passes through the sinusoidal side of the hepatocyte membrane and back into the blood. If this C-bilirubin remains unbound to protein, it is quickly excreted by the kidney via glomerular filtration. A portion of the C-bilirubin in the blood may become covalently bound to albumin and is termed biliprotein or delta bilirubin.[122] This form of C-bilirubin does not pass through the glomerulus and remains in the blood for a longer period of time. (The implications of delta bilirubin in the assessment of cholestatic disease are discussed later.)

Conjugated bilirubin that is secreted into bile canaliculi passes with the bile into the small intestine where it is converted to urobilinogen by bacterial reduction. Approximately 90% of the urobilinogen is excreted with the feces as stercobilinogen. The remaining 10% of urobilinogen is reabsorbed and enters the blood. A portion of this urobilinogen then is removed from the blood by the hepatocytes and is re-excreted. Another portion of the urobilinogen is excreted in the urine by glomerular filtration.

Abnormalities of bilirubin metabolism

Three main pathologic processes can cause increased serum bilirubin concentration (hyperbilirubinemia). These include increased bilirubin production (due to accelerated erythrocyte destruction), decreased bilirubin uptake or conjugation by hepatocytes, and decreased bilirubin excretion (cholestasis).

Increased production of bilirubin most often occurs due to hemolytic disease (extra- or intravascular hemolysis), but can also result from massive internal hemorrhage and subsequent breakdown of erythrocytes in the area of that hemorrhage. During the process of extravascular hemolysis, macrophages remove and destroy erythrocytes just as they do senescent erythrocytes, but at an accelerated rate. Hemoglobin breakdown and bilirubin delivery to the liver then occurs in the normal way. During the process of intravascular hemolysis, the free hemoglobin that is released into the blood forms complexes with haptoglobin. These complexes are removed from the circulation by mononuclear phagocytes with subsequent breakdown of hemoglobin and U-bilirubin production. Whatever the specific underlying cause, increased erythrocyte destruction and production of increased amounts of U-bilirubin may overwhelm the liver's capacity for U-bilirubin uptake or C-bilirubin excretion, resulting in an increased serum bilirubin concentration. This is often referred to as prehepatic hyperbilirubinemia.

By contrast, hepatic hyperbilirubinemia may result from decreased uptake or conjugation of bilirubin by hepatocytes. (Intrahepatic cholestasis, discussed later, is also a cause of hepatic hyperbilirubinemia.) Decreased functional hepatic capacity due to acute or chronic hepatic disease can cause both decreased bilirubin uptake and decreased bilirubin conjugation. Hereditary defects in conjugation due to enzyme deficiencies occur in people, but have not been confirmed in animals. There has been one report of persistent hyperbilirubinemia in a horse that appeared to have a congenital defect in an enzyme required for bilirubin conjugation, similar to Crigler-Najjar syndrome in people; the exact enzymatic defect was not characterized.[46] Two forms of inherited hyperbilirubinemias have been identified in sheep. Mutant Southdown sheep can have hyperbilirubinemia associated with defective hepatocyte uptake of bilirubin from the serum producing increased serum U-bilirubin concentration; this is similar to Gilbert syndrome in humans.[96] Mutant Corriedale sheep can have hyperbilirubinemia associated with defective hepatic excretion of conjugated bilirubin producing increased serum C-bilirubin concentration; this is similar to Dubin-Johnson syndrome in humans.[97]

Decreased uptake of bilirubin occurs in some species secondary to fasting. This type of hyperbilirubinemia is most obvious in horses. It can result in serum bilirubin concentration that plateaus at 5–6 mg/dL by 64–136 hours after initial food deprivation; bilirubin concentration up to 8.5 mg/dL has been reported due to fasting alone.[100,103] Fasted cattle develop milder hyperbilirubinemia (<1.4 mg/dL).[117] Small increases in the serum bilirubin concentration occur in other species when deprived of food. These increases result from an increased serum concentration of U-bilirubin, but they do not appear to relate to increased bilirubin production. The mechanisms responsible for fasting hyperbilirubinemia have not been determined, but increased blood fatty acid concentrations correlate with hyperbilirubinemia. Increased fatty acids in the blood may compete for binding to Y and Z proteins in hepatocytes, or may compete with membrane protein transport proteins.[48,100,103]

Decreased bilirubin excretion (cholestasis) can be either hepatic or posthepatic in origin, and usually results from a blockage (partial or complete) in the biliary system that causes accumulation of bile (i.e., bile inspissation). Blockage of bile flow results in regurgitation of C-bilirubin into the blood. Blockages are often caused by processes directly affecting the biliary tree, such as infections or neoplasms that compress or damage bile ducts or biliary calculi. However, diseases that primarily affect the hepatic parenchyma can also result in cholestasis by causing hepatocyte swelling that blocks small bile canaliculi and prevents the

normal flow of bile. Extrahepatic bile duct obstruction can also occur secondary to small intestinal or pancreatic lesions, which may cause severe cholestasis and hyperbilirubinemia. Leakage of bile into the abdominal cavity resulting from rupture of the gall bladder or bile duct can also result in hyperbilirubinemia.

Another type of intrahepatic cholestasis results not from obstruction but from impaired excretion of C-bilirubin secondary to extrahepatic bacterial infection. This has been termed functional or sepsis-associated cholestasis. Functional cholestasis has been well described in people, but is likely underrecognized in animals. The pathogenesis involves the production of inflammatory cytokines (TNF, IL-6, IL-1β) that reduce bile flow by inhibiting hepatocellular transport mechanisms.[57]

Whenever there is increased serum C-bilirubin concentration, a portion of the C-bilirubin may become tightly (covalently) bound to serum protein (biliprotein or delta bilirubin). Delta bilirubin is removed from circulation relatively slowly; it is eliminated at a rate approximately equal to the half-life of albumin (8–20 days).[88] Delta bilirubin is included in the serum total bilirubin as measured by routine wet chemistry methods. Therefore, total bilirubin concentration can occasionally be misleading since delta bilirubin may persist for a period of weeks in the serum of animals with resolved cholestatic disease. Such animals may have increased serum total bilirubin concentration, but normal urinary bilirubin concentration, since tightly protein-bound delta bilirubin does not easily cross the glomerulus. Practical laboratory methods for the measurement of delta bilirubin concentration exist; dry chemical methods allow calculation of delta bilirubin concentration. However, delta bilirubin is not currently routinely measured in clinical practice.

Historically, the measurement of serum bilirubin involved not only the measurement of total bilirubin, but also the determination of the concentrations of both conjugated and unconjugated bilirubin. In theory, hyperbilirubinemia associated with hemolysis or reduced hepatic uptake of bilirubin should produce marked increases in U-bilirubin and smaller, if any, increases in C-bilirubin. Similarly, cholestasis or leakage of bile should produce marked increases in C-bilirubin and smaller, if any, increases in U-bilirubin. Such determinations, however, have proved unreliable in differentiating between causes of hyperbilirubinemia.[121,122] If hyperbilirubinemia is detected, the patient history, physical findings, and results of other laboratory tests can be helpful in differentiating the potential causes. A flow chart for the evaluation of hyperbilirubinemic animals is presented in Figure 26.7.

Species differences should be considered when evaluating serum bilirubin concentration. If cholestasis is the cause of hyperbilirubinemia, the serum ALP and GGT activities may also be increased and are considered more sensitive than serum bilirubin concentration for cholestasis in dogs and cattle, but not cats and horses.[133,136] Most species have a relatively low renal threshold for bilirubin, and because C-bilirubin is efficiently excreted by the kidney, bilirubinuria often precedes hyperbilirubinemia.[137] Healthy dogs, however, frequently exhibit mild bilirubinuria, likely due to the ability of the canine renal tubules to form and conjugate bilirubin.[106] Bilirubinuria in dogs should be interpreted in conjunction with the urine specific gravity; concentrated urine (specific gravity >1.025) may normally contain a small amount of bilirubin.[137]

Serum total bilirubin concentration in healthy horses tends to be greater than in other domestic species, so it is important to use species-specific reference intervals when interpreting test results. Hepatic necrosis, neoplasia, cirrhosis, lipidosis, fasting, and hemolysis are reported to cause hyperbilirubinemia in horses; biliary obstruction is a relatively uncommon cause.[151] Hyperbilirubinemia in horses with hemolysis can be marked; serum bilirubin concentration approaching 50 mg/dL has been reported in foals with neonatal isoerythrolysis.[116] As noted earlier, anorexia or starvation can result in increased serum bilirubin concentration in horses. Regardless of the cause of hyperbilirubinemia in horses, most of the bilirubin in the blood is unconjugated.[2]

Hyperbilirubinemia is not consistent in ruminants with liver disease. Diffuse hepatic diseases such as hepatic lipidosis or chronic liver failure are most likely to cause hyperbilirubinemia.[21,153] Primary diseases of the biliary tract and gallbladder are uncommon in ruminants. Significant hyperbilirubinemia most often results from hemolysis. Cattle that are ill with a variety of nonhemolytic, nonhepatic diseases may have hyperbilirubinemia associated with rumen stasis and anorexia.[90]

Bile acids

Serum bile acids

Measurement of serum bile acid (SBA) concentration is a routine diagnostic test for hepatic function, cholestasis, and abnormalities of portal circulation. It has replaced the more difficult to perform dye excretion tests such as bromosulfophthalein (BSP) and indocyanine green (ICG). Bile acids are synthesized in hepatocytes from cholesterol (Fig. 26.8). Cholic acid and chenodeoxycholic acid are the primary bile acids in most animals. After their synthesis, bile acids are conjugated to amino acids (primarily taurine in most animals) before secretion into bile. Bile acids are stored and concentrated in the gallbladder (in those species that have one). At the time of a meal, hormonal and neurohormonal factors stimulate gallbladder contraction and passage of bile acids into the small intestine, where dehydroxylation by anaerobic microorganisms results in the conversion of the primary bile acids to secondary bile acids. Thus, cholic acid is converted to deoxycholic acid, and chenodeoxycholic acid

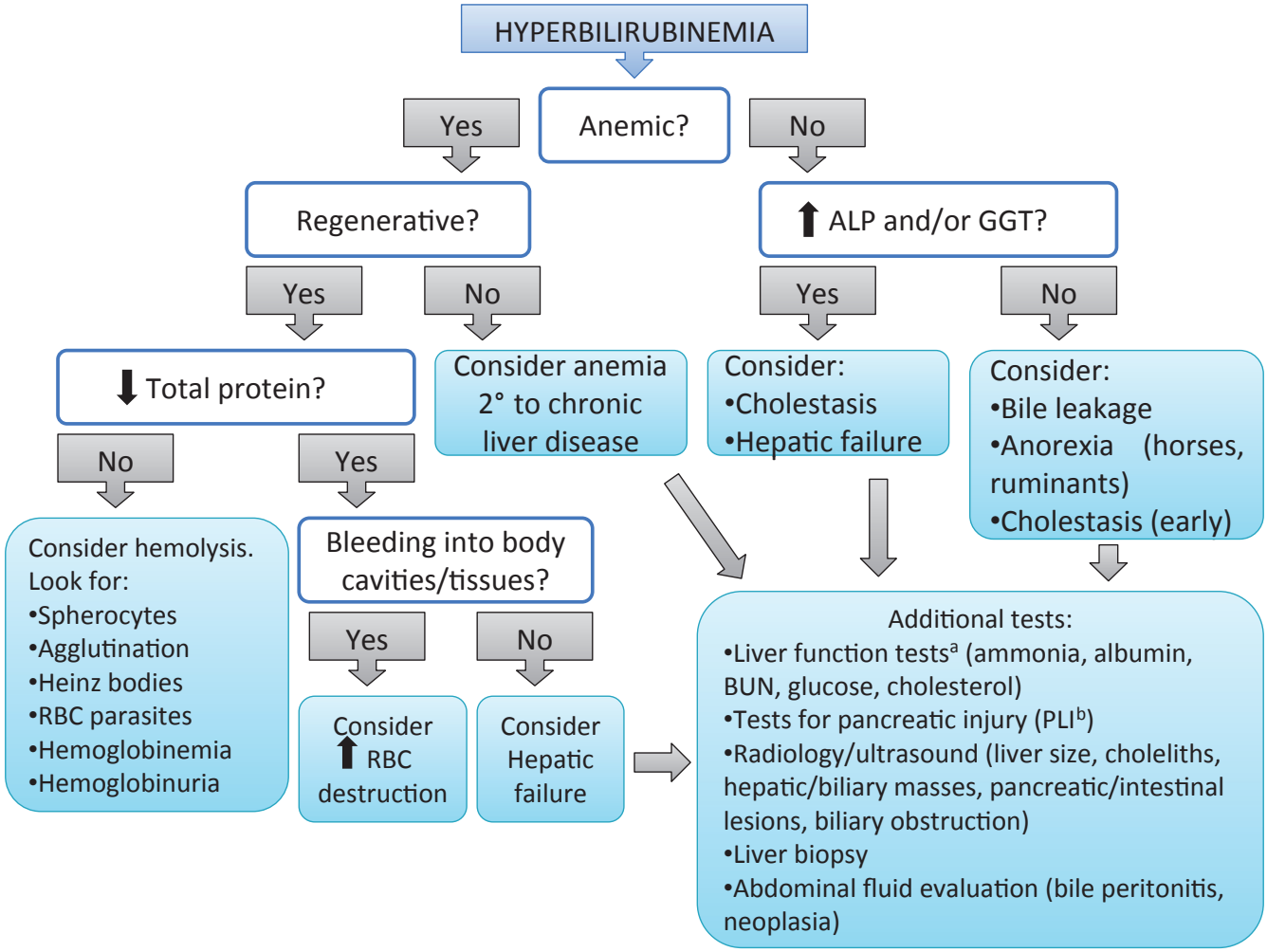

Figure 26.7 Flow chart for the evaluation of hyperbilirubinemic animals. [a]Serum bile acids are not usually useful in hyperbilirubinemic animals, but may be helpful in anorexic horses or anemic animals (see text for details). [b]PLI, pancreatic lipase immunoreactivity.

is converted to lithocholic acid. Bile acids emulsify fat and, therefore, promote both the digestion and absorption of fat as well as of fat-soluble vitamins. Most of the bile acids are reabsorbed from the ileum into the portal circulation (<5% of the bile acid pool is lost in the feces each day).[26] Normally, bile acids are efficiently cleared from the portal circulation on their first pass through the liver; as a result, only a slight postprandial increase in serum bile acid concentration is seen in healthy animals. Bile acids that are cleared by hepatocytes are secreted into the biliary system and recirculate; a bile acid molecule recirculates several times after a meal.

There are three main pathologic processes involving the liver that result in increased SBA concentration. Decreased SBA concentration does not occur with hepatic disease, but may occur with some intestinal disorders.[26]

1. Abnormalities of portal circulation (e.g., congenital portosystemic shunts, hepatoportal microvascular dysplasia, acquired shunts due to severe cirrhosis). In these situations, blood is shunted away from hepatocytes impairing first-pass clearing of bile acids from portal circulation; bile acids then enter the systemic circulation.

2. Decreased functional hepatic mass. This is a major factor in many diffuse liver diseases (e.g., hepatitis, necrosis, glucocorticoid hepatopathy) that result in sufficient hepatocyte damage that uptake of bile acids from portal blood is impaired.

3. Decreased bile acid excretion in bile. This can result from hepatic or posthepatic cholestasis from any cause (obstruction, hepatocyte swelling, neoplasia, inflammation), functional or sepsis associated cholestasis, or leakage from the bile duct or gallbladder.

Bile acid assays are most useful for animals in which liver disease is suspected, but not unequivocally proven on the basis of routine biochemical profile tests. A good example is when serum liver enzyme activities are increased, but serum total bilirubin concentration is normal. Serum bile

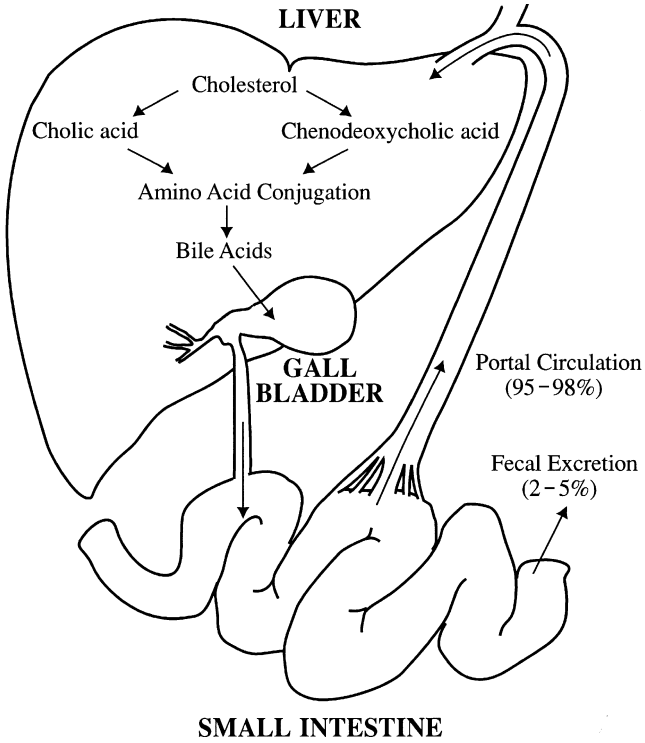

Figure 26.8 Normal production and circulation of bile acid.

acid concentration is a sensitive indicator of cholestasis, and it is important to recognize that patients already icteric due to cholestasis will always have increased SBA concentration as well. However, measuring SBA concentration may be helpful in differentiating hemolytic hyperbilirubinemia from hepatic or cholestatic hyperbilirubinemia in anemic patients for which a hemolytic cause is not obvious and liver enzyme activities are equivocal. Bile acids do not compete with bilirubin for uptake or metabolism by hepatocytes; therefore, a hemolytic hyperbilirubinemia can occur without a concurrent increase in SBA concentration.[26] However, severe anemia can cause hepatocellular hypoxia leading to hepatic dysfunction, with subsequent increases in SBA concentration.[26] In horses, SBA concentration may also be helpful in discriminating fasting hyperbilirubinemia from hepatic or cholestatic hyperbilirubinemia. Bile acid concentration does increase 2–3 fold after three days of fasting, but generally stays below 25 µmol/L, and so may be only slightly outside of laboratory reference intervals.[49,68] By contrast, horses with experimental bile duct obstruction or diffuse hepatic necrosis have SBA concentration increases exceeding 8–10 fold (50–100 µmol/L) after three days.[68]

Bile acid assays are readily available. Bile acids are stable in serum at room temperature for several days, and serum for bile acid assays can be frozen. Depending upon the test method, hemolysis may cause false decreases and lipemia false increases in SBA concentration.[26]

In dogs and cats, both fasting (preprandial) and postprandial samples are recommended for bile acid assays in order to provide the most reliable interpretation. A standard procedure is the following:

1. The patient is fasted for 12 hours before collection of the first (fasting) serum sample.

2. A fat-containing diet of adequate volume and adequate fat to stimulate cholecystokinin secretion by the small intestine and subsequent gallbladder contraction is fed. Growth diets with higher fat content are recommended. In animals with potential hepatic encephalopathy, a restricted-protein diet can be used but should be supplemented with corn oil to increase the fat content to approximately 5%.

3. A serum sample is collected at 2 hours after feeding (postprandial sample).

4. Both fasting and postprandial bile acid concentrations are measured.

Fasting bile acid concentrations of >20 µmol/L and postprandial bile acid concentrations of >25 µmol/L are very specific for liver disease in dogs and cats.[26] A fasting bile acid concentration of <5 µmol/L is normal in dogs and cats, and concentrations between 5–20 µmol/L are suggestive of hepatic disease. However, a fasting bile acid concentration as great as 20 µmol/L occasionally occurs in normal dogs and cats. Fasting bile acid concentrations between 5–20 µmol/L in dogs and cats should be interpreted in light of the patient history, clinical signs, and results of diagnostic imaging as well as other laboratory tests for hepatic disease or function.

In dogs, increased serum bile acid concentration can occur with a variety of liver diseases, including portosystemic shunt, cholestasis, cirrhosis, necrosis, hepatitis, hepatic lipidosis, glucocorticoid hepatopathy, and neoplasia.[29,33] Exaggerated increases in postprandial bile acid concentration are most consistent and marked in animals with portosystemic shunts.[26] However, defining the type of liver disease on the basis of the bile acid concentration alone is not possible. Abnormal bile acid concentration is an indication for further testing (e.g., liver biopsy, radiologic studies, ultrasound) aimed at identifying the specific type of liver disease that is present. Slight increases in SBA concentration with an increased percentage of unconjugated bile acids has been reported in some dogs with small intestinal bacterial overgrowth.[91] Additionally, there is one report of increased SBA concentration in healthy Maltese dogs.[142] The sometimes marked increases were found with the routine enzymatic assay, but not by an HPLC assay, suggesting presence of a crossreacting substance or unusual bile acid.

In cats, increased serum bile acids occur with portosystemic shunt, cholestasis, cirrhosis, necrosis, hepatitis, hepatic lipidosis, and neoplasia.[28,31] The fasting bile acid concentration in cats with these diseases is less consistently increased than is the postprandial concentration, and measuring both is desirable.

The postprandial bile acid concentration is occasionally lower than the fasting bile acid concentration in dogs and cats. This may result from spontaneous emptying of the gallbladder during the fasting period or to differences in gastrointestinal variables (gastric emptying time, intestinal transit time, intestinal flora) or the release of/response to cholecystokinin.[26]

In horses, ruminants, and llamas, a single sample is usually collected for a bile acid assay. Reference intervals in these species tend to be wider than those in dogs and cats. Interpretation of SBA concentration in cattle is hampered by the periodically interrupted flow of ingesta into the duodenum, which results in considerable hourly variation (differences up to 60 µmol/L) in SBA concentrations.[112] Differences also occur between dairy and beef breeds, and among age groups and stages of lactation.[40] However, despite relatively wide reference intervals, SBA concentration is still the most sensitive test for hepatobiliary diseases in cattle.[153] Fluctuations in SBA concentration are less in llamas than in cattle, and increased SBA concentration has been reported in llamas with hepatic lipidosis and portosystemic shunt.[3,73,144] Reported reference intervals for llamas ≤1 year of age are 2–50 µmol/L, and those for llamas >1 year of age are 1–23 µmol/L.[3]

In horses, the SBA reference interval has varied in different studies, but the upper limit of this interval is <20 µmol/L.[68,152] Horses continuously secrete bile into the intestinal tract because of their lack of a gallbladder and apparent weakness of the sphincter of the common bile duct. Increased SBA concentration is a sensitive indicator of hepatobiliary disease in horses with a variety of disorders including hepatic necrosis, hepatic lipidosis, neoplasia, and cirrhosis.[151,152] The increase in SBA concentration observed with these diseases is often marked (40 to >100 µmol/L).

Urine bile acids

Because the liver efficiently removes bile acids from the portal circulation, in health only small amounts of bile acids enter systemic circulation to be eliminated in the urine. However, when SBA concentration is increased there are increased amounts of bile acids excreted in urine. In theory, a one-time measurement of urine bile acid (UBA) concentration compared to urine creatinine concentration (UBA : creatinine ratio) might provide information similar to assays measuring SBA without the need for fasting and postprandial blood samples. Preliminary investigations of urine sulfated and nonsulfated bile acid : creatinine ratios have been performed in dogs and cats.[7,146] In dogs, the ratio of unsulfated UBA : creatinine had excellent specificity (100%), but relatively poor sensitivity (63%) for liver disease.[7] In cats, the ratio of unsulfated UBA : creatinine had good specificity (88%) and sensitivity (87%) for liver disease.[146] The clinical utility of these ratios for diagnosis of various hepatic disorders awaits further study.

Plasma ammonia

Ammonia (predominantly ammonium, NH_4^+) is produced largely by bacteria in the GI tract during normal digestion and absorbed from the intestinal tract into the blood. It is removed from portal circulation by the liver, where it is used for urea and protein synthesis. Alterations in blood flow to the liver or markedly decreased numbers of functional hepatocytes can result in an increased blood ammonia concentration. Blood ammonia measurement or the ammonia tolerance test may be used to assess liver function. One advantage of measuring ammonia concentration over SBA concentration is that blood ammonia levels are not altered by cholestasis. Additionally, increased blood ammonia concentration is considered evidence for hepatic encephalopathy, although this is not a consistent finding. Increased blood ammonia concentration is considered fairly specific, but relatively insensitive for serious hepatic disease. Increased plasma ammonia concentration is most common in animals with portosystemic shunting of blood (either congenital shunts or shunting secondary to severe cirrhosis). Increased blood ammonia concentration also can occur with the loss of 60% or more of the hepatic functional mass.[22]

In addition to decreased clearance of ammonia from portal circulation, as occurs with portosystemic shunting or decreased functional hepatic mass, there are additional situations in which blood ammonia concentration may be increased. Increased ammonia intake and/or production have been documented in cattle with urea toxicosis or ingestion of contaminated feed.[62,147] Strenuous exercise has been shown to increase ammonia levels in dogs and horses.[58,98,128] Intestinal disease in horses has occasionally been associated with increased ammonia concentration and signs of hepatic encephalopathy.[113,126,135] Irish wolfhound puppies may have transient hyperammonemia that disappears in adulthood; however, Irish wolfhounds also have an increased incidence of inherited portosystemic shunts.[95] Finally, there are rare instances of inherited or acquired urea cycle defects that may cause hyperammonemia.[137]

Ammonia concentration is typically measured in plasma using an enzymatic method available in commercial laboratories. Ammonia concentration in blood is very unstable after collection, however, which has been a deterrent to routine use of this test.[66] A procedure for the collection and storage of plasma for an ammonia assay is as follows:[137]

1. Simple-stomached animals are fasted for at least 8 hours before sampling.

2. Blood is collected and placed into EDTA or ammonia-free heparin anticoagulant, placed in an ice bath, and the plasma separated immediately (within 10 minutes). There should be minimal exposure to air. Delayed separation of plasma or storage at room temperature will cause falsely increased ammonia concentration.

3. Plasma is refrigerated (4°C) and assayed within 30–60 minutes.

Point-of-care analyzers that utilize whole blood samples have increased the usefulness of blood ammonia assays in clinical situations by eliminating the issues related to proper sample handling for timely delivery of plasma to a commercial laboratory.[59,134] However, reference intervals for these point-of-care methods may need to be adjusted in order to minimize false negative test results.[59]

Assaying blood ammonia concentration following administration of ammonium chloride (ammonia tolerance test) increases the diagnostic accuracy of the test.[22,94] The ammonia tolerance test is usually performed on animals in which portosystemic shunt or decreased hepatic function are suspected, but other tests are equivocal and fasting ammonia concentration is normal. Ammonia tolerance tests should never be performed on animals with fasting hyperammonemia, as dangerously high blood ammonia concentration may result, causing acute ammonia toxicity. Ammonia tolerance tests involving both oral and rectal administration of ammonium chloride have been described.

A suggested procedure for the oral ammonia tolerance tests is as follows:[22]

1. A fasting (preadministration) heparinized (ammonia free heparin) blood sample is obtained and processed as previously described.
2. Ammonium chloride solution (20 mg/mL) at a dosage of 100 mg/kg body weight is administered via a stomach tube.
3. A total dose of 3 g should not be exceeded.
4. A 30-minute postadministration heparinized blood sample is collected and processed.

The preadministration to postadministration increase of blood ammonia in normal dogs is from 2.0 to 2.5 fold. Most dogs with portosystemic shunts or severe hepatic insufficiency have postadministration increases of 3–10 fold.

A postprandial ammonia tolerance test has also been described for dogs, in which food instead of ammonium chloride is used as the challenge material.[149] This test had a sensitivity of 91% for detection of portosystemic shunts in dogs when the postadministration sample was collected 6 hours after feeding. However, it was not useful for detecting other liver diseases.

Albumin

Liver is the site of all albumin synthesis. Hypoalbuminemia usually is not noted until 60–80% of hepatic function is lost. There appear to be some species differences, however, in the incidence of hypoalbuminemia accompanying liver disease. Hypoalbuminemia is quite common in dogs with chronic liver diseases (>60% have hypoalbuminemia), but it does not appear to be as common in horses with chronic liver diseases (~20% have hypoalbuminemia).[89,109,125] Many nonhepatic factors can influence blood albumin concentration (see Chapter 29).

Globulins

Liver is the site of synthesis for the majority of globulins, with the exception of immunoglobulins synthesized in lymphoid tissue. Hepatic failure can result in decreased synthesis and, therefore, decreased serum concentration of these globulins. However, globulin concentration usually does not decrease as much as the albumin concentration, and so the albumin:globulin ratio commonly decreases in hepatic failure. In many cases, globulin concentration may increase with chronic liver disease, either as a result of increased acute phase protein production or immunoglobulin production.[25] This has been especially well documented for horses, in which more than 50% of those with chronic hepatic disease also have increased globulin concentration.[109] In animals with severe liver disease, the clearance of foreign proteins by the Kupffer cells of the liver is theorized to be decreased. Such foreign proteins are thought to be absorbed from the intestine and carried to the liver by the portal circulation. Thus, when Kupffer cells fail to efficiently clear these proteins on their first passage through the liver, they come in contact with the immune system in other parts of the body resulting in an immune response and hyperglobulinemia.

Glucose

The liver plays a key role in glucose metabolism. Glucose that has been absorbed by the small intestine is transported to the liver via the portal circulation and then enters hepatocytes. The hepatocytes convert glucose to glycogen, which helps to regulate the blood glucose concentration. Hepatocytes also synthesize glucose via gluconeogenesis and release stored glucose via glycogenolysis. In animals with hepatic failure, glucose concentration can vary from decreased to increased. Increased glucose concentration may occur because of decreased hepatic glucose uptake, resulting in prolonged postprandial hyperglycemia. Conversely, decreased glucose concentration may occur because of reduced hepatocytic gluconeogenesis or glycogenolysis. The liver has tremendous reserve capacity for maintaining normal blood glucose levels; 70% hepatectomy does not result in hypoglycemia.[25]

Urea

Urea is synthesized by hepatocytes from ammonia. In animals with liver failure, the decrease in functional hepatic mass results in decreased conversion of ammonia to urea. Consequently, the blood ammonia concentration increases, and the blood urea (also known as BUN) concentration decreases. However, blood urea concentration also may decrease because of numerous other disorders (see Chapter 23).

Cholesterol

Bile is a major route of cholesterol excretion from the body. Therefore, interference with bile flow (cholestasis) can result

in increased serum cholesterol concentration (hypercholesterolemia). Many other nonhepatic disorders, however, also can result in hypercholesterolemia (see Chapter 31).

The liver is also a major site of cholesterol synthesis. In some forms of hepatic failure, decreased cholesterol synthesis can lead to decreased blood cholesterol concentration (hypocholesterolemia). The balance between decreased cholesterol synthesis and decreased cholesterol excretion varies with different types of liver disease. If decreased synthesis of cholesterol is the major alteration in hepatic failure, hypocholesterolemia can result; if cholestasis is the major alteration, hypercholesterolemia may occur. Many dogs and cats with portosystemic shunts (60–70%) have hypocholesterolemia.[32] However, many animals with liver failure have normal serum cholesterol concentration.

Coagulation factors

The liver plays a central role in the regulation of coagulation as the sole source of synthesis for the majority of coagulation factors; it also produces anticoagulants such as antithrombin, protein C, and protein S.[42] In addition, the blockage of bile flow can result in decreased absorption of vitamin K leading to decreased function of the vitamin K–dependent coagulation factors (factors II, VII, IX, and X) and anticoagulants (proteins C and S). Therefore, defects in both hemostasis and fibrinolysis may occur in animals with liver disease.[25] Accordingly, animals with liver disease may have abnormalities in a variety of coagulation tests including prothrombin time, activated partial thromboplastin time, antithrombin activity, protein C activity, and fibrinogen concentration.[4,5,76,87,104,145] Although coagulation test abnormalities are frequent, clinical bleeding tendencies are recognized less often.[76,104,139] Platelet abnormalities including thrombocytopenia and decreased platelet function may also be associated with liver disease.[25,42] Animals with evidence of liver disease and coagulation abnormalities should be fully evaluated using the tests discussed in Chapter 16 due to the potential for serious complications such as disseminated intravascular coagulation (DIC).

Patterns of laboratory abnormalities for specific diseases

The spectrum and potential magnitude of changes in laboratory test results for selected liver diseases are summarized in Table 26.1. The most common changes in different types of liver diseases are listed, but one should be aware that there is a great deal of overlap.

Portosystemic shunt

Portosystemic shunting of blood can be acquired because of severe cirrhosis, and if this is the case, test results similar to those described for end-stage liver disease are expected. Early congenital portosystemic shunts usually do not produce much active hepatocyte damage. Consequently, leakage enzyme activities often are normal or only slightly increased. Cholestasis is not a feature of congenital portosystemic shunts; consequently increased production of ALP and GGT does not occur. However, because congenital shunts most commonly occur in young animals with growing bones, mildly increased serum ALP activity (due to BALP) is common. Because portal circulation to the liver is impaired, increased fasting or postprandial bile acid concentration and increased blood ammonia concentration are common. While fasting SBA increases may be marginal, postprandial SBA increases are often marked. The decreased hepatic blood flow can cause hepatic atrophy and decreased functional hepatic mass. Therefore, other tests of hepatic function may become abnormal in more chronic cases. Microcytosis, with or without mild anemia, is a relatively common hematologic finding in dogs with portosystemic shunts. The pathogenesis is uncertain, but abnormal iron metabolism associated with altered iron transport has been implicated.[19,83,127]

Hepatic necrosis

Hepatic necrosis can vary from focal to multifocal to diffuse. Focal to multifocal hepatic necrosis can result in increased activities of leakage enzymes, but these increases are less frequent and of lesser magnitude than those resulting from diffuse necrosis. Focal necrosis usually does not cause significant cholestasis, and induced enzyme activities usually remain normal. Diffuse necrosis is more likely to compromise the flow of bile and cause cholestasis, resulting in induced enzyme activity increases. Bile acid concentration usually is not affected by focal necrosis, but diffuse necrosis can produce increased SBA concentration because of decreased hepatocyte removal of bile acids from the portal circulation as well as cholestasis. Similarly, other tests of hepatic function are not affected by focal necrosis, but if more than 60–80% of the hepatic mass is lost because of diffuse necrosis, results of liver function tests (i.e., albumin, BUN, glucose, cholesterol, coagulation) may be abnormal. Infiltrative disease, such as lymphoma or other hematopoietic cell neoplasia, can cause laboratory changes similar to those seen with diffuse necrosis. Modest increases in leakage enzyme activities may occur, with variable increases in induced enzyme activities depending on the degree of cholestasis. Liver function may eventually become impaired in advanced cases of infiltrative disease.

Hypoxia or mild toxic damage

Hypoxia (due to anemia or hepatic congestion) or mild toxic damage (possibly secondary to endotoxins, mycotoxins, or other toxicants) can result in mild injury to many hepatocytes. As a result, leakage enzyme activities can be mildly to moderately increased. Generally cholestasis does not occur, and the activities of induced enzymes usually are normal.

Table 26.1 Common laboratory findings for various hepatic diseases.

Disorder	Leakage Enzymes (ALT, AST)	Induced Enzymes (ALP, GGT)	Bilirubin	Serum Bile Acids	Other Function Tests	Miscellaneous
Congenital portosystemic shunt	N to ↑	ALP = N to ↑ (due to BALP in young animals)	N	Fasting = N to ↑↑ Postprandial = ↑↑ to ↑↑↑	Ammonia = N to ↑ Albumin = N to ↓ BUN = N to ↓ Glucose = N to ↓ Cholesterol = N to ↓ Protein C = ↓ PT = N to prolonged	RBC microcytosis (60–70% of dogs) Ammonium biurate crystalluria
Necrosis—focal to multifocal	N to ↑↑	N	N	N	N	
Necrosis—diffuse, or infiltrative disease	↑↑ to ↑↑↑	N to ↑↑	N to ↑↑	Fasting = N to ↑↑ Postprandial = N to ↑↑	Variable	
Hypoxia or mild toxic insult	↑ to ↑↑	N to ↑	N	Fasting = N to ↑ Postprandial = N to ↑	N	
Focal abscesses, infarcts, neoplasms	N to ↑	N to ↑↑	N to ↑	Fasting = N to ↑ Postprandial = N to ↑	N	
Hepatic lipidosis (diffuse, cats)	N to ↑↑↑	ALP = N to ↑↑↑ GGT = N to ↑	N to ↑↑↑	Fasting = N to ↑↑↑ Postprandial = ↑ to ↑↑↑	PT, APTT = N to prolonged BUN = N to ↓	RBC poikilocytosis
Steroid hepatopathy (dogs)	N to ↑↑	↑ to ↑↑↑	N to ↑	Fasting = N to ↑ Postprandial = N to ↑	N	
Bile duct obstruction, cholangiohepatitis, cholangitis	↑ to ↑↑	ALP = ↑ to ↑↑↑ GGT = N to ↑↑↑	N to ↑↑↑	Fasting = N to ↑↑↑ Postprandial = ↑ to ↑↑↑	Variable PT, APTT prolonged if Vit. K deficient	
Chronic liver disease or diffuse neoplasia	N to ↑↑	N to ↑↑↑	N to ↑↑	Fasting = N to ↑↑↑ Postprandial = N to ↑↑↑	Variable	
End-stage liver (Liver failure)	N to ↑↑	N to ↑↑↑	↑↑ to ↑↑↑	Fasting = N to ↑↑↑ Postprandial = N to ↑↑↑	Ammonia = N to ↑ Albumin = N to ↓ BUN = N to ↓ Glucose = N to ↓ Cholesterol = N to ↓ Protein C = ↓ PT, APTT prolonged	

N, Normal; PT, prothrombin time; APTT, activated partial thromboplastin time.

However, cell swelling may occur and if it is severe enough, swollen hepatocytes can impinge on bile canaliculi and cause cholestasis as well as increased activities of induced enzymes. This cholestasis usually is not severe enough to result in increased serum bilirubin concentration, but may cause a mild increase in SBA concentration.

Focal lesions

Focal lesions such as abscesses, infarcts, or localized neoplasms may only cause local hepatocyte damage, in which case the activities of leakage enzymes are normal or mildly increased. The activities of these enzymes depend on the time and the extent of hepatocyte damage. Expansion of abscesses or neoplasms into the surrounding tissue may be slow and result in only a few hepatocytes being damaged during any given period of time. Activities of induced enzymes usually are normal but may be increased if the focal lesion causes significant cholestasis. Serum bilirubin or SBA concentrations occasionally are increased, however the pathogenesis is not clear; focal lesions seldom occlude bile ducts that are large enough to interfere significantly with bile flow. Other tests of hepatic function usually are normal,

because 60–80% of the hepatic mass is not lost with focal lesions.

Hepatic lipidosis

Hepatic lipidosis occurs in many species, but the syndrome has been documented best in cats.[23] Serum activities of leakage enzymes (ALT, AST) are mildly to markedly increased in 70–90% of cats with hepatic lipidosis, likely from marked lipid accumulation in hepatocytes. More than 80% of cats with hepatic lipidosis have increased serum ALP activity, varying from mild to marked, whereas only approximately 16% have increased serum GGT activity.[30] However, cats with underlying necro-inflammatory disorders may have relatively greater GGT activity compared to ALP activity.[23] Serum activities of these induced enzymes are most likely increased because lipid-laden hepatocytes impinge on bile canaliculi, with resultant cholestasis. The serum bilirubin concentration is increased to some degree in the majority of cats (75–95%), probably because of cholestasis, and most cats have increased SBA concentration. Other tests of hepatic function are inconsistently abnormal. If diabetes mellitus is the underlying problem in cats with hepatic lipidosis, blood glucose concentration may be very high. Coagulation abnormalities (prolonged prothrombin time or activated partial thromboplastin time) are not unusual, occurring in 25–40% of cats with hepatic lipidosis.

Steroid hepatopathy

Steroid hepatopathy is most common in dogs and can produce moderate damage to hepatocytes, largely due to distention of hepatocytes from glycogen accumulation. The serum activities of leakage enzymes usually are mildly increased in dogs with steroid hepatopathy, while serum activities of induced enzymes may be markedly increased because of corticosteroid-mediated induction of their synthesis. Serum bile acids may be modestly increased, but total bilirubin concentration is rarely increased, and other tests of hepatic function are usually normal.

Biliary abnormalities

Cholangitis, cholangiohepatitis, and extrahepatic bile duct obstruction can occur in many different species. Because lesions usually are centered in the portal areas of the liver or outside of the liver, increased serum activities of leakage enzymes usually are mild and result from secondary damage to hepatocytes caused by increased intrabiliary pressure. The serum activities of induced enzymes are markedly increased and become progressively higher as the disease becomes more severe. Increased intrabiliary pressure induces hepatocytes and biliary epithelial cells to produce increased amounts of these enzymes. Serum bilirubin concentration is moderately to markedly increased because of the blockage of bile flow. Both fasting and postprandial serum bile acid concentration is usually increased and sometimes markedly so, resulting from the blockage of bile flow. Other tests of hepatic function are usually normal, unless these diseases progress to end-stage liver disease.

Chronic progressive liver diseases

Chronic progressive liver diseases can occur in many species but are most common in dogs. Moderate to severe inflammation is a common feature, and variable degrees of hepatocyte necrosis, fibrosis, and cirrhosis may also occur.

Some cases of chronic hepatitis are associated with abnormal copper accumulation in the liver. Bedlington terriers have a well-described hereditary disorder resulting in hepatic copper accumulation and chronic hepatitis; the molecular defect in this disorder has been characterized.[81] A variety of other dog breeds have been identified that seem to have a predisposition to hepatic copper storage and chronic hepatitis, including West Highland white terriers, Skye terriers, Doberman pinschers, Dalmations, and Labrador retrievers.[67,141] Certain drugs (e.g., anticonvulsants) and infectious agents also may cause chronic hepatitis in dogs. Serum activities of leakage enzymes often are mildly or moderately increased because of progressive hepatocyte damage. If progression of the disease is slow, the release of these enzymes within a given period of time may be minimal, and serum activity may be normal. Many of these diseases ultimately result in varying degrees of hepatic fibrosis which may compromise bile flow. Therefore, serum activities of induced enzymes are often mildly or moderately increased. Serum bilirubin concentration is normal in animals with the early, less severe forms, but can be increased in those with later, more advanced disease. Fasting and postprandial SBA concentrations are inconsistently increased, depending on how far the disease has advanced. These increases probably relate to impaired blood flow to the liver, impaired clearance of bile acids by hepatocytes, and cholestasis. Other tests of hepatic function are normal, unless the disease has resulted in the loss of 60–80% of functional capacity.

End-stage liver disease

End-stage liver disease occurs when more than 60–80% of the hepatic functional mass has been lost. The serum activities of leakage enzymes are normal to moderately increased. Normal serum activities of these enzymes may result from markedly decreased numbers of hepatocytes or minimal active hepatocyte damage. Serum activities of induced enzymes are moderately to markedly increased because of cholestasis. Serum bilirubin concentration is moderately to markedly increased. Fasting or postprandial SBA concentrations are increased, and sometimes markedly so, resulting from decreased hepatic blood flow, impaired hepatocyte uptake of bile acids from portal blood, and cholestasis. Many other hepatic function tests are often abnormal, including increased blood ammonia concentration, decreased blood

glucose concentration, decreased blood urea (BUN) concentration, and decreased serum albumin concentration. Serum globulin concentration varies from mildly decreased to increased. Coagulation tests are also often abnormal in animals with end-stage liver disease.

Summary

Biochemical testing can suggest three basic categories of liver disease depending upon the pattern of abnormalities observed: hepatocellular injury, cholestasis, and decreased function. However, characterization of the specific type of liver disease usually requires additional tests (e.g., radiographic studies, ultrasound, liver fine needle aspirate, liver biopsy). Additionally, careful attention must be paid to the patient's clinical history, current medications, and physical examination findings in order to rule-out underlying conditions that could affect test results. Repeated biochemical testing is often useful for evaluating disease progression or response to therapy.

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27 Laboratory Evaluation of the Pancreas and Glucose Metabolism

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The pancreas is a compound organ with both exocrine and endocrine functions. The exocrine pancreas is composed of glandular epithelium that forms acinar lobules comprising about 80% of the pancreas,[66] and the endocrine cells are concentrated in the islets of Langerhans.

The exocrine pancreas

The primary function of the exocrine pancreas is the synthesis and secretion of digestive enzymes. These enzymes include proteases that are stored in acinar cell zymogen granules and secreted as inactive proenzymes (e.g., trypsinogen, chymotrypsinogen, proelastase, and procarboxypeptidases), lipase, which hydrolyzes lipids; and amylase, which hydrolyzes starches.[12] The inactive proenzymes become activated by enzymatic cleavage of a small peptide (activation peptide). Normally, trypsinogen is cleaved by enterokinase in the intestine to form trypsin and trypsinogen activation peptide (TAP); trypsin then activates other proenzymes.[124] Unlike the proteases, amylase and lipase are secreted in active form.[12]

Two major disorders of the exocrine pancreas can be detected by laboratory evaluation:

- Injury to the pancreatic parenchyma usually due to pancreatitis. Inflammation may result in the premature activation and leakage of pancreatic enzymes into the pancreatic interstitium, peritoneal cavity, and vasculature. Pancreatitis is recognized most commonly in dogs and cats, and may be acute or chronic. Intraperitoneal release of pancreatic enzymes causes tissue damage in the area of the pancreas, thereby increasing both the severity and the extent of the inflammation. Subsequent release of inflammatory mediators can result in a systemic inflammatory response.[96]
- Exocrine pancreatic insufficiency (EPI), a disorder resulting in insufficient production and secretion of pancreatic enzymes. EPI is due to loss of pancreatic acinar cells, and results in inadequate digestive function (maldigestion). The clinical signs are similar to intestinal disorders that result in inadequate absorption of adequately digested nutrients (malabsorption). Laboratory testing to differentiate maldigestion and malabsorption is discussed in Chapter 28.

Detection of pancreatic injury

The diagnosis of pancreatitis can be extremely difficult to establish, especially in cases of chronic or mild disease. Dogs with acute pancreatitis frequently exhibit vomiting and abdominal pain, but these clinical signs are less common in cats.[96] Cats seem to develop chronic pancreatitis more frequently than acute disease.[127] Recent necropsy studies indicate subclinical chronic pancreatitis occurs more often in both dogs and cats than previously appreciated.[20,117,129] Although most cases of pancreatitis are considered idiopathic, various risk factors have been identified. Some dog breeds (miniature schnauzers, Yorkshire terriers) seem to be at increased risk.[129] Idiopathic hyperlipidemia is also common in miniature schnauzers, and hyperlipidemia frequently occurs with acute canine pancreatitis; whether hyperlipidemia is a cause or effect of the pancreatitis is not clear.[124] Other risk factors in dogs include obesity, high fat diets, a wide variety of drugs, zinc toxicosis, hypercalcemia, trauma, ischemia, biliary tract obstruction, neoplasia, and infectious agents.[124] In cats, many cases of pancreatitis have been associated with inflammatory diseases of the bowel and biliary tract (often referred to as triaditis).[127] Trematode infections of the liver or pancreas can cause pancreatitis.[43,88,114] Other risk factors in cats are similar to those in dogs.[103,129]

Because clinical signs are nonspecific and highly variable depending upon disease severity, laboratory testing, imaging studies, and sometimes pancreatic biopsy are employed to confirm the diagnosis. Many laboratory tests for pancreatitis

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have been developed, but most have significant limitations. Historically, serum activities of enzymes such as amylase and lipase were measured, but such tests have poor sensitivity and specificity for pancreatitis (discussed later). However, recently developed immunodiagnostic methods appear more promising, as detailed below.

Pancreatic lipase immunoreactivity (PLI)

These tests are species-specific immunoassays that use antibodies to measure serum concentrations of lipase originating specifically from the pancreas.[102,104] By contrast, older tests (discussed later) used enzymatic methods to measure serum enzyme activity of lipase, which includes lipase originating from many tissue sources (i.e., not pancreas-specific lipase).[93] Recently, radioimmunoassays have been developed to detect canine (cPLI) and feline (fPLI) pancreatic lipase immunoreactivity,[102,104] and these assays are now available commercially (Spec cPL™ and Spec fPL™, IDEXX Laboratories, Westbrook, Maine). There is also a rapid in-clinic test available for cPLI (SNAP® cPL™, IDEXX Laboratories). In dogs, the sensitivity of cPLI for the detection of pancreatitis is 65–82%, depending upon disease severity,[101] with a specificity >95%.[96] Limited studies suggest that cPLI concentrations are minimally increased with renal failure and not affected by prednisone administration,[98,99] in contrast to enzymatic serum lipase assays; however, further studies in this area are needed. In cats, the sensitivity of fPLI for the detection of pancreatitis is 54–100%, depending upon disease severity, with a specificity of 91%.[33] These assays are most reliable for detection of moderate to severe pancreatitis, and to date they are the most useful laboratory tests for diagnosis of pancreatitis in dogs and cats.[96]

Serum trypsin-like immunoreactivity

Trypsinogen is synthesized only by the pancreas, and it is converted to the active proteolytic enzyme, trypsin, in the small intestine. The TLI assay uses species-specific antibodies to detect both trypsinogen and trypsin in serum (hence, trypsin-like immunoreactivity). Currently, TLI assays are readily available for dogs and cats, and have been used experimentally in horses.[40,100,123] In healthy animals, a small amount of trypsinogen leaks into the extracellular space and then diffuses via the lymphatics into the blood. Thus, a normal serum TLI concentration is a good indicator of adequate pancreatic trypsinogen production.[123]

Increased serum TLI is expected with pancreatitis due to leakage from damaged acinar cells; however, trypsinogen is cleared by glomerular filtration;[36] thus any disorder causing a decreased glomerular filtration rate (GFR) can increase serum TLI concentration. Activated trypsin, on the other hand, is quickly complexed with protease inhibitors in the blood, and these complexes are removed by the mononuclear phagocyte system.[124] The sensitivity of increased serum TLI concentration for diagnosis of pancreatitis in dogs and cats is 33–36%,[96,127] less than that of PLI. Specificity has been reported between 65–90%,[96] also less than that of PLI. As a result, the serum TLI concentration is now principally applied to diagnosis of pancreatic exocrine insufficiency (see Chapter 28).

Acute and chronic pancreatitis have been recognized in horses, albeit infrequently, and an assay for equine TLI has been described.[40,58] Information on the utility of this assay for diagnosis of equine pancreatitis awaits clinical trials. In one study, five of seven horses with strangulating intestinal obstructions had increased serum TLI, with the highest values in two horses that did not survive.[40] Serum TLI was not increased in three of three horses with nonstrangulating obstructions. Pancreatic histopathology was not performed.

Serum lipase activity

Enzymatic assays that measure serum lipase activity detect lipase from pancreas as well as other tissues.[93] Thus, increases in serum lipase activity are not specific for pancreatic injury. The utility of measuring serum lipase activity to detect pancreatitis varies between species. Serum lipase activity is frequently normal in cats with spontaneous pancreatitis, and therefore is not considered useful for the diagnosis of pancreatitis in this species.[46,96,127] Similarly, it is not considered helpful in the diagnosis of pancreatitis in horses or cattle, although there are rare reports of increased serum lipase activity with acute pancreatitis in these species.[7,70,115] Serum lipase activity has some utility as a screening test for detection of pancreatitis in dogs, and is frequently included on standard biochemical profiles. However, it is neither sensitive nor specific for canine pancreatitis.[96,101] Generally, increases of serum lipase activity of greater than 3–5× the upper reference limit (URL) are interpreted as suggestive of pancreatitis in dogs, and should prompt further evaluation (cPLI, imaging, biopsy).[96] However, in one study of dogs with fatal acute pancreatitis, serum lipase activity was increased in only 16 of 41 cases.[44] In dogs, increased serum lipase activity can result from a variety of conditions other than pancreatitis, including:

- Decreased GFR. Dogs with prerenal, renal, or postrenal azotemia can have increased serum lipase activity due to decreased renal excretion and/or inactivation of lipase.[50,76,108] Usually the increase is <4× URL, but increases up to 10× URL have been reported.
- Corticosteroid administration. Dexamethasone and, to a lesser extent prednisone, can cause increased serum lipase activity in dogs without pancreatitis.[32,73] Increases are typically <2× URL, but may be as much as 5× URL.
- Neoplasia. A variety of neoplasms involving the pancreas (carcinoma, adenocarcinoma), liver (hepatocellular carcinoma, bile duct carcinoma, lymphoma), gastrointestinal tract (lymphoma, adenocarcinoma), and heart (hemangiosarcoma) have been associated with increased serum lipase activity in dogs.[77,108]

• Hepatic disease. In addition to neoplasia, hepatic necrosis and fatty degeneration have been associated with increased serum lipase activity in dogs.[108]
• Other. Gastrointestinal and hepatic tissues can be a source for serum lipase activity.[97] Increased serum lipase activity up to 5× URL has been reported in dogs with acute enteritis; however, pancreatitis was not ruled out in those dogs.[78] Mild transient increases in serum lipase activity (3-fold baseline values) were reported in dogs following exploratory laparotomy that included manipulation of viscera; no histologic evidence of pancreatitis was present.[8]

Serum amylase activity

Assays that measure serum amylase activity, similar to those for serum lipase activity, detect amylase from a variety of tissue sources in addition to the pancreas.[93,105] Thus, increased serum amylase activity is not specific for pancreatic injury. In dogs, four amylase isoenzymes have been identified including amylase complexes bound to proteins (macroamylases), which have a longer serum half-life than uncomplexed amylase.[18,105]

Although serum amylase activity is readily available on standard biochemical profiles, its utility for the diagnosis of pancreatitis is limited. Cats with spontaneous or experimental pancreatitis typically have normal to minimally increased serum amylase activity, although decreased activity has also been reported.[46,62] Therefore, serum amylase activity is not useful for diagnosis of pancreatitis in cats.[127] Increased serum amylase activity has rarely been reported with pancreatitis in cattle or horses,[7,70,115] and may also occur with intestinal mucosal injury.[74] In dogs, increased serum amylase activity is neither sensitive nor specific for pancreatitis, and generally considered inferior to serum lipase activity as a screening test.[11,51,69,96,108] Increases of 3–5× URL may be interpreted as suggestive of pancreatitis, prompting further evaluation (cPLI, imaging, biopsy). However, in dogs without pancreatitis many of the same conditions that cause increased serum lipase activity (discussed earlier) can also cause increased canine serum amylase activity.[8,50,51,96,108,129] The main exception is corticosteroid administration, which does not increase serum amylase activity and may actually decrease it.[32,73]

Peritoneal fluid amylase and lipase activities

If peritoneal fluid can be obtained from animals suspected of having pancreatic injury, measurement of amylase and lipase activities in this fluid may be diagnostically useful. With active pancreatic damage, these enzymes leak into the cavity resulting in increased fluid enzyme activity. Peritoneal fluid amylase or lipase activity that is higher than serum amylase or lipase activity is suggestive of pancreatic injury.[7,41,74] However, duodenal perforation can also result in increased peritoneal fluid amylase and lipase activities. The sensitivity and specificity of peritoneal fluid amylase and lipase activities for detecting pancreatic injury have not been determined.

Other laboratory abnormalities associated with pancreatic injury

None of the routine laboratory tests typically performed as part of the minimum database (CBC and biochemical profile) is diagnostic for pancreatic injury, but the presence of several of these abnormalities in addition to physical findings suggestive of pancreatitis should prompt further evaluation using more sensitive and specific tests (cPLI, imaging, etc.). Laboratory abnormalities that can accompany pancreatic injury are discussed here. It is important to realize that some cases of pancreatitis, particularly chronic pancreatitis in cats, may have normal CBC and biochemical profile results.[127,129]
• Leukocytosis and neutrophilia with or without a left shift may be present. Hematologic evidence of inflammation occurs more often with severe pancreatitis in dogs (~55% of cases), and less often with pancreatitis in cats.[96,127] Because pancreatitis can be very painful, neutrophilia induced both by epinephrine (excitement) and corticosteroids (stress) may also occur. Lymphopenia may also be present, due to either inflammation or stress.
• Increased hematocrit, hemoglobin concentration, and red blood cell count may be present if the animal is significantly dehydrated, which may occur secondary to vomiting and reduced fluid intake. Mild anemia, regenerative or nonregenerative, occurs occasionally in dogs and cats with pancreatitis.
• Azotemia, usually prerenal, is common in severe cases of pancreatitis and is caused by a combination of factors, including dehydration and hypovolemia that result in decreased GFR.[96,127] Tubular concentrating ability is usually normal, and urine specific gravity is usually high. Urine specific gravity helps to differentiate prerenal azotemia accompanying pancreatitis from renal azotemia associated with renal failure. This is an important distinction, because pancreatitis and renal failure can cause increases in serum amylase and lipase activities of similar magnitude. In addition, the clinical signs of pancreatitis and renal failure can be similar. Analysis of urine collected at the time of blood sampling is important for animals in which pancreatitis or renal failure (or both) are possibilities; keep in mind that acute renal failure can occur in severe cases of pancreatitis. Details of differentiating prerenal from renal azotemia, including other potential causes of dilute urine in azotemic animals, are discussed in Chapter 23.
• Hyperglycemia is common in animals with acute pancreatic injury and, acutely, is the result of increased serum concentrations of corticosteroids, epinephrine, and glucagon.[124] In patients with chronic or recurring pancreatitis, hyperglycemia may be caused by diabetes mellitus resulting from islet cell injury.
• Mild to moderate hypocalcemia is inconsistently present in animals with pancreatic injury. The exact pathogenesis of

this hypocalcemia is not known, but is likely multifactorial. Proposed mechanisms include calcium binding with fatty acids in plasma or those freed from peripancreatic fat by the action of pancreatic lipase (fat saponification), hormonal imbalances involving PTH, glucagon, or calcitonin, and intracellular translocation of calcium.[1] In dogs with marked hypoproteinemia, hypoalbuminemia resulting in decreased protein-bound calcium also may contribute to the hypocalcemia. In one study of cats with acute pancreatitis, 19 of 46 cats had low total serum calcium but 28 of 46 had low ionized calcium, and low ionized calcium was associated with a poorer clinical outcome.[61]

- Increased serum activity of leakage (alanine aminotransferase, aspartate aminotransferase) or induced (alkaline phosphatase, and γ-glutamyltransferase) liver enzymes occurs frequently.[124,127] Increased serum activity of leakage enzymes results from ischemic or toxic damage to hepatocytes secondary to pancreatic damage and release of pancreatic enzymes. Increased serum activity of induced enzymes may result from blockage of the common bile duct secondary to inflammation of tissue near both the pancreas and the bile duct. Hepatic lipidosis can occur with pancreatitis in anorexic cats and contribute to hepatic enzyme activity increases. Serum bilirubin concentration may be increased in dogs and cats with pancreatitis, particularly those with acute disease; potential causes include cholestasis (intra- or extrahepatic) and secondary hepatocyte injury.[44,46]
- Hypercholesterolemia and hypertriglyceridemia, often with gross plasma lipemia, are common in dogs with pancreatitis. Although the pathogenesis is not clear, altered lipoprotein processing is suspected, and cholestasis may contribute.[107] Hypertriglyceridemia may be either a cause or an effect of pancreatitis.[128] Hypercholesterolemia, and less commonly lipemia, have been reported in cats.[127]
- Serum and plasma protein concentrations are variable in patients with pancreatitis. Exudation of protein-rich fluid into the peritoneal cavity, as a component of peritonitis, can decrease the serum protein concentration, but dehydration tends to increase the serum protein concentration. In some cases, these changes counterbalance each other.
- Disseminated intravascular coagulation can be a sequela to acute pancreatitis. Alterations in hemostatic function tests that occur with disseminated intravascular coagulation are discussed in Chapter 16. Because bile flow is essential for absorption of fat-soluble vitamins in the intestine, bile duct obstruction caused by pancreatic disease occasionally leads to vitamin K deficiency, causing altered hemostasis and abnormal coagulation test results.

The endocrine pancreas

The islets of Langerhans contain the cells of the endocrine pancreas (Fig. 27.1). There are a variety of specialized endocrine cells present in the islets, including α cells, which secrete glucagon, δ cells, which secrete somatostatin, and PP cells, which secrete pancreatic polypeptide. However, the most common functional abnormalities of the endocrine pancreas involve the β cells, which comprise 60–80% of all islet cells and secrete insulin.[130] Both deficient and excessive insulin production may result in serious abnormalities of glucose metabolism. Many factors in addition to the endocrine pancreas play key roles in glucose metabolism. This section reviews the major factors affecting glucose metabolism, discusses the causes of decreased blood glucose concentration (hypoglycemia) and increased blood glucose concentration (hyperglycemia), and describes tests for evaluating the status of glucose metabolism.

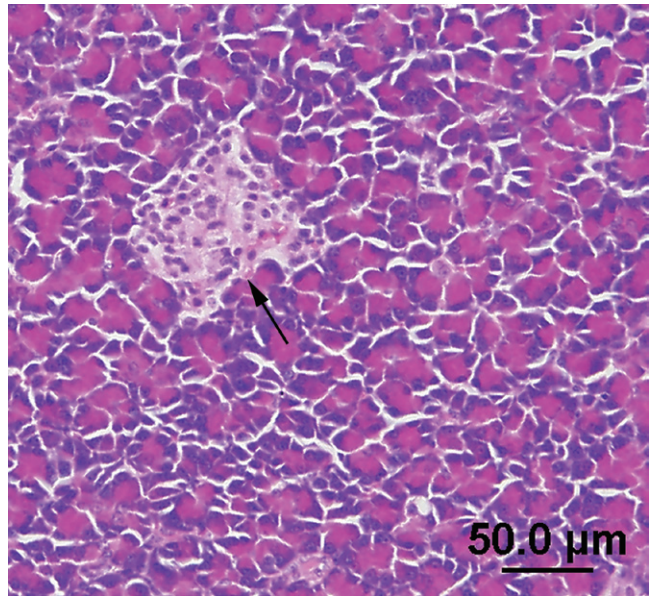

Figure 27.1 The islets of Langerhans (arrow) are the endocrine portion of the pancreas. Both deficient and excessive production of insulin by islet β cells result in abnormalities of glucose metabolism.

Normal glucose metabolism

Sources of blood glucose

Glucose in blood is derived from three sources:

- Intestinal absorption. Dietary carbohydrates are broken down and absorbed in the intestine. Intestinal absorption of glucose can increase blood glucose concentrations in monogastric animals for 2–4 hours after a meal.
- Hepatic production. Hepatic production of glucose results from gluconeogenesis and glycogenolysis. Gluconeogenesis is the formation of glucose from noncarbohydrate sources, primarily amino acids (from protein) and glycerol (from fat) in monogastric animals. Ruminants absorb volatile fatty acids rather than carbohydrates, and gluconeogenesis from propionic acid is a major source of blood glucose in ruminants. Glycogenolysis is the hydrolysis of glycogen to glucose.

• Kidney production. Although the liver is considered the primary source of glucose production, gluconeogenesis has also been documented in renal epithelial cells. One study in dogs demonstrated the kidney is responsible for about 30% of glucose turnover during fasting.[15] Renal gluconeogenesis occurs in the proximal tubule, and is now understood to have a significant effect on glucose metabolism in both normal and abnormal physiological states.[68]

Regulation of blood glucose concentration

Blood glucose concentrations are dependent on multiple interacting factors, including time since last meal, hormonal influences, and use of glucose by peripheral tissues such as skeletal muscle. Time since the last meal is important only in monogastric animals, in which food ingestion is followed by an increase in the blood glucose concentration.

Hormones affect the blood glucose concentration by regulating hepatic production and peripheral use of glucose (Table 27.1). Insulin is secreted by pancreatic islet β cells. Insulin lowers blood glucose concentrations by promoting glucose uptake by liver, skeletal muscle, and fat; by inhibiting gluconeogenesis in the liver; and by promoting the formation and storage of liver glycogen. Glucose uptake into myocytes and adipocytes is facilitated by a glucose transport protein called GLUT-4, which is translocated to plasma membranes after insulin binds to cell surface insulin receptors.[130] After a meal, approximately one-third of absorbed glucose is stored as glycogen within the liver, and approximately two-thirds is used as energy by other tissues.[125] Insulin also accelerates the conversion of glucose to fat, accelerates glucose oxidation, and promotes protein and glycogen synthesis in muscle. The net effect of these actions is increased hepatic and peripheral uptake and use of glucose, with decreased hepatic synthesis of glucose.

Table 27.1 Effects of various hormones on glucose metabolism and blood glucose concentrations.

Hormone	Actions	Effect on blood [Glucose]
Insulin	Promotes tissue glucose uptake Inhibits gluconeogenesis Promotes glycogen synthesis	Decrease
Glucagon	Promotes gluconeogenesis Promotes glycogenolysis Inhibits glycogen synthesis	Increase
Glucocorticoids	Promotes gluconeogenesis Promotes glucagon release Inhibits tissue glucose uptake	Increase
Catecholamines	Promote glycogenolysis Inhibits insulin secretion Stimulates growth hormone release	Increase
Growth hormone	Inhibits tissue glucose uptake Inhibits insulin action Promotes glucose production	Increase

Glucagon is secreted by α cells of the pancreatic islets in response to insulin-induced hypoglycemia. In direct contrast to insulin, glucagon increases blood glucose concentrations by stimulating hepatic gluconeogenesis and hepatic glycogenolysis, and inhibiting hepatic glycogen synthesis.[55]

Glucocorticoids increase blood glucose concentrations by promoting glucagon release, hepatic gluconeogenesis, and inducing a state of insulin resistance by affecting the ability of membrane proteins (such as GLUT-4) to transport glucose into cells.[106] The net effect of these actions is decreased peripheral use of glucose and increased hepatic synthesis of glucose.

Catecholamines (i.e., epinephrine and norepinephrine) increase blood glucose concentrations by increasing hepatic glycogenolysis, inhibiting insulin secretion, and stimulating growth hormone release.[106] The net effect of these actions is decreased peripheral use of glucose and increased hepatic synthesis and release of glucose.

Growth hormone increases blood glucose concentrations by inhibiting insulin-mediated uptake of glucose by hepatocytes, muscle cells, and adipose cells; by increasing hepatic production of glucose; and by exerting a post receptor influence within cells that inhibits the action of insulin on glucose metabolism.[47,87] The net effect of these actions is decreased peripheral use of glucose and increased hepatic synthesis of glucose.

Extreme physical activity might result in a decreased blood glucose concentration because of increased use of glucose by tissues such as skeletal muscle. In normal animals, hormonal influences keep the blood glucose concentrations stable during most types of physical activity.

Causes of hypoglycemia

Conditions that can cause hypoglycemia are listed in Table 27.2.

• Drugs. Therapeutic insulin overdose may occur when treating an animal that has diabetes mellitus. Similarly, sulfonylurea medications such as glipizide and glyburide, which act by stimulating insulin secretion, may cause hypoglycemia. An increased insulin concentration decreases gluconeogenesis and glycogenolysis and increases cellular uptake and use of glucose.

• Extreme exertion. Hypoglycemia may occur in hunting dogs and endurance horses, if glycolysis demands more glucose than gluconeogenesis or glycogenolysis can produce.[106]

• Glycogen storage diseases. These rare diseases are congenital deficiencies of the enzymes required for glycogenolysis, causing intracellular glycogen accumulation and possibly

Table 27.2 Causes of hypoglycemia.

Drugs
Insulin overdose
Sulfonylurea medications
Extreme exertion
Glycogen storage diseases
Hepatic insufficiency or failure*
Hypoadrenocorticism*
Hypopituitarism
Juvenile and neonatal hypoglycemia
Lactational hypoglycemia*
Neoplasia
β-cell tumor (insulinoma)*
Non-β-cell tumors
Pregnancy hypoglycemia
Sepsis*
Starvation or malabsorption
Xylitol toxicosis

* Relatively common.

hypoglycemia. A variety of specific enzyme deficiencies have been reported in cattle, dogs, cats, and horses.[34,59,90,111,116]

• Hepatic insufficiency/failure. Severe hepatic insufficiency or hepatic failure resulting from the loss of >70% of functional hepatic mass may cause hypoglycemia due to decreased gluconeogenesis and glycogenolysis. Other laboratory evidence of decreased hepatic function is expected to be present, such as hypoabluminemia, decreased BUN concentration, and increased serum bile acid concentration (see Chapter 26, Laboratory Evaluation of the Liver).

• Hypoadrenocorticism. Hypoglycemia occurs inconsistently in dogs with hypoadrenocorticism, likely caused by a lack of cortisol. Hypoglycemia is usually mild and probably results from decreased gluconeogenesis and increased insulin-mediated uptake of glucose by muscle tissue.[67,109]

• Hypopituitarism. Lack of ACTH secretion from the pituitary results in hypocortisolemia, which may cause mild hypoglycemia. Lack of growth hormone secretion may also contribute to hypoglycemia.

• Juvenile and neonatal hypoglycemia. Neonatal hypoglycemia is especially common in pigs, but it can occur in other species. It is usually associated with poor nursing secondary to diarrhea, dehydration, or hypothermia in the piglets, or agalactia in the sow.[22,85] Hypoglycemia during periods of decreased food intake in neonates results from inadequate storage pools of glycogen and protein, which could be used for glucose production. Juvenile hypoglycemia is a syndrome that usually is seen in toy breed puppies younger than 6 months.[110,113] Clinical signs often are triggered by stressors such as diarrhea, fasting, or parasitism. As in neonatal hypoglycemia, inadequate storage pools of glycogen and protein probably play an important role in this syndrome. Inadequate levels of hepatic enzymes for gluconeogenesis also may contribute.

• Lactational hypoglycemia. This syndrome, also known as spontaneous bovine ketosis, occurs in cattle during periods of marked milk production.[5] Hepatic gluconeogenesis is unable to meet the demand for glucose production, and ketosis develops due to increased fat mobilization.

• Neoplasia. Neoplasms of the β cells of the pancreatic islets (insulinomas) are the most common tumors associated with hypoglycemia. Insulinomas have been reported in dogs, cats, and ferrets.[16,28] Excess insulin production by the neoplastic β cells causes increased glucose utilization by tissues and decreased hepatic gluconeogenesis and glycogenolysis. Hypoglycemia may be sporadic, but is often of sufficient magnitude to cause clinical signs of weakness and seizures. Non-β cell tumors of several types have also been associated with a paraneoplastic hypoglycemia. In dogs, many of these tumors have been mesenchymal (leiomyoma, leiomyosarcoma), but epithelial tumors (hepatic carcinoma, renal carcinoma, and others) and round cell tumors (lymphoma, plasma cell tumor) have also been reported.[28] Proposed mechanisms for the hypoglycemia include liver dysfunction, glucose utilization by neoplastic cells, and neoplastic cell production of insulin-like growth factor.[131] In horses, hypoglycemia has been reported in association with hepatic and renal neoplasia, peritoneal mesothelioma, and gastrointestinal stromal tumor.[6,37,42,65,86]

• Pregnancy hypoglycemia. A syndrome of hypoglycemia and ketonemia may occur during late pregnancy in dogs and sheep.[56,91] There is a decreased ability to produce glucose via gluconeogenesis, glycogenolysis, and lipolysis due to blunting of the normal responses to hypoglycemia. In sheep, this is referred to as pregnancy toxemia, which may be related to the number of fetuses and quality/quantity of feed. Pregnancy hypoglycemia appears to be uncommon in dogs.

• Sepsis. Hypoglycemia occurs inconsistently with sepsis, most often associated with endotoxemia. Experimentally, hyperglycemia occurs early, followed by hypoglycemia.[71] The causes of hypoglycemia that occurs in association with sepsis are not completely understood. Possible causes include impaired gluconeogenesis and glycogenolysis and increased use of glucose by tissues, including leukocytes.

Hypoglycemia secondary to glucose consumption by large numbers of hemotropic mycoplasmas, bacteria that parasitize erythrocytes, has been reported in pigs, sheep, llamas, and calves. However, rapid bacterial glycolysis in vitro may also cause artifactually decreased blood glucose concentrations.[13]

• Starvation or malabsorption. Decreased glucose absorption from the intestine is a rare cause of hypoglycemia. Hypoglycemia only occurs after long-term starvation or malabsorption, because gluconeogenesis helps to maintain a

normal blood glucose concentration at the expense of other substances, principally protein.

• Xylitol toxicosis. Xylitol is used as a sugar substitute in various products, and is a strong promoter of insulin release in dogs. Severe hypoglycemia has been reported in dogs following ingestion of xylitol-containing sugar-free products.[23,24] Xylitol also causes marked changes in liver leakage enzymes, as described for diffuse hepatic necrosis (Chapter 26).

Causes of hyperglycemia

Conditions that can cause hyperglycemia are listed in Table 27.3.

• Drugs or toxins. A variety of drugs are associated with transient mild hyperglycemia. The mechanisms of action differ. Detomidine, xylazine, propanolol, and thyroxine inhibit insulin release. Progestins and morphine stimulate growth hormone release; ketamine stimulates epinephrine release. Megestrol acetate acts as a steroid and also stimulates growth hormone release. The Somogyi effect is a paradoxical hyperglycemia that may occur in a diabetic animal in response to excess insulin administration. The actions of glucagon and glucocorticoids were described earlier (see Regulation of Blood Glucose Concentration).

Table 27.3 Causes of hyperglycemia.

Drugs or toxins
Detomidine
Ethylene glycol
Glucocorticoids
Glucagon
Insulin (Somogyi effect)
Intravenous glucose
Ketamine
Megestrol acetate
Morphine
Progestins
Propanolol
Thyroxine
Xylazine
Physiologic
Diestrus (progestins)
Exertion/excitement/pain (catecholamines)
Postprandial (monogastrics)
Stress (corticosteroids)
Pathologic
Diabetes mellitus
Hepatocutaneous syndrome (dogs)
Hyperammonemia (horses and cattle)
Metabolic syndrome (horses)
Milk fever (cattle)
Moribund animals
Neoplasia—acromegaly, glucagonoma, hyperadrenocorticism, pheochromocytoma, hyperthyroidism, hyperpituitarism
Pancreatitis
Proximal duodenal obstruction (cattle)

• Physiologic. Mild hyperglycemia can occur secondary to several physiologic responses. During diestrus, progesterone stimulates release of growth hormone, which decreases tissue glucose utilization. Catecholamine release (epinephrine and norepinephrine) associated with excitement, pain, or strenuous exertion stimulates growth hormone release, inhibits insulin secretion, and stimulates glycogenolyis. Cats frequently exhibit transient hyperglycemia related to struggling during blood collection; the magnitude of the hyperglycemia may reach 300 mg/dL or greater, and it may persist for 1.5–2 hours.[75,80] A stress response, caused by endogenous corticosteroid release, stimulates gluconeogensis, glucagon release, and causes a state of insulin resistance. Corticosteroid and/or catecholamine release likely play a role in many different disease processes in which hyperglycemia occurs secondarily. Monogastric animals experience a normal postprandial increase in blood glucose concentrations that typically subsides within 4 hours. The magnitude of increase may be either constrained within the reference interval or result in an interpreted mild hyperglycemia.

• Diabetes mellitus. Diabetes mellitus is caused by a deficiency of insulin production or an interference with the action of insulin in target tissues, thereby resulting in abnormal glucose metabolism. Altered protein and lipid metabolism also occurs in diabetes mellitus. Diabetes is typically associated with the greatest degrees of hyperglycemia. Therefore, animals with diabetes mellitus usually have blood glucose concentrations greater than the renal threshold resulting in glucosuria. Glucosuria occurs less commonly with other causes of glucose intolerance. Diabetes mellitus has been classified according to the underlying cause as either type 1 or type 2, and by the dependence of the affected animal on insulin therapy as either insulin dependent (IDDM) or noninsulin dependent (NIDDM). These two classification schemes overlap, causing confusion regarding the types of diabetes mellitus occurring in animals. Type 1 diabetes mellitus results from immune-mediated destruction of pancreatic β cells, and animals with type 1 diabetes mellitus are insulin dependent. Type 1 diabetes is the most frequent cause of diabetes in dogs, but it has not been well documented in cats.[48] Insulin dependent diabetes mellitus can also occur secondary to other disease processes that destroy β cells (such as pancreatitis[48]), or cause β cell hypoplasia (genetic diabetes mellitus in Keeshond dogs,[64] and juvenile pancreatic atrophy in greyhounds.[10]) Type 2 diabetes mellitus is characterized by a sluggish insulin response to hyperglycemia (i.e., decreased capacity to produce insulin) and a poor tissue response to insulin (i.e., insulin resistance). Animals with type 2 diabetes mellitus may be either insulin or noninsulin dependent. This is the most common type of

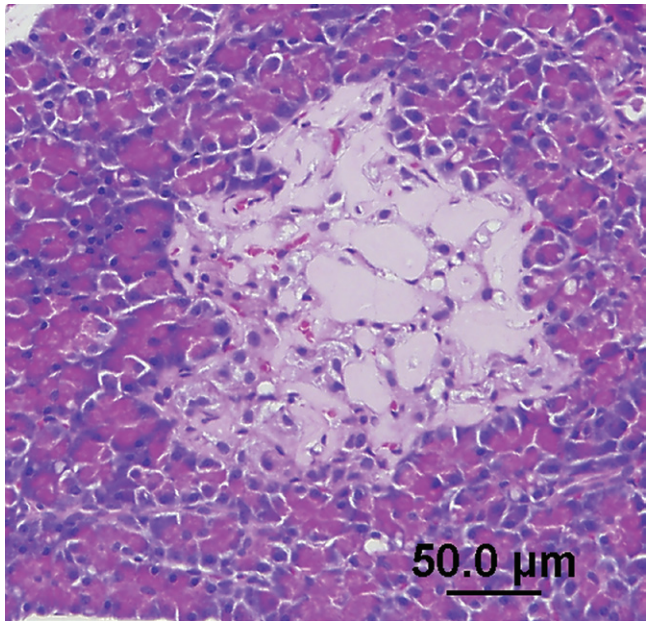

Figure 27.2 Pancreatic amyloidosis. Amyloid deposition surrounds β cells and has enlarged this islet of Langerhans. Amyloid deposition is toxic to β cells and hampers insulin secretion.

diabetes mellitus in cats, but it can occur in dogs as well.[48] Approximately 70% of cats with type 2 diabetes mellitus are insulin dependent.[30]

The pathogenesis of type 2 diabetes mellitus in cats is complex and incompletely understood. A consistent finding in over 90% of diabetic cats is deposition of islet amyloid, derived from islet amyloid polypeptide (IAPP, or amylin)[72] (Fig. 27.2). Pancreatic amyloidosis is toxic to β cells, causing cell death and decreased insulin secretion.[72] IAPP is secreted by β cells along with insulin, and states of insulin resistance (e.g., obesity) cause increased secretion of both insulin and IAPP. In turn, circulating IAPP may contribute to peripheral insulin resistance. Obesity causes insulin resistance in several ways (down-regulates insulin receptors, impairs receptor affinity for insulin, causes postreceptor defects in insulin action) and is considered a major risk factor for diabetes mellitus in cats.[9,30]

- Hepatocutaneous syndrome. This uncommon syndrome in dogs is characterized by liver disease in combination with superficial necrolytic dermatitis. Hyperglycemia is common, but the pathogenesis is not clear.[39]
- Hyperammonemia. Hyperglycemia may occur in horses and cattle with hyperammonemia that is unrelated to liver disease (e.g., excess ammonia production in the intestine, urea toxicosis, ammonia toxicosis). Proposed mechanisms include stimulation of gluconeogenesis and reduced tissue uptake of glucose.[2,31]
- Metabolic syndrome. Serum glucose concentrations may be increased or normal in horses with metabolic syndrome, which is a complex disorder that mimics Cushing's disease.[57] Affected horses are typically obese and insulin resistant, and are prone to develop laminitis.
- Milk fever. Hyperglycemia, along with hypocalcemia and hypophosphatemia, is often present in cattle with milk fever (parturient paresis).[89] Hypocalcemia suppresses insulin release;[126] catecholamine and/or corticosteroid release in "down" cows may also contribute to the hyperglycemia.
- Moribund animals. Hyperglycemia may occur in moribund animals, usually ruminants. Likely causes include catecholamine and/or corticosteroid release, and decreased peripheral use of glucose.
- Neoplasia. A variety of neoplastic diseases can predispose to development of diabetes mellitus. Acromegaly is typically caused by a pituitary adenoma that secretes growth hormone, and occurs most commonly in cats. Excess growth hormone promotes insulin resistance. Glucagonoma is a pancreatic α cell tumor that secretes glucagon, which increases hepatic glucose production. Hyperadrenocorticism, whether due to pituitary or adrenal neoplasia, results in excess cortisol production that increases hepatic gluconeogenesis and causes insulin resistance. Hyperadrenocorticism is a fairly common concurrent disorder in dogs diagnosed with diabetes mellitus.[45] Pheochromocytomas secrete catecholamines, which inhibit insulin secretion and stimulate glycogenolyis. A small percentage of cats with hyperthyroidism are persistently hyperglycemic, theorized to be due to insulin resistance; the mechanism is unknown.[49] Hyperpituitarism may be due to pituitary hyperplasia or neoplasia, with excess secretion of growth hormone or ACTH that causes insulin resistance and increased cortisol concentrations. Pituitary pars intermedia dysfunction in horses causes increased ACTH secretion and hyperglycemia.[92]
- Pancreatitis. Destruction of β cells due to pancreatitis can lead to development of insulin dependent diabetes mellitus. This may be the underlying cause in up to 30% of canine IDDM cases.[48]
- Proximal duodenal obstruction. Cattle with proximal duodenal obstruction may have marked hyperglycemia, up to 1000 mg/dL.[35] The proposed pathogenesis is a combination of stress and decreased peripheral glucose utilization. By contrast, cattle with abomasal volvulus have a much milder hyperglycemia, usually attributed to stress.

Laboratory evaluation of glucose metabolism

Blood glucose

Measurement of the blood glucose concentration is the initial step in evaluating glucose metabolism. After detection of either hyperglycemia or hypoglycemia, tests for more specific evaluation of glucose metabolism may be required. Analysis of blood glucose concentration can be performed by a reference laboratory and is usually part of the standard biochemical profile. Serum or plasma is the sample required by reference laboratories, and it must be separated from

erythrocytes within 30 minutes of blood collection. Glycolysis in erythrocytes results in loss of 10% of glucose per hour if the serum or plasma remains in contact with erythrocytes. Sodium fluoride anticoagulant inhibits glycolysis and should be used if serum or plasma cannot be separated from cells promptly. All in-clinic clinical chemistry analyzers also have glucose methods, either as single tests or included in panels. Portable blood glucose meters (PBGMs) are also available that allow rapid and repeated measurements of whole blood glucose concentrations in clinic situations; some pet owners utilize these instruments to monitor diabetic pets at home.[14,95,118] Several of these instruments have been evaluated for use in animals; most of them provide results that differ to some degree from reference methods.[17,119] In most cases (but not all), glucose concentrations determined by PBGMs are lower than those determined by reference methods. Therefore, it is important to consider test methodology when comparing results from any individual patient.

Because blood glucose concentrations in monogastric animals are increased for 2–4 hours postprandially, glucose concentrations should be measured after fasting. Dogs and cats should be fasted for 12 hours before sampling to avoid postprandial influences. Potentially hypoglycemic animals should not be fasted before sampling, however, because severe hypoglycemia may result. Horses usually are not fasted before collecting blood samples for glucose analysis; however, blood glucose concentrations might increase during a period of 2–4 hours after eating high-energy supplements. It is not necessary to fast ruminants before blood glucose analysis, because they primarily absorb volatile fatty acids rather than glucose from the gastrointestinal tract.

Artifactual hypoglycemia may occur due to in vitro consumption of glucose in cases of extreme leukocytosis and marked erythrocyte parasitemia with hemotropic mycoplasmas.

Urine glucose

Urine glucose measurement is discussed in Chapter 23. Glucosuria occurs when the blood glucose concentration exceeds the renal threshold, which varies by species. Renal thresholds are between 180 and 220 mg/dL in dogs,[29] 200 to 300 mg/dL in cats,[30] 180–200 mg/dL in horses,[60] and 100 mg/dL in cattle.[60] Concurrent measurement of blood glucose is important when interpreting glucosuria; diabetic animals typically have both persistent hyperglycemia and glucosuria. Glucosuria can occur in the absence of hyperglycemia if the renal glucose threshold is decreased. Decreased renal thresholds usually result from proximal tubular abnormalities, which may be acquired or congenital. Acquired abnormalities include those caused by ischemia, nephrotoxins, and amyloidosis;[21] congenital disorders include primary renal glucosuria and Fanconi syndrome.[38]

Serum insulin

Insulin levels can be determined in serum or heparinized plasma. These are usually immunoassays using antibodies developed to detect porcine or human insulin, but there is good crossreactivity with canine insulin; assays should be validated for the species of interest. Serum insulin is stable for a week if kept refrigerated, and for several months if frozen.[106]

Insulin levels are most frequently measured in hypoglycemic animals when insulinoma is suspected. Because animals with insulinoma may be euglycemic on a random blood sample, it is important to document inappropriate insulin levels at the same time that hypoglycemia is present. Normally, insulin concentrations should be very low when glucose concentrations are low. In dogs with a blood glucose <60 mg/dL, detection of insulin concentrations that are above the reference interval (usually >20 μU/mL) is strong evidence for insulinoma.[28] Insulinoma is possible in hypoglycemic dogs with insulin concentrations in the mid to upper reference interval (10–20 μU/mL). Fasting, with hourly evaluation of blood glucose concentrations, may be required to achieve the desired hypoglycemic state for accurate results. Dogs must be carefully monitored during this process to avoid life-threatening hypoglycemia. Following the test, the dog should be fed several small meals over several hours. Calculated ratios (insulin : glucose or amended insulin : glucose) are not reliable for diagnosis of insulinoma and are not recommended.

In theory, measurement of insulin levels in diabetic animals could help to classify their disease as IDDM or NIDDM. Practically, however, this has not proved to be very useful. The vast majority of dogs have IDDM with low serum insulin concentrations. Most cats with type 2 diabetes mellitus (insulin resistant) also have low serum insulin and require insulin therapy, although some only transiently. Prolonged hyperglycemia and glucose toxicity, which impairs β cell function, is thought to be responsible for this finding.[30]

Fructosamine

Fructosamine is a general term that refers to any glycated protein (i.e., a protein with attached carbohydrate). Fructosamine is formed when glucose is linked irreversibly to amine groups of albumin and other proteins in the blood.[4] The serum fructosamine concentration is an indicator of blood glucose concentrations during the previous 2–3 weeks (based on the average life-span of the proteins involved in this complex).[83] Fructosamine provides more reliable information regarding the long-term state of glucose metabolism than the blood glucose concentration, which may be transiently increased in some situations. Fructosamine, therefore, has potential in establishing the diagnosis of diabetes mellitus and in monitoring therapy for diabetics.

Serum fructosamine assays are available at reference laboratories. Fructosamine appears to be quite stable in serum

kept refrigerated (~10 days) or frozen (~30 days).[54,63] Hemolyzed samples may give erroneous results, and should be avoided. Hyperproteinemia and hyperbilirubinemia do not appear to affect test results.[82]

Increased fructosamine concentrations

Increased fructosamine concentrations are indicative of persistently increased blood glucose concentrations and, in diabetic animals receiving insulin treatment, of a lack of therapeutic control of blood glucose concentrations during the previous 2–3 weeks. Because hyperglycemia is relatively common even in well-controlled diabetics, the cut-off value used for a determination of poor glycemic control is greater (typically >500 µmol/L) than the upper reference limit for nondiabetic animals (typically 365 µmol/L).[29,30]

Fructosamine also is useful in distinguishing excitement-induced hyperglycemia from diabetic hyperglycemia in cats. Fructosamine concentrations are usually within the reference interval in cats with hyperglycemia caused by excitement, since hyperglycemia must be present for approximately four days before increased fructosamine concentrations are detected. The reported sensitivity of increased fructosamine concentration for detection of diabetes mellitus in cats is 93%, with a specificity of 86%.[19] The reported sensitivity and specificity of increased fructosamine concentration for detection of diabetes mellitus in dogs is 88% and 99%, respectively.[53]

Mildly increased fructosamine concentrations have been reported in some dogs with hypothyroidism; however, those dogs were not hyperglycemic.[81] Prolonged albumin half-life due to decreased protein turnover is the proposed mechanism.

Decreased fructosamine concentrations

Decreased fructosamine concentrations are expected with persistent hypoglycemia, as occurs with insulinoma. However, because fructosamine is a measure of glycated proteins, hypoproteinemia may be a cause of decreased fructosamine concentrations. In one study, normoglycemic dogs had decreased fructosamine concentrations that correlated best with the degree of hypoalbuminemia, while normoglycemic cats had decreased fructosamine concentrations that correlated best with the degree of hypoproteinemia.[82] Based on these correlations, formulae have been suggested to correct fructosamine concentrations for protein abnormalities in dogs and cats. In these formulae, the reference interval median value is used for the "normal" albumin and total protein concentrations.

Dogs: Corrected fructosamine = fructosamine × (normal albumin ÷ patient albumin)

Cats: Corrected fructosamine = fructosamine × (normal total protein ÷ patient total protein)

Decreased fructosamine concentrations have been reported in normoglycemic cats with hyperthyroidism, likely due to increased protein turnover.[84] Decreased fructosamine concentrations in the absence of hypoglycemia or hypoproteinemia have also been reported in some animals with parasitic infections, including dogs with *Angiostrongylus vasorum*[122] and sheep with *Teladorsagia circumcincta*[94] infections. Increased protein turnover is suspected in these cases. Decreased fructosamine concentrations were reported in normoglycemic, normoproteinemic dogs (but not cats) that were hyperlipidemic or azotemic.[82]

Glycated hemoglobin

Glycated hemoglobin (GHb) is formed in erythrocytes by an irreversible reaction between carbohydrates (especially glucose) and hemoglobin. Glycated hemoglobin forms continuously during the life span of an erythrocyte; therefore, older erythrocytes usually contain more GHb compared with younger erythrocytes. The amount of GHb that is formed is proportional to the blood glucose concentration during the life span of the erythrocyte. The blood GHb concentration reflects glucose status during a longer period of time than does the serum fructosamine concentration, because of relatively long erythrocyte life spans (approximately 110 days in dogs, 70 days in cats, 150 days in cattle and horses).[52] Increased GHb concentrations do not immediately return to normal after reestablishing more normal blood glucose concentrations, because this requires the removal of senescent erythrocytes with high GHb concentrations. Such decreases in GHb concentrations might be delayed for several weeks. Glycated hemoglobin can be used in the same situations as fructosamine.[26,27] However, fructosamine concentrations change faster with changes in blood glucose concentrations, which may be an advantage in many situations.

Glycated hemoglobin is measured in EDTA-anticoagulated whole blood, and is stable for 7 days when refrigerated.[29] GHb will be decreased in anemic animals, due to decreased hemoglobin concentrations and/or increased numbers of reticulocytes present as part of a regenerative response.[27] Conversely, GHb will be increased in polycythemic animals.

Serial glucose curve

In diabetic animals receiving initial insulin therapy, measurement of blood glucose concentrations at 1–2 hour intervals throughout the day helps to assess the efficacy and appropriateness of the insulin dosage. These results, which are known as the serial glucose curve, are analyzed to ensure that the insulin therapy has lowered the blood glucose concentrations, that the lowest glucose concentration after insulin treatment (nadir) is in an appropriate range, and that the duration of the insulin effect is appropriate (Fig. 27.3). Serial glucose curves are also useful when animals with previously well-controlled IDDM show clinical signs of hyperglycemia or hypoglycemia.

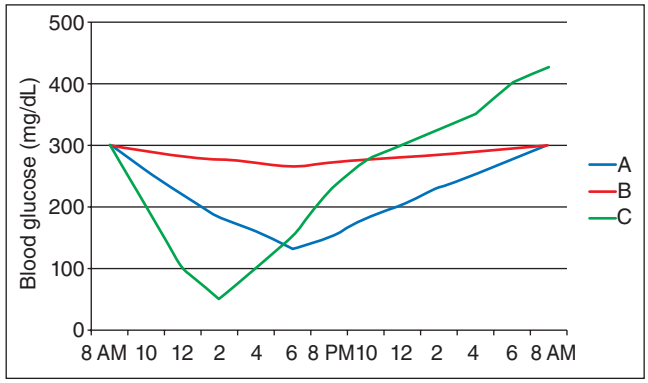

Figure 27.3 Hypothetical serial glucose curves in three diabetic cats receiving insulin at 8 am. Cat A appears well-controlled, with blood glucose concentrations reaching a nadir of about 125 mg/dL and staying between 125 and 300 mg/dL over a 24-hour period. Cat B exhibits a poor response to insulin, which may be related to insulin underdosage. Cat C becomes rapidly hypoglycemic, with rebound hyperglycemia. This is known as the Somogyi effect, which is due to hormonal responses following excess insulin administration.

In diabetic dogs, the goal is to keep glucose concentrations between 100 and 250 mg/dL.[29] In diabetic cats, the goal range is 100–300 mg/dL.[30] Ideally, the blood glucose nadir should be 100–125 mg/dL for both dogs and cats. Many factors must be taken into account when interpreting serial glucose curves, including the type and duration of insulin being administered, time of feeding, and stress and/or excitement induced by hospitalization during the procedure. Portable blood glucose meters are sometimes used by owners of diabetic pets to generate serial glucose curves at home, under the supervision of their veterinarian, to avoid the effects of stress or excitement.[118]

Continuous glucose monitoring

New technological developments are providing advances in glucose monitoring of diabetic animals. A continuous glucose monitoring system (CGMS) utilizes a subcutaneous sensor that measures interstitial fluid glucose concentrations and stores up to 288 measurements in a 24-hour period.[120] Interstitial fluid glucose concentrations correlate well with blood glucose concentrations. A commercially available CGMS has been tested on dogs, cats, and horses.[121] Use of this system avoids limitations associated with traditional serial glucose curves such as repeated blood collection, patient restraint and hospitalization, and provides more detailed information about glucose metabolism since measurements are taken every 5 minutes.

Glucose tolerance tests

Oral or intravenous glucose tolerance tests can be performed to provide more information about glucose metabolism in animals suspected of having insulin resistance. These tests are labor and time intensive and rarely used in clinical small animal practice, but are occasionally performed in horses that are suspected to have metabolic syndrome[79] and are used in research settings.[112] As discussed previously (see Serum Insulin), it is rarely clinically useful to document insulin resistance in animals that have been diagnosed with diabetes mellitus.

Basically, these tests involve administration of a glucose solution followed by blood collection at predetermined intervals; blood samples are analyzed for glucose concentrations and sometimes insulin levels. Intravenous tests are considered superior to oral tests because gastrointestinal factors are eliminated. Decreased glucose tolerance is suggested if glucose concentrations fail to return to baseline within the expected time period or if the calculated fractional glucose turnover rate is low. Insulin response tests can also be performed,[3] and a test combining glucose tolerance with insulin response (combined glucose-insulin test) has been developed for horses.[25]

Other laboratory abnormalities associated with diabetes mellitus

- CBC findings may include increased PCV/HCT and increased plasma protein concentrations due to dehydration. The leukogram may indicate stress or inflammation.
- Azotemia and dilute urine. Glomerular lesions have been reported in diabetic dogs and cats, but the occurrence of clinical renal disease in such animals is not well documented. Urine specific gravity usually is low in animals with glucosuria, generally because of the osmotic effect of glucose rather than from a defect in the ability of the tubules to concentrate urine. If dehydration is present, there may be a prerenal azotemia in addition to dilute urine, mimicking renal failure. The serum phosphorus concentration also may be increased in azotemic animals because of the decreased glomerular clearance of phosphorus. Some diabetic animals have hyperphosphatemia, but hypophosphatemia may occur in others (discussed later).
- Pyuria, hematuria, and proteinuria. Urinary tract infection is common in diabetic animals. Such infection can result in increased numbers of leukocytes, erythrocytes, and bacteria in the urine as well as in an increased concentration of protein. Increased urine protein concentration without evidence of inflammation could result from glomerular damage, which commonly occurs in humans with diabetes, but is not well documented in animals with diabetes mellitus.
- Ketonuria. Ketones include acetoacetate, β-hydroxybutyrate, and acetone. Deficient insulin production in diabetes mellitus results in decreased incorporation of fatty acids into triglycerides (i.e., decreased lipogenesis). Fatty acids then are converted to acetyl–coenzyme A (acetyl-CoA). Almost all acetyl-CoA is converted to acetoacetate in animals with severe diabetes mellitus. Some of this acetoacetate then is converted to β-hydroxybutyrate and acetone. Increased

blood ketone concentration (i.e., ketonemia) and increased urine ketone concentration (i.e., ketonuria) can result. Ketones are acids that dissociate into hydrogen ions and respective unmeasured anions. Their metabolic production therefore results in development of acidosis and increased anion gap (see below). The common method of detecting ketones used by urine dipsticks (nitroprusside reaction) detects acetoacetate and acetone, but it does not detect β-hydroxybutyrate. In some ketoacidotic patients, production of β-hydroxybutyrate can predominate, thereby resulting in failure to detect ketonuria. Impaired peripheral use of ketones because of insulin deficiency also contributes to ketonemia and ketonuria in diabetes mellitus. The renal threshold for ketones is low, and ketonuria often precedes ketonemia. Causes of ketonemia and ketonuria, in addition to diabetes mellitus, include starvation, bovine ketosis, pregnancy toxemia in sheep, and hepatic lipidosis syndrome of cattle.

- Electrolyte abnormalities. Osmotic diuresis and ketonuria cause the loss of sodium, chloride, potassium, and phosphorus in the urine. Hyponatremia, hypochloremia, and less commonly, hypokalemia and hypophosphatemia may result. The serum potassium concentration may be normal or increased in diabetic animals, especially if the animals are acidotic, but the whole-body potassium concentration is often depleted. Potassium depletion results from hypoinsulinemia, which allows intracellular potassium to shift out of cells and into blood; this potassium then is lost via the urine. This has an important therapeutic implication because administration of insulin in treatment of the acute stage of diabetes will drive potassium back inside cells, which may cause severe hypokalemia. Phosphorus depletion results from multiple factors, including increased renal excretion, increased tissue catabolism, and in animals treated with insulin, shifting of phosphorus from the serum into cells. Serum phosphorus concentrations of less than 1.5 mg/dL may occur in diabetic dogs and cats, especially after the initiation of insulin therapy. Severe hypophosphatemia may potentially result in hemolysis, leukocyte or platelet dysfunction, neurologic disorders, and abnormal muscle function.
- Metabolic acidosis (ketoacidosis). Ketones are acidic, and increased concentrations lead to metabolic acidosis, which can be life threatening.
- Increased anion gap. An increased anion gap usually results from increased ketoacid concentrations in the blood. Increased blood lactate concentration also can contribute to this gap.
- Hyperosmolarity. Hyperosmolarity usually occurs in animals with extremely high blood glucose concentrations (>600 mg/dL). A serum osmolarity of >350 mOsm/L can cause neurologic and gastrointestinal abnormalities.
- Increased hepatic and pancreatic enzyme activities. Metabolic alterations in hepatocytes can lead to the leakage of enzymes. Fatty change in hepatocytes results from the increased liberation of fatty acids from adipose tissue, influx of these fatty acids into hepatocytes, and incorporation of fatty acids into triglycerides. Activities of induced enzymes also increase if these alterations result in hepatocyte swelling and cholestasis. Pancreatitis can cause diabetes mellitus as a result of islet damage, and if active pancreatitis is present, serum activities of PLI, amylase or lipase may be increased.
- Increased serum bilirubin concentration. Cholestasis secondary to the hepatocyte swelling that is associated with fatty change may lead to hyperbilirubinemia. Moreover, hemolysis resulting from Heinz-body formation can occur in diabetic cats and result in increased serum bilirubin concentrations.
- Hyperlipidemia. Increased blood concentrations of several lipids, including triglycerides, cholesterol, and free fatty acids, result from decreased incorporation of triglycerides into fat deposits, decreased hepatic degradation of cholesterol, and increased hepatic production of very low-density lipoproteins. Increased concentrations of these proteins often result in visible lipemia.

Other laboratory abnormalities associated with hyperinsulinism

In addition to hypoglycemia, the only laboratory abnormality that is frequently associated with hyperinsulinism is hypokalemia, which may result from insulin-mediated shifting of extracellular potassium into cells.

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28 Laboratory Evaluation of Digestion and Intestinal Absorption

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Introduction

Diarrhea, vomiting, and weight loss are clinical signs that are frequently seen with diseases of the digestive system. These signs are not, however, indicative of a specific disease or cause. Laboratory tests that specifically evaluate the digestive system can provide important diagnostic information in these cases. Because many underlying disease processes can secondarily affect the gastrointestinal tract, basic laboratory tests such as a CBC, biochemical profile, urinalysis, and routine fecal examination are usually performed in animals with evidence of gastrointestinal disease prior to more specialized tests.

Eventually biopsy of the affected area of the GI tract may be required to obtain a definitive diagnosis, but it must be borne in mind that there are a number of functions of the bowel such as motility, absorption, secretion, permeability, visceral sensitivity, and oral tolerance that may be compromised without evidence of structural or morphological anomalies.[30] The choice of laboratory tests to evaluate the digestive system depends on whether clinical signs are suggestive of acute or chronic disease.

There are a number of breed-specific predispositions to various GIT disturbances. Knowledge of these diseases may help in the assimilation of a differential list but should not be used in isolation to make a definitive diagnosis. These are listed in Table 28.1.

Two important syndromes that cause signs of chronic gastrointestinal disease are maldigestion and malabsorption. Maldigestion is a failure to adequately digest food and usually results from extensive atrophy of exocrine pancreatic acinar cells, causing inadequate secretion of digestive enzymes. This is known as exocrine pancreatic insufficiency (EPI), which results secondarily in the inadequate absorption of nutrients. By contrast, malabsorption is failure of the intestinal tract to absorb adequately digested nutrients and results from a variety of small intestinal lesions. Clinical signs of these two syndromes can be similar, including increased fecal volume and poorly formed feces; however, the treatment for these conditions differs. This chapter describes the use of laboratory tests to differentiate maldigestion from malabsorption in animals showing signs of weight loss, and to differentiate between EPI and other small intestinal disorders. In addition, several other laboratory tests to evaluate the digestive system are discussed.

Maldigestion and malabsorption in dogs and cats

Because the treatment and prognoses differ in maldigestion and malabsorption, distinguishing these two syndromes is important in small animals. Since malabsorption may result secondary to maldigestion, the clinical signs are similar regardless of which syndrome is the primary disease process.

Exocrine pancreatic insufficiency (EPI) or maldigestion

Although EPI can occur at any age, it is usually recognized in young dogs (1–5 years of age). Dogs are thin, have a ravenous appetite, and typically have voluminous greasy, rancid, gray stools (steatorrhea), often with diarrhea. Steiner[77] reports that lesions suggesting feline exocrine pancreatic insufficiency (EPI) have been found to be 0.2% of the feline pancreata that he has examined. The most frequently reported clinical signs of EPI in cats include loss of weight, copious amounts of loose stools and oily soiling of the hair coat.[77]

A number of specific conditions are recognized:

- Idiopathic pancreatic acinar atrophy is the most common cause of EPI in dogs but has not been reported in cats.[89]

Veterinary Hematology and Clinical Chemistry, Second Edition. Edited by Mary Anna Thrall, Glade Weiser, Robin W. Allison, and Terry W. Campbell.

Table 28.1 Common breed-specific predispositions to various gastrointestinal disorders found in dogs and cats.

Gastrointestinal disorder	Breed
Inflammatory bowel disease (IBD)[28]	German shepherds Shar Peis Basenjis[57] Soft-coated Wheaten terriers[43] Siamese cats
Lymphangiectasia	Yorkshire terriers Rottweilers Norwegian Lundehunds[30]
Protein losing enteropathies (PLE)	Basenji Chinese Shar-Pei Soft-coated Wheaten terriers (the latter have also been documented with protein-losing nephropathy[34]
Exocrine pancreatic insufficiency (heritable)	German shepherds Rough collies
Small intestinal bacterial overgrowth (antibiotic-responsive diarrhea)	Young, large-breed dogs, especially German shepherds
Lymphoplasmacytic enteritis and hypergammaglobulinemia	Basenji[7,57]
Histiocytic ulcerative colitis (HUC)[27]	Boxers French bulldogs Mastiffs Alaskan malamute Doberman pinscher[12]
Gluten-sensitive enteropathy	Irish setter (clinical onset between 4–7 months of age)[4,23,24]

Table 28.2 Causes of malabsorption syndromes in dogs.

Disease syndrome	Breeds associated with Disease syndrome and comments
Chronic inflammatory small intestinal enteropathies	Yorkshire terriers
Eosinophilic enteritis	Basenji[57]
Lymphocytic-plasmacytic enteritis	Norwegian Lundehund
Granulomatous enteritis	Soft-coated Wheaten terriers[34,43]
Histoplasmosis	
Pythiosis	
Giardiasis	
Protothecosis	
Lymphangiectasia	
Intestinal lymphoma / adenocarcinoma	Occult blood may be useful to evaluate gut bleeding in these cases
Defective brush-border enzymes	
Villous atrophy	
Wheat sensitive enteropathy	Hereditary in Irish setters[23]
Bacterial overgrowth	

• Juvenile pancreatic acinar atrophy is thought to be caused by hereditary immune-mediated lymphocytic pancreatitis and has been reported in German shepherd dogs and rough-coated collies.[1a,86]

• Exocrine pancreatic insufficiency may be acquired in dogs and cats secondary to chronic pancreatitis due to atrophy and /or fibrosis of the pancreas. There may be subsequent development of diabetes mellitus if there is concurrent islet cell destruction.[84] Chronic pancreatitis occurs more commonly in cats than dogs,[79] and chronic pancreatitis is thought to be the most common cause of EPI in cats.[89]

• Pancreatic duct obstruction can occur in dogs and cats and may impair secretion of pancreatic enzymes into the intestine. This is usually associated with acute inflammation but animals do not necessarily develop maldigestion. However, some cases of pancreatic neoplasia that cause obstruction of the pancreatic duct may lead to pancreatic atrophy.[77,89]

• Infection with the fluke *Eurytrema procyonis*, has also been reported to cause EPI in cats without previous evidence of pancreatitis, although this is extremely rare.[24]

The serum TLI assay (discussed later) is the most common test used to diagnose EPI. There are no consistent hematological or biochemical changes with maldigestion and amylase and lipase values are usually normal in cases of EPI. Undigested fats may be found in the feces, but this is an inconsistent finding.

Malabsorption

There are many possible underlying causes of malabsorption, including inflammatory, infectious, and neoplastic processes. A list of possible causes of malabsorption syndromes in dogs can be seen in Table 28.2. Specific function testing for intestinal absorption is not always necessary as syndromes such as osmotic diarrhoea can usually be recognized clinically and signs generally tend to cease when the animal is fasted. Osmotic diarrhea is associated with water retention in the GIT, which results from the presence of osmotically active solutes in the intestine that are not absorbed. TLI testing can be used to differentiate osmotic diarrhea due to malabsorption from that due to maldigestion[30] (see later). Examples of osmotic and secretory diarrheas can be found in Table 28.3.

Table 28.3 Examples of osmotic and secretory diarrheas.

Osmotic Diarrheas	Secretory Diarrheas
Johne's disease Granulomatous colitis Eosinophilic gastroenteritis Intestinal lymphosarcoma Lymphangiectasis Protein-losing enteropathies Proximal enteritis (horses) Magnesium cathartics Other maldigestion/malabsorption syndromes	Salmonellosis (depends on protein loss, i.e., higher protein loss = higher gap) Enterotoxic colibacillosis Endotoxemia

A variety of specialized tests, discussed later in this chapter, may be useful in cases of suspected malabsorption. Diagnostic tests such as endoscopy and intestinal biopsy rather than tests for intestinal absorption are usually recommended. Intestinal absorption tests that may be useful in the diagnosis of malabsorption include the breath hydrogen test (dogs)[73,75,83] or vitamin B_{12}/folate levels (dogs and cats). There are no consistent hematological or biochemical changes in cases of malabsorption, but more chronic cases may have decreased serum protein concentrations due to protein losing enteropathy.

A failure of oral tolerance may result from food allergy that can cause acute or chronic gastrointestinal disease, which is difficult to differentiate from inflammatory bowel disease (IBD) without dietary elimination-challenge tests. Dietary trials should be a routine part of all gastrointestinal work-ups,[30] but details are beyond the scope of this text.

Maldigestion and malabsorption in horses

Maldigestion alone is a rare cause of malassimilation in horses and maldigestion syndromes are uncommon in horses as compared with other domestic species. EPI is not recognized in cattle and horses,[79] but Carlson[96] reported a few cases in ponies and draught horses where horses showed chronic weight loss and intermittent colic. Definitive diagnosis of chronic pancreatic necrosis was made on necropsy.

Malabsorption is more common in horses, and many horses with malabsorptive disease develop protein losing enteropathy and subsequent hypoproteinemia. A list of causes of protein losing enteropathies in horses can be found in Table 28.4.

Table 28.4 Causes of protein losing enteropathy in horses.

Syndrome	Examples
Cellular Infiltrates	Granulomatous enteritis[10] Eosinophilic gastroenteritits[49] enterocolitis[14,58] Lymphocytic, plasmacytic or monocytic
Biochemical or genetic abnormalities	Congenital or acquired lactase deficiency (lactose intolerance)[61] Monosaccharide transport defects
Metabolic	Congestive heart failure, intestinal ischemia
Microbiological Bacterial	Chronic infectious granulomatous enterocolitis due to tuberculosis[50] Paratuberculosis[31] Salmonellosis *Rhodococcus equi* *Lawsonia intracellularis*[6,33,40] Multiple abscessation *Clostridium*[62]
Fungal (may be secondary to antibiotic[16] or corticosteroid therapy[52])	*Aspergillus fumigatus*[52] *Histoplasma capsulatum*[16]
Viral (rotavirus, coronavirus)	Villous damage or atrophy[10]
Parasitic	*Strongylus vulgaris* larvae,[2] and small strongyles—*Strongyloides westeri* (foals) causing ischemia and damage due to migration Cryptosporidia[9]
Neoplastic	Intestinal mural lymphoma[50] Leiomyoma, leiomyosarcoma[35] Squamous cell carcinoma[60] Adenocarcinoma[26]
Nutritional	Dietary-induced enteropathy Zinc deficiency
Immune mediated	Amyloid A-associated gastroenteropathy[25]
Toxic	Heavy metal toxicity
Pancreatitis	

Maldigestion and malabsorption in ruminants

In ruminants, maldigestion syndromes are poorly understood and are generally uncommon. Main causes of maldigestion may be associated with changes in the rumen microflora or gastric function, overgrowth of small intestinal bacteria, or lactase deficiencies.[52] Variations in bile salt concentrations

may exacerbate diarrhea in milk-fed neonates, but this does not interfere with digestion in the adult ruminant.

Malabsorption syndromes in cattle are poorly documented, but villous atrophy in calves secondary to viral infection (rotavirus, coronavirus), or cryptosporidia leads to maldigestion and malabsorption. Maldigestion results from villous destruction with subsequent hydrolytic enzyme deficiency (such as lactase).[52] Other causes of malabsorption include congestive heart failure, which may result in localized or generalized ischemia, lymphatic obstruction, parasitism (trichostrongylosis of sheep and cattle), protein malnutrition, tuberculosis, and Johne's disease (the latter in ruminants over 2 years of age).

Screening tests in veterinary practice

Several tests can be performed in a veterinary practice on animals with clinical signs and histories that are suggestive of digestive system disease. The results of these tests can be supportive of, but are not always definitive for a specific diagnosis or etiology. Further confirmatory tests may need to be performed at a reference laboratory (discussed later).

Fecal assessment

Optimal fecal assessment involves systematic formulation of a differential list based on signalment, history, and clinical signs. The options for fecal testing surpass simple flotation for parasite ova, and it is essential to select appropriate tests for specific etiologies and to interpret them based on their relative sensitivity and specificity for the specific disease process.[10]

In small animals with diarrhea, fecal examination should include assessment for intestinal parasites (including *Giardia*, coccidia, hookworms and whipworms) and culture for potentially pathogenic bacteria (including *Salmonella* and *Campylobacter*).[2] In ruminants, *Yersinia* should also be considered in addition to the previously mentioned bacterial pathogens. There are numerous other nematodes and pathogens that are more species and age specific but these are beyond the scope of this text.

Feces start deteriorating from the time a stool is passed and cells undergo degenerative changes, which makes identification difficult. Organisms such as *Giardia* and trichomonads are fragile and undergo rapid deterioration with time, refrigeration, or processing, and fecal samples that are more than 5 minutes old are inadequate for the detection of these organisms.[10,88,94] Marks[46] suggests that feces should be less than 2 hours old, and if there is a delay in examination, the sample should be refrigerated.[40] Nematode eggs undergo development or hatch, which makes identification difficult. Hookworm eggs tend to hatch within 24 hours in warm humid weather, giving rise to motile larvae. Toxascaris eggs embryonate within a few days in older fecal samples.[94] Variations in bacterial flora lead to overgrowth and sporulation of some species.[10]

The preferred fecal collection method is by means of digital rectal examination, but fecal loops are more practical for smaller animals where digital collection is inappropriate.[10]

Rectal lavage may be useful for cytology, but the technique results in less fecal material with relatively more mucous secretion from the mucosal surface, and thus the amount of fecal material may be insufficient for some techniques. Flush samples have high yields of motile protozoa and bacteria as these organisms are usually more ubiquitous at the mucosal surface, whereas eggs and cysts are more common in fecal material.[39]

Voided fecal samples provide larger samples, which are required for fecal flotation and sedimentation techniques. Defecation should occur in uncontaminated areas, followed by timely collection and appropriate storage.

Adequate amounts of feces are required for various tests: 1–2 g feces is required for fecal floatation or sedimentation, 2–3 g feces are necessary for fecal culture,[10] and up to 10 g of feces should be used for the Baermann technique.[39,95] Feces can be refrigerated for up to 24 hours for floatation or sedimentation techniques,[10] but can be preserved in formalin for longer periods. Fecal antigen (mainly for PCR) can be preserved by freezing feces[10] but culture requires transport medium.[39]

Fecal parasites

Fecal examination for confirmation of potential GI parasites should be a routine part of laboratory testing in animals with chronic diarrhea. Fecal examination should preferably be performed on fresh feces, but if there is a delay for more than 2 hours after collection, the feces should be refrigerated at 4°C.[46] Basic methods for the detection of parasitic ova, larvae, oocysts, cysts, and trophozoites are discussed here, but a parasitology textbook should be consulted for more detailed descriptions and interpretations of these techniques.

Wet preparations

Wet mounts can be used to detect motile parasites, and are performed by mixing a small amount of feces with a few drops of isotonic saline. Direct smears should be carried out on fresh feces, which is ideally less than 5 minutes old.[88]

A small sample should be mixed with a drop of warm saline on a glass slide with a wooden applicator stick, which is then covered with a cover glass. The smear preparation can then be examined on low power (×10) for eggs, cysts, and larvae. Other organisms may be found using higher magnification (×40). Motile organisms seen on wet mounts can also be assessed on dry mount, stained slides, but it is important not to make dry smears too thick.[10] A list of potential fecal pathogens identifiable on wet and dry fecal

Table 28.5 Potential fecal pathogens identifiable on wet and dry mounts with other selected tests available for diagnosis.

Wet Preparations	Parasites	Other tests available for diagnosis
Protozoa	Trophozoites of *Giardia*[8,37]	Zinc sulfate centrifugation
		Coproantigen ELISA kit[11,37]
		Snap *Giardia* from IDEXX (Snap *Giardia* Antigen Test Kit; IDEXX Laboratories, Westbrook, ME)
		Prospect T kit (Prospect T *Giardia* Microplate Assay; Remel Microbiology Products, Lenexa, KS).[63]
	Tritrichomonas foetus[8]	In pouch culture systems[18]
		Polymerase chain reaction (PCR)for *T fetus* antigen in feces[3]
	Balantidium[8]	
	Entamoeba sp.[8]	*Entamoeba histolytica* antigen in enzyme-linked immunosorbent assay (ELISA)[47]
	coccidial oocysts[8,41]	
	Cryptosporidium[8]	Modified Ziehl-Neelsen stain (stain red)
		Immunofluorescent antibody staining (MeriFluor *Cryptosporidium* Direct Immunofluorescence Test Kit)[37,42,46,51,63]
		ProSpecT® *Cryptosporidium* Microplate Assay[36]
		Coproantigen ELISA kit[11,37]
		Polymerase chain reaction PCR)[15]
Nematodes	*Strongyloides* larvae[8,20]	Fecal floatation
	Nanophyetus salmincola ova[8,48]	Fecal floatation
Bacteria	*Campylobacter*[29]	Culture, PCR

mounts, including selected tests available for diagnosis can be seen in Table 28.5.

Fecal flotation and sedimentation

In many cases, low concentrations of parasites preclude their detection in direct fecal smears, and fecal flotation is required for examination of parasitic ova and oocysts. Fecal floatation is the method of choice for the detection of whipworm eggs (*Trichuris vulpis*). In dogs with large intestinal disease, presence of *Trichuris* can only be ruled out by testing at least 3 fecal samples, as whipworms produce eggs sporadically and in low numbers.[14] *Giardia* cysts are also shed intermittently, and thus for improved sensitivity, examination of feces from three[32] nonconsecutive stools is required over a period of 6–10 days.[45]

A further list of other test methods available for the detection of *Giardia* can be found in Table 28.6.

Parasites from other organ systems may also be detected by fecal floatation such as ova of *Capillaria aerophila*, (a lungworm of cats) *Oslerus osleri* (tracheal worm of dogs), and *Filaroides hirthi* (a lungworm of dogs).

The typical fecal flotation technique involves mixing feces with water, removing large pieces of debris by straining the mixture, centrifuging the strained feces, followed by mixing the resulting sediment with flotation solutions composed of varying concentrations of sugar or salts, including sodium chloride,[39,94] magnesium sulfate (35%),[10] zinc sulfate (33%),[10,95] or sodium nitrate.[39,56] Flotation solutions are commercially available. The fecal sediment/flotation solution mixture is then centrifuged for 5–10 minutes or is allowed to stand for 30 minutes. The correct specific gravity (s.g.) should be maintained for flotation solutions to be effective.[39] Zinc sulfate (with an s.g. of 1.20) is a superior flotation solution for maintainence of the morphology of *Giardia* cysts as compared with other floatation solutions.[46] Sheather's solution[10,95] is a sugar solution with a specific gravity high enough to float any ova. It is considered superior for for isolation of most eggs and oocysts as it generally causes little distortion and it does not crystallize, but it does however tend to distort *Giardia* and some lungworm larvae.[10,46]

A modified Sheather's solution with a higher specific gravity (s.g. of 1.270) gives increased recovery of species

Table 28.6 Other test methods available for detection of *Giardia*.

Test		Sensitivity and Specificity
ELISA (ProSpecT® Microplate ELISA Prospect T *Giardia* Microplate Assay; Remel Microbiology Products, Lenexa, KS	Antigen	Sensitivity 91.2% Specificity greater than 99.4%[19]
Zinc sulfate centrifugation	Organism	Sensitivity 85.3%[42] Specificity greater than 99.4%[19]
Snap *Giardia* Test Kit; IDEXX Laboratories, Westbrook, ME	Antigen	Sensitivity 85.3%[42] 92%[19] Specificity greater than 99.4%[19]

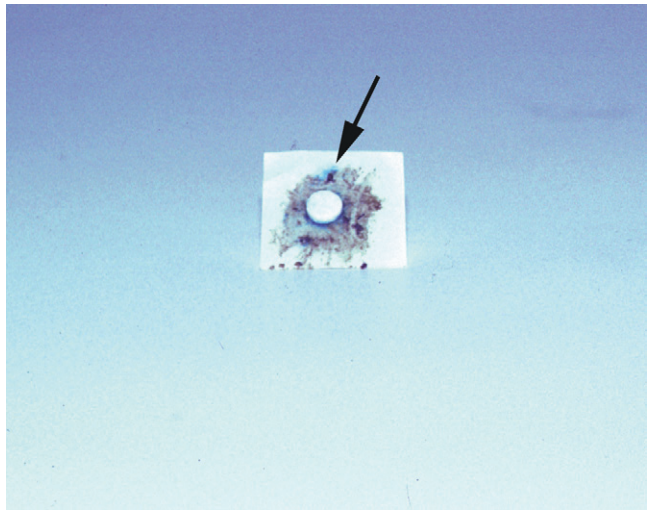

Figure 28.1 The occult blood test. This test is positive for occult blood, as indicated by the blue color on the filter pad.

with heavier eggs such as *Taenia* spp.[46] Most parasitic ova and oocysts float to the surface of the mixture as they have a lower density than the flotation solution. They can be harvested by touching a coverslip to the surface. Microscopic observation of the material collected (×10 objective) reveals the presence of parasitic ova or oocysts, but the technique can be modified for counting ova and oocysts to assess their concentrations in feces.

The centrifugation flotation technique is reported to have less false-negative results and to recover more parasite eggs, cysts, and oocysts than passive (gravitational) flotation techniques (e.g., Ovassay®, Fecalyzer®, Ovatector®).[20]

Fecal sedimentation is superior for the detection of fluke ova, even though they float in flotation solutions such as zinc sulfate. Sedimentation can also be used for detection of embryonated nematode eggs, such as *Physaloptera* spp. and *Spirocerca lupi*,[10] and the method involves mixing feces with water or another appropriate floatation solution, straining off large pieces of debris, and centrifuging the strained feces at 1200 rpm (280×g) for 5 minutes.[46] Centrifugation of the mixture sediments the fluke ova, and the presence or absence of the parasite ova can then be demonstrated by microscopical examination of a few drops of the sediment. Saline is superior for sedimentation of fluke eggs, as they hatch in water.[39]

Some GI parasites (e.g., *Strongyloides* sp.) produce larvae rather than ova. These larvae are not easily detected using flotation methods, but they may be detected using sedimentation techniques. The Baermann technique is the most sensitive for the detection of fecal larvae. The technique involves placing warm water in a glass funnel that is plugged by a stopcock or rubber hose clamped at its end. A small amount of feces is wrapped in a double layer of gauze and placed in the water for 8 hours. During this time, larvae that are present in the feces pass into the water, and descend to the bottom of the funnel. After 8 hours, a small aliquot of fluid is collected from the base of the funnel and centrifuged. The resulting sediment is then examined microscopically for the presence of larvae. This method can be used for detection of various larvae such as lungworm (ie, *Aelurostrongylus* and *Filaroides*) and small intestinal threadworms (i.e., *Strongyloides*). Hookworm eggs (ie, *Ancylostoma* and *Uncinaria*) can hatch in fresh feces, producing active larvae.[39]

Fecal occult blood

The test for occult blood is a simple test, which is available for in-practice use. The test detects the pseudoperoxidase activity of fecal hemoglobin and picks up minute amounts of fecal blood at concentrations as low as 20× to 50× times less than those where blood is visible grossly.[27] A loss of 30–50% of the blood volume into the GI tract can occur without gross blood being visible in the feces. The test procedure involves application of the feces to the test paper, and when blood is present, the peroxidase activity results in the formation of a blue color (Fig. 28.1).

There are two types of fecal occult blood test available. The modified guaiac slide test is based on the detection of a conjugate product called quinone by chemical oxidation of guaiaconic acid, and the orthotolodine tablet test is based on the oxidation of tetramethylbenzidine. In both tests, a positive fecal blood is evidenced by the development of a blue

color. In dogs, some authors report a higher threshold for peroxidase detection in the orthotolidine (o-tolidine) test.[15,61] In a study by Rice *et al.*,[61] the o-tolidine test was shown to be more specific as compared to the guaiac test, but these tests appear to have similar sensitivities. The clinical applications of the fecal occult blood test include testing animals with unexplained acute or chronic diarrhea, those with loose stools, or in cases of microcytic anemias, where the cause of chronic blood loss is not apparent.

The test can also be used to monitor animals that are at risk of developing GI hemorrhage due of treatment with ulcerogenic drugs (e.g., nonsteroidal anti-inflammatory compounds [NSAIDs]) or those with a history of GI neoplasia.[27]

The fecal occult blood test is extremely sensitive, and thus false positive results may be seen with meat or fish diets which contain myoglobin and hemoglobin, and some vegetable diets including plants such as brassicas.[61] The guaiac slide test is reported to be more likely to give false positive results than the orthotolodine tablet test, but this difference also depends on the composition of different diets.[61] It is important to observe strict dietary restriction for at least 3–5 days prior to performing the occult blood test as this decreases the number of false-positive results.[10,32] Recommended feed restrictions include meat-free, low-peroxidase diets (e.g., rice or pasta with cottage cheese or egg as a protein source).

Cimetidine is reported to cause a false-positive hemoccult reaction in gastric juice but has not been shown to be associated with false-positive hemoccult reactions in feces.[55]

Positive results on the fecal occult blood test in the absence of grossly visible blood in the feces suggests the possibility of upper or lower (colon) GI tract inflammation, ulceration, or neoplasia. Blood from the upper GI tract is usually digested and is not always grossly visible in the feces, but blood from the lower GI tract is undigested, and is normally evident grossly. Loss of large amounts of blood in the upper GI tract can cause rapid transit times, and occasionally, results in grossly visible blood in the feces. At least three tests for fecal occult blood should be carried out[10] to make a definitive diagnosis, as the sensitivity of the test increases when three tests are done as compared with a single test result. In a study by Smith,[71] the fecal occult blood test in ruminants (Hematest, Miles Laboratories, Inc, PO Box 70, Elkhart, IN 46515) was reported to have a sensitivity of 77% and a specificity of 97% for abomasal ulceration, which were confirmed at surgery or necropsy.[70,71]

Fecal cytology

Fecal cytology has the potential to provide definitive diagnoses for some animals with signs of GI disease, although there are mixed opinions on the usefulness of fecal cytology.

Single smears may not be entirely representative and there may be a variation of cells or organisms seen depending on how dilute/watery the stool sample is at the time of sampling.

Examination of stained fecal smears can be useful for the detection of numerous pathogens such as trophozoites of *Balantidium coli, Entamoeba histolytica*, and *Giardia*.

Thin fecal smears may be stained with routine hematology stains (e.g., Wright-Giemsa or Diff-Quik stain [Dade Diagnostics of P.R., Inc., Aquada, PR]). Bacterial pathogens such as *Campylobacter* species, clostridial spores, inflammatory cells (neutrophils and macrophages) and occasionally other intracytoplasmic intestinal pathogens such as *Histoplasma and Leishmania*)[28] can be seen with routine stains. Bacteria and cell morphology are best assessed with oil immersion at 500 to 1000× magnification. Special stains may be required for different organisms, e.g., *Cryptosporidium* stains red with a modified Acid Fast stain. Various stains such as iodine (for *Giardia*), methylene blue (*Entamoeba histolytica*), or acid methyl green (*Balantidium coli*) help to optimize recognition of some organisms.

In birds, Gram positive cocci and bacilli predominate in cloacal films from noncarnivorous birds, and thus Gram stains may be useful in these species. In avian feces, occasional *Candida*-like yeasts or gram negative bacterial rods (or even partial Gram positive rods) per 1000× oil are regarded as normal. Gram negative bacteria (bacilli) that are present in large numbers, increased numbers of filamentous gram positive bacteria or increased numbers of *Candida*-like yeasts, protozoa, or parasite ova are regarded as abnormal. Staining cloacal smears with carbol fuchsin or iodine may facilitate detection of *Giardia* on cytology.[13]

The initial step in fecal cytology is assessment of the bacterial flora, which should be mixed in normal small domestic animals (Fig. 28.2).

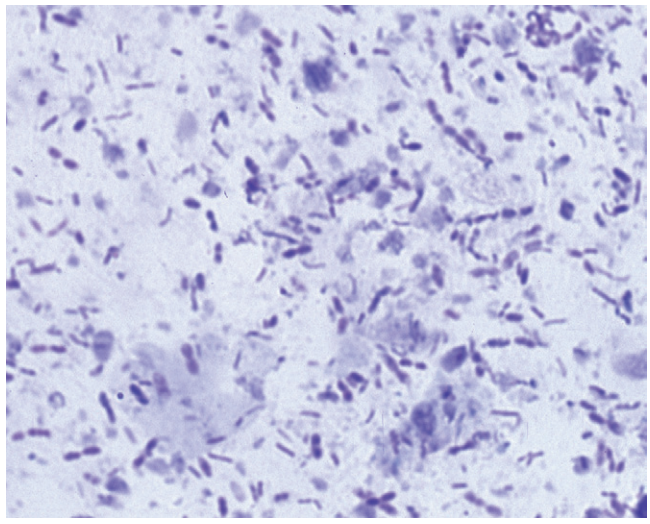

Figure 28.2 Wright-Giemsa-stained fecal smear from a dog showing a mixture of bacteria representing the mixed bacterial flora typical of normal animals. ×1000.

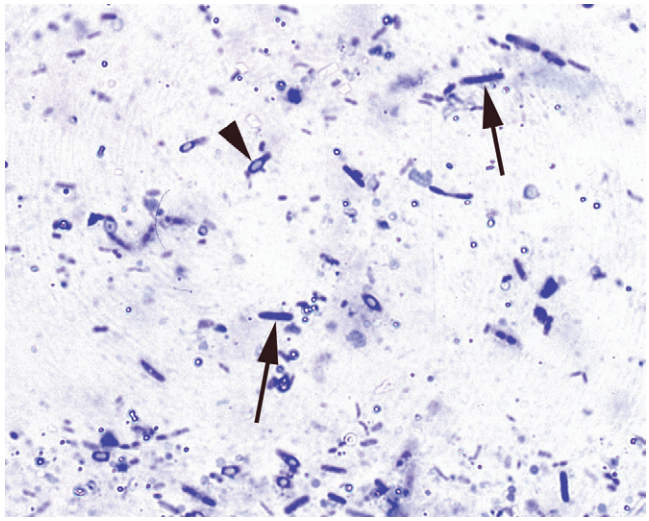

Figure 28.3 Wright-Giemsa-stained fecal smear from a dog showing an overgrowth of *Clostridium* sp. (arrows) that are recognized in the sporulated form ("safety pin" form, arrowhead). ×1000.

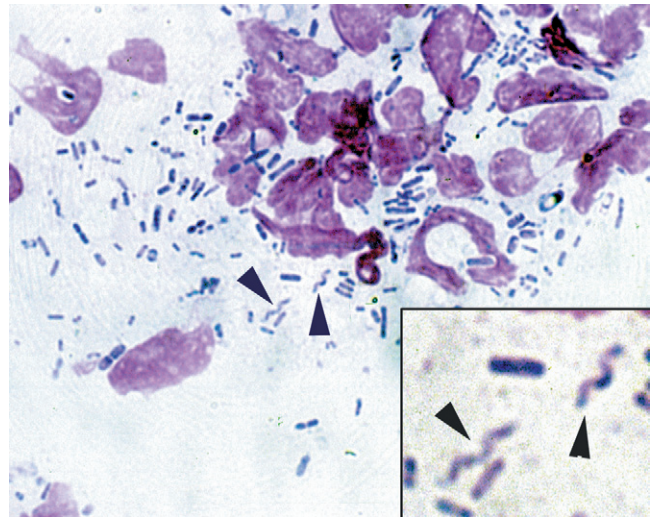

Figure 28.4 Wright-Giemsa-stained fecal smear from a dog showing an overgrowth of *Campylobacter* sp., which are recognized by a distinctive "sea gull" morphology (arrows). ×1000.

If there is an obvious predominance of a single type of bacterium, this organism may be pathogenic, and bacterial culture is indicated. In animals with maldigestion or malabsorption, mixed flora is usually observed. *Clostridium* and *Campylobacter* spp. can predominate and cause GIT disease.

Clostridia are bacilli, which, in the sporulated form have a "safety pin" appearance (Fig. 28.3) and can be identified microscopically. The spore causes distension of the bacillus, which then appears swollen and clear. It has been suggested by some that more than five sporulated bacteria per 1000× oil immersion field are excessive and suggestive of clostridial overgrowth. However, recent studies have documented a poor correlation between fecal endospore numbers and the presence of enterotoxin.[47] Healthy cats can have large numbers of *C. perfringens* endospores in their stools, and thus one should be aware of the possibilty of overinterpreting the presence of *C. perfringens* endospores in fecal smears obtained from cats with diarrhea.[44] *Campylobacter* sp. are recognized by their "seagull" or "W" shape (Fig. 28.4) Pathogenic protozoa (e.g., *Giardia* sp.) may occasionally be seen in direct fecal preparations (Fig. 28.5). Fecal films or scrapings from the colon may also reveal other infectious agents (e.g., fungal organisms such as histoplasmosis, *Aspergillus, Pythium*, and *Candida*).[32]

Small numbers of epithelial cells (Fig. 28.6) can be found in fecal films from normal animals. The presence of neutrophils (Fig. 28.7) in fecal films are abnormal and are suggestive of inflammation[10] of the colon. Viral disease is a consideration in puppies with hemorrhagic diarrhea if fecal neutrophils are absent.[10] Neutrophils often appear degenerate as they undergo degenerative changes during their migration into the lumen of the small intestine, in transit to the terminal colon. Invasive bacteria (e.g., *Salmonella* and *Campylobacter* sp.) should be considered as possible etiological agents when neutrophils are present in feces.

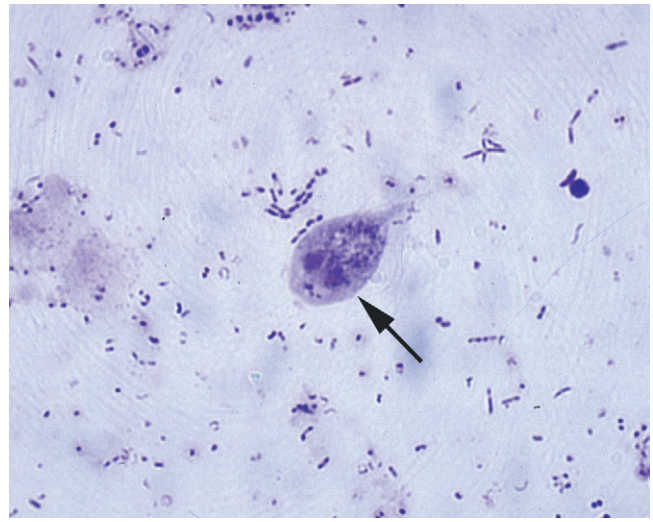

Figure 28.5 Wright-Giemsa-stained fecal smear from a dog showing a *Giardia* organism (arrow). ×1000.

Eosinophils are also abnormal in fecal films and, when present, are suggestive of eosinophilic colitis or chronic endoparasitism. They may also be associated with GI lymphoma and mast cell tumor. Occasionally malignant lymphocytes can be seen in cases of gastrointestinal tract lymphoma.[32]

Grindem *et al.*[16] suggest that fecal cytology may be of use in horses for the evaluation of variations to the GI flora or

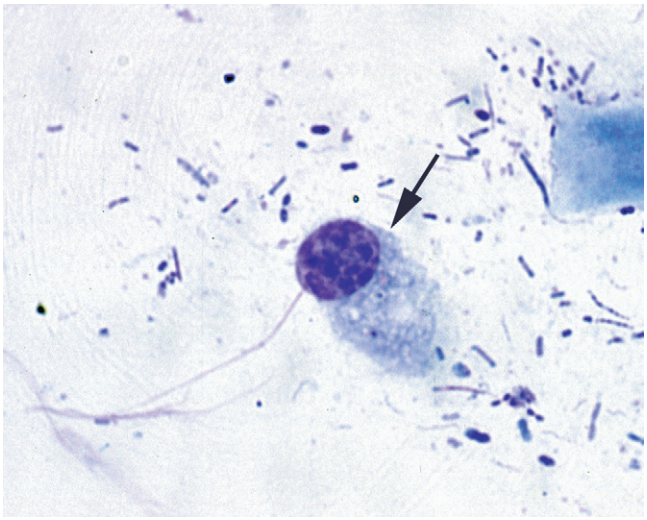

Figure 28.6 Wright-Giemsa-stained fecal smear from a dog. Epithelial cells (arrow) are interspersed with a variety of bacteria and amorphous material. Small numbers of epithelial cells are a normal finding in fecal smears. ×1000.

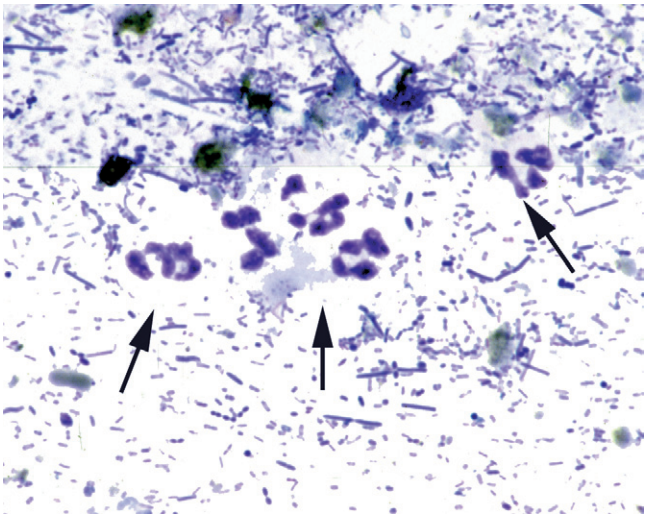

Figure 28.7 Wright-Giemsa-stained fecal smear from a dog. Large numbers of degenerate neutrophils are present (arrows). Neutrophils are abnormal in fecal smears from all species. ×1000.

for the identification of precise etiological agents, which may cause gastrointestinal disease. These authors prefer Romanowsky stains over Gram stains for detection of the presence of inflammatory cells or infectious agents during the initial evulation of smears, as Romanowsky stains give better differentiation than Gram stains. With Gram stains, most cellular structures tend to stain Gram negative (red). Fecal cytology may also be used to make a tentative diagnosis of chronic inflammatory bowel disease in horses. These infiltrative bowel diseases include eosinophilic lymphoproliferative disorders and granulomatous gastroenteritis (the latter may be associated with mycobacteriosis, histoplasmosis, or parasitic larvae).[58] Eosinophilic gastroenteritis may be part of a complex multisystemic epitheliotropic syndrome which may be associated with eosinophilic dermatitis and eosinophilic granulomatous pancreatitis.[49,58]

Rectal biopsies in healthy horses may often contain eosinophils, thus making a definitive diagnosis of eosinophilic infiltrative disease difficult.[42] Ciliated protozoa such as *Tritrichomonas* and nonciliated protozoa such as *Eimeria* have been associated with chronic diarrhea in horses, but their pathogenicity is uncertain.[9]

Eimeria leuckarti has been reported in North American horses and a study demonstrated a prevalence in foals in Kentucky, but the organism appears to be relatively harmless and clinical significance is questionable.[8]

Rectal scrape cytology

Rectal wall scrapes[32] may be useful in cases with suspected infectious diseases of the gastrointestinal tract (e.g., *Aspergillus, Candida, Histoplasma, Pythium,*[32] *Prototheca*, and *Leishmania*) or infiltrative disease such as inflammation or neoplasia. Cell harvest from the rectal wall can be obtained by means of moistened cotton swabs, a gloved fingernail, or spatula (similar to those used for conjunctival scrapes). Slides are made by gently rolling the harvested material onto glass slides,[32] which are subsequently stained with Diff-Quik or Wright-Giemsa stains.

Cytology of biopsy samples

Intestinal biopsies[32] can be used to make scrape or impression smears for cytologic preparations. These can then be used to make rapid or tentative diagnoses of various disease syndromes such as malignant lymphoma (lymphosarcoma) or inflammatory infiltrates such as lymphoplasmacytic or eosinophilic enteritis.

Negative results do not rule out any differentials and full thickness gut biopsies for histopathology are the preferred sample in most cases for definitive diagnosis and confirmation of the cytological findings.[37]

Digestion/absorption screening tests

Historically there have been numerous screening tests performed on feces to try to evaluate maldigestion and/or malabsorption. These tests have become obsolete as they are subjective, imprecise, and interpretation is complicated by numerous factors amongst which include the variation associated with different diets and intestinal transit times. They have low sensitivity and specificity for diagnosis of GI disease, and are regarded by current leading experts and researchers as diagnostically useless and are not recommended for clinical use.[2,32,80] Some of these tests include microscopic examination for fecal starch, fat, and muscle protein, and tests for fecal proteolytic activity. This latter test

is fraught with numerous false positive and negative results due to the daily fluctuations in fecal protease activity and the presence of protease inhibitors in the feces, and is not recommended even as a crude screening test.[34,93]

The plasma turbidity test for fat absorption has poor sensitivity (i.e., 80% or more of ingested fat is still absorbed in dogs with EPI). There can also be marked variation in the degree of lipemia that develops and in the length of time required for lipemia to develop in normal animals.

Tests in a reference laboratory

Serum trypsin-like immunoreactivity (TLI)

Serum TLI (canine and feline specific TLI respectively)[93] is regarded as the most sensitive and specific laboratory test available for diagnosing exocrine pancreatic insufficiency (EPI).[91] It should be part of any standard work-up for canine small intestinal diarrhea.[30] Trypsin-like immunoreactivity assay (TLI) utilizes species-specific antibody that detects cationic trypsin and trypsinogen, which are bound to protease inhibitors.[79] Immunoassays are currently available for dogs and cats. In healthy animals, trypsinogen is constantly produced by pancreatic acinar cells and small amounts leak continually into the peripheral circulation, and thus most of the serum TLI measured is trypsinogen. Trypsinogen is converted in the small intestine to trypsin, which is the active form of the proteolytic enzyme, but this is not reabsorbed into circulation. In animals with EPI, TLI levels are severely decreased due to the marked depletion of functional exocrine pancreatic tissue. EPI can be treated successfully, which results in the resolution of the changes that usually occur in the small intestine, and thus EPI should be ruled out as a differential before considering a diagnosis of primary intestinal disease.

EPI can also cause cobalamin (vitamin B_{12}) malabsorption, which in turn, can encumber interpretation of serum cobalamin levels and thus the diagnosis of intestinal disease.[2]

The following information should be considered when performing TLI:

- Animals should be fasted for a minimum of 12 hours prior to collection of a blood sample, as recent feeding may falsely elevate TLI levels.
- 1 mL of nonhemolyzed serum is preferred, but EDTA or heparinized plasma may be used.[79] Severe lipemia will interfere in the radioimmunoassays commonly used for the measurement of TLI.[80,91]
- Serum TLI is stable for several days at room temperature and for several years when frozen. Serum samples for TLI analysis can thus be sent to a laboratory through the mail, but high temperatures will destroy TLI. Samples should be stored at 4°C or −20°C.[79]
- Oral supplementation with pancreatic extracts (generally extracted from porcine pancreatic tissue) do not interfere with TLI assays or affect results.[80]

Normal dogs have serum TLI concentrations >5 µg/L (5–35 µg/L). Serum TLI concentrations are dramatically decreased in dogs with EPI (<2.5 µg/L).[75] In dogs with clinical signs of maldigestion due to EPI, the diagnostic sensitivity, specificity and accuracy of fasting serum TLI is high (approaching 100%).[91] Values between 2.5 and 5.0 µg/L are seldom associated with signs of EPI, but may reflect subclinical pancreatic disease (such as subtotal pancreatic acinar cell destruction secondary to ongoing immune-mediated lymphocytic pancreatitis).[87] The TLI can decrease from the "gray" zone to a confirmatory level within a few weeks. In these cases the TLI assay should be repeated after one month ensuring that food is withheld for 12–15 hours prior to blood sample collection.

Other possible causes for results in this "gray" zone (2.5–5.0 µg/L) include:

- The dog is in the process of recovering pancreatic function after an episode of pancreatitis, and the results may be normal at retesting.
- The sample had a normal TLI when collected but was exposed to excessive heat during transit. Results may be normal at retesting.
- Food was not withdrawn for an adequate length of time prior to testing. Retesting may indicate that the dog has EPI.

In cats, values ≤8.0 µg/L are diagnostic for EPI.[75,91]

Values between 8.0 and 12.0 µg/L are equivocal and retesting in a month (as for dogs) is recommended.[78]

TLI concentrations are reported to be normal in cases where EPI is due either to obstructive pancreatic duct tumors or to congenital deficiencies of the enzymes other than trypsinogen.[22]

Diseases causing decreased GFR are reported to increase TLI,[76] and could potentially mask an abnormally low serum TLI concentration.

Assays for serum cobalamin (vitamin B_{12}) and folate are strongly recommended whenever serum TLI is assayed as serum vitamin abnormalities are common in dogs and especially cats with EPI.

Fecal elastase 1

A fecal Elastase 1 ELISA test (ScheBo®, BiotechUS) was developed and claims a sensitivity of 97% and a specificity of 98% for detection of canine pancreatic insufficiency.[72] The one advantage of this test over cTLI testing is this test does not require a 12-hour fast, but a disadvantage is that a feces sample is required. There are also marked daily variations in fecal elastase results, with no clear cut differentiation in results between subclinical and control dogs.[87]

Fecal α_1-proteinase inhibitor concentration (α_1-PI)

Many gastrointestinal disorders can be associated with protein loss. In small animals the most common causes of GI protein loss include inflammatory bowel disease, intestinal lymphoma, and lymphangiectasia.[2]

α1-Proteinase inhibitor (PI) is a protein in feces that is not degraded by digestive or bacterial proteinases. It is lost at about the same rate as albumin and other plasma proteins into the GI lumen.[51] α1-Proteinase inhibitor is normally found in plasma, lymph, and interstitial fluid but is not normally present in the intestinal lumen.[54] In both dogs and cats with gastrointestinal protein loss, increases in fecal α_1PI concentrations may be seen before the protein loss become severe enough to observe hypoalbuminemia.[50]

In dogs and cats, confirmation of gastrointestinal protein loss, i.e., protein losing enteropathy (PLE), can be made by measurement of fecal α1-proteinase inhibitor by means of a validated ELISA assay.[51]

There are daily fluctuations in fecal α_1-PI levels and preferably excretion should be measured over 24 hours, but a more realistic approach involves collection and evaluation of three individually voided fecal samples.[10] Fecal blood is reported to falsely increase α_1-PI.[40] Fecal samples should be frozen immediately and shipped to the reference laboratory frozen,[10] on dry ice for testing. Time and increased temperature decrease concentrations.[51] Samples kept at room temperature for 72 hours were shown to have only 66% of the prestorage concentrations.[51] Currently, this assay is only available through the Gastrointestinal Laboratory at Texas A&M University (College Station, TX).[51]

Serum vitamin B_{12} and folate

In dogs and cats that have unexplained weight loss or chronic small intestinal diarrhea, serum vitamin B_{12} and folate concentrations may be useful to evaluate intestinal function,[6,32] but it must be remembered that a precise etiological diagnosis based on these tests results is unlikely.[32]

Serum is the preferred sample for both vitamin B_{12} and folate assays, but EDTA plasma may be used for folate only in some assays,[79] Assays should be validated for the species of interest.

Cobalamin is stable in serum for 12 hours at 8°C, and for up to 8 weeks at −20°C. Folate is stable for 24 hours at 4°C and for up to 8 weeks at −20°C.[79] Exposure to light may cause false decreases in cobalamin values,[32,79,82] whereas hemolysis falsely increases folate concentrations.[32,79,80,82]

Serum vitamin B_{12}

Cobalamin is a large molecule that cannot traverse the intestinal epithelial barrier either by diffusion or by carrier-mediated transport, and thus vitamin B_{12} (cyanocobalamin/cobalamin) has a highly complex homeostasis which primarily involves stepwise metabolism by means of enterohepatic recirculation.[2]

Secretions of pepsin and gastric acid (HCl) in the stomach mediate the release from dietary proteins. Free cobalamin then binds to specialized proteins (known as R proteins), which in turn, renders cobalamin unavailable for absorption. This complex passes into the small intestine, where it subsequently becomes digested by pancreatic proteases, which in turn releases the vitamin B_{12}.[75] Intrinsic factor is produced primarily by the pancreas in dogs and cats,[25] and also by the gastric mucosa in dogs,[79] and consequently binds to the free vitamin $B_{12.}$ This vitamin B_{12}-intrinsic factor complex is later absorbed in the distal small intestine, especially the ileum.[2,38]

The three main causes for decreased serum B_{12} (cobalamin) concentrations are:

- Exocrine pancreatic insufficiency: Either cobalamin is not released from R proteins because of insufficient secretion of bicarbonate rich fluid into the duodenum, or there is decreased intrinsic factor production, especially in cats as they lack gastric intrinsic factor.[68] Low serum cobalamin levels are an indication for checking serum TLI.
- Small intestinal bacterial overgrowth (SIBO); especially anerobes. The amount of cobalamin bound to intestinal bacteria is increased, decreasing free cobalamin for absorption. SIBO has been defined as $>10^5$ colony forming units/mL of duodenal juice).[92] However, this definition is controversial as healthy dogs may have bacterial counts higher than this.[32]
- Decreased absorption of cobalamin in the ileum of dogs and cats due to diseases damaging the ileum. Underlying diseases include many types of inflammatory bowel disease and neoplasia.

Additionally, congenital selective cobalamin malabsorption and cobalamin deficiency has been documented in border collies,[57,90] giant schnauzers,[26,35] Australian shepherd dogs, and beagles with defective ileal cobalamin–intrinsic factor receptor.

In an ongoing study at the GI lab at Texas A&M, they report that approximately 70% of the serum samples tested from Shar Peis with GI disease have serum cobalamin B_{12} levels below the reference interval,[29,32] but these dogs do not appear to have abnormal pancreatic function. They also report that in nearly half of these dogs tested, the serum cobalamin has not been detected by normal methods.[32]

Increased levels of serum cobalamin concentrations are relatively uncommon in dogs, but they may occur with supplementation of cobalamin, or they can also arise from hepatic parenchymal damage, as hepatocytes store cobalamin.

Serum folate

Folate is ingested in the diet (green leafy plants) in the form of folate polyglutamate. It is conjugated with glutamic acid residues but is poorly absorbed. In the proximal small intestine, folate polyglutamate is deconjugated by folate deconjugase enzyme to folate monoglutamate,[75] which is then

absorbed by specific folate carriers in the proximal small intestine, especially the jejunum. Enteric bacteria also produce folate.

The main cause of decreased serum folate concentrations is the decrease of intestinal folate absorption due to proximal small intestinal disease. Many potential underlying causes are possible, such as inflammatory bowel disease or infiltrative neoplasia such as lymphoma. Ingested antigens are in increased concentrations in the lumen of the proximal small intestine and this area is thus vulnerable to damage by specific diets, e.g., gluten enteropathy in Irish setters.[2,4] Overuse of antibiotics with subsequent sterilization of the intestine can also lead to decreased serum folate levels.

A functional folate deficiency may also occur in cases of cobalamin deficiency, and serum folate concentrations may be normal or be potentially increased in those animals due to the decreased utilization of folate.[79]

There are several potential causes for increased serum folate concentrations, including excess supplementation or high dietary intake. Many different bacterial species synthesize folate and thus small intestinal bacterial overgrowth can lead to significant increases in serum folate concentrations. Bacterial overgrowth may result secondary to EPI, or a variety of underlying intestinal diseases that cause defects in the mucus barrier or decreased peristalsis. IgA deficiency in German shepherds has also been associated with intestinal bacterial overgrowth.[3]

Greater folate absorption occurs at lower pH, which could be caused either by excess gastric acid secretion or decreased bicarbonate secretion. The latter can be seen in cases of EPI.

Interpretation of vitamin B_{12} and folate concentrations

Results are only meaningful if pancreatic function is normal and if the condition is sufficiently chronic for body reserves of vitamin B_{12} and folate to have been depleted. Dietary intake is a consideration as prolonged anorexia can affect serum concentrations. Misleading results can occur in cats or dogs with EPI and in patients who have bacterial overgrowth or are receiving vitamin supplements. In a survey by Hall *et al.*,[33] the authors reported that 74% of dogs with EPI had decreased vitamin B_{12} and 32% had increased folate.[33]

The combination of decreased vitamin B_{12} and increased folate concentrations with normal pancreatic exocrine function suggests small intestinal bacterial overgrowth (SIBO),[32] also known as antibiotic responsive diarrhea or bacterial dysbiosis.[5]

In dogs, decreased B_{12} and increased folate concentrations have a low sensitivity (5%) for detecting bacterial overgrowth, but have high specificity (almost 100%).[66]

SIBO is not a clinical condition that is commonly seen in cats, but serum cobalamin concentrations can be decreased due to binding by intestinal bacteria. In cats, low concentrations of serum cobalamin are most frequently associated with small intestinal disease, that is, if pancreatic insufficiency (EPI) has been excluded.[2]

A decrease in serum concentrations of both vitamin B_{12} and folate suggests severe, chronic diffuse disease involving the entire small intestine (generalized malabsorption).

Decreased vitamin B_{12} and folate concentrations have been reported in cats with EPI. In a study by Simpson,[67] in more than 50% of the cats tested at the time that presented with GI disease, the cobalamin concentration was below normal, and some of the cats with GI lymphoma were also found to have concurrently low folate levels.[67]

It is hypothesized that the reduced serum vitamin B_{12} concentration results from decreased secretion of pancreatic intrinsic factor, which is necessary for vitamin B_{12} absorption in cats. Decreased folate concentrations are thought to result from concurrent intestinal disease with EPI and the resultant decrease in folate absorption. Detection of decreased vitamin B_{12} and folate concentration in cats warrants consideration of EPI in addition to intestinal disease.

A decrease in vitamin B_{12} with normal folate concentration but normal pancreatic function suggests distal small intestinal disease, whereas decreased folate with normal B_{12} concentrations suggests proximal small intestinal disease. A decrease in vitamin B_{12} with or without increased folate in dogs (due to bacterial overgrowth) suggests EPI, and testing TLI is indicated. If both vitamin B_{12} and folate concentrations are increased, vitamin supplementation prior to sampling is the most likely explanation, because there is no disease process that should give rise to this change.

A summary of the interpretation of vitamin B_{12} and folate levels is described in Table 28.7.

A list of other tests that may be used for the evaluation of gastrointestinal disease can be seen in Table 28.8.

Cobalamin in large animals

Cobalt is required for synthesis of cobalamin by ruminal bacteria, and thus cobalt deficiencies in ruminants can result in decreased serum cobalamin.[74] Cobalamin deficiencies are shown to have repercussions on production in large animals, with manifestations of diarrhea, weight loss, ill thrift, pica, etc.[69]

Table 28.7 Summary of the interpretation of vitamin B_{12} and folate levels.

IBO—↓ Vitamin B_{12}, folate ↑
EPI—slight ↓ Vitamin B_{12} folate normal / ↑
Cats EPI—most ↓ Vitamin B_{12}, >50% folate ↓
Vitamin supplementation—↑ Vitamin B_{12}, folate ↑
Severe, diffuse long-standing SI disease – usually ↓ Vitamin B_{12}, folate ↓
Upper SI disease—usually normal B_{12}, folate low
Malabsorption—low folate

Table 28.8 Other tests that may be useful for the evaluation of gastrointestinal disease.

Test type	Purpose of test
Fecal PCR or bacterial or fungal culture	Evaluation of microorganisms associated diarrhea and or vomition such as *Campylobacter* spp.,[32,46a,88] *Clostridium perfringens*,[47,88] *Clostridium difficile*,[32,46a] pathogenic strains of *Escherichia coli*[6a,32,66a,88] and *Salmonella* spp.,[10,32,45,46a,88] *Helicobacter*,[54a,68a] *Heterobilharzia Americana*,[23a] *Tritrichomonas*,[2,27a] *Histoplasma capsulatum*[32]
Fecal antigen detection methods	Evaluation of microorganisms associated with diarrhea and/or vomition, e.g., ELISA for viruses (parvo,[10,32] rota), *Giardia*,[32] and *Cryptosporidium* antigen[88]
ELISA for fecal bacterial enterotoxins	*Clostridium perfringens*[10] and *Clostridium difficile*[10,46a]
Serum pepsinogen	Screening for abomasal ulceration/damage[40a] in ruminants and screening test for ostertagiasis[1] in young calves <2 years of age
Serum gastrin	Evaluation for Gastrinoma (Zollinger-Ellison syndrome)[66b]
Hydrogen breath test[73,75,83]	Investigation of bacterial overgrowth and carbohydrate malabsorption secondary to EPI in dogs and cats[30a,52a]
Abdominal ultrasound	Evaluation of GIT/abdominal abnormalities
Intestinal biopsy	Evaluation of etiology of GIT disease[32]/causes of malabsorption/maldigestion[2]

Evaluation of ruminal fluid

It is important to ensure that the first part of the ruminal content is not contaminated with saliva (which has an alkaline pH).

Ruminal pH varies according to the type of feed, and time interval between feeding and sampling. The maximal decrease in pH occurs 5–6 hours post prandially due to production of volatile fatty acids. pH increases on exposure to air and thus should be measured immediately after sampling.[19]

Normal rumen pH is quoted to be between 5.5 and 7.2, with an average of 6.5–6.8 (on roughage).[19,21]

Acidosis is evidenced by a pH less than 5.5–6.0 (usually due to grain overload or high concentrate diets),[41] although an animal with lactic acidosis with protracted anorexia may have a normal rumen fluid pH if there is persistent saliva production.[69]

Alkalosis is associated with pH above 7 (8–10) and is due to high protein diets, saliva contamination of rumen content, or rumen putrefaction due to rumen stasis.[19]

Microscopic examination of rumen fluid includes evaluation of the types of bacteria and types and motility of the protozoa present. It is important to mix the sample adequately prior to examination.

Protozoal motility can be evaluated on wet preparations at low power that should be kept warm.[64]

Numbers and morphology can be examined on wet mounts without stains (or by adding a drop of Lugol's iodine to the rumen content) on a slide and covered with a coverslip. Normally a minimum of five to seven active protozoa should be seen per low power field.[19]

Ruminal bacteria

Predominant ruminal bacteria are Gram negative but these may be replaced by Gram positive organisms in cases of ruminal acidosis.[19]

Normal rumen chloride

<30 mEq/L (10–25 mEq/L) (mmol/L) in cattle and <15 mEq/L) (mmol/L) in sheep),[19] and that of the abomasum is >90 mEq/L) (mmol/L).[64]

Increases in rumen chloride are associated with abomasal reflux of HCl, ileus, or high salt intake.[19,69]

Absorption tests

The functional assessment of small intestinal disease in small animals by measuring absorption of various substrates such as glucose, lactose, and starch have, for the most part, been discarded.[32] This is because concentrations of plasma glucose after dosing of these compounds depends on more than mucosal hydrolysis of starch and lactose, and absorption of glucose, making these tests unreliable.[32] Other insensitive tests which have also become redundant include vitamin A testing (previously used to assess malabsorption), D-xylose testing in small animals and triglyceride absorption tests.[32]

Some of these absorption tests however, are still used in large animals, such as xylose absorption, and is occasionally used in horses when malabsorption is suspected.[63,65] This has largely been replaced by glucose absorption tests, which are used to assess chronic weight loss in horses.[62]

Both tests are influenced by the rate of gastric emptying, small intestinal transit time, diet and fasting period. Both

tests require adequate fasting prior to testing, but this may be contraindicated in debilitated animals.

Xylose absorption tests depend largely on absorption of xylose whereas glucose absorption tests depend on intestinal absorption and utilization of glucose by hepatocytes and other tissues. Theoretically the xylose absorption test is preferred because xylose is not a normal metabolite, although the test has a number of disadvantages. The test is more expensive, xylose assays are less accessible, and may be difficult to interpret.[19] Results are also affected by diet (horses on high energy diets show lower absorption curves). The glucose absorption test is more practical as glucose is readily available, less expensive, and assays are more accessible.

Glucose absorption tests can be used in monogastrics and preruminant calves to assess intestinal absorption, but cannot be used in adult ruminants as sugars are degraded in the rumen.

Oral D-xylose absorption tests in horses

Protocol:

- The animal is fasted for 12–18 hours.[11]
- D-Xylose is administered orally via nasogastric tube at a dosage of 0.5–1.0 g/kg (10% solution) in horses.[19]
- Blood samples are collected into heparinized tubes for D-xylose determinations, and are collected before D-xylose administration and at 30 minute intervals for up to 5 hours (30, 60, 90, 120, 180, 240, and 300 minutes) after administration. For routine diagnostic purposes, the 60- through 180-minute samples are the most important.

D-xylose concentrations should represent a bell-shaped curve, but should peak at greater than 20–30 mg/dL (1.34–2.01 mmol/L) at 90–180 minutes (Fig. 28.8).[19] In normal horses, peak values should be greater than 15 mg/dL (1.00 mmol/L) above baseline values. In normal foals the xylose peak is reached at 30–60 minutes, but peak concentrations are reported to vary with age.[19] A flattened D-xylose absorption curve is suggestive of malabsorption.[19]

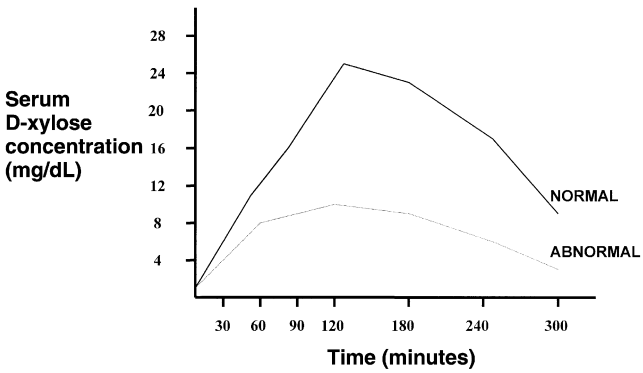

Figure 28.8 The D-xylose absorption curves from normal and abnormal horses. A normal D-xylose absorption curve in a horse peaks at greater than 20–30 mg/dL at 90–180 minutes after D-xylose administration.

The D-xylose absorption test is insensitive, and thus a normal D-xylose absorption test does not rule out malabsorption.

D-Xylose absorption curves can be affected by many factors not directly related to malabsorption such as diet, anorexia, age, decreased renal clearance, infections, anemia, hypoxia, and in foals, the IgG concentration. D-Xylose absorption can be falsely decreased (i.e., a falsely flattened curve or delay in reaching peak value) with delayed gastric emptying, bacterial overgrowth causing intraluminal bacterial breakdown of xylose, and sequestration of xylose in patients with abnormal extravascular fluid accumulation (e.g., edema, hydrothorax, or ascites).

Oral glucose absorption/tolerance test (OGTT) in horses

Protocol[62,79]

- The animal is fasted overnight (18–24 hours), and a baseline (0 hour) blood sample for glucose is taken into a sodium fluoride tube.
- Glucose (20% solution) is administered at 1 g/kg body weight via nasogastric tube.
- Blood samples for glucose determination are collected into sodium fluoride tubes at 30, 60, 90, 120 and 180 minutes after glucose administration.

Normal horses should show increases in blood glucose concentrations of greater than 85–100% above the baseline concentration at 120 minutes.[79,81] Results may be affected by a number of factors such as age and diet,[36,53] bacterial overgrowth, delayed gastric emptying (excitement),[43] small intestinal obstruction, reduced intestinal circulation, and sequestration in ascitic fluid. Anorexia or prolonged fasting may delay or decrease peak glucose concentrations, causing flatter curves due to decreased peristalsis.

The specificity of the oral glucose tolerance test was assessed in 42 cases of horses with chronic weight loss.[43]

Specificity was found to be very good in cases with normal OGTT and was found to correlate well with normal histopathological findings of the small bowel, but severe infiltrative disease of the small intestine (such as lymphoma or granulomatous enteritis) were associated with a complete malabsorptive response.[43]

Histopathological findings correlate poorly with a partial malabsorption response.

Tests for maldigestion in horses

Oral lactose tolerance test in horses

Maldigestion syndromes are infrequently observed in horses as compared with other species. In foals and young adults less than 3 years of age, lactase enzyme (which is found in the brush border of the small-intestinal enterocytes), hydrolyzes lactose into its two component sugars D-glucose and galactose before absorption occurs. Acquired lactase deficiency can be

seen in foals and preruminant calves, secondary to a number of causes of intestinal mucosal damage. These include viral (rotavirus), protozoal, or bacterial enteritis (*C. difficile* enterocolitis in foals)[85] or other less specific causes of small intestinal disease.[79] Preruminant calves and lactose-deficient foals may develop osmotic diarrhea due to the presence of osmotically active particles (lactose) and subsequent retention of water and electrolytes in the small intestine.

These two types of diarrhea may be difficult to differentiate clinially.[60]

The lactose tolerance test does not distinguish maldigestion from malabsorption, but is used principally in foals to identify lactase deficiency and for young foals and calves with diarrhea or poor growth. The test is inappropriate for adult ruminants and horses (the latter greater than three years of age as they are lactose-intolerant).

Protocol[48,79]

- Grain and hay should be withdrawn from the dam and foal for 18 hours and water removed 2 hours prior to testing. Muzzling of the foal is recommended 4 hours prior to testing and the foal should remain muzzled for the duration of the test. A baseline (0 hour) blood sample for glucose is taken.
- Lactose monohydrate (20% solution) is dosed at 1 g/kg body weight via nasogastic tube.[19]
- Blood samples for glucose assay are collected into sodium fluoride tubes at 30, 60 and 90 minute (120 minutes optional) intervals after lactose administration.

In healthy foals, the glucose concentration is quoted to be 150–250% of baseline concentration at 60 or 90 minutes;[48,81] or should peak at least 35 mg/dL (1.94 mmol/L) greater than the baseline concentration.[48] Maldigestion or malabsorption will usually result in an inappropriate increase in the blood glucose concentrations after the administration of lactose. If the lactose tolerance test is abnormal, then either a glucose or D-xylose absorption test is recommended to evaluate for possible malabsorption. Casein hypersensitivity and lactose intolerance can be differentiated by evaluation of the foal's response to enzymatically treated and untreated milk. A definitive diagnosis of lactase deficiency can be confirmed by direct measurement of mucosal lactase activity in the intestinal tissue, but this is rarely performed in practice, as surgical biopsy of the mucosa is required.

Starch digestion test in horses

This is a test of small intestinal and pancreatic function.

Protocol[19]

- The horse is fasted for 18 hours after which a baseline sample for blood glucose is taken into a sodium fluoride tube.
- Cornstarch is dosed at 1 kg in 4 L of water or 2 g/kg body weight via nasogastric tube.
- Samples for blood glucose determinations are collected in sodium fluoride tubes at 15, 30, 60, 90, 120 minute intervals and then hourly for 6 hours.

Normal horses are reported to should show increases in blood glucose concentrations of approximately 30 mg/dL (1.67 mmol/L) with a peak at 60 minutes, and the curve should return to pretreatment levels within 3 hours. This pattern of response closely approximates results obtained by oral glucose absorption tests.[31]

Other laboratory abnormalities associated with digestive system diseases

Laboratory test abnormalities associated with gastrointestinal disease vary with the area of the system affected, the disease etiology, and the speed of onset and duration of the disease. Common abnormalities associated with acute or chronic diarrheas are discussed here.

Other laboratory abnormalities associated with acute or chronic diarrhea (more than three weeks' duration) or vomiting

1. Increased hematocrit, hemoglobin concentration, and erythrocyte count, as well as increased plasma and serum protein concentrations, can occur with acute diarrhea or vomiting. These occur due to the loss of fluid via the GI tract and the resulting dehydration and hemoconcentration.

2. Variable abnormalities in the leukocyte concentration can occur with acute diarrhea. If the diarrhea results from an infectious agent that produces toxins, then sequestration of neutrophils as well as strong tissue demand can result in marked neutropenia and leukopenia with or without a left shift. Less severe endotoxemia or tissue demand can result in neutrophilia with a left shift.

- Eosinophilia may or may not be seen with hypersensitivity—allergy or intestinal parasitism, intestinal mast cell tumor, GI lymphoma, hypoadrenocoticism, eosinophillic enteritis, or hypereosinophilic syndrome.
- Lymphopenia may be seen with immunodeficiency, stress or lymphangiectasia.[2,12,32]
- Thrombocytopenia, coagulopathies, and DIC may be features of endotoxemia secondary to sepsis.

3. Acid-base and electrolyte abnormalities can occur in animals with diarrhea or vomiting. These abnormalities, however, are variable and unpredictable. Assessment of acid-base and serum electrolyte status is important in such animals. In patients with secretory diarrheas, loss of Na, Cl, and occasionally K can result in decreased serum concentrations of these electrolytes. Hypokalemia is common in horses with long-standing colic with impaction of the large colon, or in horses that have been anorexic for a few days.[19]

Increases in potassium with pseudohypoadrenocorticism (pseudoaddisons) is noted in dogs with secretory diarrhea,

especially with whipworm (*Trichuris*) infection.[10,18] Bicarbonate is also lost in diarrhea, which can cause a metabolic acidosis. This in turn can result in a shift of K from intracellular to extracellular spaces as well as retention of K by the kidneys. Potassium shifts can lead to a normal or increased serum K concentration despite a loss of K in the feces. Vomiting animals may lose significant amounts of HCl in the vomitus, which often leads to hypochloremia and metabolic alkalosis. If the vomitus includes alkaline small intestinal contents, however, such animals may have normal acid-base results or metabolic acidosis. Metabolic alkalosis in vomiting animals is associated with obstructive GI disease, due to loss of HCl without loss of alkaline intestinal contents. No significant association was found by Boag *et al.*,[7] between electrolyte or acid base abnormalities and the site of foreign body; metabolic alkalosis with hypochloremia (disproportionate to sodium) and hypokalemia can be seen with both proximal and distal gastrointestinal foreign bodies. Linear as compared with discrete foreign bodies were more likely to be associated with lowered sodium concentrations.[7]

A disproportionate decrease in chloride as compared to sodium may also be associated with left displacement of the abomasum (LDA) in ruminants with alkalosis and hypokalemia. This may be accompanied by paradoxic aciduria.[19]

4. Increases in blood urea nitrogen (serum urea) and creatinine can result, secondary to dehydration (i.e., prerenal azotemia).

5. Increased activities of hepatic leakage enzymes (mainly ALT (alanine aminotransferase) or ALP (alkaline phosphatase)[32] in small animals and GLDH (glutamate dehydrogenase) in large animals) can occur with acute diarrhea, possibly because of hepatocyte damage resulting from toxins absorbed from the injured GI tract.

Serum amylase and lipase may be variably increased with gastrointestinal disease in dogs, including gastrointestinal space occupying lesions such as foreign body or neoplasia.

6. Hypocholesterolemia, although nonspecific, may be present in some cases of intestinal disease. Many dogs with PLE have hypocholesterolemia, which may be secondary to lymphangiectasia,[32] and presumably results from fat malabsorption associated with failure of chylomicron transport.

7. Panhypoproteinemia can be seen with protein losing enteropathies (PLE).[12,32] This should be differentiated from hypoalbuminemia secondary to protein losing nephropathies and liver failure.[32]

8. Horses with severe colic may show coagulation defects characterized by low levels of antithrombin III and prolonged PT and APTT.[17]

Other laboratory abnormalities associated with EPI or malabsorption syndrome

Hematologic and serum biochemical tests usually are not helpful in establishing the diagnosis of EPI. Routine hematologic tests and biochemical profiles, however, may help to differentiate EPI from other disorders. Serum amylase and lipase activities may or may not decrease slightly with EPI, but these decreases usually are not recognized as being significant. Increased alanine aminotransferase (ALT) activities and decreased cholesterol concentrations occasionally are seen in dogs with EPI.

Other laboratory abnormalities that can occur with malabsorption syndrome include:

1. Microcytic anemia associated with iron deficiency, which commonly results from chronic blood loss via the GI tract.[12] Chronic GI blood loss can be associated with a variety of underlying conditions including neoplasia and parasites.

2. An inflammatory leukogram, which may be suggestive of significant inflammation or deep ulceration in the intestinal wall.

3. Neutropenia with or without a left shift. If, in addition, neutrophils are toxic, then endotoxin absorption from the GI tract secondary to Gram negative enteritis, intestinal stasis, septicemia, severe bacterial peritonitis secondary to intestinal perforation, or viral enteritis are possible.

4. Eosinophilia, which may be associated with eosinophilic gastroenteritis or parasitism, but is an inconsistent marker of eosinophilic gastroenteritis.[12,32] Lack of eosinophilia does not rule out these differentials. Hall[32] states that in his experience less than 50% of cases of biopsy-proven eosinophilic enteritis have peripheral eosinophil and that mild eosinophilia can also be seen with other forms of IBD.[32]

5. Abnormal serum protein, albumin, or globulin concentrations. Serum albumin and globulin concentrations are important in screening for protein-losing enteropathies.[12] In these cases, both albumin and globulin concentrations are usually decreased. In other types of malabsorption or maldigestion, the only decrease, if any, occurs in the albumin concentration. An exception is immunoproliferative enteropathy of Basenjis,[32] in which globulin concentrations increase as part of an immune response. Similar immunoproliferative enteropathies with hyperglobulinemia may occur in other breeds of dogs, especially German shepherds.

6. Prolonged prothrombin (PT) times, prolonged activated partial thromboplastin times (APTT), and prolonged activated clotting times (ACT) due to vitamin K deficiency may be seen in animals with malabsorption syndrome. A suspected vitamin K–deficient bleeding syndrome has been reported in cats with malabsorption syndrome. Malabsorption of vitamin K, which is a fat-soluble vitamin, probably plays an important role in this syndrome, but the vitamin K deficiency in such animals also is potentiated by secondary hepatic diseases, thereby resulting in decreased production of vitamin K–dependent clotting factors; possible antibiotic therapy, thereby altering small intestinal bacterial flora and reducing bacterially derived vitamin K_2 production; and in some cases, severe dietary fat restriction, thereby reducing vitamin K uptake still further, because it is depen-

dent on fat absorption. Before these changes are seen, the activity of vitamin K dependent clotting factors decrease to less than 35% of normal by which time these animals are markedly deficient and should receive parenteral vitamin K supplementation.[23]

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29 Laboratory Evaluation of Plasma and Serum Proteins

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Laboratory evaluation of plasma and serum protein concentrations is a part of both basic hematology and biochemistry testing in animals. Protein alterations occur commonly as secondary changes in a large number of diseases, and may be the major abnormal finding in a few disease processes. Measurement of plasma or serum protein concentrations often yields important information that can be helpful in narrowing the list of diseases to be considered and, in some cases, in revealing the presence of a specific disease. This chapter discusses the types of proteins that are normally present in plasma and serum, the methods for analyzing these proteins, and the significance of abnormal protein concentrations.

Classification of plasma proteins

The two major types of proteins in plasma are albumin and the globulins. Albumin is one of the smallest of these proteins and the single most abundant, accounting for approximately 75% of the oncotic pressure (colloidal osmotic pressure) of plasma within the vasculature, which regulates water from diffusing from the blood into the tissues.[41] Albumin is an important carrier protein and plays a role in the transport of free fatty acids, bile acids, bilirubin, calcium, hormones, and drugs.[69] Albumin is synthesized by the liver, enters the blood, and is catabolized by most tissues. The half-life of a circulating albumin molecule varies in different species, ranging from approximately 8 days in dogs to approximately 20 days in horses.[21,55]

Globulins are a heterogeneous group of proteins that are variable in size, but usually larger than albumin. Hundreds of different types of globulins are present in plasma, including the immunoglobulins (e.g., IgG, IgM, IgA), complement proteins, clotting factors, many different enzymes, and a variety of proteins that carry lipids, vitamins, hormones, extracellular hemoglobin, and metal ions (e.g., iron, copper).[41] The majority of the globulins are produced in the liver, with the exception of immunoglobulins (antibodies) that are produced in lymphoid tissues. Globulins typically are classified as alpha, beta, or gamma on the basis of their electrophoretic mobility. (The separation and measurement of these globulins are discussed later.) A relatively small number of globulins are present in sufficient quantities to affect the electrophoretic pattern. Some of the major contributors to each fraction are listed below.

The alpha globulin fraction includes α1-fetoprotein, α_1-acid glycoprotein (orosomucoid), α_1-antitrypsin (protease inhibitor), α_1-antichymotrypsin (protease inhibitor), α_1-lipoprotein (HDL; transports lipid), ceruloplasmin (transports copper), haptoglobin (binds hemoglobin), α_2-macroglobulin (protease inhibitor), and serum amyloid-A.[76,81] The beta globulin fraction includes β_2-lipoprotein (LDL; transport lipids), transferrin (transports iron), ferritin (iron storage), complement components (C3 and C4), and fibrinogen (in plasma but not serum).[76,81] Immunoglobulin molecules of the IgM and IgA type occasionally migrate in the beta fraction. The gamma globulin fraction is composed primarily of immunoglobulins (all types). Immunoglobulins are produced by plasma cells in the lymphoid tissues in response to antigenic stimulation. C-reactive protein migrates in this fraction in dogs, but migrates between the beta and gamma fractions in horses.[78,90]

Acute phase proteins

Concentrations of a number of plasma proteins change significantly during the acute systemic response to inflammation; collectively these proteins are referred to as acute-phase proteins (APP). The magnitude of this response typically does not cause hyperproteinemia. It is detected by either the protein electrophoretogram pattern, or less commonly by measurement of selected protein concentrations. The

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acute-phase response occurs due to release of a variety of cytokines (e.g., IL-1, IL-6, and TNF-α) from the site of inflammation.[10] These cytokines affect the production of APP by the liver. The plasma concentrations of most APP increase; these proteins are called positive acute-phase proteins, and they generally reach maximal serum concentrations within a day or two after initiation of the response. Concentrations of some APP actually decrease; these are called negative acute-phase proteins. The specific pattern and magnitude of protein alterations during the acute-phase response is species-specific; however, the concentration of serum albumin (a negative acute-phase protein) is consistently decreased by 10–30%.[63] Transferrin, measured in serum as the total iron-binding capacity (TIBC), is another negative acute-phase protein. The positive acute-phase proteins are globulins; important ones in veterinary species include haptoglobin, fibrinogen, C-reactive protein, serum amyloid-A, and α_1-acid glycoprotein.[10,63] Serum amyloid-A is a major APP of dogs, cats, pigs, and horses; haptoglobin is a major APP of cattle, sheep, and pigs; C-reactive protein is a major APP of dogs, horses, and pigs.

Measurement of plasma and serum proteins

Plasma versus serum

The two types of samples commonly used for clinical biochemistry analyses are plasma and serum. Plasma is the liquid portion of blood that has not clotted, thus the blood must be collected in an anticoagulant. Plasma contains all the proteins described earlier. Serum is the liquid portion of the blood that remains after clotting. When a blood sample is collected without use of an anticoagulant, the subsequent clotting in that sample results in the conversion of all fibrinogen to fibrin. Therefore, serum is devoid of fibrinogen, but contains albumin and the remaining globulins.

Total protein concentration

The total plasma or serum protein concentration can be estimated using a refractometer. Protein molecules in plasma or serum increase the refractive index of that fluid in proportion to their concentration. However, there are other molecules potentially present in plasma or serum that can increase the refractive index and artifactually increase the estimated protein concentration. Substances most likely to cause significant interference include lipoproteins (such as in lipemic serum), cholesterol, urea, and glucose.[30] Note that marked elevations in cholesterol concentrations will not result in visible lipemia, but can artifactually increase the protein estimate. Plasma protein may be falsely increased by 0.6 g/dL by serum urea concentrations of 300 mg/dL or glucose concentrations of 700 mg/dL.[76] Synthetic colloid solutions, sometimes given as volume expanders, will artifactually increase the refractometer estimate of total protein. Hyperbilirubinemia and hemolyzed serum typically do not interfere with the total protein estimate, although hemolysis may obscure the line of demarcation on the refractometer.[30]

The total protein concentration of serum is measured routinely in reference laboratories and in-clinic chemistry analyzers by spectrophotometry, most commonly using the biuret method, which detects peptide bonds and is considered very specific. The serum total protein concentration obtained by this method will be less than the plasma protein estimation from a refractometer, due in part to the absence of fibrinogen from serum. Differences in protein measurements performed by different methods (refractometry versus spectrophotometry) should be expected, even for the same type of sample. In avian species, marked differences in total protein concentrations have been found depending upon the methodology used, with lower values determined with the biuret method versus refractometry.[49,72] Use of method and species-specific reference intervals are necessary for accurate interpretation.[13]

Albumin concentration

The albumin concentration is routinely measured spectrophotometrically using dye-binding methods, usually with bromcresol green (BCG). However, BCG is not specific for albumin and may bind to some globulins. As a result, the BCG method may overestimate the albumin concentration when it is very low (<1 g/dL). Other dye-binding methods sometimes used in human laboratories (BCP, HABA methods) are unreliable for accurate measurement of albumin concentrations in animals due to species variations. Albumin concentrations measured by the BCG method may be overestimated as much as 1.2 g/dL in canine heparinized plasma samples compared to serum samples, especially when fibrinogen concentrations are increased; this interference may be eliminated by changes in the specific method protocol.[77] In avian species, albumin concentrations measured by the BCG method often correlate poorly with results obtained by electrophoretic methods (discussed later).[50,72,73]

Globulin concentration

Calculated globulin concentration

The serum total protein and albumin concentrations are measured routinely as part of serum biochemical profiles. The globulin concentration as reported on these profiles is not measured, however, but rather is calculated by subtracting the serum albumin concentration from the total protein concentration.

Serum protein electrophoresis

Both serum albumin and globulin concentrations can be determined by serum protein electrophoresis (see Chapter 1). Electrophoresis is performed by placing a small amount

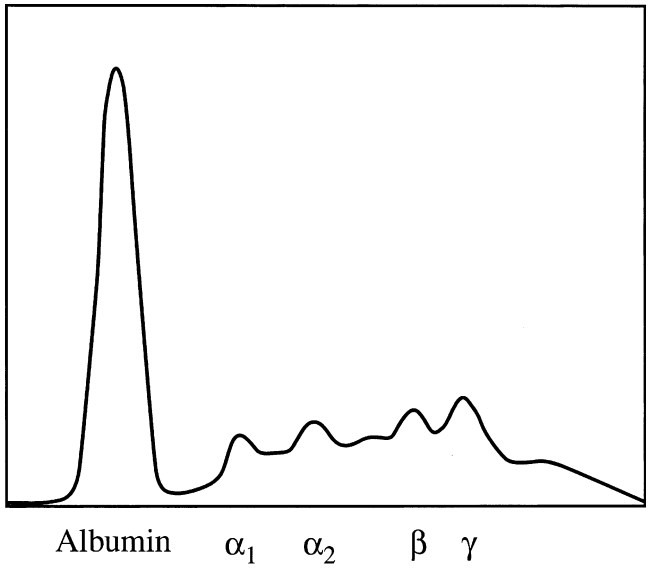

Figure 29.1 An electrophoretogram from a serum protein electrophoresis separation.

of serum near one end of a supporting substance such as cellulose acetate or agarose gel. An electrical current is applied, causing the serum proteins to migrate at variable rates as determined by the net negative charge and size of each type of protein. Staining of the gel reveals the various protein bands, which are then scanned by a densitometer to produce an electrophoretogram (i.e., a hard copy depiction of the distribution of the proteins) (Fig. 29.1).

Modern scanning densitometers also calculate the concentration of protein in each fraction after the operator inputs the total protein concentration of that sample. This method separates globulins into several fractions, including alpha, beta, and gamma globulins. Albumin and globulin concentrations that are derived using this method do not necessarily match those obtained using spectrophotometric methods. The number of fractions separated by serum protein electrophoresis varies both with the species and with the type of supporting substance used; high resolution agarose gels are capable of resolving more protein fractions than low resolution agarose or cellulose acetate gels. Albumin and the alpha, beta, and gamma globulin fractions can be separated in specimens from all species with all types of support substances. In some species, the alpha, beta, and gamma globulins are separated into $alpha_1$ and $alpha_2$, $beta_1$ and $beta_2$, or $gamma_1$ and $gamma_2$ fractions, respectively. (Causes for altered concentrations of these protein fractions are discussed later.)

Another type of electrophoresis, capillary zone electrophoresis, has recently been developed and is now beginning to be investigated for use with animal sera.[4,16,24] In this method protein fractions are separated in solution within a narrow-bore capillary exposed to high voltage. No staining of proteins is required; protein fractions are detected and quantified by ultraviolet light absorbance. Potential advantages of capillary zone electrophoresis include smaller sample size, better resolution and reproducibility, and the ability to automate the procedure. The visual appearance of the electrophoretogram produced by this method differs from that of traditional methods, and quantitative results may also differ; thus experience and method-specific reference intervals are necessary for proper interpretation of results.[31]

Qualitative and semiquantitative estimation of immunoglobulin concentrations

Several different types of rapid screening tests for the estimation of immunoglobulin concentrations are available. These tests may be utilized in large animal clinical practice when other methods are not available, and they can provide qualitative or semiquantitative estimates of immunoglobulin concentration. They primarily are used to screen neonates (especially calves, foals, and crias) for possible failure to ingest colostrum or to absorb immunoglobulins from colostrum; this failure results in increased susceptibility to infection and is referred to as failure of passive transfer (FPT). These tests are not as sensitive or specific as more sophisticated tests such as radioimmunodiffusion (RID; considered the gold standard), but the results are usually available immediately and allow for treatment decisions to be made without delay. Results are most valid as indicators of adequate passive transfer or FPT when tests are performed within a few days of birth. General guidelines have been established for minimum IgG concentrations that indicate adequate passive transfer; >800 mg/dL for foals and >1000 mg/dL for calves and crias.[14,84,87]

Total protein measurement by refractometry

Immunoglobulins absorbed from colostrum are the major determinant of the total serum protein concentrations in neonates. Total protein concentrations in calves increase by approximately 2 g/dL after the ingestion of colostrum.[60] Measurement of the total protein concentration by refractometry, therefore, has been evaluated as an indicator of serum immunoglobulin concentration and as a gauge for the adequacy of passive transfer.

In calves, use of the serum total protein concentration as an indicator for the serum immunoglobulin concentration has been evaluated using different cut-off values or decision thresholds. A serum total protein concentration of 5.2 g/dL correlates with an IgG concentration of 1000 mg/dL and adequate passive transfer.[84] A decision threshold of either 5.0 or 5.5 g/dL classifies >80% of calves correctly; however, sensitivity and specificity of these thresholds are different.[84] Sensitivity and specificity of the 5.0 g/dL threshold are 0.59 and 0.96, respectively, indicating few normal calves would be incorrectly classified as FPT (false positives). Sensitivity and specificity of the 5.5 g/dL threshold are 0.94 and 0.74, respectively, indicating few calves with FPT would be

incorrectly classified as normal (false negatives). Because many sick calves with FPT are also dehydrated, which results in a relative hyperproteinemia, the higher decision threshold may be more appropriate for those individuals.[88]

In crias, one study found that serum total protein concentrations of <4.5 g/dL measured by refractometer indicated FPT, and concentrations >5.5 g/dL indicated adequate passive transfer; however concentrations between those values could not be accurately interpreted.[86]

In foals, using the serum total protein to estimate IgG concentration appears to be unreliable and is not recommended as a sole indicator of FPT.[14] The poor performance of serum total protein might result, in part, from wide variations in precolostral protein concentrations in foals.

Turbidity and coagulation assays

These tests are based on the ability of different substances to either precipitate or form insoluble complexes with serum immunoglobulins. Solutions used in these assays can be made in the clinic or purchased in kit form from several different manufacturers. In general these are inexpensive and rapid assays that are easy to perform, but sensitivity and specificity for the diagnosis of FPT varies considerably when different cut-off values are used. Additionally, because assessment of the degree of turbidity or coagulation present is subjective, results can vary between users.

- *Sodium sulfite precipitation test.*[66] The sodium sulfite precipitation test is based on the fact that immunoglobulins can be selectively precipitated from serum using concentrations of anhydrous sodium sulfite ranging from 14% to 18%. A higher sodium sulfite concentration is required to cause precipitation in serum containing lower immunoglobulin concentrations. Sera with very low immunoglobulin concentrations do not undergo precipitation when mixed with any sodium sulfite solutions in the 14–18% range. Fibrinogen is also precipitated by these concentrations of sodium sulfite; thus serum, rather than plasma samples, should be used. This test is useful for calves and crias, but does not work well for foals.[22,70,84,87] A procedure for performing the sodium sulfite precipitation test in ruminants is presented in Appendix 29.1; test kits are also commercially available (Bova-S and Llama-S, VMRD, Pullman, Washington). In this test, the immunoglobulin concentration is determined by judging the presence or absence of precipitation in three concentrations of sodium sulfite: 14%, 16%, and 18%. The test can distinguish three ranges of immunoglobulin concentrations: <500 mg/dL, 500–1500 mg/dL, and >1500 mg/dL. Using the <500 mg/dL limit makes the test more specific for detecting FPT (e.g., calves negative for precipitation are likely to have FPT), but less sensitive for detecting FPT (e.g., will miss some calves with FPT). Using the 1500 mg/dL limit makes the test more sensitive for detecting FPT, but reduces the specificity (e.g., will indicate FPT in calves with adequate transfer of immunoglobulin). Using the <500 mg/dL limit appears to correctly predict the highest percentage of calves with FPT (~86%); thus some recommend using only the 18% sodium sulfite solution.[88]
- *Zinc sulfate turbidity test.*[38,70] Immunoglobulins are precipitated from serum by zinc sulfate over a wide range of zinc sulfate concentrations. This test is most useful in calves; a procedure is presented in Appendix 29.2. Like the sodium sulfite precipitation test, a positive reaction (i.e., turbidity) in sera with low immunoglobulin concentrations occurs when a solution with a high zinc sulfate concentration is used, but not when a solution with a low zinc sulfate concentration is used. In sera with high immunoglobulin concentrations, turbidity occurs when zinc sulfate solutions of lower concentrations are used. Thus, different sensitivities and specificities for detecting FPT result when different concentrations of zinc sulfate are used (see Appendix 29.2). The highest proportion of calves correctly classified as having FPT (i.e., true immunoglobulin concentration <1000 mg/dL) occurs when either 350 or 400 mg/L concentrations of zinc sulfate are used (83% and 88% correctly classified, respectively).[38] The actual concentrations most appropriate for this test depend on whether high sensitivity or high specificity is most important in the specific situation.

A procedure for the zinc sulfate turbidity test in foals is presented in Appendix 29.3; commercial kits are also available (Equi-Z, VMRD Inc., Pullman, Washington). Observing any visible turbidity in the reaction solution after 1 hour of incubation is a good indication that the foal has a serum immunoglobulin concentration of >400 mg/dL. This procedure, however, does not distinguish foals with immunoglobulin concentrations of between 400 and 800 mg/dL, which are considered evidence for partial failure of passive transfer. Correlations between zinc sulfate turbidity results and those of more specific tests for immunoglobulin concentrations in foals are not strong.

- *Glutaraldehyde coagulation test.*[3,79] The glutaraldehyde coagulation test is based on the ability of glutaraldehyde to form insoluble complexes with immunoglobulins, resulting in coagulation of the test mixture. Glutaraldehyde also forms insoluble complexes with fibrinogen; therefore, serum rather than plasma is preferred. This test has been evaluated in neonatal calves and foals. A procedure for this test in ruminants is presented in Appendix 29.4; commercial kits are also available (Gamma-Check®-B, Plasvacc USA Inc., Templeton, California), but are unreliable when used with whole blood.[83] In neonatal calves, use of a 10% glutaraldehyde solution results in no coagulation in almost all calf sera with immunoglobulin concentrations of less than 400 mg/dL and complete or partial coagulation in almost all calf sera with immunoglobulin concentrations of greater than 600 mg/dL. Calves with immunoglobulin concentrations of between 400 and 600 mg/dL have results that vary from no coagulation to complete coagulation. However, the accepted cutoff limit for adequate passive transfer in calves is 1000 mg/

dL, thus this test has poor sensitivity (FPT in calves with immunoglobulin concentrations between 400 and 1000 mg/dL will not be identified).

A procedure for performing the glutaraldehyde coagulation test in foals is presented in Appendix 29.5; a commercial kit is also available (Gamma-Check®-E, Plasvacc USA Inc., Templeton, California). In horses, FPT is defined as serum IgG concentrations <200 mg/dL, and partial FPT occurs at serum IgG concentrations of 200–800 mg/dL.[14] The glutaraldehyde coagulation test distinguishes three ranges of immunoglobulin concentrations: >800 mg/dL, 400–800 mg/dL, and <400 mg/dL. Reported sensitivities and specificities for detection of IgG concentrations <400 mg/dL have ranged from 95 to 100% and 80 to 89%, respectively.[11,14] For detection of IgG concentrations <800 mg/dL, sensitivities and specificities have ranged from 93 to 100% and 59 to 94%, respectively.[11,17] Lower test specificity using the 800 mg/dL cutoff value indicates a greater chance of false positive results (diagnosing FPT in a normal foal), suggesting the need for additional confirmatory tests prior to treatment.

Antibody-based detection kits

A number of manufacturers have developed commercial kits for detection of FPT in foals and calves that use antibody-based methods (e.g., latex agglutination, enzyme-linked immunoassays). Examples include the Quick Test® calf or foal IgG kits (Midland Bio-Products, Boone, Iowa), Foal-check® (Centaur, Overland Park, Kansas), and SNAP® Foal test (Idexx, Westbrook, Maine). Accuracy, sensitivity and specificity of these assays vary with the individual test and cutoff values.[14,17,18] These commercially available tests are not necessarily superior to the screening tests described earlier. To minimize false negative results and ensure detection of a high percentage of animals with FPT, screening tests should have high sensitivity. In foals, the glutaraldehyde coagulation test may be equal or superior to commercially available semiquantitative tests in terms of sensitivity (depending on the cutoff value used).[11,17] However, more specific confirmatory tests may be desired in some situations.

Measurement of immunoglobulin concentrations by reference laboratories

Reference laboratories offer more sophisticated antibody-based methods for quantitating specific immunoglobulins (e.g., radial immunodiffusion, immunochemistry). Use of these methods is indicated when a detailed examination regarding the status of the immune system is desired. These methods are more expensive, however, and the results usually are delayed (incubation periods of 18–24 hours are required) compared with those of the screening methods discussed earlier.

Fibrinogen concentration

Plasma fibrinogen concentrations can be determined by two methods. One assesses the conversion of fibrinogen to fibrin in the presence of thrombin (thrombin time) and requires instrumentation that is somewhat expensive for routine use in clinical practice. Such measurement of plasma fibrinogen concentrations also requires citrated plasma that has been harvested from a mixture of nine parts fresh, whole blood and one part 3.8% sodium citrate anticoagulant. Special evacuated blood collection tubes containing sodium citrate anticoagulant are available for this purpose; these tubes draw the appropriate amount of blood to ensure a 9:1 ratio of blood to anticoagulant. This method is not routinely used to measure plasma fibrinogen concentrations, but may be included in a coagulation profile.

In clinical practice situations, the most common method for measuring plasma fibrinogen concentration is heat precipitation. This method is less expensive than the method described earlier and requires minimal equipment; it is summarized in Appendix 29.6. The heat precipitation method provides an estimate of the plasma fibrinogen concentration that is adequate for evaluation of hyperfibrinogenemia, but lacks the analytic sensitivity needed for evaluation of hypofibrinogenemia. (The significance of abnormal fibrinogen concentrations is discussed later.)

Abnormal protein concentrations

Both decreased and increased total protein concentrations are commonly detected laboratory abnormalities in animals. These findings may result from alterations in the albumin or globulin concentrations (or both). In plasma, an increased concentration of fibrinogen, which is a globulin, can occasionally produce an increased protein concentration. Interpretation of altered protein concentrations depends on determining which major protein constituents of the serum or plasma are abnormal. A decreased or increased albumin or globulin concentration does not always result in detectable alterations of the total protein concentration. Therefore, albumin and globulin as well as total protein concentrations should be assessed. Causes of decreased or increased total protein, albumin, globulin, and fibrinogen concentrations are summarized here. It is often helpful to consider alterations in albumin and globulin concentrations together for interpretation.

Causes of decreased protein concentrations

Decreased total protein concentrations can result from decreased concentrations of albumin, globulin, or both. A diagnostic algorithm for evaluating variations in these decreases is presented in Figure 29.2.

Hypoalbuminemia with hypoglobulinemia

Concurrent hypoalbuminemia and hypoglobulinemia can result from overhydration (e.g., excessive fluid therapy, excessive water intake) or from loss of both protein fractions.

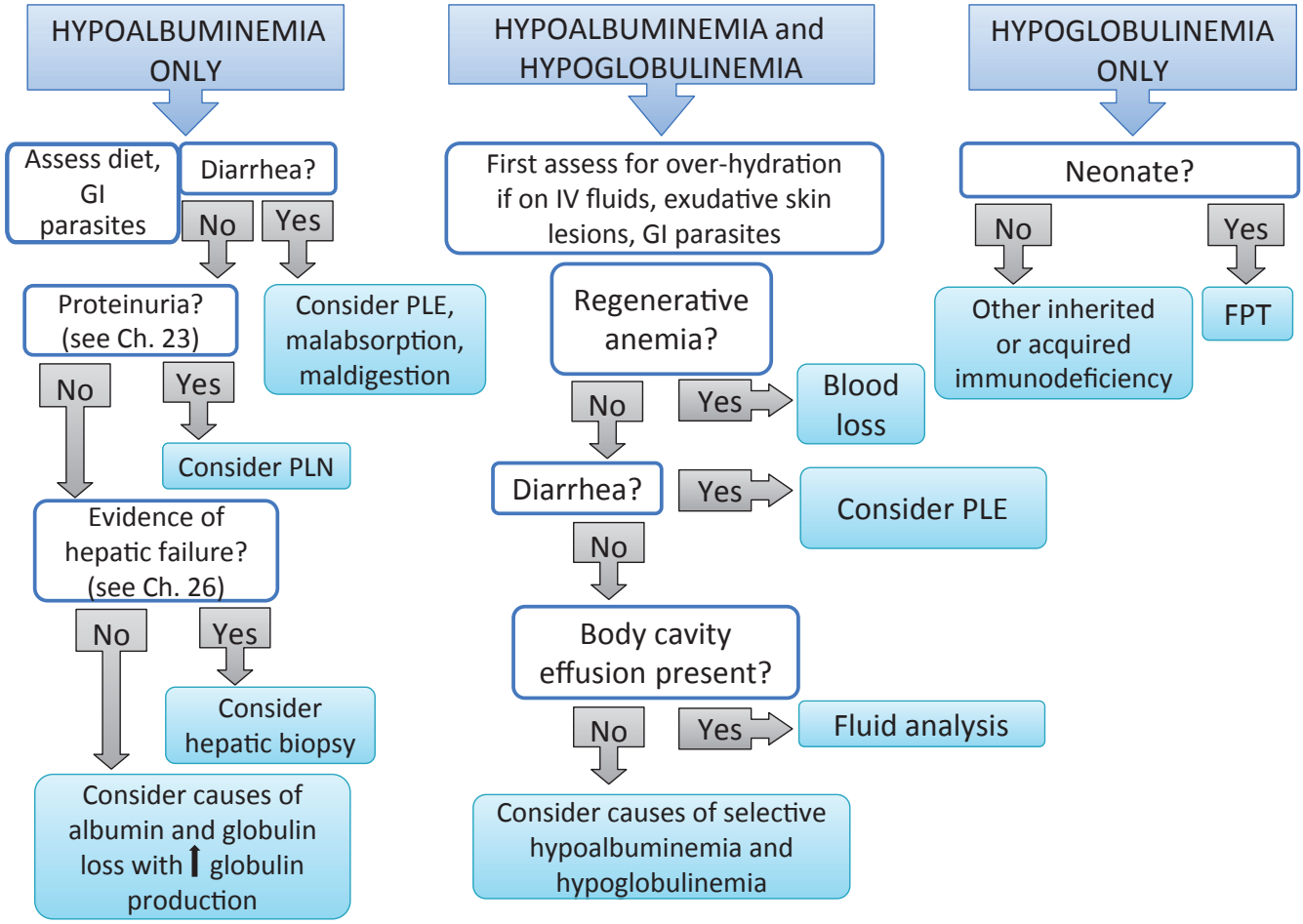

Figure 29.2 An algorithm for evaluation of decreased serum protein concentrations. FPT = failure of passive transfer; PLE = protein losing enteropathy; PLN = protein losing nephropathy. Refer to text for details.

The latter is much more common and occurs in the following disorders:

- Blood loss. This results in proportional loss of all blood constituents. Albumin and globulin, therefore, are lost in concentrations equal to their concentrations in the blood. After blood loss, fluid moves from the extravascular space to the intravascular space and dilutes the remaining blood constituents, including the proteins (and erythrocytes). It is important to remember that this water shift takes time to develop, and will not be evident for the first few hours following acute hemorrhage. Hypoproteinemia due to blood loss is generally caused by external (rather than internal) hemorrhage, and may also be caused by bloodsucking parasites (external or internal).
- Protein-losing enteropathy. This may result from a variety of generalized intestinal lesions, including inflammatory bowel disease, lymphangiectasia, infectious diseases, neoplasia, severe prolonged starvation or cachexia, and gastrointestinal hemorrhage.[39,48,65] Hypoproteinemia can develop whenever protein leakage into the intestinal lumen exceeds the rate of protein synthesis. In some instances a concurrent immune response causes increased instead of decreased globulin concentration; gastrointestinal lymphoma may also be associated with hyperglobulinemia.[52]
- Severe skin disease. Generalized exudative skin disease or burns can result in loss of plasma proteins due to increased vascular permeability.[43] Concurrent immune responses may increase globulin concentration.
- Effusive disease. This results in the accumulation of body-cavity fluids with high protein concentration that can result in decreased serum albumin and globulin concentrations.[76] Such decreases depend on the degree of increased vascular permeability accompanying these disorders.

Selective hypoalbuminemia

Decreased albumin concentration that is not accompanied by decreased globulin concentration can result from either decreased production or increased loss of albumin. If the globulin concentration is concurrently increased, total

protein concentration may be within the reference interval despite hypoalbuminemia.

Decreased production of albumin can occur in the following disorders:

- Hepatic failure. The liver is the site of albumin production. Because of the liver's reserve capacity, most types of liver damage do not result in decreased albumin production. If more than 60–80% of the functional liver capacity is lost, however, decreased albumin production and hypoalbuminemia can occur. In such cases, other evidence of hepatic failure is also present (see Chapter 26). Serum globulin concentrations are not usually decreased because immunoglobulin production in lymphoid tissues is not hampered; globulin concentrations may actually be increased in patients with hepatic failure (discussed later).
- Starvation or cachexia. Marked malnutrition or starvation results in less hepatic protein production due to a deficiency of available amino acids. In cachectic states associated with neoplasia or chronic infections, a prolonged negative protein balance causes increased catabolism of body proteins that exceeds protein production. Body fat and muscle mass are lost in both cases, resulting in weight loss. Usually these conditions result in selective hypoalbuminemia; rarely there is concurrent hypoglobulinemia.
- Gastrointestinal parasitism. This can cause hypoalbuminemia by at least two mechanisms. If the parasites absorb significant amounts of nutrients, including amino acids, the animal is deprived of the amino acids needed to produce albumin. If the parasites attach to the gastric or the intestinal wall and consume the host's blood, albumin and globulin are lost. Gastrointestinal parasitism seldom results in a deficiency of amino acids that is severe enough to lead to hypoglobulinemia. Fecal examination for parasite ova is helpful in establishing the diagnosis of this potential cause of hypoalbuminemia.
- Intestinal malabsorption or maldigestion. Decreased albumin production can occur if intestinal malabsorption results in deficient absorption of amino acids. Animals with malabsorption syndrome often have a history of chronic diarrhea or loose stools. If malabsorption syndrome is considered a possible cause of hypoalbuminemia, tests to verify this syndrome should be performed (see Chapter 28).

Inadequate digestion of dietary proteins can result from exocrine pancreatic insufficiency (EPI), in which amino acids are not liberated by protein digestion in the intestine and, therefore, are not available for absorption, thus resulting in amino acid deficiency and decreased albumin production. Animals with EPI often have a history of chronic diarrhea or loose stools. If EPI is suspected, tests to verify this disease should be performed (see Chapter 28). Hypoglobulinemia does not typically occur with malabsorption/maldigestion syndromes.

- Inflammation. Because albumin is a negative acute phase protein, albumin synthesis is decreased during acute inflammation. Globulin concentrations are typically mildly increased due to increased positive acute phase protein synthesis. Because albumin and globulin concentrations change in different directions, total protein concentrations may be within the reference interval.

Increased loss of albumin can occur in the following disorders:

- Glomerular disease. Because albumin molecules are small and more negatively charged than globulin molecules, they leak more readily through damaged glomerular membranes. Severe glomerular disease, therefore, can result in selective hypoalbuminemia. Both urinary protein concentrations and urinary protein : creatinine ratios will be increased in animals with glomerular disease.[9,33]
- Gastrointestinal parasitism (discussed earlier).
- Diseases listed as being possible causes of hypoalbuminemia with hypoglobulinemia (discussed earlier). Loss of both albumin and globulin typically occurs with these diseases, but a concurrent immune response may cause increased production of globulins resulting in normal to increased globulin concentration. These diseases also should be considered when hypoalbuminemia with normal to increased globulin concentration is detected.

Selective hypoglobulinemia

Hypoglobulinemia in the absence of hypoalbuminemia usually results from a decreased beta or gamma globulin concentration. A decreased alpha globulin concentration alone does not result in a decreased globulin concentration. A selective decrease in beta or gamma globulin concentrations is usually due to a decreased immunoglobulin concentration. Such a decrease can occur in the following disorders:

- Failure of Passive Transfer (FPT). Ingestion of colostrum and absorption of immunoglobulins from colostrum are termed passive transfer. Because most animals are born with minimal immunoglobulin concentrations, this process plays an important role in transferring resistance to infection during the neonatal period. Failure to ingest colostrum or to absorb immunoglobulins from colostrum is termed failure of passive transfer (FPT) and is well documented in domestic animals.[14,87,88] Several screening tests are available to assess the adequacy of passive transfer (discussed earlier).
- Inherited or acquired immune deficiency. Immune deficiency involving B lymphocytes or plasma cells can result in low concentrations of immunoglobulins and, in some cases, hypoglobulinemia. Immune deficiencies resulting in low globulin concentration has been reported in foals (e.g., severe combined immunodeficiency, selective IgM deficiency, Fell pony immunodeficiency, transient hypogammaglobulinemia, agammaglobulinemia),[14] calves (e.g., selective IgG2 deficiency, severe combined immunodeficiency, transient hypogammaglobulinemia),[76] and puppies

(e.g., severe combined immune deficiency, selective IgA deficiency, selective IgM deficiency, selective IgA and IgG deficiency).[76]

Causes of increased protein concentrations

Increased total protein concentration can result from increased concentrations of albumin, globulin, or both. An increased albumin or globulin concentration, however, does not always produce detectable increases in total protein concentration. A diagnostic algorithm for evaluating the variations in these increases is presented in Figure 29.3. In clinical practice, serum protein electrophoresis is often reserved for patients with moderate to severe hyperglobulinemia (>5 g/dL), for which an underlying cause for inflammation or chronic antigenic stimulation has not been identified (discussed later).

Hyperalbuminemia

The primary cause of hyperalbuminemia is dehydration. Loss of plasma water results in a relative increase in albumin, which may be of sufficient magnitude to cause hyperproteinemia. Globulin concentration also may be increased in some patients with dehydration (discussed later).

Rarely, administration of drugs (glucocorticoids) has been associated with mild transient hyperalbuminemia.[59]

Hyperalbuminemia with hyperglobulinemia

Concurrent increases in albumin and globulin concentrations most commonly result from dehydration, which causes loss plasma water and a relative increase in both protein fractions. The albumin : globulin ratio is not altered, because both fractions are concentrated equally. The hematocrit is often increased as well, unless there was a preexisting

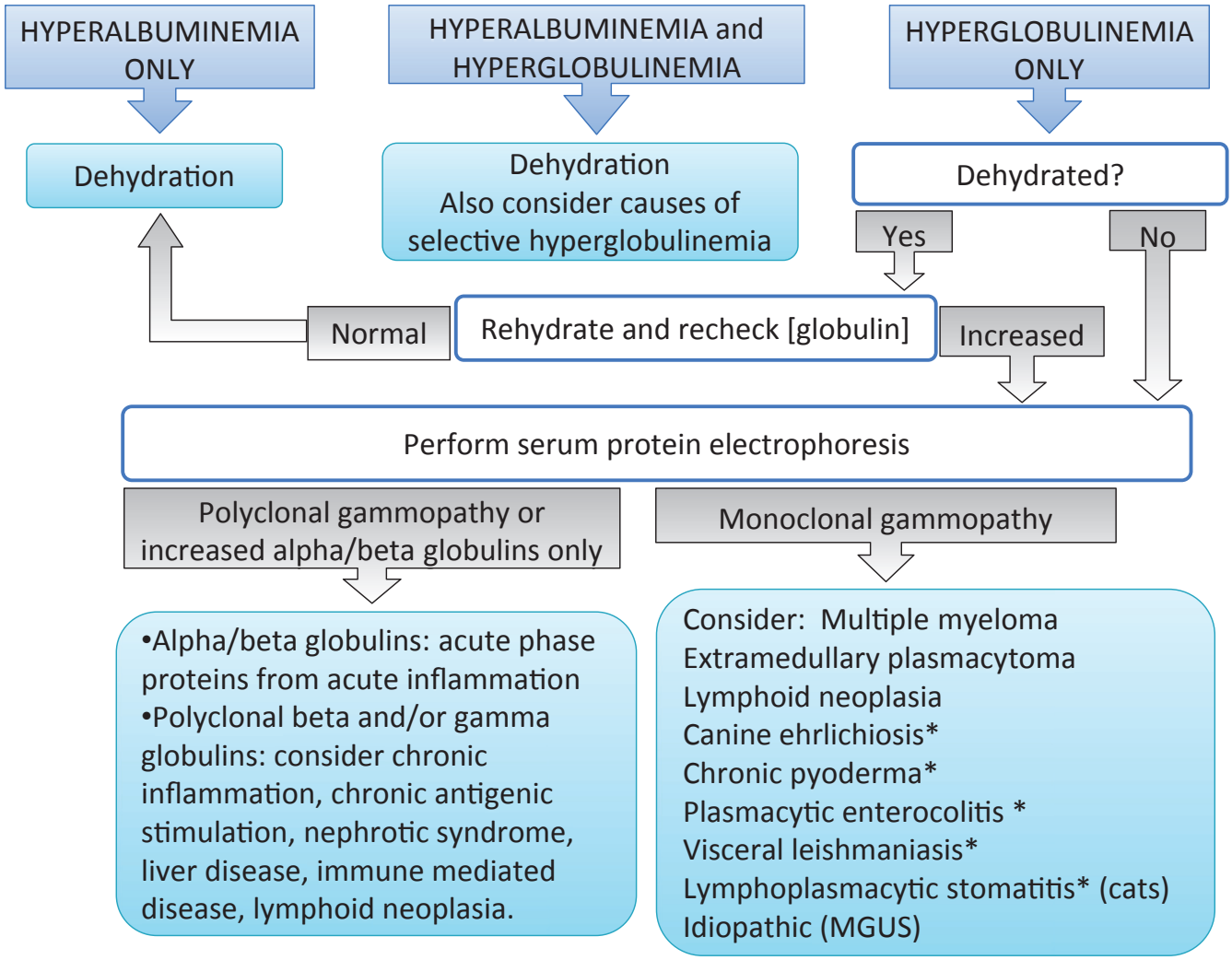

Figure 29.3 An algorithm for evaluation of increased serum protein concentrations. MGUS = monoclonal gammopathy of undetermined significance. *Typically causes polyclonal, not monoclonal, gammopathy. Refer to text for details.

anemia. Other potential causes of hyperglobulinemia should also be considered (discussed later).

Selective hyperglobulinemia

The significance of hyperglobulinemia depends on the magnitude and the type of globulin that is increased, which can be determined by serum protein electrophoresis. No matter what the underlying cause, mild to moderate hypoalbuminemia is often also present. Common disorders and typical electrophoretic patterns are discussed below.

Increased alpha/beta globulin concentrations

Acute/chronic inflammation. During acute inflammation, increased synthesis of acute phase proteins may cause hyperglobulinemia, which is generally mild. The acute phase proteins are located in the alpha and beta globulin regions of the electrophoretogram (except for fibrinogen, which is absent from serum). There are numerous acute phase proteins, and generally many of them must be increased in order to visualize an electrophoretic abnormality or result in hyperglobulinemia; however, fibrinogen or haptoglobin alone can be increased to a degree sufficient to cause hyperglobulinemia and an increase in total protein.[76] Because albumin is a negative acute phase protein, albumin concentrations usually decrease due to decreased hepatic production during acute inflammation. The magnitude of the decrease is usually <30%.[63]

Increased gamma globulin concentrations

The gamma globulin fraction includes immunoglobulins of all types. Increases in gamma globulin concentrations are termed gammopathies. Gammopathies may be monoclonal or polyclonal, which may be distinguished presumptively on the basis of the homogeneity of the globulin peak on an electrophoretogram. Polyclonal gammopathies have broad-based peaks (i.e., wider than the base of the albumin peak, with a slope that is less steep than the albumin peak) on the electrophoretogram (Fig. 29.4). These polyclonal peaks represent increased quantities of a mixture of immunoglobulin types produced by a heterogeneous population of B lymphocytes, plasma cells, or both, each secreting its own immunoglobulin molecule specific for one particular antigen epitope. Monoclonal gammopathies, however, have narrow-based peaks (i.e., similar in width to the base of the albumin peak, with a slope that is as steep or steeper than the albumin peak) on the electrophoretogram (Fig. 29.5), and they result from increased production of a single type of immunoglobulin by a single clone of B lymphocytes or plasma cells. Proliferation of a single clone of lymphocyte results in overproduction of its specific immunoglobulin molecule. Uncommonly, two narrow-based peaks may be seen on the electrophoretogram; this is termed a biclonal gammopathy. Biclonal gammopathies can occur when a single neoplastic clone produces immunoglobulin molecules that migrate separately. Examples include polymerization of immunoglobulin molecules (such as dimer formation), production of incomplete molecules (such as free light chains in addition to intact immunoglobulins), or production of an immunoglobulin that undergoes isotype switching.[67,89] Rarely, two clones of plasma cells or B lymphocytes may proliferate, resulting in production of two separate, but homogeneous,

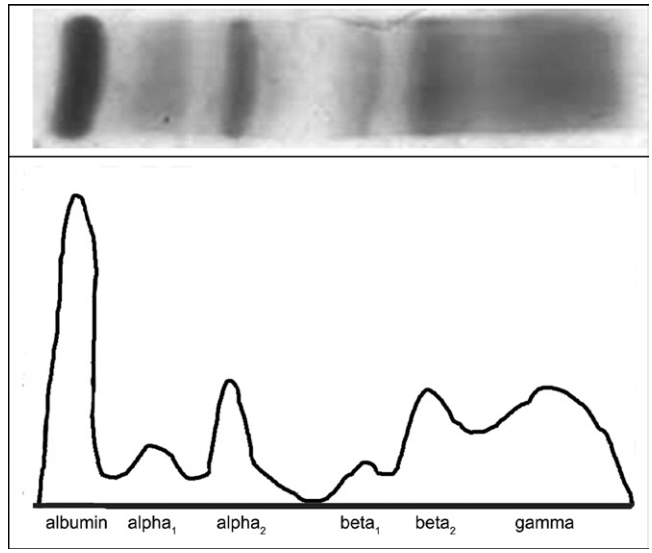

Figure 29.4 An electrophoretogram and corresponding gel from a dog with a polyclonal gammopathy. There is an increase in $alpha_2$-globulins and a broad-based peak in the $beta_2$ and gamma regions.

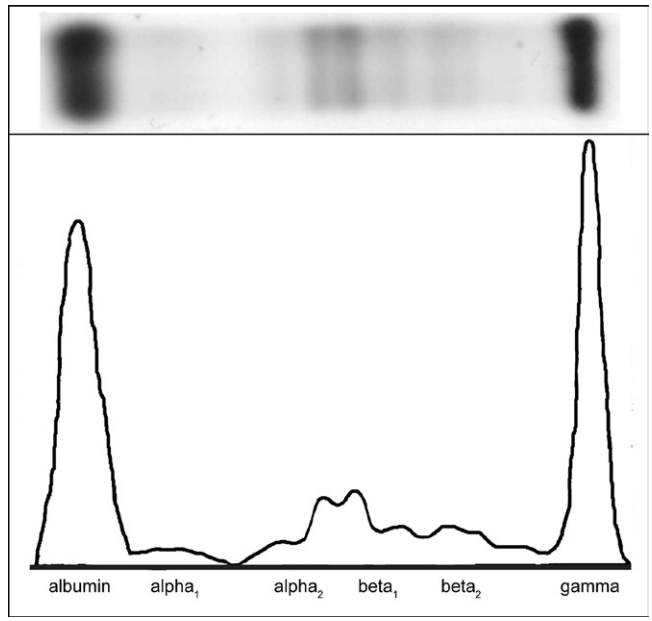

Figure 29.5 An electrophoretogram and corresponding gel from a cat with a monoclonal gammopathy due to multiple myeloma. There is a narrow-based peak in the gamma region; the corresponding monoclonal band is apparent on the gel.

types of immunoglobulins.[64] It is possible for a monoclonal gammopathy to be obscured by a concurrent polyclonal peak; in these cases visual inspection of the stained electrophoretic gel may suggest presence of a monoclonal band. More sensitive and specific techniques may be required to confirm presence of a monoclonal gammopathy (i.e., immunoelectrophoresis or immunofixation).[2,42]

Hyperviscosity of the blood can result from high concentrations of immunoglobulins, especially in association with monoclonal gammopathies. Hyperviscosity syndrome might cause the initial clinical signs observed in animals with monoclonal gammopathies. These signs include epistaxis, ocular abnormalities (e.g., visual impairment, distension and tortuosity of retinal veins, retinal hemorrhage and detachment), cardiovascular abnormalities (e.g., gallop rhythm, left ventricular hypertrophy), and neurologic dysfunction (e.g., disorientation, seizures).[26] Hyperviscosity syndrome is often associated with monoclonal gammopathies involving IgM (because of their large size) or IgA (because of dimer formation), but can also occur with IgG.

Monoclonal cryoglobulinemia is a rare variation of monoclonal gammopathy that has been reported in dogs, cats, and horses.[35] In this disorder, the monoclonal globulins are soluble at 37°C, but become reversibly insoluble at lower temperatures, causing formation of a gel-like precipitate. To demonstrate cryoglobulins, serum must be harvested from the blood at 37°C. If blood samples are stored at refrigerator temperature before harvesting the serum, the cryoglobulins are not harvested, and cryoglobulinemia is not detected. Cryoglobulins are rarely associated with polyclonal disorders.

Conditions typically associated with polyclonal gammopathies include:

• Chronic inflammation or antigenic stimulation. As an inflammatory response becomes chronic (>1 week), production of immunoglobulins and complement proteins may be increased; acute phase proteins may remain increased as well. Chronic antigenic stimulation from any cause, including immune-mediated diseases, can cause similar abnormalities. Immunoglobulins usually migrate in the gamma globulin region, although some (IgA and IgM) occasionally migrate in beta globulin region along with complement proteins. The magnitude of the hyperglobulinemia that occurs with chronic inflammation is variable, but can be marked in some cases (>10 g/dL).[74]

Gammopathies associated with chronic inflammation are usually polyclonal, exemplified by canine ehrlichiosis and feline infectious peritonitis. However, exceptions to this rule have been recognized. In particular, apparent monoclonal gammopathies have been reported in dogs with chronic ehrlichiosis, chronic pyoderma, plasmacytic enterocolitis, visceral leishmaniasis, and in cats with lymphoplasmacytic stomatitis.[5,7,20,25,51] In some of these cases, the apparent monoclonal spike was not a true monoclonal gammopathy because it consisted of a several subclasses of IgG or heterogeneous light chains.[58,85] Infection with *Bartonella henselae* has been reported to cause a monoclonal gammopathy (confirmed by immunofixation) in people, but this has not been described yet in animals.[44]

• Liver disease. Especially when chronic, liver disease may lead to increased globulin production, which has been well-documented in horses, but also occurs in other species.[61] The globulins are frequently immunoglobulins that migrate in the beta or gamma region of an electrophoretogram and can obscure the border between the beta and gamma regions, known as beta–gamma bridging. It is theorized that mononuclear phagocytes (Kupffer cells) in the diseased liver fail to clear antigens from the portal circulation. The antigens subsequently reach the general circulation, where they stimulate B lymphocytes to mount an immune response. In liver failure, albumin concentrations may be decreased concurrently due to decreased hepatic synthesis. Although historically believed to be pathognomonic for hepatic disease, recent studies have shown that beta-gamma bridging is frequently seen with infectious diseases as well.[8]

• Lymphoma and lymphocytic leukemia. Polyclonal gammopathy occasionally occurs with lymphoma and lymphocytic leukemia, sometimes because of increased production of heterogeneous immunoglobulins by multiple clones of proliferating, neoplastic lymphoid cells.[41] However, monoclonal gammopathies are more commonly associated with lymphoid neoplasia (discussed later). Secondary infectious processes may stimulate immunoglobulin production in animals with lymphoma and lymphocytic leukemia.

Conditions typically associated with monoclonal gammopathies include:

• Multiple myeloma. This is a malignant, proliferative disease of plasma cells that involves the bone marrow at multiple sites and, often, other tissues (e.g., spleen, liver). Infiltration of visceral organs appears to be relatively common in cats.[62] Multiple myeloma typically results from the proliferation of a single clone of plasma cells that produce a homogeneous type of protein that is referred to as paraprotein or M-component. This protein most commonly is IgA or IgG; IgM paraproteinemias occur with lymphoma and lymphocytic leukemia, but are rare with multiple myeloma.[32,46] Primary macroglobulinemia (Waldenström macroglobulinemia) results from neoplastic proliferation of less differentiated B lymphocytes and is an uncommon cause of IgM monoclonal gammopathy; this disease may be difficult to distinguish from multiple myeloma.[28] Multiple myeloma paraproteins can be composed of entire immunoglobulin molecules or of just heavy or light chains of these molecules.[12,23,36,89] Paraproteins typically are found as a monoclonal peak in the beta or gamma region and, more rarely, in the alpha region of the electrophoretogram.[27] As discussed previously, biclonal peaks are also possible, but rare. Light chains also may be detected in the urine and are

referred to as Bence-Jones proteins. The diagnosis of this disease is usually established on the basis of finding at least three of the following four features:

1. Monoclonal gammopathy.

2. Excessive numbers of plasma cells on a bone marrow film. The percentage of plasma cells that is considered to be suggestive of myeloma varies with different authors (>5% to >20%). Chronic antigenic stimulation also can result in greater than 5% plasma cells on a bone marrow film. Other features that are suggestive of plasma cell neoplasia, such as the presence of plasma cell aggregates, poorly differentiated plasma cells, or both, are helpful in differentiating myeloma from antigenic stimulation in bone marrow films with increased numbers of plasma cells.

3. Radiographic evidence of osteolytic bone lesions.

4. Bence-Jones proteinuria. Bence-Jones proteins are light chains of immunoglobulins that are produced in some gammopathies. Because of their small size, these proteins readily pass the glomerulus. If the concentration of Bence-Jones proteins in the urine exceeds the tubular reabsorptive capacity, they are excreted in the urine. Bence-Jones proteins rarely are detected by urine dipstick tests for proteins, because dipsticks primarily detect albumin. Bence-Jones proteins can be detected by several techniques, including the heat precipitation test, electrophoresis, and immunoelectrophoresis. The heat precipitation test can be performed in a practice laboratory, but this test is difficult to perform and interpret.[75] Bence-Jones proteins are detectable in approximately 30% of dogs and cats with multiple myelomas and have been reported in a horse with multiple myeloma.[23,53] Bence-Jones proteins have also been detected in animals with other neoplastic and nonneoplastic monoclonal gammopathies.[7,19,47,51]

• Extramedullary plasmacytoma.[6] Extramedullary plasmacytomas are proliferations of plasma cells originating from a site other than bone. They are usually solitary, cutaneous, benign lesions that most commonly occur in dogs but have also been reported in cats. Plasmacytomas that occur in the digestive tract are more likely to be malignant. In cats, there is evidence that extramedullary tumors may progress to multiple myeloma.[57] Monoclonal gammopathies rarely occur in association with these tumors. A biclonal gammopathy has been reported in a cat with two extramedullary plasmacytomas.[45]

• Lymphoma and lymphocytic leukemia.[56,68,82] Monoclonal gammopathies can occur with lymphoma and lymphocytic leukemia. Approximately 5% of dogs with lymphoma and lymphocytic leukemia have monoclonal gammopathies.[82] The incidence appears higher in dogs with chronic lymphocytic leukemia, however, with studies indicating an incidence of greater than 50% in such cases.[47] The immunoglobulin most commonly increased is IgM, especially in cases of chronic lymphocytic leukemia, but IgG and IgA monoclonal gammopathies also have been reported.[47]

Less common causes of apparent monoclonal gammopathies include:

• Canine ehrlichiosis.[5] Although polyclonal gammopathies are more common, monoclonal gammopathies have been reported in dogs with ehrlichiosis. Infrequently, polyclonal gammopathies progress to monoclonal gammopathies. Typically, the monoclonal gammopathies are composed of IgG and result from an unexplained proliferation of one plasma cell clone. Monoclonal spikes disappear after treatment for ehrlichiosis. The serum hyperviscosity syndrome (discussed later) has also been reported in these dogs.

• Chronic pyoderma.[7] An IgG monoclonal gammopathy with Bence-Jones proteinuria has been reported in a dog with chronic pyoderma. Treatment and resolution of the pyoderma were followed by disappearance of the monoclonal gammopathy.

• Plasmacytic enterocolitis.[20] Monoclonal gammopathy has been reported in a dog with this disease. The monoclonal gammopathy disappeared after treatment and resolution of the inflammation.

• Visceral leishmaniasis (in dogs).[25] Most dogs with visceral leishmaniasis have polyclonal gammopathies. In a few such dogs, a single clone of plasma cells may proliferate and result in IgG monoclonal gammopathy.

• Lymphoplasmacytic stomatitis (in cats).[51] Monoclonal gammopathy with Bence-Jones proteinuria occurs infrequently in cats with this disease.

• Idiopathic monoclonal gammopathy.[19,37] Unexplained monoclonal gammopathies among animals in which known causes have been eliminated are termed idiopathic, or monoclonal gammopathy of undetermined significance (MGUS). These animals are asymptomatic and may have stable production of the monoclonal immunoglobulin for a prolonged period of time (i.e., months to years); Bence-Jones proteinuria occurs in some of these cases. These gammopathies may relate to antigenic stimulation of a B-lymphocyte clone. "Idiopathic" monoclonal gammopathy, however, may precede the onset of overt multiple myeloma.

Hyperfibrinogenemia

Increased plasma fibrinogen concentrations are most often associated with inflammatory conditions and dehydration, but have also been recognized with pregnancy and neoplasia.[29,34,54,76]

• Dehydration. With dehydration, fibrinogen increases in proportion to other plasma proteins. To eliminate the effect of hydration status, a plasma protein:fibrinogen ratio (PP:Fib) can be calculated as follows:[76]

$$\text{PP : Fib} = \frac{\text{Plasma protein (g/dL)} \times 1000}{\text{Plasma fibrinogen (mg/dL)}}$$

The PP:Fib should not change with changes in hydration status.

As a general rule, a PP:Fib <10 is considered consistent with hyperfibrinogenemia due to inflammation (discussed below), and a ratio >15 is considered normal or consistent with dehydration.[80] Some authors suggest slightly different cut-off values for horses; <15 for inflammation and >20 for normal or dehydration.[76] These are rough guidelines for use in adult animals, and do not take into account other factors that could influence plasma protein and fibrinogen concentrations.

- Inflammation. Fibrinogen is a positive acute phase protein, therefore plasma concentrations increase with inflammation. Although other acute phase proteins may be better indicators of inflammatory disease, fibrinogen continues to be used because it is easy to measure in practice situations.[15,40,63] It is often included in routine equine and bovine complete blood counts. With inflammation in those species, fibrinogen concentrations may sometimes be increased in the absence of an inflammatory leukogram.[1] In dogs, it offers no advantage over leukocyte counts for evaluation of inflammation.[71]

Table A29.1 Interpretation of sodium sulfite precipitation test results.

Estimated immunoglobulin concentration	Sodium sulfite concentration		
	14%	16%	18%
<500 mg/dL	–	–	+
500–1500 mg/dL	–	+	+
>1500 mg/dL	+	+	+

– No precipitation after one hour (cloudiness without visible flakes is a negative test).
+ Flakes of precipitation after one hour (regardless of flake density).
Adapted from Pfeiffer NE, McGuire TC (1977) A sodium sulfite-precipitation test for assessment of colostral immunoglobulin transfer to calves. *J Am Vet Med Assoc* 170: 809–11.

Table A29.2 Zinc sulfate turbidity test performance for detection of FPT in ruminants.

Zinc sulfate concentration	Sensitivity	Specificity
200 mg/L	100%	25%
250 mg/L	100%	42%
300 mg/L	98%	65%
350 mg/L	94%	76%
400 mg/L	83%	91%

Adapted from Hudgens KA, Tyler JW, Besser TE, Krytenberg DS (1996) Optimizing performance of a qualitative zinc sulfate turbidity test for passive transfer of immunoglobulin G in calves. *Am J Vet Res* 57: 1711–13.

Appendix 29.1

Sodium sulfite precipitation test: Application in ruminants

1. Prepare three solutions of sodium sulfite (14%, 16%, and 18%) from anhydrous sulfite and distilled water.
2. Place 1.9 mL of sodium sulfite solution into each of three 13 × 100 mm test tubes.
3. Add 0.1 mL of serum into each of the three tubes.
4. Mix immediately, and then incubate at room temperature for 1 hour.
5. After 1 hour, examine the tubes for evidence of precipitation.
6. Interpret as described in Table A29.1.

Appendix 29.2

Zinc sulfate turbidity test: Application in ruminants

1. Prepare a solution of zinc sulfate ($ZnSO_4 \cdot 7H_2O$) by mixing 350 mg of zinc sulfate in 1 L of distilled water that has been previously boiled to remove CO_2. Note that lower concentrations of zinc sulfate might be appropriate in some cases. Lower concentrations have a higher sensitivity but a lower specificity; higher concentrations (e.g., 350 mg/L) have a lower sensitivity and a higher specificity (Table A29.2).
2. The solution should be stored in an air-tight bottle that is connected to a CO_2 trap to prevent CO_2 absorption.
3. Add 0.1 mL of serum (hemolysis might interfere with the test) to a tube (13 × 100 mm) containing 6 mL of the zinc sulfate solution. Cap the tube to prevent absorption of CO_2, which adds to turbidity.
4. Mix the contents of the tube and incubate at room temperature (23°C) for 1 hour.
5. After the incubation period, mix the contents of the tube, and then hold the tube in front of newsprint.
6. Cloudiness sufficient to obscure newsprint when viewed through the tube is considered to be a positive reaction.
7. Interpret a negative reaction as being suggestive of the failure of passive transfer.

Appendix 29.3

Zinc sulfate turbidity test: Application in horses

1. Prepare a solution of zinc sulfate ($ZnSO_4 \bullet 7H_2O$) by mixing 208 mg of zinc sulfate in 1 L of distilled water that has been previously boiled to remove CO_2.
2. The solution should be stored in an air-tight bottle that is connected to a CO_2 trap to prevent CO_2 absorption.
3. Add 0.1 mL of serum to a 13 × 100 mm test tube containing 6 mL of the zinc sulfate solution. Cap the tube to prevent absorption of CO_2, which adds to turbidity.
4. Mix the contents of the tube and incubate at room temperature (23°C) for 1 hour.
5. After the incubation period, mix the contents of the tube, and then observe for turbidity.
6. Interpret as follows:
(a) Visible turbidity indicates immunoglobulin concentration is at least 400 mg/dL.
(b) This test can be made semiquantitative by using a spectrophotometer and reading absorbance at 600 nm, which requires the use of standards.

Appendix 29.4

Glutaraldehyde coagulation test: Application in ruminants

1. Prepare a 10% solution of glutaraldehyde (usually prepared via dilution of a 25% solution to a 10% solution).
2. Place 0.5 mL of serum into a 13 × 100 mm test tube.
3. Add 50 µL (0.05 mL) of the 10% glutaraldehyde reagent to the tube.
4. Mix immediately, and then incubate at room temperature.
5. Examine the tube at intervals for as long as 1 hour, looking for evidence of coagulation.
6. Interpret as follows:
(a) Complete coagulation indicates immunoglobulin concentration is more than 600 mg/dL.
(b) Semisolid gel indicates immunoglobulin concentration is 400 to 600 mg/dL.
(c) No coagulation indicates immunoglobulin concentration is less than 400 mg/dL.

Appendix 29.5

Glutaraldehyde coagulation test: Application in horses

1. Perform steps 1 through 4 as outlined in Appendix 29.4.
2. Examine the tube at 5, 10, 15, 20, 30, 45, and 60 minutes.
3. A positive reaction is solid coagulation (i.e., does not move when the tube is tilted).
4. Interpret as follows:
(a) Coagulation within 10 minutes indicates immunoglobulin concentration is more than 800 mg/dL.
(b) Coagulation by 60 minutes indicates immunoglobulin concentration is 400 to 800 mg/dL.
(c) No coagulation by 60 minutes indicates immunoglobulin concentration is less than 400 mg/dL.

Appendix 29.6

Fibrinogen determination by heat precipitation

1. Fill two microhematocrit tubes with ethylenediamine tetraacetic acid (EDTA)–anticoagulated blood.
2. Sediment blood in both tubes using a microhematocrit centrifuge.
3. Break one tube at the bottom of the plasma column, apply the plasma to a refractometer, and read the protein concentration.
4. Place the second microhematocrit tube in a waterbath at 56 to 58°C for 3 to 5 minutes, which denatures and precipitates the fibrinogen in the sample. Note that hot tap water frequently is in the 56 to 58°C range. If so (check with a thermometer), such tap water placed in an insulated container can replace the waterbath as an incubation chamber.
5. After incubation, re-centrifuge the second microhematocrit tube in the microhematocrit centrifuge to sediment the precipitated fibrinogen.
6. Measure the protein concentration in the second tube using a refractometer.
7. Subtract the protein concentration of the second tube from that of the first tube. The difference is the estimate of the plasma fibrinogen concentration. For example, if the protein concentration in the first tube is 7.1 g/dL and that in the second tube is 6.7 g/dL, then the fibrinogen concentration is 0.4 g/dL.
8. Fibrinogen concentrations usually are converted to mg/dL (e.g., 0.4 g/dL = 400 mg/dL).

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30 Laboratory Detection of Muscle Injury

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Routine laboratory tests that evaluate muscle are primarily aimed at detecting muscle injury. These tests include assays that measure the serum activities of enzymes and other proteins that leak from injured muscle cells and the urine concentrations of myoglobin, which also leaks from injured muscle cells and is excreted via glomerular filtration.

Creatine kinase

Creatine kinase (CK) is an enzyme present in highest concentrations in skeletal muscle, cardiac muscle, smooth muscle, and brain, with lesser amounts present in various organs such as intestine, liver, and spleen.[4,12] Creatine kinase is found free in the cytoplasm of muscle cells and leaks from these cells when they are damaged. Creatine kinase is considered a muscle-specific leakage enzyme. Although CK is present in the brain, brain injury causes increased CK activity in the cerebrospinal fluid instead of the blood because of the blood-brain barrier. Increases in CK activity following muscle injury occur rapidly (peaking in 6–12 hours) but also decline rapidly (a day or two) because CK has a short half-life of about 2 hours (See Fig. 30.1).[7] Thus, persistent increases in CK activity indicate ongoing muscle damage. Although the specificity of CK activity for muscle injury is high, sensitivity is fairly low, likely related to its short half-life.[3]

Creatine kinase exists as a dimer, composed of different combinations of two subunits designated B (brain) and M (muscle). A total of four isoenzymes have been identified. There is some variation by species, but in dogs CK-BB (CK-1) predominates in brain and organs such as spleen and kidney, CK-MB (CK-2) is mainly in intestine, lung, and spleen with a small amount in myocardium, and CK-MM (CK-3) is the major form in skeletal and cardiac muscle.[4] The fourth isoenzyme, CK-Mt, exists within mitochondria of many tissues. The reported distributions of isoenzyme activities in the blood of normal dogs vary, but CK-MM and CK-BB together are responsible for the majority of the CK activity with a small contribution from CK-MB.[3] Because of the different tissue locations, CK isoenzymes have the potential to be tissue specific. In people, increased serum CK-MB activity has historically been considered a reliable marker of myocardial injury, but has been replaced by other cardiac biomarkers in recent years (see discussion of cardiac troponins).[23] Measurement of CK isoenzyme activities requires electrophoresis or specifies-specific antibodies, and these assays are not routinely available for animals.

Creatine kinase activity can be measured in serum or plasma, but the activity is reportedly about 2.5 times greater in serum, most likely due to release of CK from platelets during clotting. In dogs, plasma CK activity is stable for a week when refrigerated and a month when frozen at −20°C. Serum CK activity has been reported to be greater in young puppies compared to adult dogs, with four times adult levels found in puppies under a month of age.[2] A falsely increased CK activity can occur as a result of hemolysis, hyperbilirubinemia, and muscle fluid contamination of the blood sample during a difficult venipuncture. Extremely high serum CK activities (>100× URL) occasionally are detected in animals with muscle injuries. These markedly increased CK activities may be greater than the linearity limits for the assay. Technicians analyzing such serum for CK activity may have difficulty reaching an endpoint in the assay because the serum CK activity continues to increase with serial dilution. Various theories have been postulated to explain this phenomenon, including that dilution of serum CK inhibitors is responsible.[8,11,26] In these cases CK activity may be reported as greater than the limit of linearity for that particular assay.

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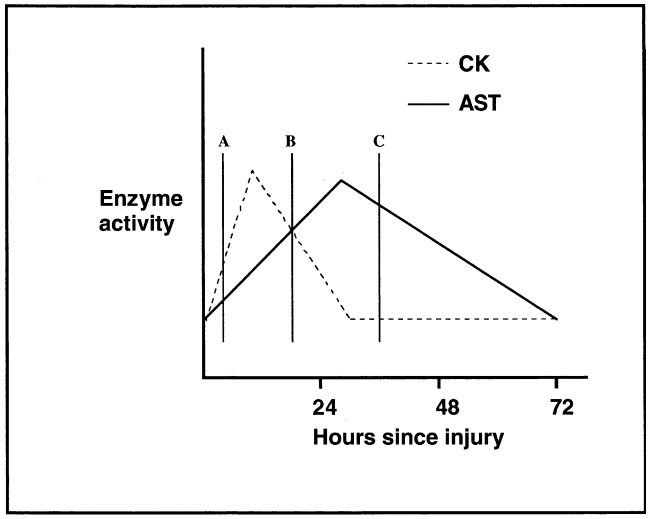

Figure 30.1 Serum activities of both AST and CK increase as a result of muscle injury, but rise and fall at different rates. Evaluation of these two enzymes together can help estimate when a muscle injury occurred and indicate whether such injury is still occurring. An increase in only the serum CK activity (line A) suggests very acute muscle injury. Increased serum activities of both AST and CK (line B) suggest active or recent muscle injury. An increase in only the serum AST activity (line C) suggests that muscle injury stopped more than 2 days earlier, and that the serum CK activity returned to normal as a result of the short half-life of CK. An increase in only the serum AST activity may also result from liver injury.

Increased serum CK activity results from:

- Skeletal muscle injury. Injury to skeletal muscle is the most common cause of increased CK activity, which may result from such minor procedures as physical restraint and intramuscular injections. Muscle necrosis and ischemia, strenuous exercise or seizures, and trauma during shipping can result in an increased serum CK activity. The underlying causes for muscle injury are numerous and varied, including trauma, toxins, exertional rhabdomyolysis, inflammatory myopathies due to bacterial, viral, or parasitic diseases, and inherited conditions such as muscular dystrophy.[3,6] So-called downer cattle will have increased CK activity due to ischemic muscle necrosis. Increased CK activity has also been reported in dogs with endocrine diseases (hypothyroidism and hyperadrenocorticism).[3] Depending on the underlying cause, the magnitude of the increase may be mild to marked and correlates somewhat with the extent of muscle injury.
- Cardiac muscle injury. Increases in CK activity can occur with injury to cardiac muscle. In dogs, this is due to increases in both CK-MM and CK-MB activities.[13] Because of the relatively small volume of cardiac muscle compared to skeletal muscle, CK activity increases with cardiac muscle injury are unlikely to reach the magnitude seen with severe injury to skeletal muscle.
- Smooth muscle injury. In theory, injury to tissues containing abundant smooth muscle could cause increased serum CK activity, but this is rarely recognized in practice. However, the bovine uterus has been shown to contain relatively high concentrations of CK, and serum CK increases have been documented in cattle with endometritis.[25]
- Muscle catabolism. Increased CK activity can occur in anorexic cats that have diseases not directly involving muscle. A median serum CK activity of 2529 IU/L, with some activities being greater than 10,000 IU/L (reference interval = 10–100 IU/L), has been reported in such cats.[9] Muscle catabolism to supply amino acids for protein synthesis and gluconeogenesis is theorized to result in the leakage of CK from muscle cells. The CK activity in these cats decreased rapidly after nutritional support was initiated.

Aspartate aminotransferase

Aspartate aminotransferase (AST), previously known as serum glutamic oxaloacetic transaminase (SGOT), is present at highest concentrations in hepatocytes as well as in skeletal and cardiac muscle cells.[4] Aspartate aminotransferase is present in both the cytoplasm and mitochondria of these cells.[12] Serum AST activity increases not only from muscle injury but also hepatocyte injury, and may be mildly increased in dogs due to drug induction (see Chapter 26). Serum AST activity increases more slowly than serum CK activity after muscle injury (Fig. 30.1). It peaks at approximately 24–36 hours after acute muscle injury, and it decreases more slowly than serum CK activity after the muscle injury ceases. The half-life of AST in the blood has been estimated between 4 and 12 hours in dogs, 77 minutes in cats, and 7 to 8 days in horses.[4,7,31]

The relative serum activities of both CK and AST can be used to estimate when muscle injury occurred and whether active muscle injury is ongoing (Fig. 30.1). An increase in only the serum CK activity (Fig. 30.1, line A) suggests very acute muscle injury (i.e., there has not been sufficient time since the injury occurred for the serum AST activity to increase). Increased serum activities of both AST and CK (Fig. 30.1, line B) suggest active or recent muscle injury. An increase in only the serum AST activity (Fig. 30.1, line C) suggests that muscle injury stopped more than 2 days earlier, and that the serum CK activity returned to normal as a result of the short half-life of CK. This latter combination of results also can occur with liver injury (i.e., if liver is the source of the AST, the CK activity would be normal).

Alanine aminotransferase

Alanine aminotransferase (ALT), previously known as serum glutamic pyruvic transaminase (SGPT), is a leakage enzyme that is free in the cytoplasm. This enzyme is primarily used to detect hepatocyte injury (see Chapter 26), but it is not

totally liver specific.[29] The ALT activities in skeletal and cardiac muscles are approximately 5% and 25%, respectively, of the liver ALT activity.[4] Muscle should be considered as a potential source of increased serum ALT activity, because the total mass of muscle is much greater than that of liver. Measuring the serum activity of an enzyme with greater muscle specificity (CK) is preferable for detecting muscle damage.

Lactate dehydrogenase

Lactate dehydrogenase (LDH) is located in the cytoplasm of most cells in the body.[4] Injury to most tissues results in leakage of LDH into the extracellular space and the blood; therefore, LDH is a very nonspecific enzyme.

Lactate dehydrogenase isoenzymes

Five LDH isoenzymes exist, which can be identified by electrophoretic separation. Each isoenzyme is present in a limited number of tissues and, therefore, is more tissue specific than the serum total LDH activity.[15] Lactate dehydrogenase molecules are composed of four components, which are either muscle (M) or heart (H) subunits. The five isoenzymes are LDH_1 (H_4), LDH_2 (MH_3), LDH_3 (M_2H_2), LDH_4 (M_3H), and LDH_5 (M_4). The designations H_4, MH_3, and so on refer to the number of each subunit (M or H) in the LDH isoenzyme molecule. Although there is considerable species variation, generally the LDH_1 (H_4) isoenzyme predominates in cardiac muscle and the LDH_5 (M_4) isoenzyme predominates in skeletal muscle.[4] The remaining three isoenzymes are found in variable quantities in several different tissues. Although measurement of specific isoenzyme activities may provide information about skeletal versus cardiac muscle injury, isoenzyme assays are not routinely performed by most veterinary laboratories and more specific serum markers are now available for evaluating myocardial injury (see discussion of cardiac troponins).

Cardiac troponins

Troponins are structural proteins of striated muscle. In people, antibody assays developed against cardiac troponins I (cTnI) and T (cTnT) have identified that these proteins are released from injured cardiac muscle and enter the peripheral blood.[24] Assays for cTnI and cTnT have largely replaced assays for CK-MB activity as markers for myocardial injury in people, and are being investigated for similar purposes in animals.[23,27] Because the cardiac troponins are well-conserved between species, immunoassays designed for use in people have been used to detect these proteins in plasma from a variety of species including dogs, cats, horses, and cattle.[16,20,21,27] However, there are multiple commercial immunoassays available for assaying these proteins, and not all have been used successfully in animals.[19,32] Additionally, values obtained with one type of assay may not be directly comparable to values obtained with another.[1] Although regarded as highly sensitive and specific markers for cardiac disease in people, how cardiac troponin concentrations will correlate with specific diseases in animals is the subject of ongoing investigations. Several studies have shown that cTnI concentrations are greater in dogs with acquired cardiac diseases compared to normal healthy dogs; conditions included mitral valve disease, dilated cardiomyopathy, and acute myocardial damage secondary to gastric dilatation-volvulus or blunt chest trauma.[5,18,28] However, cTnI concentrations were also increased in cases of renal failure, noncardiac systemic disease, and noncardiac dyspnea.[10,22] Recently, greyhound dogs were shown to have cTnI concentrations significantly greater than those of non-greyhound control dogs.[14] The specificity of cardiac troponins for cardiac injury in animals will depend on development of appropriate decision limits for the particular assay being used.

Myoglobinemia and myoglobinuria

Myoglobin is released from dead or dying muscle cells into the blood as a result of severe, usually acute muscle injury.[30] Because myoglobin has a low molecular weight (MW = 17,000) and is not significantly bound to proteins in the blood, it quickly passes through the glomerulus and is excreted in the urine.[17] The urine will be brown to red-brown if the urinary myoglobin concentration is high enough (Fig. 30.2). Myoglobin is detected as a positive reaction on the urine dipstick test for blood or hemoglobin because of its peroxidase activity. Therefore, myoglobinuria must be differentiated from hemoglobinuria (see Chapter 23

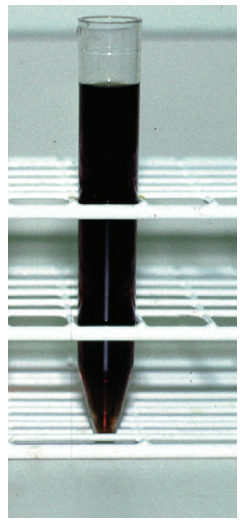

Figure 30.2 Urine from a horse with exertional rhabdomyolysis and myoglobinuria. High concentrations of myoglobin result in brown to red-brown urine.

for further discussion of red urine). This differentiation can be aided by observing the packed cell volume and color of the serum. Hemoglobin released into the plasma because of hemolysis is quickly bound to a carrier protein, haptoglobin. Hemoglobin–haptoglobin complexes are large and do not readily pass through the glomerulus. If haptoglobin becomes saturated with hemoglobin, free hemoglobin dimers (MW = 32,000) in plasma are cleared by the kidney, resulting in red urine.[17] Since hemoglobin tends to be retained in the plasma after hemolysis, it imparts a red color to the plasma and serum. Myoglobin, however, is readily excreted by the kidneys, and does not typically cause a color change in the serum. Colorless to yellow serum in animals with evidence of muscle injury (increased CK or AST activity) and a positive reaction for hemoglobin on a urine dipstick test suggests myoglobinuria; red serum in an anemic animal is suggestive of hemolysis and hemoglobinuria.

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31 Laboratory Evaluation of Lipids

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Lipids play diverse roles in normal physiology. The most obvious use is as an energy source that can be stored as triglycerides within adipocytes during times of nutritional plenty and mobilized when needed. Lipid stores within brown fat cells can be rapidly oxidized via uncoupling protein pathways to provide heat (thermogenesis). Fat pads provide thermal insulation and act as shock absorbers. Lipids function as structural components of cell membranes and organelles, as mediators of intracellular signal transduction pathways, as a constituent of surfactant in the lung, and as electrical insulators (myelin in the nervous system). Cholesterol is an important component in cellular membranes of animals and is the precursor for the synthesis of steroid hormones, vitamin D, and bile acids. Recent studies have suggested a role for cell membrane cholesterol in innate immunity and in the pathogenesis of some infectious agents through regulation of microbial entry, intracellular survival, and exit.[1] Volatile fatty acids (propionate, acetate, and butyrate) are major products of rumen microbial fermentation of carbohydrates and play a significant role in ruminant energy metabolism. While often thought of as indicators of metabolic disturbances, ketones are normally present at low levels in the circulation and are an important energy source during times of negative energy balance.

A variety of lipids are present in the circulation. Alterations in their concentrations reflect energy balance and metabolic disturbances. Lipid abnormalities can contribute to development of serious clinical syndromes such as insulin resistance, hepatic lipidosis, and atherosclerosis. Measurement of different types of lipids is predicated on available test methodologies as well as clinical relevance. Circulating lipids of clinical interest that can be readily assessed include triglycerides, cholesterol, nonesterified fatty acids (NEFA), lipoproteins, and ketone bodies. Prior to discussion of laboratory evaluation and diagnosis of lipid abnormalities, a brief overview of lipid metabolism is presented. More detailed discussions lipid chemistry and metabolism are available elsewhere.[2,3]

Dietary absorption of lipids

Lipids may be obtained from the diet or by synthesis. Dietary fat entering the small intestine stimulates release of cholecystokinin. Cholecystokinin causes the gall bladder to contract, releasing bile into the intestinal lumen. Bile salts and lecithin in the bile emulsify dietary fat to form micelles that consist of fatty acids, triglycerides, cholesterol, and the fat-soluble vitamins A, D, E, and K. Cholecystokinin also stimulates the exocrine pancreas to secrete lipases which interact with the micelle and break down the lipid into forms that may be absorbed by the intestinal enterocytes. Pancreatic lipase and colipase are responsible for hydrolysis of triglyceride to two fatty acids and a monoglyceride. Dietary cholesterol esters are hydrolyzed by cholesterol esterase to release cholesterol and a fatty acid. Long chain fatty acids (LCFA, fatty acids having more than 12 carbons), monoglycerides, cholesterol, and fat-soluble vitamins diffuse from the micelle across the brush border into the enterocyte, leaving the bile salts within the intestinal lumen (Fig. 31.1). Short and medium chain fatty acids having fewer than 12 carbons may be absorbed without the need of micellar emulsification and may be transferred from the enterocyte directly to the portal blood. LCFA must be re-esterified to triglycerides and packaged into lipoproteins called chylomicrons for transport in the lacteals and blood.

Maldigestion and malabsorption of dietary fats can result in steatorrhea and deficiency of essential fatty acids and fat soluble vitamins. If pancreatic exocrine deficiency results in inadequate release of lipases into the intestine, maldigestion will result in high concentrations of triglycerides (also called neutral fats) in the feces. If the stool contains increased fatty

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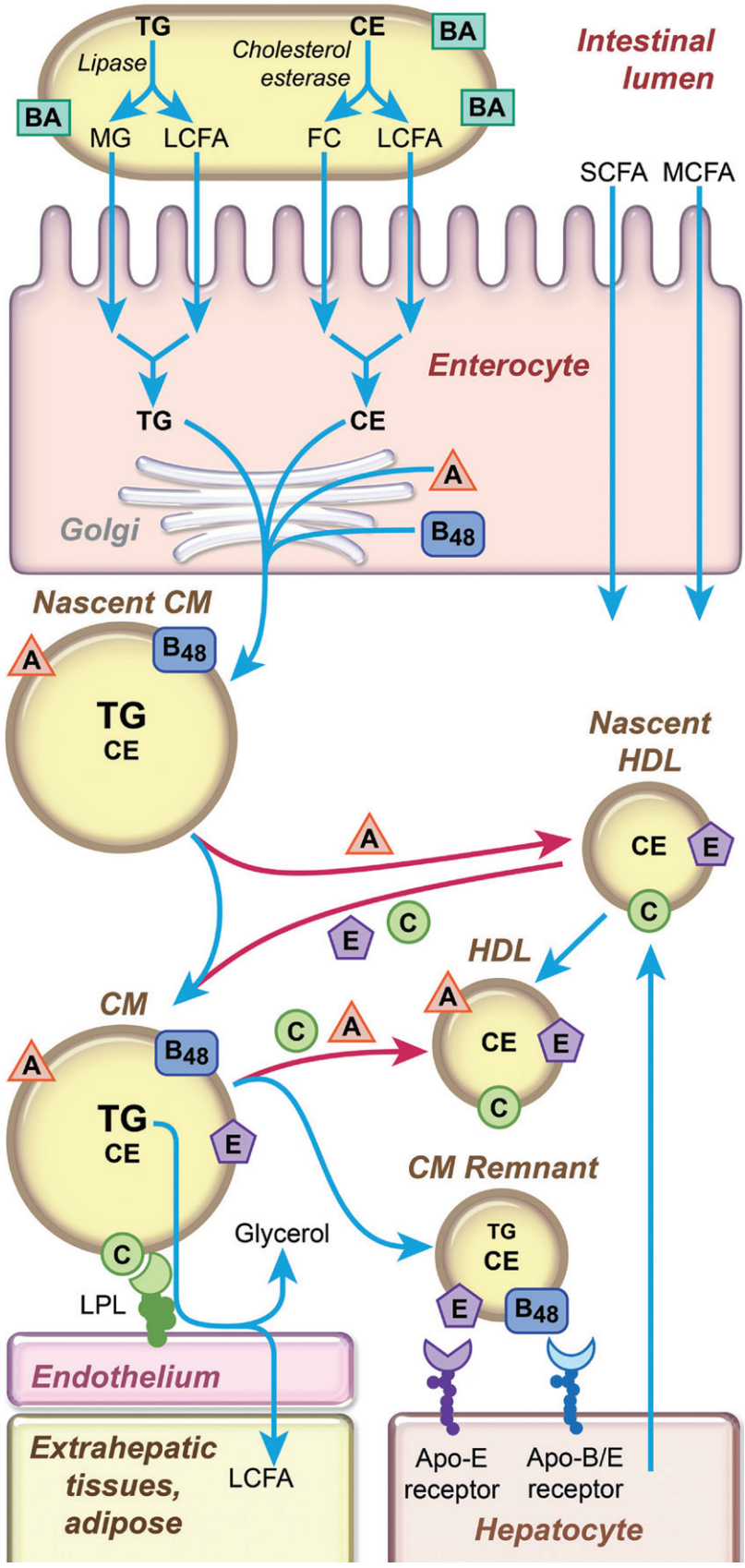


Figure 31.1 Dietary lipids in the intestinal lumen are solubilized by emulsification with bile acids (BA) to form micelles. Pancreatic lipases associate with the micelles and hydrolyze triglycerides (TG) to monoglycerides (MG) and long chain fatty acids (LCFA). Cholesterol esters (CE) are hydrolyzed by cholesterol esterase to free cholesterol (FC) and a LCFA. Short chain fatty acids (SCFA) and medium chain fatty acids (MCFA) do not require micellar emulsification for intestinal absorption. Following absorption by the enterocyte, MG, FC and LCFA are re-esterified and assembled along with apoprotein-A and apoprotein-B48 into nascent chylomicrons (CM). In the blood, CM exchange apoproteins-A, -C, and -E with high density lipoproteins (HDL). TG in the CM are hydrolyzed to LCFA and glycerol by lipoprotein lipase (LPL) on the surface of the endothelial cells. This process requires Apoprotein-C. LCFA may be used by adipocytes to form TG while glycerol is released into the blood for use by the liver or other extrahepatic tissues. Apoprotein-A and apoprotein-C from CM remnants are transferred back to HDL. Removal of the cholesterol-enriched CM remnant from the blood is mediated by Apo-B/E and Apo-E receptors on hepatocytes.

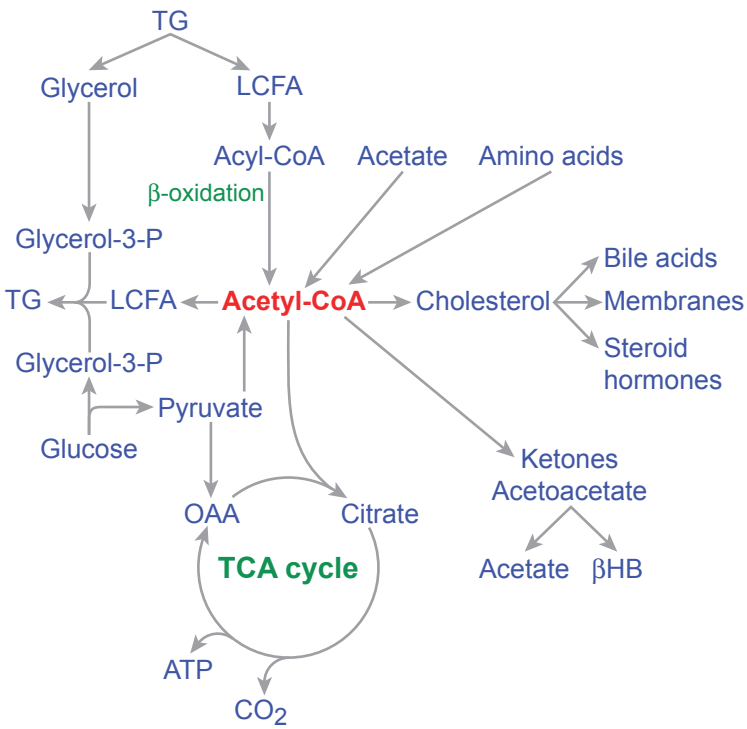


Figure 31.2 Acetyl-coenzyme A (Acetyl-CoA) is central to both catabolism and synthesis of long chain fatty acids (LCFA) in the hepatocyte. LCFA derived from lipolysis are converted to acyl-CoA which undergoes β-oxidation to acetyl-CoA. Other sources of acetyl-CoA include acetate, amino acids, and glucose. Acetyl-CoA may be used in the synthesis of LFCA or cholesterol. TG are synthesized by esterification of LCFA to glycerol-3-phosphate (glycerol-3-P) derived from either lipolysis or glucose metabolism. A major route for utilization of Acetyl-CoA is for energy production through the tricarboxylic acid (TCA) cycle. This process requires combination of acetyl-CoA with oxaloacetate (OAA) to form citrate. OAA is obtained from glucose metabolism. If there is excess acetyl-CoA and/or insufficient OAA, acetyl-CoA may be shunted into ketogenesis to form acetoacetate. Acetoacetate is then converted to acetone and β-hydroxybutyrate (βHB). This last pathway is stimulated in conditions of negative energy balance characterized by excess lipolysis and undersupply of glucose.

acids and glycerol (referred to as split fats), this implies that sufficient lipase is present to hydrolyze triglyceride and that malabsorption of fats is occurring.

Lipids present in the blood

Fatty acids

Unesterified fatty acids are referred to as free fatty acids or nonesterified fatty acids (NEFA). Most mammalian fatty acids contain more than 12 carbon atoms and are referred to as long chain fatty acids (LCFA). Fatty acids may be obtained from the diet or may be synthesized. Because they are hydrophobic, LCFA must be attached to plasma proteins, primarily albumin, for transport in the blood.

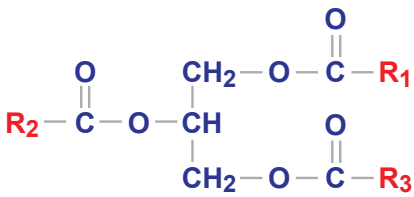
In nonruminants, biosynthesis of fatty acids occurs at the highest rates in liver, with lesser amounts produced in adipose tissue and mammary glands. In ruminants, adipocytes are the primary site for fatty acid formation, and the liver plays a less important role in fatty acid synthesis. During lactation, the mammary gland is a major synthetic site. Other tissues are capable of producing fatty acids but at much lower rates. Fatty acids are synthesized from acetyl-coenzyme A (acetyl-CoA) (Fig. 31.2). Glucose is the main precursor for acetyl-CoA in nonruminants, while acetate serves this function in ruminants. Amino acids also can be used as precursors for formation of acetyl-CoA. Synthesis of fatty acids is stimulated by insulin and inhibited by glucagon and epinephrine via modulation of activity of acetyl-CoA carboxylase, the rate limiting enzyme of fatty acid synthesis. As a result, the rate of fatty acid synthesis is responsive to diet and metabolic state. For example, fatty acid synthesis is stimulated by high carbohydrate/low fat diets (high insulin and availability of glucose as a precursor). Fatty acid synthesis is decreased by fasting (low insulin, high glucagon), high fat/low carbohydrate diets (increased availability of preformed LCFA), and diabetes mellitus.

Fatty acids are an important energy source for peripheral tissues such as skeletal muscle and are oxidized back to acetyl-CoA as part of this process. Acetyl-CoA produced by mitochondrial β-oxidation of LCFA may be used to generate ATP and CO_2 via the tricarboxylic acid (TCA) cycle (Fig.

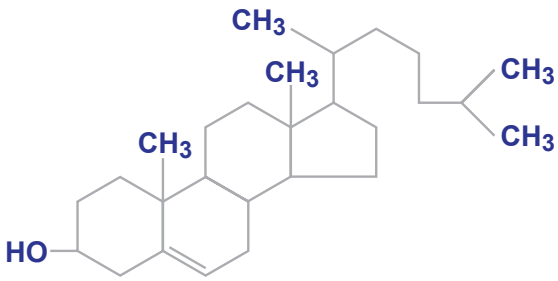
31.2). This process initially requires conversion of acetyl-CoA and oxaloacetate to citrate. If there is inadequate oxaloacetate, such as may occur with a low carbohydrate diet or diabetes mellitus, acetyl-CoA may be directed into ketogenesis. Acetyl-CoA also may be used for production of cholesterol.

Triglycerides

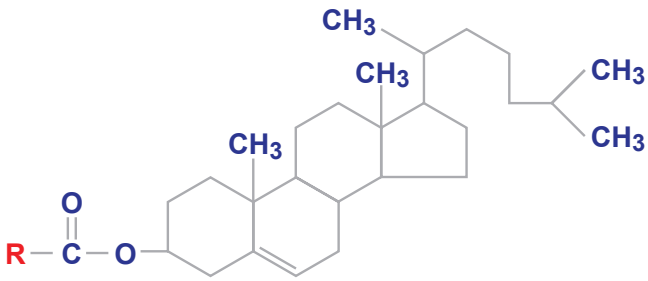
Triglycerides are formed by esterification of three LCFA to glycerol-3-phosphate (Fig. 31.3). Triglyceride synthesis occurs in the intestinal mucosal cells, adipocytes, hepatocytes, mammary epithelial cells, and kidneys. Within the intestinal mucosal cell, dietary fatty acids and monoglycerides are re-esterified to form triglycerides. Triglycerides also may be produced by adding dietary LCFA to glycerol-3-phosphate that is newly synthesized from glucose by the enterocyte. Control of triglyceride synthesis by the enterocytes is largely dependent on dietary availability of fatty acids. Once formed, triglycerides are not stored to any great extent in enterocytes but are packaged into chylomicrons and released into the lacteals.



Triglyceride



Cholesterol



Cholesterol Ester

Figure 31.3 The structure of triglycerides, cholesterol, and cholesterol esters. R represents the carbon chain of a long chain fatty acid. The hydroxyl group on cholesterol confers some water solubility, allowing free cholesterol to be part of the outer shell of lipoproteins. Cholesterol esters are hydrophobic and contained within the center of lipoprotein particles.

Hepatocytes use LCFA obtained from the plasma or from *de novo* synthesis for production of triglycerides. Glycerol may be taken up from the plasma or may be synthesized from glucose. Synthesis of triglycerides by hepatocytes is decreased in conditions with high glucagon and low insulin (fasting, diabetes mellitus) and is stimulated by increased availability of LCFA. Under normal circumstances, triglycerides are released from the hepatocytes into the circulation as a component of lipoproteins called very low density lipoproteins (VLDL).

Adipocytes may synthesize LCFA or may obtain them via lipolysis of blood triglycerides present in chylomicrons or VLDL. The enzyme responsible for hydrolysis of chylomicron or VLDL triglycerides is lipoprotein lipase which is located on the surface of capillary endothelial cells. Unlike hepatocytes, adipocytes lack the enzymes to use glycerol derived from lipolysis. Triglyceride synthesis by adipocytes depends on *de novo* production of glycerol-3-phosphate from glucose or gluconeogenesis. Insulin is an important regulator of adipocyte triglyceride synthesis through stimulation of activity of lipoprotein lipase (Table 31.1). Insulin also enhances glucose uptake by increasing membrane expression of the GLUT4 glucose transporter, thus increasing intracellular availability of glucose for glycerol-3-phosphate synthesis. Once triglycerides are formed, they are stored for future use as fat droplets in adipocytes.

Mobilization of triglycerides stored in adipocytes is mediated by the enzyme, hormone sensitive lipase (HSL). Hydrolysis of triglycerides results in the release of LCFA and glycerol to the blood for transport to tissues. A variety of hormones directly and indirectly affect lipolysis through modulation of the activity of HSL (Table 31.1). Catecholamines rapidly activate HSL by promoting phosphorylation of the enzyme. This permits a rapid increase in lipolysis to supply fatty acids for energy production. Thyroid hormone acts synergistically with catecholamines by increasing the number of receptors for catecholamines on adipocytes. Glucocorticoids facilitate lipolysis by increasing gene transcription and synthesis of HSL. Insulin and insulin-like growth factor inactivates HSL by promoting dephosphorylation of the enzyme. Insulin also opposes the effect of glucocorticoids on HSL gene transcription.

Cholesterol

Cholesterol may be in the form of free cholesterol or may be esterifed with a fatty acid to form a cholesterol ester (Fig. 31.3). Because it is not synthesized by plants or microbes, only carnivores or omnivores may obtain cholesterol from

Table 31.1 Effect of hormones on key regulatory steps in lipid metabolism.

	LPL Hydrolysis of triglycerides from chylomicrons and VLDL	**HSL** Lipolysis of triglycerides to LCFA and glycerol	**Acetyl CoA Carboxylase** Rate limiting enzyme in the synthesis of fatty acids	**HMG CoA Reductase** Rate limiting enzyme in the synthesis of cholesterol	**LDL receptors** Clearance of LDL from the blood
Insulin	↑ enzyme activity; ↑ synthesis of the enzyme; ↑ translocation of the enzyme to the endothelium	↓ enzyme activity; ↓ cortisol-induced gene transcription	↑ enzyme activity	↑ activity	↑ synthesis of the receptor
Glucagon		↑ activity	↓ enzyme activity	↓ activity	
Cortisol		↑ gene transcription and synthesis of enzyme		↓ gene transcription and synthesis of the enzyme	↓ synthesis of receptor due to secondary decrease in thyroid hormones
Thyroid hormones		↑ synthesis of adrenergic receptors which increases the effect of catecholamines		↑ synthesis of the enzyme	↑ synthesis of the receptor
Catecholamines		↑ enzyme activity	↓ enzyme activity		
Growth hormone		↑ enzyme activity			↓ synthesis of receptor due to secondary decrease in thyroid hormones

CoA, coenzyme A; HMG CoA, 3-hydroxy-3-methylglutaryl coenzyme A; HSL, hormone sensitive lipase; LDL, low density lipoproteins; LPL, lipoprotein lipase; VLDL, very low density lipoproteins.

the diet. Herbivores must synthesize their own cholesterol. The primary site for cholesterol synthesis is the liver, and the rate limiting enzyme is 3-hydroxy-3-methylglutaryl-CoA (HMG-CoA) reductase. Several hormones modulate HMG-CoA reductase activity and consequently cholesterol synthesis (Table 31.1). HMG-CoA reductase activity is increased by insulin and decreased by glucagon. Thus, cholesterol synthesis increases following a meal (high insulin) and decreases with fasting (high glucagon, low insulin) or with diabetes mellitus. Thyroid hormone increases HMG-CoA reductase activity by increasing synthesis of the enzyme. Glucocorticoids have the opposite effect, decreasing synthesis of HMG-CoA reductase and consequently cholesterol synthesis. Statins, a class of cholesterol lowering drugs, have their effect on serum cholesterol by inhibiting HMG-CoA reductase.

Once formed, cholesterol may be utilized through several routes. The liver can export cholesterol and cholesterol esters to the blood as a constituent of lipoproteins. Cholesterol is a structural component of cell and organelle membranes and is a precursor for vitamin D synthesis. Cholesterol also is used for production of steroid and sex hormones by tissues such as the adrenal gland and gonads. Alternatively, cholesterol may be used by hepatocytes to synthesize bile acids. Bile is a major route for elimination of cholesterol from the body.

Lipid transport in the blood

Because lipids are largely immiscible in water, transport in the blood must be accomplished by binding to carrier proteins. LCFA bind to albumin, while triglycerides, cholesterol, cholesterol esters, and phospholipids are transported by lipoproteins. Lipoproteins consist of a shell of apoproteins, cholesterol, and phospholipids oriented so that amphoteric portions of the molecules are on the outside, facing the aqueous environment of the blood. The hydrophobic ends

are oriented toward the center of the particle. Triglycerides and cholesterol esters constitute the hydrophobic core of lipoprotein particles. Lipoproteins are traditionally named based on their density as determined by ultracentrifugation and are further characterized by lipid and apoprotein constituents. Apoproteins may be integrated into the shell of the lipoprotein or be more loosely associated with the surface of the lipoprotein. Integrated apoproteins include apoprotein-B48 (Apo-B48) of intestinal origin and apoprotein-B100 (Apo-B100) of hepatic origin. Peripheral apoproteins such as apoprotein-A (Apo-A), apoprotein-C (Apo-C), and apoprotein-E (Apo-E) are exchanged between lipoproteins in circulation (Figs. 31.1 and 31.4).

Enterocytes package fat from the diet into lipoproteins called chylomicrons which are released into the lacteals with eventual delivery into the blood. Chylomicrons contain mostly triglycerides with lesser amounts of cholesterol, cholesterol ester, fat soluble vitamins, Apo-B48, and Apo-A. In the circulation, Apo-C and Apo-E are transferred from high density lipoproteins (HDL) to the chylomicron. Chylomicrons are the largest and least dense of the lipoproteins. When present in large quantity, they confer a visible haziness to the serum, contributing to the appearance of lipemia. Apo-C is a co-factor for lipoprotein lipase which is found on the surface of endothelial cells within tissue beds such as adipose and muscle. Lipoprotein lipase is synthesized by extravascular tissues and transferred to the surface of endothelial cells where it is anchored by heparan sulfate. Injection of heparin can cause release of lipoprotein lipase into the circulation and this technique has been used to clear serum of lipemia. Lipoprotein lipase hydrolyzes triglycerides to LCFA and glycerol for use by extrahepatic tissues. In adipose and muscle, insulin increases lipoprotein lipase activity, facilitating hydrolysis of chylomicron triglycerides and absorption of LCFA. The remaining lipoprotein, now depleted of triglycerides, is called a "chylomicron remnant" and is subsequently removed from the circulation by hepatocytes. Uptake of chylomicron remnants is mediated by binding of Apo-B48 and Apo-E on the chylomicron remnant to either Apo-E or Apo-B/E receptors on the hepatocyte.

Triglycerides synthesized by hepatocytes are packaged into very low density lipoproteins (VLDL) for transport in the blood (Fig. 31.4). VLDL contain a large quantity of triglyceride along with lesser amounts of cholesterol, cholesterol esters, and Apo-B100. Apo-C and Apo-E are obtained from HDL in the circulation. If present in high quantities, VLDL also can contribute to a lipemic appearance of the blood. Like chylomicrons, binding of VLDL to lipoprotein lipase in the tissues is facilitated by Apo-C, and triglycerides are hydrolyzed to LCFA and glycerol for utilization by extrahepatic tissues.

After the VLDL is depleted of triglycerides, the remaining lipoprotein is termed an intermediate density lipoprotein (IDL). IDL may be taken up by hepatocytes, a process mediated by binding of Apo-B and Apo-E to hepatocyte receptors. Alternatively, additional hydrolysis of triglyceride by hepatic lipase converts IDL to low density lipoproteins (LDL). As the lipoprotein loses triglyceride, Apo-C and Apo-E are transferred back to HDL while Apo-B100 is retained. The primary function of LDL is transport of cholesterol to the liver and other tissues. Removal of LDL from circulation is receptor mediated and depends on the presence of Apo-B. LDL receptor expression is stimulated by insulin and thyroxine. Most cells have receptors for LDL and can acquire cholesterol by binding LDL; however the liver plays the major role in LDL clearance.

Cholesterol transport is mediated by high density lipoproteins (HDL). Nascent HDL are synthesized by hepatocytes and contain phospholipids, a small amount of cholesterol, Apo-C, and Apo-E. As previously described, HDL provide a source of Apo-C and Apo-E for exchange with chylomicrons and VLDL. As HDL mature, Apo-A is acquired by exchange with chylomicrons. The small intestine also produces HDL that initially contain Apo-A, but lacks Apo-C and Apo-E. Intestinal HDL must pick up Apo-C and Apo-E from hepatic-origin HDL in the blood. Importantly, HDL incorporate excess cholesterol from extrahepatic tissues in a process called "reverse cholesterol transport." Cholesterol is then esterified to cholesterol esters by lecithin cholesterol acyl transferase (LCAT) in the HDL, an enzyme which requires Apo-A for activation.

There is species variation in the relative amounts of HDL and LDL (Table 31.2).[4,5] In species that typically have high HDL and low LDL in the blood, such as dogs and cats, HDL are the primary transporters of cholesterol and cholesterol esters to the liver. In low HDL/high LDL species such as humans, cholesterol esters may be transferred by cholesteryl ester transfer protein (CETP) to VLDL remnants and LDL for subsequent delivery to the liver. High LDL species are at risk for the development of atherosclerosis because macrophages are capable of scavenger receptor-mediated removal of LDL from the circulation. Accumulation of LDL cholesterol in macrophages results in subendothelial lipid deposits or atherosclerotic plaques. High cholesterol diets can favor increased concentrations of LDL and exacerbate development of atherosclerosis.

Ketones

Ketogenesis is another option available for metabolism of fatty acids by the liver. As described above, LCFA may be repackaged into triglycerides and released as VLDL. Alternatively, LCFA may undergo β-oxidation to acetyl-CoA and subsequently be used for energy production via the TCA cycle, for cholesterol synthesis, or for ketogenesis (Fig. 31.2). Under conditions of adequate nutrition, low levels of ketone bodies normally are produced by the liver. The rumen epithelium also appears to be capable of synthesizing β-hydroxybutyrate which may account for the higher

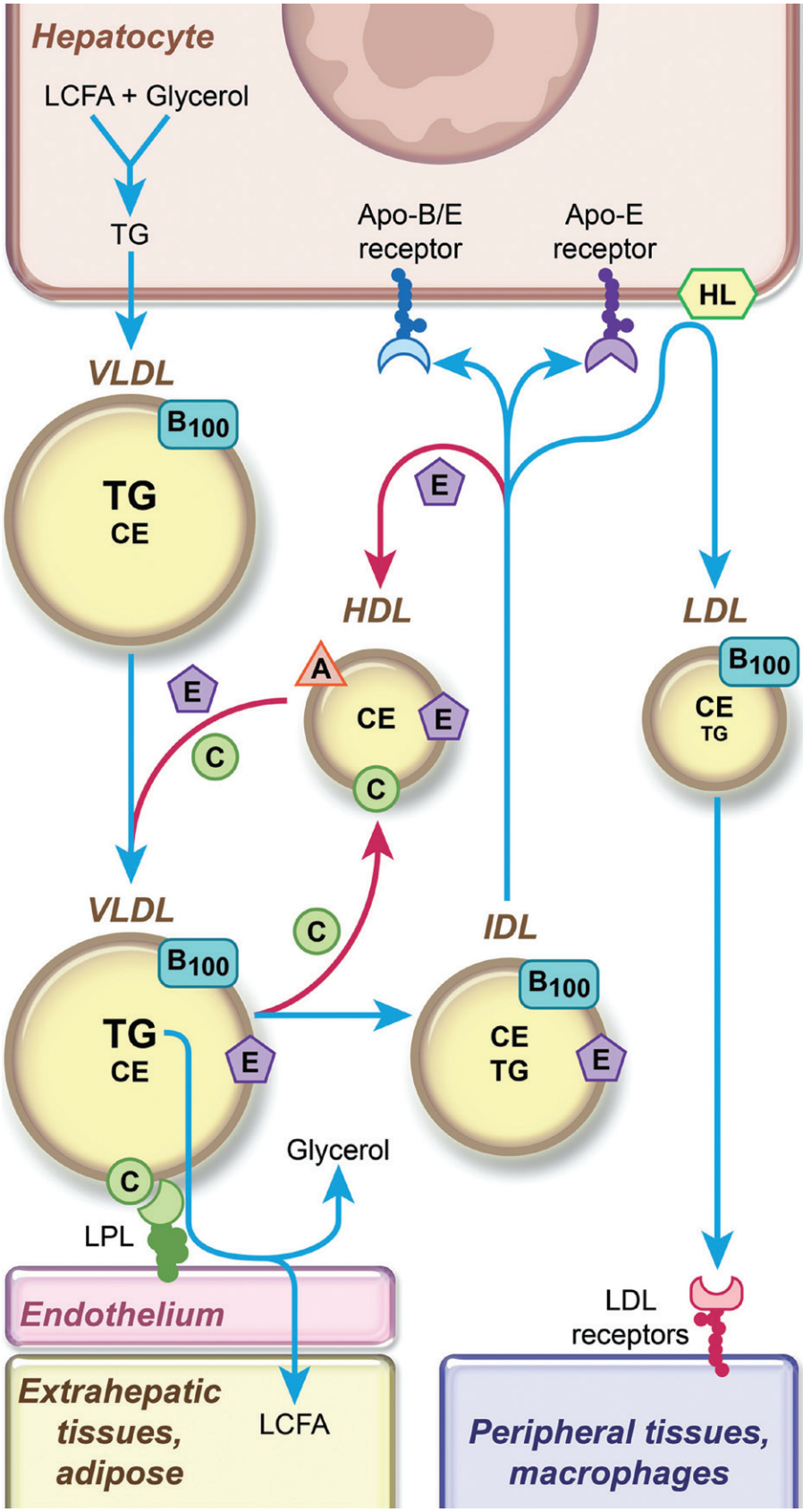


Figure 31.4 Triglycerides (TG) synthesized in hepatocytes are packaged along with cholesterol, cholesterol esters (CE), and apoprotein-B100 into very low density lipoproteins (VLDL) for transport in the blood. VLDL pick up apoprotein-C and apoprotein-E from high density lipoproteins (HDL). TG are hydrolyzed to long chain fatty acids (LCFA) and glycerol by lipoprotein lipase (LPL) on the surface of endothelial cells. LCFA may be used by adipocytes to form TG while glycerol is released into the blood for use by the liver or other extrahepatic tissues. Depletion of TG results in formation of an intermediate density lipoprotein (IDL). Apoprotein-C and apoprotein-E recirculate back to HDL. IDL may be removed from the circulation by hepatocytes, a process mediated by Apo-B/E and Apo-E receptors. Alternatively, IDL may be further depleted of TG by hepatic lipase (HL) and converted to low density lipoproteins (LDL). Uptake of LDL by peripheral tissues is mediated by binding of LDL to LDL receptors. Macrophages are also capable of removing LDL from circulation through scavenger receptors.

Table 31.2 Examples of HDL mammals and LDL mammals.[4,5] HDL mammals are defined as those having HDL as >50% of total lipoproteins while LDL mammals have >50% LDL. Age, strain, breed, and diet may affect the relative distribution of lipoproteins.

HDL Mammals	LDL Mammals
Dogs	Guinea pigs
Cats	Hamsters
Ferrets	Pigs
Horses	Camels
Cattle	Rabbits (some strains)
Sheep	Spider monkey
Mice	Humans
Rats	
Chimpanzee	
Most Old World monkeys	

HDL, high density lipoprotein; LDL, low density lipoprotein.

concentrations of ketone bodies in fed ruminants compared to fed monogastric species. The main ketone bodies are acetone, acetoacetate, and β-hydroxybutyrate. Acetyl-CoA is metabolized to acetoacetate, which subsequently is converted to acetone and β-hydroxybutyrate. These small lipids are water soluble and may be transported by the blood to other tissues for use as an energy source. Because they are not bound to albumin, ketone bodies easily enter cells and cross the blood brain barrier. Tissues such as the heart and brain readily utilize ketone bodies as an energy source.

Ketogenesis increases in conditions of negative energy balance and is a normal response to fasting. As plasma glucose falls, the concentrations of insulin decreases and glucagon increases. This stimulates lipolysis by hormone sensitive lipase, mobilizing fatty acids from triglycerides stored in adipocytes. Fatty acids delivered to the liver are converted to acetyl-CoA. At the same time, enhanced gluconeogenesis consumes the available oxaloacetate without which acetyl-CoA cannot enter into the TCA cycle. As a result, acetyl-CoA is directed to ketogenesis.

Transient ketosis may develop following intense exercise and has been seen dogs and horses. During exercise, lipolysis by hormone sensitive lipase is stimulated by catecholamines, cortisol and thyroxine, and the released fatty acids are consumed by muscle for energy production. Ketones formed during exercise are rapidly used, and circulating levels remain low to undetectable. In the postexercise period, muscle metabolism switches from oxidation of fatty acids to gluconeogenesis and glycogen synthesis to clear lactate and replenish glycogen stores. Metabolism of acetyl-CoA through the TCA cycle declines as a result of decreased availability of oxaloacetate. Excess circulating fatty acids are cleared by the liver, and the resultant acetyl-CoA may be shunted into ketone production. The degree of postexercise ketosis appears related to intensity and duration of exercise, conditioning of the athlete, and diet.

Measurement of lipids

Because of the technical challenges associated with measurement of lipids, relatively few types of lipids are measured in routine biochemistry panels. Triglycerides and cholesterol are both measured using spectrophotometric assays which are readily adapted to automated analyzers and are commonly part of biochemical profiles. Measurement of free fatty acids or NEFA has been used to assess metabolism in ruminants and camelidae. However, these assays are not as commonly available on routine biochemical profiles.

Ketones may be measured on automated analyzers or at the cage side by dry reagent methods such as dipsticks, tablets, or nitroprusside powder. Measurement of ketones in the urine using dipsticks based on the nitroprusside test is frequently used as a less invasive, semiquantitative means to evaluate for accelerated ketogenesis in diabetes mellitus in small animals or in ketosis in cattle. The disadvantage of this method is that these dipsticks are more sensitive to acetoacetate than acetone or β-hydroxybutyrate and may underestimate ketones in some stages of diabetic ketoacidosis. The presence of some drugs or compounds in the urine also may produce a false positive result. One "point of care" instrument has been evaluated for measurement of whole blood β-hydroxybutyrate in dogs and cats.[6] In cattle, test strips are available for measurement of β-hydroxybutyrate in milk which may prove more practical to obtain than urine.

Measurement of lipoproteins in veterinary species requires more sophisticated methods such as density gradient centrifugation or electrophoresis. Autoanalyzer and "point of care" methods designed to quantitate HDL-cholesterol using precipitation and calculation techniques in humans have not been validated in veterinary species. As a result, lipoprotein analyses are not routinely measured in veterinary medicine and require shipment of samples to specialized reference laboratories.

The term "hyperlipidemia" refers to increased circulating lipids. This may be due to hypertriglyceridemia and/or hypercholesterolemia. Lipemia or hyperlipemia refers to a visible haziness to overt latescence of the serum or plasma. Lipemia is caused by increased triglycerides in chylomicrons and/or VLDL. Hypercholesterolemia without concurrent hypertriglyceridemia will not cause a sample to appear lipemic. The refrigeration test is a simple means to distinguish between chylomicrons and VLDL as the cause of

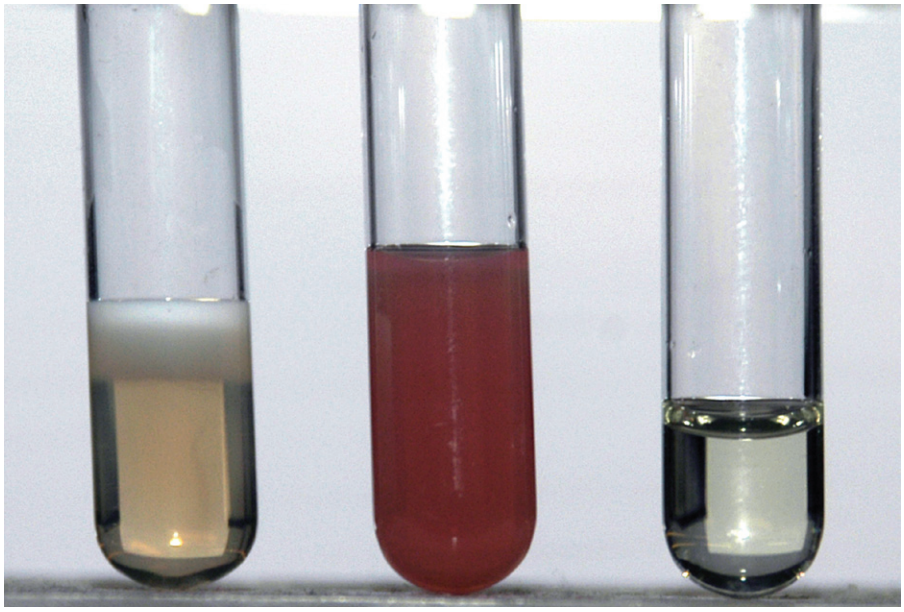


Figure 31.5 Lipemia is characterized by haziness to overt lactescence of a serum sample due to increased triglycerides in the form of chylomicrons and/or very low density lipoproteins (VLDL). Formation of a cream-like layer on the top of a sample indicates the presence of chylomicrons (left). Failure of a sample to separate indicates the presence of VLDL (middle). Normal samples are clear (right).

lipemia (Fig. 31.5). To perform this test, a sample is left upright in a refrigerator overnight. Chylomicrons will float to the top of the sample and form a milky or cream-like layer at the top of the sample. If the underlying serum clears, then chylomicrons are the cause of the lipemia. On the other hand, if VLDL are the cause of the lipemia, VLDL will not separate out into a cream layer, and the sample will remain hazy to turbid. If the lipemia is due to increases in both chylomicrons and VLDL, a cream layer will form over a sample that remains turbid.

Clinically relevant changes in serum lipids include hypertriglyceridemia, hypercholesterolemia, hyperketonemia, and hypocholesterolemia. Hypotriglyceridemia is of uncertain clinical relevance and may be most indicative of nutritional state.

Hyperlipidemias

Postprandial hyperlipidemia

Postprandial hyperlipidemia is due to a transient increase in triglycerides in the form of chylomicrons. Hyperlipidemia becomes apparent within 1–2 hours of consuming a meal that contains fat and usually peaks by 6–8 hours. Because postprandial hyperlipidemia is primarily due to increases in triglycerides, blood samples may appear hazy to grossly lipemic. For dogs and cats, fasting for 12 hours should allow sufficient time for clearance of the hyperlipidemia. Because of continuous rumenal digestion and diet composition, ruminants do not show significant postprandial effects and so need not be fasted prior to sampling.

Persistence of hyperlipidemia after a 12-hour fast in dogs and cats suggests an alternative pathogenesis for the hyperlipidemia. In monogastric animals, consumption of a high fat diet may contribute to higher fasting and postprandial serum lipids compared to normal or low fat diets. The time it takes for serum lipids to decrease to fasting levels may be prolonged following a high fat meal. As seen in Table 31.1, many key regulatory steps in lipid metabolism are influenced by hormones. It is not surprising that pathologic hyperlipidemia is commonly due to secondary causes such as hormonal or metabolic disturbances. Primary or idiopathic hyperlipidemias are rare and likely have a genetic basis.

Secondary pathologic hyperlipidemias

Secondary pathologic hyperlipidemias are caused by a variety of diseases (Fig. 31.6). While described separately below, it is important to realize that there may be overlapping effects promoting abnormalities in lipid metabolism in any given patient. For example, pancreatitis may be complicated by diabetes mellitus due to damage to pancreatic parenchyma. Diabetes mellitus commonly occurs in conjunction with hyperadrenocorticism in cats as a result of corticosteroid-related insulin resistance. Many of these conditions also have an inflammatory component resulting in increased local and systemic release of pro-inflammatory cytokines capable of modulating lipid metabolism.

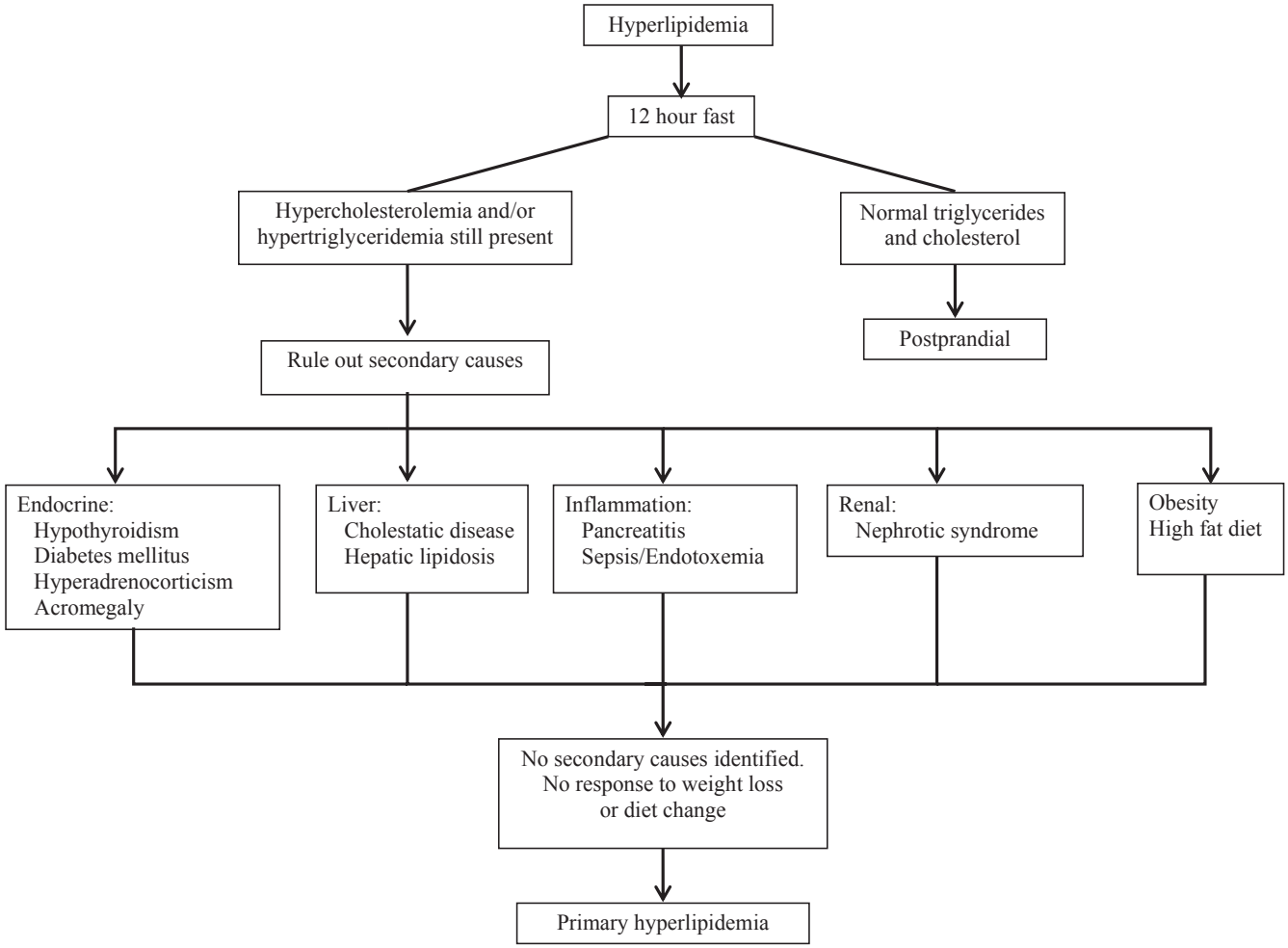


Figure 31.6 Approach to the hyperlipidemic patient.

Hypothyroidism

Hypothyroidism in dogs is a common endocrinopathy and is frequently accompanied by hypercholesterolemia due to increases in LDL and HDL.[7–10] Hypertriglyceridemia also is common and is due to increased VLDL and sometimes chylomicrons. In contrast to dogs, spontaneously occurring hypothyroidism is rare in cats but may develop as a sequella to treatment for hyperthyroidism. Congenital hypothyroidism has been described in kittens.[11] Hypercholesterolemia may be a feature of both spontaneous and iatrogenic feline hypothyroidism.[11,12] In adult horses, the actual incidence of naturally occurring hypothyroidism is uncertain. Cases initially diagnosed as hypothyroidism may actually be attributable to equine metabolic syndrome or hyperadrenocorticism secondary to a tumor or dysfunction of the pituitary pars intermedia.[13] Experimental ablation of the thyroid glands of adult horses will result in hypercholesterolemia and hypertriglyceridemia characterized by increased LDL and VLDL, respectively.[14]

In humans with hypothyroidism, decreased LDL receptor synthesis and activity contributes to the hypercholesterolemia by impairing LDL clearance.[15] Biliary excretion of cholesterol also is decreased. Decreased lipoprotein lipase activity delays clearance of triglycerides from both VLDL and chylomicrons, while decreased hepatic lipase activity slows clearance of cholesterol-enriched chylomicron remnants. Similar mechanisms have not been thoroughly examined in domestic species.

Hyperadrenocorticism

Increased serum concentrations of triglycerides and cholesterol may be observed in dogs with Cushing's disease.[10,16–19] Hypercholesterolemia also is reported in cats with hyperadrenocorticism.[20–21] As a consequence of cortisol-induced insulin resistance, hyperadrenocorticism, especially in cats, is often complicated by concurrent diabetes mellitus which exerts additional effects on lipid metabolism.[22] Horses with a tumor of the pars intermedia may develop pituitary-

dependent hyperadrenocorticism. Stimulation of lipolysis in these horses results in elevated circulating NEFA as well as increased ketogenesis.[23] Reports vary on the incidence of hypertriglyceridemia in horses with pars intermedia tumors and hyperadrenocorticism.[23–25]

A combination of direct effects of glucocorticoids and indirect effects due to steroid-induced insulin resistance contribute to the alterations in lipid metabolism in patients with hyperadrenocorticism. Hypercholesterolemia results from impaired clearance of LDL along with decreased catabolism of cholesterol secondary to steroid-induced hepatopathy and cholestasis. Decreased clearance of VLDL secondary to decreased activity of lipoprotein lipase is a factor in the development of hypertriglyceridemia. At the same time, synthesis of VLDL by hepatocytes is increased.

Diabetes mellitus

Poorly controlled diabetes mellitus due to insulin deficiency (type 1 or insulin dependent diabetes mellitus) is associated with hypertriglyceridemia, increased serum LCFA, and hypercholesterolemia in dogs.[7,26,27] Type 1 diabetes mellitus is rare in the horse but may be accompanied by hypertriglyceridemia.[28] Insulin is required for synthesis and activity of lipoprotein lipase, so insulin deficiency results in failure to clear triglycerides from chylomicrons and VLDL. In addition, circulating LCFA are increased due to a combination of increased lipolysis and decreased triglyceride synthesis by adipocytes. A lack of insulin results in increased activity of hormone sensitive lipase in adipocytes and subsequent hydrolysis of stored triglycerides and release of LCFA into the circulation. Because adipocytes require glucose for synthesis of glycerol-1-phosphate, impaired insulin-mediated glucose uptake by adipocytes results in decreased availability of glycerol-1-phosphate for esterification of LCFA to form triglycerides. LCFA are released into the blood and are subsequently taken up by hepatocytes, converted to triglycerides, and released as VLDL. If the concentration of LCFA exceeds the ability of hepatocytes to produce and release VLDL or to consume acetyl-CoA through the TCA cycle, acetyl-CoA generated from LCFA may be shunted into synthesis of ketone bodies, contributing to the development of ketoacidosis.

Insulin stimulates production of the LDL receptors so hypercholesterolemia appears to be primarily the result of a decrease in receptor-mediated uptake of LDL. Increased intestinal synthesis of cholesterol also appears to play a role in the genesis of the hypercholesterolemia in the dog.

Obesity, insulin resistance, and metabolic syndrome

Insulin resistance may vary from mild to overt type 2 diabetes mellitus. The pathogenesis of insulin resistance is complex and instigating causes are varied. Conditions associated with insulin resistance include obesity, hyperadrenocorticism, and hypersomatotropism. As a result, the effects of impaired response to insulin are superimposed on the disturbances in lipid profile caused by the original disorder. Type 2 diabetes mellitus is the most common form of diabetes mellitus in cats and horses.

Obesity is a frequently encountered problem in dogs, cats, and horses. Lipid profiles of obese individuals can exhibit a spectrum from normal to marked increases in triglycerides along with variable increases in cholesterol. Plasma NEFA often are increased. The variation observed in lipid profiles in obesity likely relates to the site of excess fat deposition, duration of obesity, and dysfunction of metabolic regulatory hormones such as insulin, cortisol, leptin, and adiponectin. Obesity is now recognized as a pro-inflammatory state, and fat pads are a source of inflammatory cytokines such as interleukin-6 and tumor necrosis factor-α. These inflammatory mediators can have a significant impact on adipocyte and hepatic lipid metabolism (see section below on inflammation) and can promote insulin resistance. Intra-abdominal obesity appears to have more severe metabolic consequences compared to peripheral adiposity and predisposes to the development of insulin resistance and metabolic syndrome.

In addition to increases in absolute concentrations of triglycerides and cholesterol, the composition of lipoproteins may change. Obese dogs show a pattern of increased triglycerides and cholesterol in both VLDL and HDL.[29,30] In cats, triglyceride and cholesterol content of VLDL are increased while HDL cholesterol is increased.[31,32] Horses exhibit increases in VLDL-triglyceride and HDL-cholesterol content.[33] Decreases in lipoprotein lipase activity has been documented in obese cats, suggesting that uptake of triglycerides by adipocytes and peripheral tissue beds is impaired.[31,34] LDL fractions appear unaffected and may account for the relative resistance of these species to development of atherosclerosis even with obesity-related hyperlipidemia.

Hypercholesterolemia has been observed in cats with acromegaly.[35] Acromegaly is the result of increased production of growth hormone. While not specifically explored in cats, there are several mechanisms that may explain the hypercholesterolemia secondary to hypersomatotropism. These cats have metabolic complications from significant insulin resistance and type 2 diabetes mellitus. In addition, growth hormone decreases release of thyroid stimulating hormone resulting in secondary hypothyroidism.

Hepatic lipidosis

Hepatic lipidosis or fatty liver occurs when triglycerides accumulate in hepatocytes. This syndrome may be precipitated by negative energy balance, hormonal or metabolic disturbances, hypoxia, or toxins. It results from an imbalance between hepatic uptake of fatty acids, synthesis of

triglycerides, formation of VLDL, and release of VLDL. Hepatic lipidosis may develop in association with conditions such as bovine ketosis, ovine pregnancy toxemia, fasting in obese cats and horses, and a variety of syndromes in camelids. In these syndromes, hormone sensitive lipase activity and subsequent adipocyte lipolysis is accelerated, increasing the supply of LCFA. The supply of LCFA outpaces the ability of the liver to oxidize LCFA through the TCA cycle so LFCA are re-esterified to triglycerides. Triglycerides accumulate as the ability to either produce VLDL or transport VLDL out of the hepatocyte is exceeded. Excess fatty acids also may be shunted to ketone body production, and lipidosis is often accompanied by some degree of ketosis. Grossly, the liver appears pale to yellow in color. Microscopically, variably-sized clear fat vacuoles are seen within the cytoplasm of the hepatocytes (Fig. 31.7).

Anorexia in horses, especially if they are obese to begin with, can prompt the development of hyperlipidemia and hepatic lipidosis. Ponies, miniature horses, donkeys, and mares are at increased risk, and the risk may be compounded by pregnancy or lactation.[36,37] The syndrome in equids is characterized by increased circulating NEFA, triglycerides, and, to a lesser degree, cholesterol.[38,39] However, ketonemia and ketonuria are not findings. Elevations in triglyceride concentrations may be marked and are primarily due to increased hepatic production of VLDL.[37] Triglyceride levels in anorexic horses have been shown to be inversely related to survival.[40,41]

Obese cats that become anorexic due to illness or are subjected to a rapid weight reduction are at risk for development of hepatic lipidosis as a consequence of increased lipolysis.[42] Hypertriglyceridemia occurs as a result of increased VLDL production as well as impaired peripheral utilization. Although export of VLDL from the liver is increased, this appears insufficient to prevent accumulation of triglycerides in hepatocytes. Hypercholesterolemia is a less consistent finding.

Hepatic lipidosis occurs in camelids secondary to conditions that increase fat mobilization.[43,44] The syndrome is accompanied by increased NEFA and, in some cases, ketonemia and ketonuria. Like other species, hepatic lipidosis may occur as a sequella to negative energy balance associated with pregnancy or lactation, in which case the dams will be hypoglycemic and ketonemic. In cases not associated with pregnancy and lactation, hyperglycemia may be observed due to the blunted insulin response and excessive gluconeogenesis typical of camelids. Hypertriglyceridemia may develop with severe, terminal lipidosis.

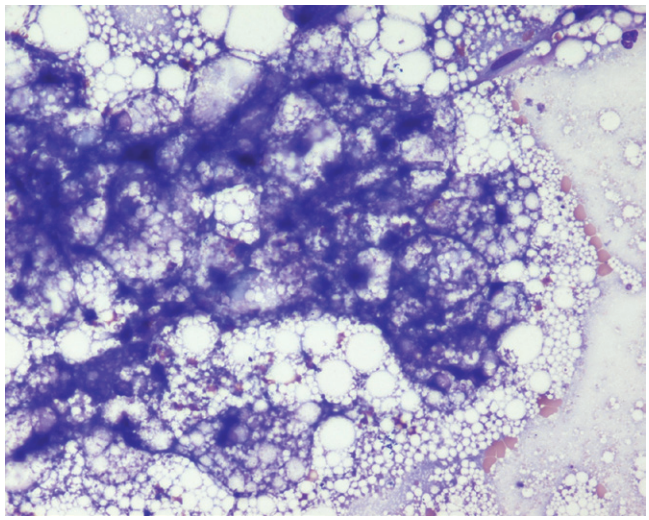


Figure 31.7 Fine needle aspirate of the liver of a cat with hepatic lipidosis. The cytoplasm of the hepatocytes contains variably-sized, clear lipid vacuoles. Lipid laden hepatocytes are often fragile and will break leaving lipid droplets evident in the background. Wright Geimsa stain, 400×.

Pancreatitis

Dogs with naturally occurring pancreatitis may have hypertriglyceridemia and hypercholesterolemia, and the serum may exhibit overt lipemia.[7,45,46] Hypercholesterolemia also is reported in cats with pancreatitis.[47] Changes in canine lipoprotein patterns include increases in VLDL, chylomicrons, and LDL with decreases in some subtypes of HDL. The hypertriglyceridemia arises from both a decrease in clearance of chylomicrons and VLDL due to decreased lipoprotein lipase activity as well as an increase in VLDL production. Hypercholesterolemia results from decreased biliary excretion due to pancreatitis-associated cholestasis as well as increased hepatic synthesis. Release of inflammatory cytokines likely contributes to the alterations in hepatic lipid metabolism. Pathogenesis of the hyperlipidemia may be further complicated by the comorbidity of diabetes mellitus as pancreatic parenchyma is damaged.

It is speculated that hypertriglyceridemia may contribute to the development of pancreatitis and may help explain the clinical impression that consumption of high fat meals can precede the onset of acute pancreatitis. The theory is that hydrolysis of chylomicron triglycerides by pancreatic lipase within the pancreatic microcirculation results in local release LCFA. LCFA have the potential to damage both the endothelial cells of the pancreatic microvasculature as well as the pancreatic ascinar cells. This provides a mechanism for perpetuating a cycle of ongoing release of pancreatic lipase and generation of damaging LCFA as well as release of other potentially harmful pancreatic enzymes into the parenchyma.

Endotoxemia and inflammation

Alterations in serum lipids and lipoproteins may be seen as a response to endotoxin and endotoxin-induced release of inflammatory cytokines. Inflammatory cytokines have been

implicated in mediating the changes in lipid metabolism in a number of pro-inflammatory conditions such as obesity and pancreatitis. Response to endotoxin is characterized by increased circulating LCFA, triglycerides, and VLDL. Stimulation of lipolysis by adipocytes and hepatic fatty acid synthesis in concert with decreased fatty acid oxidation results in increased triglyceride and VLDL synthesis.[48] Clearance of triglycerides is impaired due to decreased lipoprotein lipase activity. Decreased lipoprotein Apo-E content blunts receptor mediated removal of lipoproteins from the blood.

Cholesterol levels are more variable and likely depend on species, clinical severity, and time course in the disease. Mild hypercholesterolemia may develop secondary to endotoxin-induced decreases in biliary excretion of cholesterol as well as decreased uptake of LDL secondary to down regulation of LDL receptors.[49] As part of a negative acute phase reaction mediated by inflammatory cytokines, patients may develop mild to moderate hypocholesterolemia due to a decrease in hepatic cholesterol synthesis. HDL may both decrease in concentration and alter in composition.[50]

Cholestasis

Cholestasis arising from a variety of mechanisms may result in mild to moderate increases in cholesterol with occasional mild increases in triglycerides. This is likely due to a combination of decreased hepatic cholesterol uptake and impaired cholesterol excretion in the bile. Alterations in lipoprotein composition and distribution have been documented in dogs, cats, and horses with natural and experimentally induced cholestasis.[51–54]

Protein losing nephropathies and nephrotic syndrome

Nephrotic syndrome may develop as a result of glomerular damage and proteinuria arising from a variety of etiologies. The hallmarks of nephrotic syndrome include proteinuria, ascites, edema, hypoalbuminemia, hypercholesterolemia, and hypertriglyceridemia. Nephrotic syndrome accompanied by hypercholesterolemia and hypertriglyceridemia has been observed in dogs, cats, and horses.[55] While nephrotic syndrome with proteinuria and hypoalbuminemia has been described in cattle, effects on serum cholesterol and triglycerides were not reported.[56]

A number of mechanisms have been suggested to explain the altered lipid metabolism secondary to proteinuria and hypoalbuminemia.[57] Experimental studies in human and animal models suggest that proteinuria and hypoalbuminemia are associated with increased activity of hepatic cholesterol synthetic enzymes, resulting in increased production of cholesterol and cholesterol containing lipoproteins. Urinary loss of key enzymes such as LCAT may affect maturation of HDL and impair the mechanism of reverse cholesterol transport. Decreased LDL receptor expression as well as altered binding of LDL to LDL receptors contributes to decreased clearance of LDL. Catabolism of cholesterol and subsequent excretion of cholesterol through the bile also may be impaired due to decreased activity of enzymes involved in bile acid synthesis.

Hypertriglyceridemia results from both increased hepatic synthesis of VLDL and decreased peripheral clearance of VLDL and chylomicrons. Impaired clearance of triglycerides appears to be the result of decreased activity of lipoprotein lipase and hepatic lipase. The amount and activity of lipoprotein lipase is affected by renal loss of heparan sulfate.

Primary hyperlipidemia

Once other causes of hyperlipidemia have been excluded, a diagnosis of primary hyperlipidemia should be considered. These conditions are rare and usually believed to have a genetic basis, although pathogenesis often remains elusive. In dogs, idiopathic hyperlipidemia is most commonly observed in miniature schnauzers and is characterized by moderate to marked hypertriglyceridemia and moderate hypercholesterolemia.[58,59] Defects in lipoprotein lipase or Apo-C have been proposed as mechanisms for the increases in VLDL and chylomicrons seen in these dogs. This syndrome may be an incidental finding or may be associated with clinical signs such as seizures or abdominal pain. Idiopathic hyperlipidemia has been reported in other dog breeds as well as sporadically in mixed breeds. Primary hypercholesterolemia and hypertriglyceridemia has been described in Shetland sheepdogs[60] and beagles.[61] Idiopathic hypercholesterolemia with normal triglycerides has been observed in Briards,[62] while idiopathic hypertriglyceridemia without elevations in cholesterol have been documented in Brittany spaniels.[63]

Primary hyperchylomicronemia resulting from a mutation in the lipoprotein lipase gene has been documented in cats.[64] It is suggested that the lipoprotein lipase enzyme cannot bind to the capillary endothelium. These cats have increases in serum chylomicrons, triglycerides, and cholesterol. This condition is associated with overall decrease in body fat mass, development of xanthomata and ocular lipid accumulation.[65,66]

Ketosis and ketoacidosis

Clinically significant ketosis occurs in conditions where energy supply and demand are out of balance. This may be seen in dairy cows in early lactation when high milk production results in a negative energy balance. Clinical bovine ketosis is characterized by increased plasma NEFA, hypoglycemia, hypoinsulinemia, high glucagon, low insulin, and metabolic acidosis.[67] Increased lipolysis supplies LCFA to hepatocytes at a rate that exceeds the ability to produce and export triglycerides or to oxidize them through the TCA

cycle. As a result, fatty acids are shunted to ketone production. Hepatic lipidosis usually precedes clinical ketosis due to sluggish export of VLDL. While clinical bovine ketosis is not a fatal disease, it does result in significant loss of milk production and may predispose to other conditions such as displaced abomasum, metritis, and mastitis. Ovine pregnancy toxemia also is a consequence of negative energy balance. In this case, the energy drain is usually the result of late term pregnancy with twins. Ovine pregnancy toxemia is a severe, often fatal disease that may be precipitated by stress. Ewes typically are depressed with hypoglycemia, severe metabolic acidosis, and ketosis. In sheep with pregnancy toxemia, an inability to either produce VLDL or transport triglycerides out of the hepatocytes occurs and hepatic lipidosis ensues.

Poorly controlled diabetics may develop ketosis and ketoacidosis. Diabetic ketoacidosis is characterized by hyperglycemia, hypercholesterolemia, hypertriglyceridemia, increased NEFA, and metabolic acidosis.[26] Enhanced lipolysis and gluconeogenesis result from a lack of insulin or poor insulin responsiveness. Increased glucagon, cortisol, and norepinephrine also have been implicated in the pathogenesis of diabetic ketoacidosis in dogs.[26] Triglycerides accumulate, resulting in hepatic lipidosis, while excess acetyl-CoA is converted to ketone bodies. Ketone production exceeds the ability to utilize them as an energy substrate and ketone bodies accumulate. Because ketones are strong acids, metabolic acidosis develops as the concentrations of ketone bodies rise.

Hypolipidemias

Table 31.3 lists diseases in which hypolipidemia may occur. Mild decreases in cholesterol and triglycerides alone may be of limited clinical significance and may just reflect a fasting state. Hypocholesterolemia can be a characteristic of illnesses that result in decreased production, malabsorption, maldigestion, or increased catabolism of cholesterol. Hypotriglyceridemia often is a reflection of inadequate nutrition such as starvation or malnutrition secondary to malabsorption and/or maldigestion.

Table 31.3 Causes of hypolipidemia.

Protein losing enteropathy
Exocrine pancreatic insufficiency
Inflammatory bowel disease
Hepatic insufficiency
Hypoadrenocorticism
Hematopoietic neoplasia
Hyperthyroidism

Protein losing enteropathy, malabsorption, and maldigestion

Conditions resulting in malabsorption and/or maldigestion may be associated with hypocholesterolemia and hypotriglyceridemia, although these findings are inconsistent. Decreased serum cholesterol and triglycerides may occur with exocrine pancreatic insufficiency. In these patients, serum albumin is usually maintained within the reference interval. In contrast, patients that develop protein losing enteropathy may have concurrent hypocholesterolemia and hypoalbuminemia due to loss of both albumin and lipoproteins. Protein losing enteropathy may result from a variety of gastrointestinal pathologies including infectious, inflammatory, or infiltrative intestinal diseases as well as primary or secondary intestinal lymphangiectasia.[68] Five to 30% of cats with idiopathic inflammatory bowel disease (IBD) are reported to have hypocholesterolemia.[69] However, this finding is not consistent, and those same studies indicate that 3–5% of cats with IBD may have hypercholesterolemia. Some breeds of dogs are predisposed to protein losing enteropathy, including the soft-coated Wheaton terrier, Yorkshire terrier, Basenji, and Norwegian Lundehund.[68] In soft-coated Wheaton terriers, concurrent protein losing nephropathy and nephrotic syndrome can have counterbalancing effects on serum cholesterol and triglycerides.[70]

Hepatic insufficiency

Noncholestatic liver failure may be associated with hypocholesterolemia due to decreased cholesterol production. Hypocholesterolemia has been associated with cirrhosis, toxin-induced parenchymal damage, and portosystemic vascular anomalies. Hypotriglyceridemia also may be present. Hepatic insufficiency can be difficult to distinguish from intestinal diseases resulting in protein losing enteropathy as both may have concurrent hypocholesterolemia, hypoalbuminemia, low BUN, and gastrointestinal signs.

Hypoadrenocorticism

Hypocholesterolemia is sometimes seen in dogs with hypoadrenocorticism.[71] The incidence of hypocholesterolemia may be more common in atypical Addisonians in which there is a deficiency of glucocorticoid but not mineralocorticoid production.[72] Hypoalbuminemia and hypoglycemia also may be present and can make distinguishing atypical Addisons from other conditions such as liver or intestinal disease difficult.

Hematopoietic neoplasia

Decreased serum cholesterol is observed in some forms of hematopoietic neoplasia in humans and animals. Hypocholesterolemia was noted in 69% of dogs with hemophagocytic histiocytic sarcoma[73] and cats with multiple myeloma.[74] A recent report indicated that 24% of cats with nasal and nasopharyngeal lymphoma had hypocholesterolemia.[75] The

mechanism underlying the hypocholesterolemia is uncertain but may relate to production of inflammatory cytokines such as interleukin-6 and tumor necrosis factor-α. These cytokines suppress hepatic cholesterol synthesis and contribute to the negative acute phase reaction observed with both albumin and cholesterol. Hypoalbuminemia was a relatively common finding in the cats with multiple myeloma and dogs with hemophagocytic histiocytic sarcoma, but not in the cats with nasal and pharyngeal lymphoma. Enhanced catabolism of cholesterol has been suggested as another mechanism to explain hypocholesterolemia in some human cancer patients.

Hyperthyroidism

Hypocholesterolemia and hypotriglyceridemia have been observed in hyperthyroid humans and in some experimental models of hyperthyroidism. While hyperthyroidism is common in older cats, decreases in serum cholesterol and triglycerides are uncommon findings. Concentrations of cholesterol and triglycerides may be near but often do not dip below the lower limit of the reference interval.[76] In one report, hypercholesterolemia was observed in 8% of cases, while hypocholesterolemia was not seen in any of the 131 cats included in the study.[77]

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32 Laboratory Evaluation of the Thyroid, Adrenal, and Pituitary Glands

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Clinical endocrinology

Introduction

Before reading any further let's clarify the record that there are no perfect diagnostic tests. Endocrine or otherwise there are none. There are expectations, or at least criticisms, that tests should be perfect and that there should be agreement between testing methods. For a test to have any value, it must identify animals with the disease from normal animals or the test is useless. For diagnostic use we need tests that distinguish animals with a specific disease from the other differentials that may clinically appear similar. In the majority of cases, endocrine testing combined with other procedures are very helpful if used properly. Diseases that are fully developed are more easily recognized with diagnostic tests. There probably is a correlation that the earlier a disease is in its onset and the earlier the veterinarian is in his or her career, the more tests are needed to arrive at "certainty-of-diagnosis." For many diseases, the consequence of an incorrect diagnosis and the prescription of chemicals that kill hyperplastic tissues and require lifelong treatment are such that we must be certain. In some instances this will require multiple tests, or repeat testing at different times. We need to be prepared to defend this need for certainty to owners and colleagues. Our awareness of certain diseases means we are suspicious of these diseases and therefore may test for them early in their development. We try to recognize a disease at one point in time and sometimes this is possible, but in other cases we need to repeat our examinations at a later point. Imaging is an absolutely great procedure to complement endocrine tests. Visualization of a mass in the correct location along with supportive endocrine test results is optimal. Many times this is present and many times finances or the techniques themselves limit what is performed. Imaging is not 100% reliable, auscultation is not 100% reliable, and neither are endocrine tests. But their combined use may be very good. As newer techniques are developed they will need clinical trials on the same old diseases to determine if they improve our diagnostic capabilities.

Some endocrine tests will remain screening tests despite criticism. Total T4, low-dose dexamethasone suppression (LDDS), urine creatinine cortisol ratio (UCCR), and other tests are excellent screening tests. Total T4 is a good first step to recognize hypothyroidism in dogs and to justify additional testing to confirm or refute this differential. It is an excellent test to rule out hypothyroidism. Total T4 will rule in hyperthyroidism in the majority of cases in cats, especially those in which the disease is fully developed. Basal cortisol is excellent to rule out Addison's disease, but is not reliable to rule out or rule in Cushing's disease. LDDS has many false positives, but it is an excellent screening test to rule out hyperadrenocorticism and to rule in hyperadrenocorticism if the dog looks like a poster-card representative of Cushing's disease. Therefore how we use tests, when we use tests, what methodologies were used, and the consequences of a false positive or a false negative test will influence the utility of tests. It may be better to select a test for Cushing's disease that could miss some cases to be certain this diagnosis is not falsely ruled in and a lifelong treatment regimen started. We seem to dwell on exceptions or when a test misses a diagnosis and become critical of the test while we forget the big picture. If a test works 80–90% of the time that is terrific; we all should be so good.

The endocrine system is unique in that it has diseases that cause hyperfunction as well as hypofunction. For most systems we try to recognize failure of an organ, but in the endocrine system many diseases are due to hyperfunctional states. A primary lesion in the endocrine gland or a lesion

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in the pituitary gland can cause each of these when the endocrine organ is dependent on the pituitary axis.

The approach used to solve or diagnose endocrine diseases should involve the following sequential steps.

- Determine the historical and physical exam findings that may suggest an endocrine disease.
- Determine the routine lab results (CBC, chemistry profile, urinalysis) that may be characteristic of the suspected endocrine disease.
- Lastly, utilize appropriate screening and confirmatory tests that rule in or rule out, the suspected endocrine disease.

Each of these steps is easier to assess by understanding the possible primary lesions and the positive and negative feedback loops that contribute to the diseases. As with all diagnostic tests the results obtained for specific endocrine tests should be compared to reference intervals from the laboratory that provides the results, as opposed to reported ranges in this or any other text. This is especially important for endocrine tests that may have variable methodologies. In fact it is recommended to use laboratories that not only produce results (numbers, values), but also have experienced people that will help you interpret the results. This is especially important for substances that are measured infrequently or in situations in which clinical experience is limited with rare or uncommon diseases.

Thyroid disorders

Thyrotropin-releasing hormone (TRH) from the hypothalmus stimulates the release of thyroid stimulating hormone (TSH, thyrotropin) from thyrotrophs in the pituitary which in turn stimulates thyroid gland follicular cell hypertrophy and a cascade of intracellular events that result in the production of thyroxine (TT4, tetraiodothyronine) and smaller amounts of triiodothyronine (TT3), and trace amounts of reverse triiodothyronine (rT3). Approximately 99% of secreted TT4 is bound to plasma proteins and less than 1% is free tetraiodothyronine (fT4). However, fT4 is biologically active, enters cells, leads to intracellular TT3 production, and causes negative feedback to TSH release. Free T4 that passes into cells is metabolized into TT3 or rT3 based on physiologic needs. In normal metabolic states TT3 is produced and this is the biologically active hormone that stimulates cellular events, but when patients are sick there is preferential conversion to biologically inactive rT3. Reverse TT3 increases in nonthyroidal illness and is responsible for the decrease in TT4 seen in the euthyroid sick syndrome. Increased concentrations of serum rT3, combined with measurement of TT4 and TT3 were used to identify patients with the euthyroid sick syndrome, but measurement of rT3 and TT3 are seldom done anymore. Measurement of rT3 can help identify the euthyroid sick syndrome, or nonthyroidal illness. Although TT3 is the biologically active form of thyroid hormone it is of limited diagnostic value. TT4, the storage form of thyroid hormone, and fT4 are of greater diagnostic value. All of the serum TT4 and fT4 come from the thyroid gland and only a portion of TT3 arises in the thyroid. This may explain the greater utility of TT4 and fT4 as opposed to TT3 to indicate thyroid gland function. The majority of T3 is produced outside of the thyroid glands via deiodination of T4 in nonthyroid cells.

The major diseases of the thyroid gland are neoplasia, hyperthyroidism, and hypothyroidism. Generally, thyroid tumors in cats are benign and thyroid gland tumors that are large enough to be detected clinically in dogs are malignant. Hyperthyroidism is a very common disease of cats, but is uncommon in dogs and other species. The majority of thyroid tumors in dogs do not cause hyper or hypothyroidism. Hypothyroidism is very common in dogs and does not occur spontaneously in adult cats. Hypothyroidism in cats is almost always iatrogenically induced following treatment of hyperthyroidism. Hypothyroidism is associated with goiter, or hyperplastic thyroid glands, in ruminants, birds, and horses.

Thyroid tests

TT4

This is an excellent test to rule in hyperthyroidism in cats and rule out hypothyroidism in dogs. Increased serum TT4 in a cat is due to hyperthyroidism until proven otherwise. Serum TT4 concentration within reference interval rules out hypothyroidism in dogs. TT4 is stable at room temperature for 1 week, an unusual benefit compared to most hormones that degrade postcollection if not frozen. TT4 can be measured via RIA, chemiluminescent enzyme immunoassay and ELISA, all of which have similar diagnostic value. Point-of-care ELISA can be used in clinics and provide results within minutes. Numerous drugs and nonthyroid diseases and can suppress serum TT4, the latter being known as euthyroid sick syndrome. It can also be lower in large body size and certain breeds (see Table 32.1). Retesting for the possibility of drug-induced suppression requires cessation of most drugs for 4 weeks. The greater the severity of the nonthyroid disease the greater the suppression of TT4 in both dogs and cats. Low concentrations of TT4 in a dog should prompt consideration of fT4, TSH, and possibly other tests to distinguish primary hypothyroidism, secondary hypothyroidism, and euthyroid sick syndrome.

TT4 < 11 nmol/L, with classic clinical signs and routine lab data, may be diagnostic for some cases. When clinical signs and other routine lab data seem inconsistent, measurement of fT4 and TSH may be helpful. Markedly decreased TT4 and fT4 is considered diagnostic for primary or secondary hypothyroidism. Decreased fT4 with increased TSH is diagnostic for primary hypothyroidism. Decreased fT4 with decreased TSH is diagnostic for secondary hypothyroidism.

fT4

Although fT4 is less than 1% of total serum thyroxine, it is of excellent diagnostic value and is suppressed less by

Table 32.1 Summary of thyroid tests.

TT4	Most common test, excellent screening for dogs and cats, used to rule out hypothyroidism in dogs and rule in hyperthyroidism in cats, stable in vitro
fT4	Excellent diagnostic utility, use equilibrium dialysis to measure, biologically active hormone
TSH	Endogenous, from thyrotrophs in pars distalis, use with TT4 and/or fT4 to evaluate hypothyroidism, do not use as stand alone test
TT4 & TSH	Panel used to diagnose and distinguish type of hypothyroidism
fT4 & TSH	Panel used to diagnose and distinguish type of hypothyroidism
TT3	Most abundant thyroid hormone, biologically active but poor diagnostic value, in some canine thyroid panels but do not use, rely on TT4 and fT4
r' T3	Used infrequently, helpful to diagnose euthyroid sick syndrome
TRH stim	Substitute for TSH stimulation, GI side effects
TSH stim	Tests thyroid reserve, bovine medical grade TSH is not available; human TSH is available but expensive; endogenous TSH and TRH stim used if injectable TSH not available
T3 suppression	Used in cats with suspected hyperthyroidism
anti-T3, anti-T4, and anti-thyroglobulin autoantibodies	Antibodies produced in lymphocytic thyroiditis, increased in <5% of cases, used to explain unusual increases or decreases in TT4 or TT3, suggests lymphocytic thyroiditis is present
Thyroid testing by species	
Dog	**Cat**
TT4	TT4
fT4	fT4
fT4 & TSH; TT4 & TSH	
If needed	
Thyroglobulin, T4, and T3 autoantibodies	T3 suppression
TSH, TRH stimulation	TSH, TRH stimulation

nonthyroid diseases and drugs than is TT4. Similar to TT4, the greater the severity of the nonthyroid disease the greater is the suppression of fT4 in dogs and cats. Nonthyroid disease is also associated with increased fT4 in some cats that could interfere with interpretation when used to evaluate hyperthyroidism. fT4 is useful in dogs and cats in which the concentration of TT4 and the rest of the data are not definitive for a diagnosis. fT4 is not influenced by autoantibodies. Anitconvulsant therapy and glucocorticoids will lower fT4. fT4 within reference interval rules out hypothyroidism. Decreased values suggest, but do not prove hypothyroidism unless other data is supportive. Concentrations of fT4 correlate with the thyroid status at the cellular level and correlate very well with TSH.

Equilibrium dialysis is the technique of choice for fT4. This means that serum is dialyzed in some manner to separate protein bound from free hormone and remove nonspecific substances in the serum that may interfere with the assay. Radioimmunoassay is performed on the dialysate.

TT3 triiodothyronine

TT3 is not recommended for testing in animals. It offers no diagnostic value over TT4 or fT4 in recognizing hyper- or hypothyroidism.

TT3 is often in reference interval in hypothyroid dogs; it is interfered with more by antibodies than is TT4. It is the most abundant and biologically active thyroid hormone, but it has poor diagnostic value perhaps because most is produced outside of the thyroid gland. It is offered in some canine thyroid panels.

r′ T3 reverse triiodothyronine

Used infrequently, helpful to diagnose euthyroid sick syndrome in humans and experimentally in dogs; see euthyroid sick syndrome at end of this chapter. It is available in some labs and clinical investigations for its utility should be done. Anticipated results in euthyroid sick dogs are: decreased TT4, reference interval fT4, reference interval TSH, and increased r′T3 due to preferential conversion of T3 to r′T3.

Thyrotropin, thyroid stimulating hormone, TSH

TSH is measured in diagnostic labs with various techniques. Never measure or interpret alone; always measure in the same sample as a panel with TT4 and/or fT4, and correlate results with all the other data available. Endogenous TSH is used primarily to differentiate primary hypothyroidism, secondary hypothyroidism, and euthyroid sick syndrome. In principle it is increased in primary, decreased in secondary, and within reference interval with euthyroid sick cases. See "screening tests hypothyroidism." Unfortunately for diagnosticians, diseases are in stages of progression, degrees of severity, and there may be different treatments to consider when the samples are analyzed and therefore results may not be this clear-cut. However, increased TSH concentration combined with a decreased TT4 and fT4 results in a specificity of nearly 100%. In other words there are no or very few false positives for hypothyroidism using this combination of test results. In a study of 108 dogs this combination was seen in one euthyroid sick dog and would have lead to a false positive diagnosis of primary hypothyroidism. If TSH is used alone then it is not as diagnostically helpful and this is discouraged. Thyroid stimulating hormone may not be increased in up to 33% of hypothyroid dogs. If hypothyroidism is still the best clinical diagnosis then retesting in several weeks or performing a stimulatory test or imaging studies of the thyroid are options. Decreased TSH with decreased TT4 and fT4 is characteristic of secondary hypothyroidism, but this only represents 5% or less of canine hypothyroidism cases.

Levothyroid treatment of a dog that does not have hypothyroidism will suppress TSH production and cause secondary thyroid atrophy if the dose of is large enough. Retesting for this possibility requires cessation of levothyroid for 8 weeks. TSH can also be used to monitor treatment of true hypothyroid dogs if TSH was measured in a thyroid panel prior to treatment with levothyroid. Endogenous TSH should decrease by 33% or more posttreatment if the dose of levothyroid being administered is sufficient to break the pituitary thyroid gland axis.

Autoantibodies

These are produced by lymphocytes and plasma cells in the thyroid in lymphocytic thyroiditis. They may be detected in the serum and are used to indicate lymphocytic thyroiditis may be present, but are not predictors of thyroid gland function status. These antibodies contribute to the destruction of the gland and are directed against thyroglobulin (aaTg present in 35–50% of hypothyroid dogs), TT3 (35% of hypothyroid dogs), and TT4 (15% of hypothyroid dogs). Thyroglobulin antibodies predominate and thyroglobulin is the protein that TT4 and TT3 are attached to, hence dogs with antibodies against TT4 and TT3 will also have aaTg, but the opposite is not true. Autoantibodes directed against thyroglobulin, TT4 or TT3 may increase or decrease TT4 and TT3 concentrations as measured by RIA depending on methodologies of the separation used. Falsely increased values of TT4 or TT3 are seen if antibody coated tubes are used in a single-step separation technique. However, falsely decreased values of TT4 and TT3 are seen if less specific separation techniques are used such as activated charcoal or ammonium sulfate. Consult the reference lab for clarification of interference.

These antibodies are used primarily to explain unusual increases in TT4 in the serum of dogs being evaluated for hypothyroidism. This happens in some cases of lymphocytic thyroiditis, probably early in the disease while inflammation is present. Results are reported as positive, negative, or inconclusive. A positive result can explain an increased concentration of TT4 in a dog with clinical signs of hypothyroidism. A very infrequent finding is that the antibodies increase the TT4 into reference interval in a dog with true hypothyroidism. A positive antibody test does not prove hypothyroidism, it indicates autoantibodies are in the serum and that lymphocytic thyroiditis is the likely lesion. There may be adequate reserve such that hypothyroidism is not present and may not develop. It is recommended that TT4 and fT4 be repeated some months later if the patient is suspected of developing hypothyroidism. Many of the breeds predisposed to develop hypothyroidism are the breeds with a high prevalence of aaTg. The false positive rate for aaTg is approximately 6% and transient increases are reported post vaccination. They are not part of initial screens for hypothyroidism and generally are only requested when unusual results for TT4 (or TT3) are obtained.

Stimulation tests: TSH and TRH response tests

TSH stimulation (response) test historically was a gold standard for diagnosis of hypothyroidism. However, exogenous bovine medical grade TSH is no longer commercially available and endogenous TSH is an alternative as described above. Chemical grade bovine TSH is not recommended due to life-threatening complications. Recombinant human TSH is available, but is expensive. TSH stimulation is used to predict thyroid reserve and differentiate primary hypothyroidism, secondary hypothyroidism, and euthyroid sick syndrome by evaluating the magnitude of increase of TT4 post TSH administration. In principle TT4 will not increase in primary hypothyroidism because the thyroid gland is destroyed and there are "no" target cells for TSH to

stimulate. TT4 values that are less than reference interval (<1.5 µg/dL) pre and post TSH are diagnostic. A euthyroid sick dog should increase TT4 normally (twofold or greater increase in TT4, or an increase of TT4 >3 µg/dL). A dog with secondary, pituitary dependent hypothyroidism will have variable results depending on the degree of atrophy in the thyroid gland, but partial stimulation is expected unless atrophy is severe. Unfortunately, intermediary results occur and it is not known if these represent early stages of the three differentials or mild to moderate lesions.

TRH response test is used in place of TSH to evaluate dogs with potential hypothyroidism and cats with hyperthyroidism when results of other tests are not conclusive or waiting several weeks to months and retesting is not an option. Some euthyroid dogs do not respond to TRH and therefore this dynamic test is less reliable. Both TT4 and TSH can be measured in dogs. Dogs with primary hypothyroidism should have values for TT4 below reference interval, <1.5 µg/dL pre and post TRH. Euthyroid dogs should demonstrate an increase of TT4 >2 µg/dL or a doubling of the pre-TT4 value. If TSH is measured there should also be a doubling of the pre TSH value. The principle is similar to the TSH response test in dogs, but distinction of primary and secondary hypothyroidism is not clear. Side effects of TRH are notable and include vomiting, defecation, urination, salivation, tachycardia, and/or tachypnea. They can be reduced by using the lower dose of TRH; see protocols under hyperthyroidism.

In cats suspected of occult hyperthyroidism (TT4 in reference interval) the TRH response test checks for a failure of TT4 to increase post TRH. Normal cats should double TT4 post TRH because TRH stimulates release of TSH which in turn stimulates normal thyroid follicular cells to increase production of TT4. However, hyperthyroid cats do not increase TT4. This is because neoplastic follicular cells do not respond to the increase in TSH caused by the injected TRH and the adjacent nonneoplastic follicular cells are atrophic and cannot respond to the TSH signal. Why the neoplastic thyroid cells do not respond to TRH-TSH has not been determined, but this could be due to lack of TSH receptors.

Thyroid gland biopsy

This is not recommended to diagnose hypothyroidism or hyperthyroidism, but it may prove useful to evaluate thyroid neoplasia in dogs. Fine needle aspiration cytology is recommended for suspected thyroid tumors and biopsy is only needed on those cases in which cytology is not definitive. Approximately 80% of thyroid gland masses in dogs are malignant if an enlarged mass is found clinically and 40% or less are malignant if necropsy data is used. Size correlates with aggressive behavior and bilateral tumors are 16 times more likely to metastasize than unilateral tumors.

Hyperthyroidism

General

Hyperthyroidism or thyrotoxicosis is one of the most common diseases of cats and is the most common endocrine disease of cats. It is caused by adenomas in the thyroid gland. Multinodular adenomatous hyperplasia is another term used to describe the lesion in cats, but the lesions are best explained by neoplasia for the following reasons. A small percentage of cases progress to carcinoma and metastasize. Nonneoplastic tissue is atrophic and is adjacent to neoplastic nodules, whereas functional hyperplasia of endocrine organs produces uniform enlargement of the entire gland. Hyperplastic endocrine lesions respond to stimulatory and suppressive signals, whereas neoplastic lesions generally do not. Cats with these adenomas do not respond to these stimuli. Furthermore, there is overexpression of the c-ras oncogene in the adenomas, and a decrease in inhibitory G protein permitting uncontrolled mitosis and thyroid hormone production. A small percentage of cats will have neoplastic tissue in the anterior mediastinum from rests of thyroid tissue. Some cases have nodules in both thyroid lobes and anterior mediastinum that could suggest multicentric hyperplastic or neoplastic stimuli. Numerous attempts have tried to identify a goitrogenic etiology and none has been found. Attempts to demonstrate antibodies to thyrotropin receptors in hyperthyroid cats, as seen in hyperplastic thyroid disease in humans with Grave's disease, have been negative in multiple studies. Regardless of the term used, 99% of the lesions are benign and they need to be removed surgically, medically, or with radiation if the disease is to be reversed. Approximately 75% of cases have bilateral involvement, 20% are unilateral, and 5% have ectopic thyroid proliferation in the anterior mediastinum or thyroid carcinoma.

Hyperthyroidism is uncommon in dogs and when present is due to thyroid adenoma or more likely, carcinoma. Although hyperthyroidism is uncommon, thyroid tumors are relatively common in dogs. Occasionally thyroid tumors may induce hypothyroidism in dogs, but most dogs with thyroid tumors are euthyroid. The size of the thyroid tumor correlates with aggressiveness. Approximately 50% of dogs have rests of thyroid tissue in the anterior mediastinum and occasionally these rests become neoplastic. Even if excessive quantities of TT4 and TT3 are produced, the efficient catabolism and metabolism of thyroid hormones in the dog (up to 20 times the capability of people and cats) leads to rapid degradation of the hormones and a euthyroid status.

Hyperthyroidism is very rare in horses and ruminants. Hyperthyroidism in horses is reported with thyroid tumors and the clinical syndrome and clinical pathology is similar to that seen in hyperthyroid dogs and cats. Horses are hyperactive, have polyphagia, weight loss, and increased serum concentrations of TT4, TT3, and fT4.

Hyperthyroidism summary

Occurs in older cats; adenoma(s); weight loss; polyphagia, hyperactive; one or more liver enzymes (ALP, ALT, AST) increased in 90% of cases; increased TT4 is diagnostic, if fT4 is needed measure by equilibrium dialysis and correlate value with TT4 concentration.

Clinical problems

Hyperactivity, weight loss, and polyphagia in a middle aged to old cat are the most common clinical problems. Weight loss is the most commonly observed clinical problem and may produce cachexia in severe cases. Mean age is 13 years; fewer than 5% of cases are in cats less than 10 years old. Chronic renal failure and cancer look similar, but cats with these diseases will not be polyphagic and hyperactive. Cardiac abnormalities can be detected in over 50% of hyperthyroid cats, but only 10% are in congestive heart failure. The most common cardiac lesion is left ventricular hypertrophy. Other signs include PU PD, vomiting, tachycardia, patchy alopecia, unkempt hair coat, bulky stools, diarrhea, and apathetic signs such as decreased activity, lethargy, anorexia, and weakness. Apathetic signs may be due to concurrent illnesses in these older cats such as heart failure or renal failure. Due to the incorporation of TT4 in geriatric panels of cats and clinical awareness of this disease, a diagnosis of hyperthyroidism is often established before owners are aware of clinical signs. Clinical signs of hyperthyroidism in dogs are similar to those in cats, but are less severe.

Routine lab data

The most consistent lab abnormality is a mild to moderate increase in serum ALP that occurs in approximately 70% of the cats. There may be mild increases in ALT and AST, and one or more liver enzymes will be increased in 90% of hyperthyroid cats. This nonspecific increase in these liver enzymes is mild to moderate and the pathogenesis is not known. Approximately one-third of the increase in ALP is due to the bone isoenzyme and the rest is liver isoenzyme. Although the increase in ALP is only mild, any increase in ALP should be investigated in cats due to the short half-life of feline ALP. If the ALT is over 1000 IU/L and TT4 is not markedly increased, pursue other differentials.

Azotemia occurs in 20–50% of hyperthyroid cats and is due to prerenal or concurrent renal disease. If urine specific gravity is less then 1.025, suspect concurrent renal issues. If it is greater than 1.040 it is probably prerenal. Since cats with hyperthyroidism are geriatric, it is likely that some will have concurrent chronic interstitial nephritis. Hyperthyroid cats that are not azotemic may have decreased serum creatinine concentrations. The mechanism is not known but may be due to muscle cachexia and decreased production of creatinine. Prevalence of concurrent urinary tract infections in hyperthyroid cats is 10–20%. Most of these cats are asymptomatic for this problem, but urinalysis will reveal pyuria and culture will yield *E. coli* in the majority of infections.

Hyperphosphatemia without azotemia is seen in 25–40% of hyperthyroid cats and the mechanism is not known. Total serum calcium is usually in reference interval, but a mild decrease in ionized serum calcium without associated clinical signs is observed in up to 50% of hyperthyroid cats. Increased concentrations of parathyroid hormone are also reported in hyperthyroid cats. Hypocalcemia, hyperphosphatemia, hyperparathyroidism, and renal problems are a common series of events. The parathyroid hyperplasia may help explain why some cats do not develop postsurgical hypocalcemia and may help explain why post surgical hypocalcemia is usually not permanent. Although hypercalcemia has been reported for dogs with hyperthyroidism, it is mild and the mechanism is not known.

Nonspecific hematologic abnormalities may occur in about half of cats with hyperthyroidism. Reported abnormalities include mild polycythemia and a stress leukogram. Less frequently, lymphocytosis and eosinophilia are observed, perhaps secondary to a decrease in cortisol due to increased thyroid hormones. Heinz bodies are often present, as they are in many diseases in cats.

Serum fructosamine is decreased in hyperthyroid cats secondary to the increased protein turnover (high metabolic rate, cachexia) and presumably a decrease in available proteins to bind with glucose. Therefore fructosamine should not be relied upon to assess long-term glucose status in hyperthyroid cats being evaluated for diabetes mellitus.

Screening tests: TT4, if TT4 in reference interval fT4

When this disease was first recognized essentially 100% of the hyperthyroid cats tested had increased serum TT4. Now, basal concentrations of TT4 are increased in 90–95% of the cases. Some 5–10% of cases have TT4 within the reference interval. False positive increases in TT4 are not reported in cats; specificity is 100%. Decreased concentrations of TT4 or concentrations at the low end of reference interval rules out hyperthyroidism with 99% confidence. fT4 is increased in 98% of hyperthyroid cats and it is increased in 6–12% of cats that do not have hyperthyroidism. False positives are 6–12%; therefore the specificity is 88–94%. If a cat has some of the physical and clinical laboratory abnormalities characteristic of hyperthyroidism, and an increased TT4 concentration, it is diagnostic of hyperthyroidism and fT4 or any additional tests are not needed. This will account for the majority of cats with characteristic clinical signs. However, because the disease is common and up to 10% of hyperthyroid cats have TT4 in reference interval, a considerable effort is placed in correctly identifying this population.

Concentrations of TT4 in the middle to high end of reference may be hyperthyroid, especially if some of the clinical signs and lab data characteristic of hyperthyroidism are present. This is the diagnostically challenging group. Below are some scenario examples of correlation of TT4 with other data.

Examples

- TT4 >4.0 µg/dL rule in hyperthyroidism, if clinical signs and lab data are supportive
- TT4 3.0–4.0 µg/dL favor hyperthyroidism, if clinical signs and lab data are supportive
- TT4 2.5–3.0 µg/dL gray zone; perform another test if clinical signs and lab data are supportive
- TT4 2.0–2.5 µg/dL probably not hyperthyroidism especially if no other data are supportive; request additional tests if still suspicious
- TT4 <2.0 µg/dL rule out hyperthyroidism unless other evidence is compelling

The top two and the bottom two examples above are easy to interpret and will explain TT4 results for the majority of situations. It is the middle gray zone that is difficult and where fT4 can be then be measured as an aid. This scenario is being recognized more frequently. This may be due to more widespread testing that detects cats early in the disease progression. Wellness exams often include TT4 on chemistry panels of geriatric cats hence we may see clinical chemistry evidence of the disease before signs are easily recognized. When faced with conflicting lab data while trying to confirm a diagnosis of hyperthyroidism, consider approaches in this order, depending on the urgency of a diagnosis:

1. Measure free fT4 (via equilibrium dialysis)
2. Repeat the TT4 concentration at some other time, i.e., 1–2 weeks or later
3. Palpate and/or image the thyroid gland and find nodule(s)
4. Find the nonthyroid disease that is concurrently suppressing the concentrations of thyroid hormones (euthyroid-sick syndrome just like in dogs)
5. Perform a TT3 suppression test
6. Perform TRH stimulation; see protocols at end of this section.

Concurrent illness will suppress TT4 in dogs and cats and the greater the severity of the illness, the greater the suppressive influence (Figs. 32.1 and 32.2). Concurrent illness in a hyperthyroid cat can suppress mild or moderate increases of TT4 into reference interval and therefore explain some of the cases of hyperthyroidism that have concentrations of TT4 within reference interval. This suppression also occurs with fT4, but the effect is not as great and therefore measuring fT4 is a logical step to determine if a cat with a normal TT4 is hyperthyroid. However, fT4 may actually be increased in some cats with concurrent illness. Sampling during a pulsating period when not much hormone is being released can also explain TT4 concentrations within reference interval. Repeat sampling for this possibility should be delayed for 1–2 weeks or longer because degrees in fluctuations of thyroid hormone secretion are seen over days rather than hours.

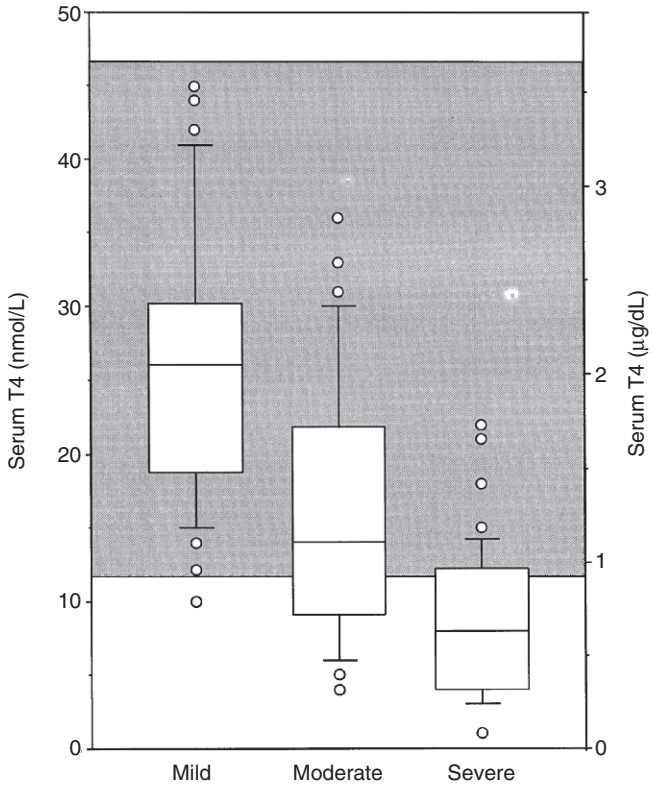

Figure 32.1 Box plots of serum total T4 concentrations in 221 cats with nonthyroidal disease, grouped according to severity of illness. Of 221 cats with nonthyroidal illness 65 had mild disease, 83 had moderate disease, and 73 had severe diseases. See Figure 32.3 for key. (Reprinted, with permission, from Peterson ME *et al.* (2001) *J Am Vet Med Assoc* 218: 529.) See Figure 32.3 for key.
Author comment: As the severity of nonthyroidal diseases increases, the serum concentrations of T4 and free T4 decrease.

Dogs

Routine laboratory data is similar to that in cats, but this is less characterized because of the much lower incidence. TT4, fT4 are increased. TSH is decreased due to negative feedback on thyrotrophs. Increased TT4 in a dog with a cervical mass and clinical signs of hyperthyroidism is sufficient for diagnosis. The cause will be a thyroid tumor. Therefore palpation, imaging of the neck and thorax, and aspiration cytology of the cervical mass are important diagnostic steps in dogs with suspected hyperthyroidism. Cytology is a preferred step as these tumors are highly vascularized and biopsy procedures will have considerable hemorrhage. Although hypercalcemia has been reported, it may not be due to hyperthyroidism.

Increased TT4 in a dog without a cervical mass and with or without clinical signs of hypothyroidism should be tested

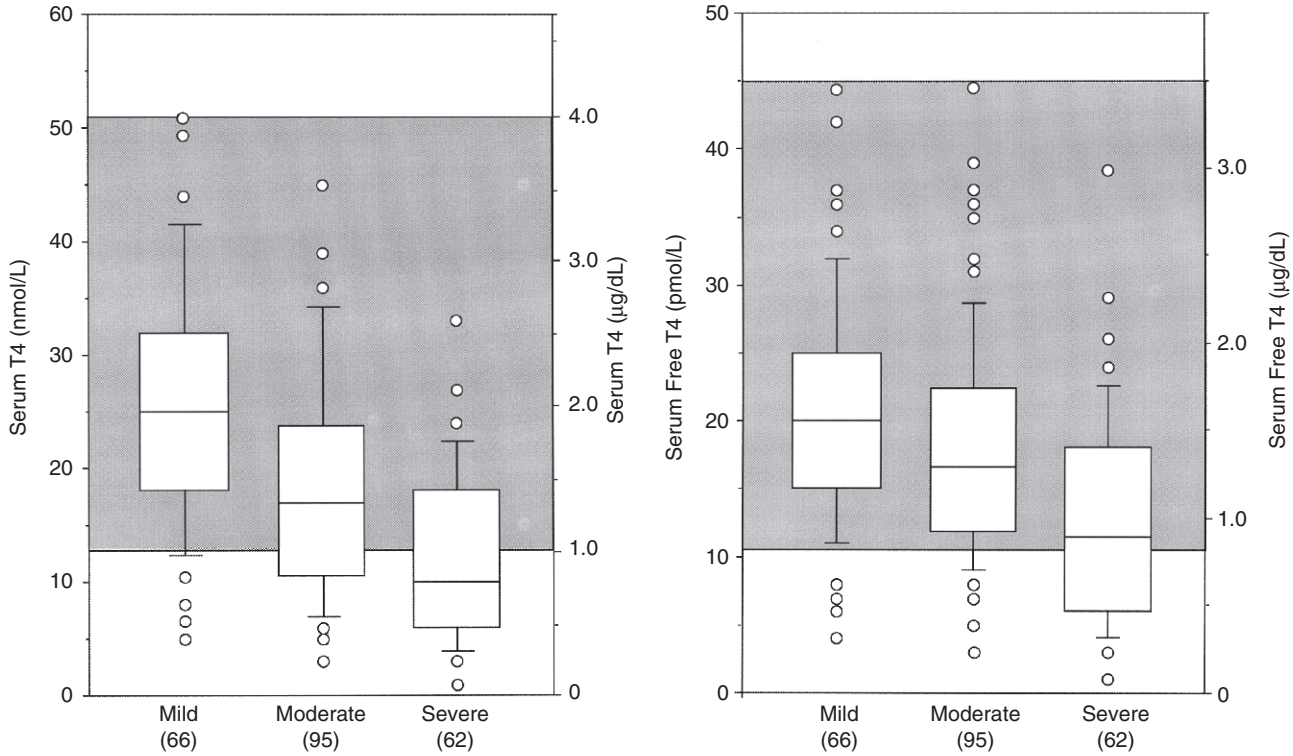

Figure 32.2 Box plots of serum total thyroxine (T4) concentrations (left) and free T4 concentrations (right) in 223 dogs with nonthyroidal disease stratified according to severity of disease (mild, moderate and severe). For each box plot, T-bars represent the main body of data, which in most instances is equal to the range. Each box represents interquartile range (25th–75th percentile). The horizontal bar in each box is the median. Open circles represent outlying data points. Numbers in parentheses indicate the numbers of dogs in each group. The shaded area indicates the reference interval. (Reprinted, with permission, with slight format modification from Kanrowitz LB *et al.* (2001) *J Am Vet Med Assoc* 219: 765.)

for thyroid antibodies that may falsely increase TT4. An increase in TT4 or especially TT3 may be due to crossreactivity of autoantibodies. Antibodies to various thyroid antigens are attributed to lymphocytic thyroiditis, some of these dogs progress into hypothyroidism and some do not. TT4, fT4, and TSH on the same serum sample should be requested to confirm hypothyroidism.

Confirmatory tests: fT4, T3 suppression, TRH or TSH stimulation

fT4

Usually TT4 is all that is needed to diagnose hyperthyroidism in cats. If the serum TT4 is increased in a cat with clinical signs of hyperthyroidism then there is no need to measure fT4 as it will also be increased in 100% of these cats. If results of TT4 are not clear-cut then measure fT4 via equilibrium dialysis and *correlate* with TT4 concentration and *clinical signs*.

Serum fT4 is not suppressed by nonthyroidal illnesses to the same degree as is TT4 and therefore fT4 is valuable when concurrent illnesses are suspected to be lowering TT4 into reference interval. In a study of over 900 hyperthyroid cats, 205 were categorized as mildly hyperthyroid and of these 125, 61% had increased TT4 and 191, 93% had increased fT4. However, increased fT4 concentrations are also present in some cats with nonthyroidal diseases that can confuse interpretation of fT4, but this false increase does not happen with TT4 (Table 32.2; Fig. 32.3). The false positive rate for fT4 is about 6–12%, likely due to nonthyroidal disease, makes it important not only to examine that fT4 is increased, but also to consider the magnitude of the increase and correlate fT4 with TT4. The increase in fT4 seen with concurrent diseases is the reason that fT4 should not be relied on solely to diagnose hyperthyroidism and why the fT4 concentration should be correlated with TT4. The greater the increase of fT4 and TT4 the more likely the diagnosis is hyperthyroidism (Fig. 32.3). The lower the TT4 the more likely the increase in fT4 is due to nonthyroidal illness.

T3 suppression test

Suppression and/or stimulation tests are recommended when repeat testing for TT4 and fT4 have not provided a diagnosis. Usually finding the concurrent illness, and treating it, or repeating the TT4 and fT4 at different time intervals, are easier ways to confirm hyperthyroidism than is performing functional thyroid tests. If a functional test is

Table 32.2 Summary of test results in cats with hyperthyroidism.

TT4:
- 90–95% of hyperthyroid cats increased concentrations; sensitivity 90-95%.
- 5–10% of hyperthyroid cats have results within reference interval = false negative; explanations:
  1. Early in the disease
  2. Fluctuations in secretion of TT4
  3. Concurrent non-thyroid diseases that decrease TT4

"Solutions"
1. Repeat TT4 (1–2 weeks, 2 months, whatever owner will tolerate)
2. Measure fT4 via equilibrium dialysis
3. Examine thyroid for nodules: palpate, US, radioactive imaging
4. Find concurrent nonthyroid disease
5. T3 suppression test
6. TRH, TSH stimulation test

freeT4:
- 98.5% of hyperthyroid cats have increased fT4
- 6–12% false positive = increased fT4 with nonthyroid disease
- Correlate fT4 with TT4, do not diagnose just on fT4
- If fT4 is increased highly probable hyperthyroidism

TT3:
- Approximately 25% of hyperthyroid cats have TT3 within reference interval

Do not use; better tests are available

desired, try T3 suppression first as it has fewer side effects and is easier to interpret then the others.

The T3 suppression principle is to administer TT3 orally and see if this decreases serum TT4 by suppressing the secretion of TSH. Normal cats will suppress and hyperthyroid cats do not suppress. Oral TT3 will suppress the secretion of TSH from thyrotrophs, which in turn decreases production and release of TT4 (and TT3) from the thyroid gland in normal cats because the thyroid pituitary axis is intact and the thyroid follicular cells are normal. Hyperthyroid cats already have increased thyroid hormones in their serum from the secreting thyroid tumors and therefore they already have decreased TSH. Therefore adding more TT3 cannot suppress this axis any further. The neoplastic thyroid follicular cells will continue secreting TT4 independent of TSH and therefore there is no decrease in TT4.

This suppression test is accomplished by administering TT3 orally for six doses; some protocols use three doses. At the start of the study, take a blood sample for a basal TT4, fT4, TT3 and 6–8 hours after the last dose of TT3 take another serum sample for the same measurements. TT3 determines if the cat successfully received TT3. The concentration of TT4, fT4 after the administration of TT3 should decrease in a euthyroid cat and they do not suppress in hyperthyroidism. If there is no suppression, even if the concentrations of TT4, fT4 are still within reference interval, it supports hyperthyroidism.

Thyrotropin releasing hormone response test—TRH response

Principle

Exogenous TRH administered to euthyroid cats will stimulate release of TSH, which stimulates increased TT4 production and secretion. This response will be blunted in hyperthyroid cats because the neoplastic follicular cells are not responding to normal physiologic stimuli and the adjacent atrophic cells are incapable of responding.

Interpretation guidelines

Increase TT4 60% or more from basal = euthyroid

Increase TT4 50–60% of basal = nondiagnostic

Increase TT4 < 50% to 0 = hyperthyroid

Side effects can be significant, occur within minutes of administration, and are transient for a few hours. Side effects include vomiting, defecation, salivation, and tachypnea.

Thyroid stimulating hormone response test—TSH response

Principle

Same as the TRH response test

Interpretation

Increase TT4 60% or > from basal = euthyroid

Increase TT4 50–60% of basal = nondiagnostic

Increase TT4 < 50% to 0 = hyperthyroid

Human recombinant TSH is expensive, but can be used in place of bovine medical grade TSH. Hyperthyroid cats with values in mid to low range of TT4 have a response to TSH that is the same as normal cats.

It is recommended to consult with your reference laboratory to obtain specific suppression and stimulation protocol information and interpretation guidelines.

Endogenous TSH

Assays for feline specific TSH are not yet available, but assays used for dogs and humans are. Consult the reference lab for which antibodies are used and more importantly the reference interval values and cut-off values used to interpret results. Measurement of TSH is not needed when TT4 is increased and is only used when TT4 and fT4 results are not definitive. When TT4 is normal to high normal, TSH that is decreased and/or below the detectable limit is diagnostic for hyperthyroidism.

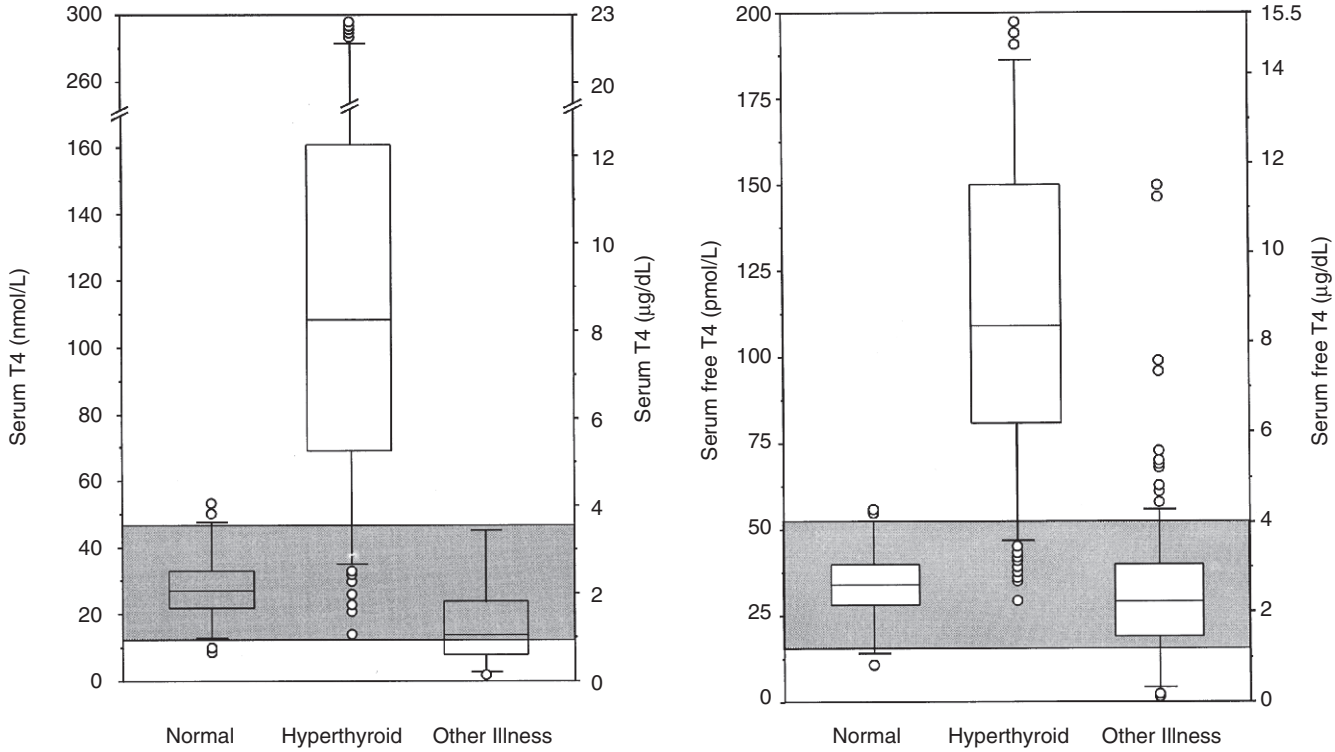

Figure 32.3 Box plots of serum total thyroxine (T4) concentrations (left) and free T4 concentrations (right) in 172 clinically normal cats, 917 cats with untreated hyperthyroidism and 221 cats with nonthyroidal disease (other illness). The box represents the interquartile range (i.e., 25th–75th percentile range or the middle half of the data). The horizontal bar in the box represents the median value. For each box plot, the T-bars represent the main body of data, which in most instances is equal to the range. Outlying data points are represented by open circles. The shaded area indicates the reference interval. (Reprinted, with permission, with slight format modification from Kanrowitz LB *et al.* (2001) *J Am Vet Med Assoc* 219: 765.)
Author comment: Some cats with hyperthyroidism can have serum concentrations of T4 and free T4 within the reference interval (false negatives). Some cats with nonthyroidal disease (other illness) can have increased concentrations of free T4 (false positives, approximately 6–12%).

Hypothyroidism

General considerations

Hypothyroidism is a common disease in dogs. About 95% of the cases are due to a primary lesion in the thyroid gland defined as lymphocytic thyroiditis and/or idiopathic follicular collapse. These lesions are part of the same disease. It starts as lymphocytic thyroiditis and ends as follicular collapse, with a continuum in between. A dog with primary hypothyroidism late in the disease will have decreased TT4 and fT4 and increased concentrations of TSH. Increased TSH is in response to decreased thyroid hormones and loss of negative feedback on the pars distalis. Other lesions in the thyroid gland that cause hypothyroidism are uncommon. These include thyroid neoplasia, aplasia, hypoplasia, and dyshormonogenesis. Secondary hypothyroidism, due to a structural or biochemical lesion in the pituitary gland that decreases TSH production, is uncommon, 5% or less of cases. A dog with secondary hypothyroidism will have decreased TT4, fT4, and TSH. Decreased TSH is due to the pituitary lesion (tumor) that is destroying or crowding out thyrotrophs. Lesions associated with this are pituitary tumors, pituitary cysts, hypoplasia, dysfunctional thyrotrophs, and an apparent deficiency of TSH production in giant schnauzers. The lack of TSH results in atrophy of the thyroid gland, which theoretically could be reversed if the primary lesion in the pituitary could be corrected. Teritary hypothyroidism due to a lesion that decreases TRH has not been reported in veterinary medicine. An increased concentration of TSH with concurrent decreases in TT4 and fT4 confirms primary hypothyroidism.

Lymphocytic thyroiditis is an immune mediated destruction of the thyroid gland directed at thyroid follicular cells, sparing C-cells. It is nonreversible and affected dogs require life long thyroid replacement therapy. Clinical signs develop gradually over several years and are detected when approximately 75% or more of the gland is destroyed. Lymphocytes and plasma cells produce antibodies directed at thyroid follicular cells, with antigens including thyroglobulin (most common), colloid, TT3, and TT4. There is a genetic component to this disease and the list of dog breeds that have an

increased prevalence of thyroid antibodies in their serum is long. These antibodies and the inflammation gradually destroy the follicular cells. Over time, the inflammation evidenced by inflammatory cells subsides. Histologically, the gland at this point appears as idiopathic follicular collapse.

Autoantibodes directed against thyroglobulin, TT4, or TT3 may increase or decrease TT4 and TT3 concentrations as measured by RIA depending on methodologies. Falsely increased values of TT4 or TT3 are seen if antibody-coated tubes are used in a single-step separation technique. Increased concentrations of TT4 in a dog with clinical signs of hypothyroidism can confuse interpretation of the results. This is uncommon, occurring in less than 5% of the cases and can be confirmed by measuring the different antibodies. Furthermore the clinical signs of hyperthyroidism versus hypothyroidism are very different. Even more confusing is the situation where the antibodies "raise" the TT4 and/or TT3 into reference interval. In either situation, if hypothyroidism is the likely differential, then the next step is to consult with the reference lab and to measure antibodies for thyroglobulin, TT3, and/or TT4. If any of these are increased then antibody interference is the most likely explanation for the confounding data. Free T4 is interfered with less than TT4 or TT3, so it is hoped that in these uncommon situations the fT4 will be decreased while TT4 is increased, which further supports antibody interference. This pattern is rarely observed (<5% of cases) and when seen is probably in an early or active phase of the disease when inflammation and antibody production are occurring. The key is to correlate clinical suspicion with the laboratory data. Recent vaccination can also produce antibodies to thyroglobulin, but there is no association with hypothyroidism.

Other casues of hypothyroidism in dogs are rare: neoplastic destruction of the gland, iodine deficiency, iatrogenic destruction (surgery, radioiodine), dyshormonogenesis, pituitary cysts (dwarfism), and congenital—giant schnauzer and an autosomal recessive form in toy fox terriers, in which there is a deficiency of thyroid peroxidase. A genetic test can recognize the carrier trait in fox terriers.

Spontaneous hypothyroidism is very rare in adult cats. An autosomal recessive form of congenital hypothyroidism is reported in Abyssinian cats. Decreased concentrations of TT4 in a cat are much more likely to be due to nonthyroidal illness than true hypothyroidism. Iatrogenic causes from surgical, chemical, or radiation-induced thyroidectomy for the treatment of hyperthyroidism is the most common cause in cats. Disproportionate dwarfism occurs in kittens and causes polyendocrinopathies including growth hormone defects and hypothyroidism. Other rare causes in kittens are defects in thyroid hormone synthesis, dysgenesis, and an autosomal recessive form of congenital hypothyroidism in Abyssinians. Iodine deficiency may cause hypothyroidism and goiter in kittens fed all meat or home designed diets.

Hypothyroidism in large animals (horses and small ruminants) is almost always due to the intake of some exogenous substance that interferes with the production of TT3 and TT4. Many substances can do this, and they interfere with the production of thyroid hormones at various stages. A few of the more common and/or high-profile substances are sulfa compounds, decreased iodine intake, increased iodine intake, plants (kale, seaweed), and various chemical substances (thiouracil). The decreased production of TT3 and TT4 due to these substances, results in reduced or no negative feedback to the hypothalamus and pituitary gland and, therefore increased TSH production. The increased TSH stimulates follicular cell hypertrophy and hyperplasia, resulting in an enlarged, goitrogenic thyroid gland. These animals have goiter, mild to massive thyroid gland enlargement, and hypothyroidism indicated by decreased TT3 and TT4. Goiter in neonates is the most common thyroid disorder in the horse and small ruminant. In small ruminants it is usually due to iodine deficiency during pregnancy and is associated with dead fetus, poor suckling, weak, hypothermia, and abnormal wool or hair coat. In foals it is associated with prolonged gestation, poor ossification, ruptured tendons, contracted tendons, prognathism, and unthrifty and weak foals. It is seen in northwestern USA and western Canada. The etiology is not known, but it is associated with lush pastures. The thyroid gland is not grossly enlarged, but it is hyperplastic microscopically. In weanlings to 2-year-olds hypothyroidism is due to ingestion of excess iodine (supplements, kelp, etc.).

Hypothyroidism is uncommon to rare in adult horses, but is often diagnosed in overweight horses and fat ponies with "cresty" necks; it is not usually confirmed with lab data. The horses may have decreased TT4 and TT3, but rarely is endogenous TSH measured or stimulatory tests performed before the horses are empirically placed on thyroid supplements. Most of these horses probably have equine metabolic syndrome and are resistant to insulin from being overweight, a form of type 2 diabetes mellitus. Horses with equine metabolic syndrome test negative for hypothyroidism and equine Cushing's disease. Furthermore drugs such as phenylbutazone and food deprivation are known to lower serum thyroid hormones in the horse.

Signalment

Canine breeds

Golden retrievers, Doberman pinschers, dachshunds, Irish setters, miniature schnauzers, Great Danes, miniature poodles, boxers, Shetland sheepdogs, Newfoundlands, chows, English bulldogs, Airedales, cocker spaniels, Irish wolfhounds, toy fox terriers, giant schnauzers, Scottish deerhounds, and Afghan hounds may be regarded as high risk breeds for hypothyroidism. The disease may also occur in all other breeds regarded to be at lower risk. High-risk breeds may present as early as 2 years of age and low-risk

breeds after 5 years of age. Both sexes may be equally affected.

History and physical examination abnormalities

These are numerous and some combination of problems may be detected in hypothyroid dogs. These include weight gain to obesity without increased feed consumption, lethargy, dull haircoat, cold intolerance detected as heat-seeking behavior, decreased libido, reproductive failure, alopecia usually at wear points with no pruritus, and hyperpigmentation in areas of alopecia. Secondary skin diseases such as seborrhea, dry coat, and pyoderma may be observed. Uncommon clinical signs include keratoconjunctivitis sicca, polyneuropathy, vestibular disease, and facial nerve paralysis. Myxedema is uncommon, but is considered pathognomonic. Thyroid hormones stimulate the immune system and there is decreased T-cell immunity in hypothyroidism that may predispose to the secondary skin infections such as pyoderma, Malassezia, generalized demodicosis, and otitis externa.

The majority of dogs with lymphocytic thyroiditis present as just hypothyroidism, but other immune mediated diseases and/or endocrinopathies may appear concurrently. These may include lymphocytic adrenalitis, lymphocytic diabetes mellitus, hypoparathyroidism, and lymphocytic orchitis. Most of these will be detected as one endocrinopathy. However, in some cases a second or third endocrine disease is recognized months or years later.

Clinical signs in cats are similar to those in dogs. Clinical signs in dwarf kittens include disproportionate growth, large head, short broad neck, lethargy, retained deciduous teeth, and retained kitten hair coat.

Routine laboratory data

Routine laboratory test abnormalities are nonspecific and may include the following. Mild nonregenerative anemia (30% of cases) due to decreased responsiveness to erythropoietin is recognized in about 30% of cases. More cases may have a decreased hematocrit, but it is still in the reference interval. Increased liver enzymes are attributed to hepatic lipidosis that is often present in these dogs. Increases in muscle enzymes (CPK, LDH) are reported, but are not consistent. Hypertriglyceridemia and hyperlipidemia occurs in a majority of cases. Hypercholesterolemia is seen in approximately 80% of hypothyroid dogs and a serum cholesterol concentration greater than 500 mg/dL is very suggestive of hypothyroidism. A concentration >600 mg/dL in a dog with appropriate clinical signs is essentially diagnostic. Perhaps as many as 20% of the cases can be diagnosed based on sufficient clinical signs in a middle aged dog combined with a cholesterol >500 mg/dL and a TT4 <2 µg/dL. This will be enough for many veterinarians to diagnose and start treatment. "Confirmation" with a panel of TT4, fT4, and TSH may not be needed in cases this fully developed. Confirming the diagnosis of hypothyroidism with additional tests depends on how advanced the disease is.

Screening tests TT4, fT4; panel TT4 and TSH or fT4 and TSH on same sample

Basal concentration of TT4 should be the initial endocrine diagnostic test utilized when hypothyroidism is suspected. For convenience, TT4 is included in some routine chemistry panels. About 95% of hypothyroid dogs have decreased concentrations of TT4, resulting in a sensitivity 95%. Approximately 20% of dogs without hypothyroidism may also have decreased TT4, resulting in a false positive rate of 20% or specificity of 80%. Euthyroid sick dogs will account for most or all of these false positives (Fig. 32.2). Therefore, TT4 is an excellent screening test to rule out hypothyroidism because only 5% of hypothyroid dogs will have TT4 in the reference interval. If a panel is selected, choose fT4 and TSH performed on the same sample, fT4 by equilibrium dialysis (see Table 32.3). Interpretive guidelines are:

fT4 > 1.5 ng/dl or 20 pmol/L = typical of euthyroid in dogs

fT4 < 0.5 ng/dl or 7 pmol/L = typical of hypothyroidism in dogs

A dog with primary hypothyroidism late in the disease should have the following abnormalities (see Table 32.4.): decreased TT4 and fT4, increased concentration of TSH, and failure to increase TT4 in response to a TSH or TRH stimulation test, if it were performed. Ninety percent of these dogs will have lymphocytic thyroiditis or idiopathic follicular collapse. The glands will never regenerate and the dog will need lifelong medication. A dog with secondary hypothyroidism will have decreased TT4, fT4, and TSH. Decreased TSH results from the pituitary lesion that is destroying or crowding out thyrotrophs resulting in thyroid gland atrophy due to the absence of trophic hormone. Findings supportive of secondary hypothyroidism include decreased TSH, indications of other endocrine diseases, and visual or CNS signs.

It is important to not diagnose hypothyroidism on one endocrine test result in isolation. The test(s) must be combined with signalment, history, physical exam, and routine lab data to determine the likelihood of hypothyroidism (Tables 32.4 and 32.5). The more pieces of the puzzle that fit with hypothyroidism, the fewer tests are needed to diagnose and start treatment. If multiple abnormalities are present, especially if the results are marked, then a diagnosis can be made with confidence. The lower the concentration of TT4 and fT4, the greater the likelihood of hypothyroidism. For example, if the TT4 and fT4 are <10 nmol/L (<0.5 µg/dL) and <7 pmol/L (<0.5 ng/dL) respectively, the best diagnosis

Table 32.3 Expected results for canine thyroid profiles.

Reference intervals	TT4 nmol/L 20–55	fT4 pmol/L 10–45	TSH ng/mL <0.5	TT4 µg/dL 1.5–4.3
Hypothyroid likely	<11	<10		<1
Hypothyroid unlikely	>20	>15		>2
Gray zone	12–20			
Primary hypothyroid	<15	<10	>1.0	<1
Primary hypothyroid	<15	<15	>1.0	<1
Secondary hypothyroid	15	<10	UD	<1

UD = undetectable, below limit of detection, <0.03 ng/mL; if a lower limit of reference is set than values below this number are also confirmatory. Reference intervals and cut-off values used should be from the laboratory performing assays.

Table 32.4 General considerations for interpreting canine thyroid profiles.

Diagnosis/Test	T4	fT4	TSH	TSH Stimulation
Rule out hypothyroidism	RI	RI	RI	Not performed
Primary late	Dec	Dec	Inc	None to suppressed
Primary early	RI	Dec	Inc	None to suppressed
Euthyroid sick	Dec	RI	RI	Increased; mild illness
Euthyroid sick	Dec	Dec	Variable	Increased; severe illness
These above scenarios cover most cases; and the number of tests selected will depend on the veterinarian's experience and how characteristic the clinical signs and routine lab data are. Not all cases require all tests. The following are less commonly observed results that we spend considerable effort trying to understand and diagnose.				
Primary early	RI	Dec	RI	None to suppressed
Primary early	RI	RI	Inc	None to suppressed
Primary <2%	Inc	Dec	Inc	None to suppressed
Autoantibodies*	Inc	RI	RI	
Secondary	Dec	Iec	Dec	Pituitary lesion
Euthyroid sick	Dec	RI	RI	Mild illness
Euthyroid sick	Dec	Dec	Variable	Severe illness
False positive inc of TSH	RI	RI	Inc	Increased, doubles or >
Drugs	Dec	Depends on drug and mode of action		If excessive ingestion
Iodine intake	Dec	Dec	Inc	
Sight hounds	Normally dec to 1/2 of RI; they need their own RI			
Obese	Inc			
Young	2–5×	Inc		

Dec = decrease; Inc = increase; RI = reference interval.
*Auto-antibodies are not a thyroid function test; they may falsely increase TT4; they indicate lymphocytic thyroiditis is likely; some of these dogs progress into the hypothyroid state and many do not.

Table 32.5 Examples, use all the case data, here are some key components

Cases	RI	Cholesterol mg/dL 130–350	TT4 nmol/L 20–55	fT4 pmol/L 10–45	TSH ng/mL <0.5	Other tests	Diagnosis
1 alopecia		>500	<10	NN	NN	NN	Hypothyroid
2 alopecia		>500	<15	<10	NN	NN	Hypothyroid
3 alopecia		>400	<20	<5	NN	NN	Hypothyroid
4 alopecia		400	<25	<15	inc	NN	Primary hypothyroid
5 alopecia		400	<20	<15	?	?	Hypothyroid
6 alopecia		>300	<20	<10	RI	TSH	Stim needed
7 lethargy		>300	>25	NN	NN	NN	Rule out
8 lethargy		>300	<20	>20	RI	NN	Rule out
9 lethargy		>300	12	20	RI	NN	Rule out
10 lethargy		>400	<15	<10	Dec	Imaging?	Pituitary lesion
11 alopecia		>500	>55	<10	Inc	Antibodies	Primary hypothyroid
12 vague		RI	8	7	NN	NN	Normal for Saluki
13 vague		RI	RI	RI	RI	Positive for antibodies	

RI = reference interval; consult laboratory doing measurements for RI and recommended cut-off values used for diagnostic, gray zone, etc.
NN = not needed, this is the veterinarian's decision; the more characteristic the clinical signs and routine lab data are for hypothyroidism the fewer tests are needed.
Examples 1–4 = hypothyroidism confident of diagnosis but have not distinguished primary vs. secondary; primary much more likely, both treated the same.
Example 5 = hypothyroidism likely; additional testing discretion of the veterinarian.
Example 6 = hypothyroidism likely, TSH did not help recommend TSH stim.
Examples 7–9 = rule out hypothyroidism; if clinical signs or other data was strongly suggestive of hypothyroidism consider a TSH stim. Examples 8 and 9 are consistent with euthyroid sick syndrome; the decrease in TT4 is disproportionately lower than the expected change in fT4.
Example 10 = hypothyroidism, secondary ruled in; consider imaging.
Example 11 = primary hypothyroidism with increased TT4 due to antibodies; <2% of cases have this profile.
Example 12 = TT4 and fT4 diagnostic for hypothyroidism unless a sight hound breed, then a profile like this is normal; diagnostic ranges for sight hounds are approximately half that of other breeds.
Example 13 = dog likely has lymphocytic thyroiditis; dog is euthyroid at this time; some develop hypothyroidism and most do not; repeat testing recommended especially if from a breed with increase prevalence of hypothyroidism.

is hypothyroidism. If cholesterol is markedly increased and TT4 and fT4 are both decreased, and the dog has multiple signs, then this is sufficient for a diagnosis without further testing.

More challenging diagnostic scenarios

The guidelines above are for classic cases that are fully developed. However, use of thyroid hormone tests to confirm hypothyroidism can be frustrating because the disease can be in various stages of development and there are situations in which the test results present conflicting or ambiguous findings. Breed, superimposed disease, drug, and age variables influence results. However, in more than 80% of situations the results are definitive and it is possible to rule out or rule in hypothyroidism. A minority of cases requires more extensive testing evaluation, perhaps over a period of weeks to months. These challenging cases may require more intensive diagnostic procedures such as measurement of antibodies to thyroid antigens, repeat testing in 4 weeks, removal of concurrent drug therapy followed by repeat testing in 4 weeks, stimulation tests (TSH or TRH), imaging of the neck (US, MRI, technetium pertechnetate), and response to T4 trial replacement therapy. Sometimes, there is merit in trial therapy in place of extensive, complex testing. Unlike Cushing's disease, hypothyroidism is a disease that can be misdiagnosed, missed and/or treated for when it does not exist and the consequences are not great. Missing a diagnosis of hypothyroidism is not life-threatening and sometimes repeat testing in a month or more will yield clearer results as the disease progresses. Treating a dog with levothyroid that does not have hypothyroidism also does not have serious biologic consequences. In fact a term has evolved to categorize dogs

that respond well to levothyroid but actually do not have hypothyroidism—"thyroid responsive disease." Thyroid hormones may benefit sick patients similar to the nonspecific stimulatory effects of steroids. Perhaps accepting some of the above before testing panels are begun will make the 10–20% of gray zone cases less frustrating. Furthermore primary and secondary hypothyroidism are treated the same, with synthetic thyroxine (T4), levothyroid supplementation. This supplement is inexpensive and a fat dog may benefit from a little T4 regardless of their thyroid status. The important clinical distinction is to differentiate hypothyroidism from the euthyroid sick syndrome and recognize the correct concurrent disease that is suppressing thyroid hormones. Differentiating primary and secondary hypothyroidism can be done as well. Summary findings of a study of endocrine tests in 108 dogs are shown in Table 32.6. The effects of some variables on thyroid hormone testing are presented in Table 32.7.

A relatively common diagnostic challenge is to determine if a dog with a decreased TT4 is really hypothyroid when only some of the physical and laboratory abnormalities of hypothyroidism are present. This is the syndrome known as "euthyroid sick." The laboratory tests are abnormal, but function of the thyroid gland and the biologic activity of the thyroid hormones is considered normal or "euthyroid." This is a syndrome that occurs in dogs and cats in which a nonthyroid disease causes the suppression of measured thyroid hormones. The decrease in thyroid hormones is a physiologic adaptive response stimulated by a variety of cytokines that lower the basal metabolic rate and reduce cellular metabolism in times of illness. There are a variety of mechanisms postulated to decrease the thyroid hormones and some of these may even decrease TSH secretion. These animals will have decreased concentrations of TT4, but are euthyroid. A variety of illnesses can cause this syndrome and the more severe the illness the more pronounced is the decrease in TT4. Approximately 20% of sick dogs that do not have hypothyroidism will have decreased concentrations of TT4, a false positive test result for hypothyroidism. Free T4 is only decreased in 5–10% of these dogs, but similar to TT4 the more severe the illness the more the suppression in fT4. If the decrease in TT4 is disproportionately greater than the decrease in fT4 then it favors the interpretation of euthyroid sick syndrome. The key feature for considering euthyroid sick is decreased TT4 without supporting changes in fT4 and TSH. Another helpful assessment is that dogs with euthyroid sick syndrome are not expected to have the more specific clinicopathologic features of hypothyroidism such as hypercholesterolemia, alopecia, and weight gain. It is important to recognize this group in order to correct the primary disease that is suppressing thyroid hormones.

Table 32.6 Thyroid hormones in hypothyroid and euthyroid dogs.

	Hypothyroid		Euthyroid	Sensitivity/Specificity
Dogs n = 108	54		54	%
TT4 dec	48 = 90% 3 RI 3 inc	**Good**	10 = 18%	90/82
TT3 dec	3/31 = 10% 23 RI 5 inc	**Not good**	3/37 = 8%	
freeT4 dec	53 = 98% 1 RI	**Great**	4 = 7%	98/93
TSH inc	41 = 76% 13 RI = 24% 5 low normal = 9%*		4 = 7%	76/93

In this study only one euthyroid sick dog had low T4, low fT4, and increased TSH = false positive.
*Some of these 5 dogs may have had secondary hypothyroidism. RI = reference interval.

	Sensitivity	Specificity	Accuracy %
free T4	98	93	95
TT4	89	82	85
TSH	76	93	84

Summary from: *JAVMA* 211: 1396 (1997).
Author comment: The more fully developed a disease the easier it is for diagnostic tests to "recognize" the disease.

Table 32.7 Interpretation of thyroid hormones in dogs with unique situations.

Consideration	Effect
Body size	
<10 kg	Higher TT4; median 31.5 nmol/L
>30 kg	Lower TT4; median 25 nmol/L
Breed	
Sight hounds	Decrease TT4 and fT4 50% less than other dogs
Nordic breeds	No effect on TSH
Age	
<3months	Increase TT4 2–5 times adult
>6 years	Decrease TT4
Nonthyroidal illness	Decrease TT4 dogs and cats; the greater the severity of illness the greater the decrease
Nonthyroidal illness	fT4 influenced less but can decrease in dogs and cats; can also increase fT4 in cats
Drugs	Decrease TT4 and fT4: glucocorticoids, sulfonamides, propylthiouracil, aspirin, Phenobarbital, carprofen, methimazole,
	Decrease TT4, little or no effect on fT4: furosemide, phenylbutazone, progestagens
	Sulfonamides may decrease TT4 and fT4 and increase TSH and cause hypothyroidism

Obese dogs have mild increases in serum TT4 concentrations; 50–75% higher.
Sight hounds have TT4 and fT4 much lower than other breeds; normal concentrations for sight hounds would be hypothyroid values for other breeds.
Pregnancy and diestrus will increase TT4.
An age-related decline occurs in serum total TT4 concentrations and response to TSH stimulation in dogs.

The euthyroid sick syndrome is also recognized in cats, but the challenge presented is in the diagnosis of hyperthyroidism. The dilemma is recognizing hyperthyroidism when the secondary disease has suppressed TT4 into reference interval. The severity of the nonthyroid disease is proportional to the decrease in TT4. This happens in older cats with concurrent diseases such as chronic interstitial nephritis, cancer, and debility. Serum fT4 is not suppressed as much as TT4, so that measurement may be helpful. However, fT4 is actually increased in some severely sick cats which adds a new diagnostic challenge. If TT4 is in reference interval, fT4 is increased it is either true hyperthyroidism or the euthyroid sick syndrome. Distinguishing these two differentials requires correlation of all the data and perhaps additional testing. See hyperthyroidism discussion for these approaches. Hopefully the clinical expression of these nonthyroid diseases is such that they are recognized or suspected.

Another diagnostic challenge occurs is when the clinical and clinical lab abnormalities support hypothyroidism, but TT4 is increased or perhaps within the mid to upper reference interval. This creates endocrine testing data that is inconsistent with the clinical picture. It's more likely such a dog is in an early or mid-phase of primary hypothyroidism with lymphocytic thyroiditis and increased concentrations of antibodies directed against thyroid antigens. The way to solve this problem is to measure antibodies for aaTg and possibly TT4 or TT3. The antibodies crossreact with reagents in the in vitro measurement of T_4 and T_3, and therefore, cause a "false increase" in T_4 and T_3. These dogs are hypothyroid, yet will "appear" to have increased measurable-concentrations of T_4 and T_3 but, most importantly, increased concentrations of T_4 and T_3 antibodies.

Diagnostic challenge may occur as a result of ongoing drug therapy. Most of the problems caused by drugs are interference with assays and usually this is observed as a decrease in TT4 and/or fT4; see Table 32.7. Sulfonamides, however, can cause hypothyroidism characterized by decreased TT4, fT4 and increased TSH. Sulfonamides block iodination of thyroglobulin and prevent production of thyroid hormones if the dose is high enough and given 4 weeks or longer. Cessation of the sulfonamides reverses the effects. Glucocorticoids affect metabolism of thyroid hormones and inhibit TSH secretion resulting in variable combinations of decreased or normal concentrations of TT4, fT4, and TSH. Phenobarbital does not cause hypothyroidism, but does decrease TT4 and fT4, and may cause a slight increase in TSH. It is beyond the scope of this chapter to review all the influences of therapy on thyroid hormones. A general recommendation is to discontinue medications that affect the thyroid gland for 4 weeks before measuring thyroid hormones or doing dynamic testing. Levothyroid should be discontinued for 8 weeks before retesting the thyroid pituitary axis.

It is occasionally useful to determine if the hypothyroidism is secondary to a pituitary lesion. The determination is somewhat academic because thyroid replacement therapy is the same, but documentation of a pituitary lesion may

uncover additional endocrinopathies and have longer term case management and prognostic implications.

Sight hounds such as Salukis, greyhounds, whippets, Scottish deerhounds, Irish wolfhounds, Sloughis, and Basenjis have low serum TT4 that is physiologic and considered normal. Salukis and greyhounds also have low fT4 that are in the range of hypothyroidism for other breeds. TT4 may be below the lower limit of reference intervals for normal dogs in 90% of greyhounds and below the limit of detection in up to 33%. Therefore, the diagnosis of hypothyroidism in one of the sight hound breeds should include tests other than TT4. For sight hound breeds it may be more important to measure TSH and fT4 concurrently no matter how low the TT4 concentration is. However, hypothyroidism can be ruled out in a sight hound breed with confidence if TT4 is within the reference interval used for all dogs. Cut-off values for TT4, fT4 and TSH for hypothyroidism in the sight hound breeds are not established. The cause for low thyroid hormones in sight hound breeds is not known.

Diagnosis in cats

Spontaneous or naturally occurring hypothyroidism is almost nonexistent in cats. Congenital hypothyroidism, lymphocytic thyroidits and secondary hypothyroidism are reported, but are rare. The most common cause of true hypothyroidism is iatrogenic from treatment of hyperthyroidism. The most common cause of decreased TT4 and/or fT4 in a cat is euthyroid sick syndrome. For true hypothyroidism, essentially all adult cats should have a history of thyroidectomy, radiation, or methimazole treatment. In most of these, thyroid hormones are being assessed periodically to gauge success of treatment. Nonregenerative anemia and hypercholesterolemia are expected if the cat is hypothyroid. TT4 is the initial step and if in reference interval the cat is euthyroid sick. If it is decreased and the history has an iatrogenic cause, then it is hypothyroid. These cats probably just need their medications adjusted. If TT4 is decreased and there is no history of thyroidectomy, it is either true hypothyroidism or more likely the euthyroid sick syndrome. The most common cause of a decreased TT4 in a cat that has not undergone thyroidectomy is a concurrent illness. Now consider finding the nonthyroid disease and/or evaluate fT4 and TSH via a canine assay. The canine assay for TSH has been validated for cats. If fT4 is decreased and TSH is increased and the clinical signs and laboratory fit then it is hypothyroid. If in doubt consider a TSH or TRH stimulation test or a trial response to levothyroxine, followed with removal of levothyroxine to see if clinical and laboratory abnormalities return.

Horses and small ruminants

Hypothyroidism in large animals is almost always due to the intake of some exogenous substance that interferes with the production of TT3 and TT4. These animals have goiter, characterized as a mild to massive thyroid gland enlargement with decreased TT3 and TT4 and associated hypothyroidism. Many substances can do this, and they interfere with the production of thyroid hormones at various stages. A few of the more common and/or high-profile substances are sulfa compounds, decreased iodine intake, increased iodine intake, plants (kale, seaweed), and various chemical substances (thiouracil). The decreased production of TT3 and TT4 due to these substances results in reduced or no negative feedback to the hypothalamus and pituitary gland and, therefore, increased TSH production. The increased TSH stimulates follicular cell hypertrophy and hyperplasia, resulting in an enlarged, goitrogenic thyroid gland. Goiter in neonates is the most common thyroid disorder in the horse and small ruminant. In small ruminants it is usually due to iodine deficiency during pregnancy and is associated with dead fetus, poor suckling, weakness, hypothermia, and abnormal wool or hair coat. In foals it is associated with prolonged gestation, poor ossification, ruptured tendons, contracted tendons, prognathisim, and unthrifty, weak foals. It is seen in northwestern USA and western Canada. The etiology is not known, but it is associated with lush pastures. The thyroid gland is not grossly enlarged, but it is hyperplastic microscopically. In weanlings to 2-year-olds hypothyroidism is due to ingestion of excess iodine (supplements, kelp etc.). It is uncommon to rare in adult horses. It is often clinically diagnosed in overweight horses with "cresty" necks, but is not usually confirmed with lab data. The horses may have decreased TT4 and TT3, but TSH measurement or stimulatory tests are rarely performed before the horses are placed on thyroid supplements, reduced feed intake, and increased exercise. It is difficult to know which of these treatments is responsible for clinical improvement.

Adrenal gland disorders

Hypoadrenocorticism: Addison's disease

Primary

Lymphocytic adrenalitis—destroys all three zones of adrenal cortex; accounts for 90% or more of cases.

Secondary

Pituitary neoplasm or prolonged exogenous steroid; either decreases ACTH causing bilateral adrenocortical atrophy of zona fasiculata and reticularis.

Hyperadrenocorticism: Cushing's syndrome

Primary

Adrenal dependent—functional adrenal neoplasm—one large adrenal and atrophy of contralateral adrenal.

Secondary

Pituitary dependent—functional pituitary neoplasm, secretes ACTH stimulating bilateral adrenal gland hyperplasia and hypertrophy; accounts for 80% or more of cases.

Adrenal gland background

The adrenal gland has a cortex and a medulla. The outer most region of the cortex is the zona glomerulosa (ZG). It produces the mineralocorticoid aldosterone that helps regulate the serum concentration of sodium and potassium, extracellular fluid volume, and blood pressure. The major regulation mechanism is via serum potassium concentrations and by the renin-angiotensin system, with a minor contribution from ACTH. Hyperkalemia will stimulate the release of aldosterone from the ZG to increase potassium excretion via many epithelial cells including renal, salivary, intestinal, and sweat glands. Concurrently aldosterone stimulates renal sodium reabsoprtion that may increase blood pressure. Renin is released from the juxtaglomerular apparatus near glomeruli in response to decreased blood pressure, decreased sodium, and several other factors. Renin then stimulates a cascade of events that leads to increased angiotensin II that stimulates vasoconstriction and release of aldosterone. The majority of the stimulus to release aldosterone comes from the steps outlined above. Approximately 10% of the overall stimulus to release aldosterone comes from ACTH and in the absence of ACTH there is mild atrophy of the ZG, but severe atrophy of zona fasiculata (ZF). The major disease of the ZG is hypoadrenocorticism, or Addison's disease, due to lymphocytic adrenalitis. An uncommon disease is hyperplasia of the ZG, primary hyperaldosteronism that increases aldosterone production.

Subjacent to the ZG is the largest region of the cortex, the zona fasculata (ZF) producing many hormones of which glucocorticoids are the most clinically important. Corticotrophs in the pituitary adenohypophysis produce ACTH that stimulates ZF to release glucocorticoids immediately. Glucocorticoids complete the regulatory loop by providing negative feedback to (1) corticotrophs which decrease ACTH secretion and (2) receptors in the paraventricular nuclei in the hypothalamus to decrease corticotrophic release hormone. The major disease of the ZF is hyperadrenocorticism or Cushing's disease. This is usually secondary to a pituitary adenoma that secretes excessive amounts of ACTH. Hyperadrenocorticism may also be caused by an adrenal cortical tumor that autonomously produces glucocorticoids or iatrogenically by the exogenous administration of glucocorticoids. Regardless of the cause, all of the clinical signs and laboratory abnormalities of hyperadrenocorticism are due to increased serum cortisol. The inner most region of the adrenal cortex is the zona reticularis (ZR) that produces glucocorticoids and sex hormones. In dogs, cats, and horses this zone has a minor role in hyperadrenocorticism. In ferrets, this zone has a significant contribution because most of the clinical abnormalities are due to increased sex steroids.

The medulla is in the middle of each adrenal gland, it produces epinephrine and norepinephrine. The major disease is neoplasia of the adrenal medulla or pheochromocytoma that causes increased serum epinephrine production and resultant hyperactivity, hypertension, and tachycardia.

Hypoadrenocorticism: Addison's disease

The following is an outline of the two types of hypoadrenocorticism with expected major abnormalities and associated diagnostic test findings (Table 32.8).

Primary

Typical: lymphocytic adrenalitis that destroys the zona glomerulosa, fasiculata and reticularis; there is decreased mineralocorticoids and glucocorticoids; hyponatremia and hyperkalemia due to decreased aldosterone. The sodium : potassium ratio is most useful in these cases. A rare variant is *atypical primary hypoadrenocoriticism* or *glucocorticoid-deficient hypoadrenocorticism*. This may be the early form of primary, but in which most of the manifestations are referable to glucocorticoid deficiency. Some of these cases will develop mineralocorticoid deficiency, but some do not.

Table 32.8 Major laboratory findings in various forms of hypoadrenocorticism.

	Primary	Atypical	Secondary
Lesion	Lymphocytic adrenalitis: ZG ZF ZR	Early?	Atrophy ZF
Basal cortisol	Dec	Dec	Dec
ACTH stimulation	No response, post stimulation cortisol <2 µg/dL for all types		
Plasma ACTH	Inc	Inc	Dec
Na	Dec	RI	RI
K	Inc	RI	RI
Cl	Dec	RI	RI
Na : K ratio	<23:1	>25:1	>25:1
Glucocorticoids	Dec	Dec	Dec
Mineralocorticoids	Dec	OK	OK
Outcome	Irreversible	Progressive?	Reversible

Secondary

Either a pituitary lesion or prolonged exogenous steroid administration followed by rapid withdrawal causes decreased ACTH and atrophy of zona fasiculata and reticularis; glucocorticoids are decreased, mineralocorticoids and sodium potassium ratio are not affected.

Primary

Primary hypoadrenocorticism accounts for approximately 90–95% of Addison's disease in the dog. The two most common lesions, lymphocytic adrenalitis and idiopathic atrophy, represent different stages of the same disease. Early, the lesion is an immune mediated lymphocytic adrenalitis and in the late stage, it is severe atrophy, similar to primary hypothyroidism. The adrenal cortex cannot regenerate from these destructive lesions and therefore these patients require lifelong replacement therapy. Lymphocytic thyroiditis, parathyroiditis, adrenalitis, and lymphocytic destruction of islets are processes that occur in dogs usually as separate diseases. It is rare that they occur in the same animal and produce polyglandular failure. Failure of multiple endocrine glands is more likely due to pituitary destruction. Infrequent other causes of primary hypoadrenocoriticism include neoplasia, granulomatous inflammation, infarction, and iatrogenic chemotherapy (mitotane, trilostane). Mitotane (lysodren) is used in the treatment of hyperadrenocorticism and it selectively causes necrosis of zona fasiculata and reticularis. In about 5% of treated dogs or in overdosed dogs destruction of the zona glomerulosa may occur. In the majority of these dogs the necrosis is permanent. The cortical zones do not regenerate and the dogs subsequently require lifelong therapy with mineralocorticoids and glucocorticoids. This is a separate situation from transient glucocorticoid deficiency associated with induction or maintenance therapy with Mitotane. Farm animals may have herpes virus induced cortical necrosis or bacterial emboli from neonatal septicemia that destroy sufficient adrenal cortex to produce hypoadrenocorticism. Additionally there is a condition referred to as relative adrenal insufficiency syndrome that is diagnosed in weak and/or septic foals.

Approximately 75–90% of both adrenal cortices must be destroyed before clinical signs are observed. Partial deficiency is probably the early stage of lymphocytic adrenalitis and is one explanation for primary atypical cases when there is cortical reserve, but the reserve is inadequate to cope with stresses such as shipping, boarding, and fights. Like all diseases it can be difficult to establish a definitive diagnosis while the disease, the clinical signs, and clinical pathology data are evolving. Eventually the lesions progress and the clinical signs and laboratory data become fully developed and more clearly diagnostic.

Atypical—glucocorticoid-deficient hypoadrenocorticism

These cases could represent either secondary hypoadrenocorticism in which the cause of the decrease in ACTH is not known or is an unusual form of primary hypoadrenocorticism. In the latter possibility they most likely represent early phases of lymphocytic adrenalitis when there is adequate cortical reserve such that some, but not all manifestations of primary hypoadrenocorticism are present. It is also associated with drugs that selectively attack zona fasiculata (mitotane and trilostane) and concurrent diseases that mask the characteristic electrolyte abnormalities. These dogs initially do not have serum sodium potassium abnormalities. Some of these dogs will progress and have a decreased sodium potassium ratio while others do not develop electrolyte abnormalities even after a year or more of follow up. Because of increased awareness of this subtype of hypoadrenocorticism, ACTH stimulation is performed on dogs with vague clinical signs coupled with suspicion of the disease. Failure of ACTH stimulation to increase cortisol >2 µg/dL is diagnostic of hypoadrenocorticism even if sodium and potassium concentrations are normal.

Secondary

The lesion of secondary hypoadrenocorticism is atrophy of zona fasiculata. Naturally occurring cases are caused by a primary pituitary lesion such as a tumor or cyst. The process destroys corticotrophs in the pars distalis resulting in decreased ACTH production (Fig. 32.4). Probably the most common cause of secondary hypoadrenocorticism is iatrogenic from the sudden withdrawal of exogenous steroid therapy. Prolonged exogenous cortisol therapy causes negative feedback to corticotrophs, decreased serum ACTH and subsequent atrophy of zona fasiculata. In contrast to irreversible lymphocytic adrenalitis, the adrenal cortex will regenerate if steroids are either gradually stopped or if the secreting pituitary tumor is surgically removed. Atrophy is in ZF and ZR, therefore there is glucocorticoid deficiency, but ZG and mineralocorticoids are spared. Atrophy may be secondary to injectable, oral, or topical administration of glucocorticoids. Cortical function usually returns approximately 2–4 weeks after these medications are stopped. Longer acting steroids are potent suppressants and may suppress the adrenal pituitary axis for 6 weeks or longer.

Clinical signs

Usually dogs are young to middle aged, 3–6 years. It can occur in dogs and cats as early as 2–3 months of age. Seventy percent are females and intact females have a greater prevalence other than for standard poodles, Portuguese water dogs, and bearded collies. There are multiple breeds associated with this disease; several with a higher risk are standard poodles, Great Danes, Rottweilers, West Highland white terriers, Saint Bernard, Nova Scotia duck trolling retrievers, bearded collies, and Portuguese water dogs. Inheritance of the disease is established for standard poodles, Portuguese water dogs, Nova Scotia duck trolling retrievers, and bearded collies; chromosomes and loci locations have even been

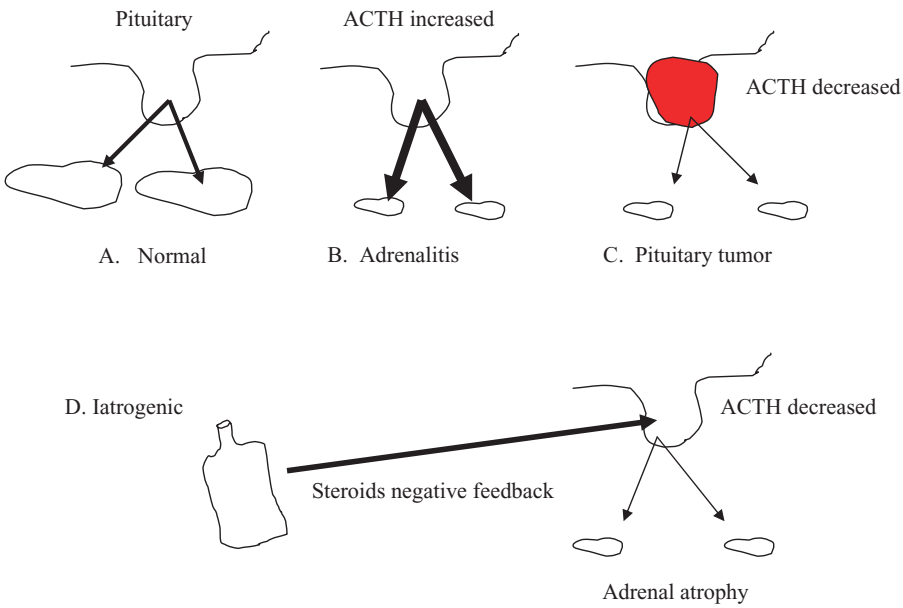

Figure 32.4 Mechanisms of hypoadrenocorticism (Addison's): Arrows represent relative amounts of hormones being secreted with a normal pituitary-adrenal axis and in three disease states. Adrenal gland sizes are relative for normal (A), primary Addison's caused by adrenalitis (B), and two types of secondary Addison's disease (C, D). In primary adrenalitis (B) an immune mediated process destroys all three zones of the adrenal cortex resulting in decreased cortisol and therefore no negative feedback to the pituitary that results in increased production and secretion of ACTH. If a pituitary tumor (C) destroys corticotrophs in the pars distalis, the result is decreased ACTH and bilateral adrenocortical atrophy. Iatrogenic Addison's (D) is also due to exogenous steroids that cause negative feedback to the pituitary resulting in decreased ACTH and bilateral adrenocortical atrophy. These latter patients may appear Cushingoid on physical examination due to excess steroids, but if the steroids are stopped too abruptly the atrophic adrenal glands cannot respond rapidly enough to stress and the patient may have an Addisonian crisis.

identified in Portuguese water dogs. The disease is rare in cats and therefore no age or sex predisposition has been recognized. Some of the clinical signs are due to deficiency in glucocortiocoids and some are due to mineralocortioids and electrolyte abnormalities or combinations. Lethargy, weakness, vomiting, diarrhea, abdominal pain, and anorexia are due to glucocorticoids. Bradycardia is due to hyperkalemia and therefore decreased mineralocortioids. Polyuria and polydipsia is due to chronic hyponatremia leading to renal medullary washout. Microcardia is due to hypovolemia and hypotension from the hyponatremia, as are decreased blood pressure, lethargy, nausea, and depression.

The history often indicates the dog has periodic bouts of not feeling good, vomiting, anorexia, lethargy, and either recovered spontaneously or required treatment with fluids and steroids. A history reporting some of the problems listed above, especially if they recur and respond to symptomatic therapy of cage rest, fluids and steroids is "classic" for chronic hypoadrenocorticism. Symptomatic treatment of a sick vomiting dog often includes fluid therapy and steroids, which, in the case of an Addisonian, is an ideal symptomatic treatment. A differential diagnosis for dogs that appear to have renal disease, gastrointestinal upset, and/or a "garbage hound syndrome" should include hypoadrenocorticism, especially if clinical symptoms are vague and recurring. Addison's and renal disease mimic each other clinically and in much of the routine lab data: 90–95% of dogs with Addison's are azotemic, the majority have hyperphosphatemia, and many cannot adequately concentrate their urine. Therefore these Addisonian patients may be clinically indistinguishable from patients with renal failure. Other signs seen in hypoadrenocorticism include weakness, muscle tremors, intestinal and gastric bleeding, shaking, and hypoglycemic seizures.

Some cases present as a medical emergency in an "Addisonian crisis": total collapse, weak pulse, dehydrated, shocky, hypothermic, and with marked bradycardia. The paradoxical combination of bradycardia and shock should trigger the differential of hypoadrenocorticism.

Dogs and cats with a pituitary lesion will have nonspecific signs and lab data referable to decreased glucocorticoids because mineralocorticoids are spared. However, patients on steroid therapy may have clinical signs and laboratory data that look like hyperadrenocorticism due to the excess cortisol they were receiving. These dogs may have alopecia, pot belly appearance, and prominent cutaneous blood vessels due to the cortisol as well as increased serum alkaline phosphatase and dilute urine. If steroids are stopped abruptly the patient may collapse, have hypoglycemia and signs of decreased cortisol. The atrophic ZF cannot respond quickly enough to produce sufficient cortisol to prevent the crisis.

Table 32.9 Laboratory abnormalities of hypoadrenocorticism.

Lab abnormality	% of dogs	Related to or caused by
Hyponatremia	60–80	Aldosterone decreased
Hyperkalemia	95	Aldosterone decreased
Na:K <23:1*	95	Aldosterone decreased
Hypochloremia	50–75	Aldosterone decreased
Hyperphosphatemia	90	Dehydration
Hypercalcemia	33	Obscure
Hypocalcemia	10	Hypoalbuminemia
Azotemia	90–95	Prerenal
Hypoglycemia	20–30	Glucocorticoids
Increased albumin	50	Dehydration
Increased serum protein		Dehydration
Decreased albumin	10+	Unknown
Urine s.g. variable; <1.030	60	Medullary washout
No stress leukogram	90	Glucocorticoids decreased
Anemia	10–30	Glucocorticoids; GI bleeding
Polycythemia		Dehydration

*Na:K <15:1, when ratio is this low Addison's is the most likely diagnosis, but it is still not pathognomonic and may be seen with other diseases.

Although steroid usage has stopped, and cortisol has decreased, it will take some time before the cortioctrophs in the pars distalis produce ACTH. Even under the influence of ACTH there is a further delay in glucocorticoid production as the ZF must regenerate. During this phase the dog or cat is susceptible to a crisis should a stressful event happen: shipping, fight, etc.

Routine clinical laboratory data

A summary of expected routine laboratory findings is presented in Table 32.9.

Some of the laboratory data are caused by deficiency of glucocorticoids, while others are caused by mineralocorticoid deficiency. Therefore dogs with a pure glucocorticoid deficiency (secondary) or those with atypical Addison's do not exhibit the classical problems of mineralocorticoid deficiency such as reduced Na:K ratio and associated bradycardia.

CBC

Absence of a stress leukogram or a totally normal leukogram in a sick/stressed dog may be the first indicator, but is often overlooked. Eosinophilia and mild lymphocytosis can be attributed to decreased glucocorticoids and therefore may be present in both primary and secondary cases. Red blood cell parameters can vary from a mild, nonregenerative anemia to a low-normal PCV that then enters the anemic range after fluid therapy. The anemia is attributed to lack of steroid stimulus to the bone marrow and gastrointestinal bleeding. If the anemia is more severe, look for gastrointestinal bleeding or a second disease. Less frequently there may be polycythemia—PCV 60–70 due to dehydration. There is no direct effect from mineralocorticoids on the CBC.

Urinalysis

The urine specific gravity is expected to be increased due to dehydration, but in about 60% of cases it is <1.030. Infrequently it is even hyposthenuric in primary Addison's due to "medullary washout" from chronic hyponatremia. The renal medulla is not saturated adequately with sodium ions and therefore tubules cannot passively reabsorb water from the glomerular filtrate. Dogs with medullary washout may not be able to concentrate their urine adequately even with concurrent dehydration. Dilute urine or at least urine with a specific gravity of 1.020 or less while the patient is dehydrated, coupled with azotemia and hyperphosphatemia, leads to a logical, but erroneous, diagnosis of chronic renal failure. If fluid therapy rapidly reverses the azotemia the patient did not have true chronic renal failure, it had prerenal azotemia. Prerenal azotemia was not recognized because the urine was dilute due to a concurrent problem of medullary washout. The remainder of the urinalysis in cases of primary hypoadrenocorticism usually has no abnormalities and if abnormalities are detected, they are unrelated to hypoadrenocorticism. Long-term steroid administration leading to secondary hypoadrenocorticism in dogs may predispose them to development of cystitis and associated inflammatory elements in the urinalysis.

Clinical chemistry

Results for clinical chemistry vary depending on if it is primary or secondary hypoadrenocorticism (Table 32.9). Hyperkalemia and hyponatremia are the classical abnormalities and the hallmark of primary hypoadrenocorticism. A Na:K ratio <23:1 is the key abnormality to indicate primary hypoadrenocortisim and is present in up to 95% of the cases. If the ratio is <15:1 it is very suggestive for hypoadrenocorticism, but still not by itself pathognomonic for the diagnosis. Other ratios reported include <27:1 or <25:1; normal ratios are 27:1 to 40:1. Most of the ratio shift is due to hyperkalemia, present in 95% of cases rather than hyponatremia, present in 80% of the cases. Cases of secondary hypoadrenocorticism and early primary ("atypical" hypoadrenocorticism) should not have these electrolyte abnormalities; therefore some estimate that up to 30% of all types of canine Addisonian's will not have abnormal Na:K ratios. Hypochloremia is also present and may be below100 mEq/L.

Azotemia is reported in up to 95% of cases and is almost always due to dehydration and prerenal azotemia. Dehydration is due to hypovolemia, fluid loss, and decreased aldosterone. Renal failure is an obvious differential diagnosis and

must be ruled out via urinalysis, UN:Ct ratio, and response to fluid therapy. Hyperphosphatemia is also due to dehydration, decreased GFR, and prerenal azotemia. The differentiation of prerenal versus renal azotemia is critical in these patients and is best done by examination of urine specific gravity and response of the azotemia to fluid therapy. If the urine s.g. is >1.030 in a dog and 1.035 in a cat that is azotemic it indicates adequate concentrating ability and prerenal azotemia. Renal azotemia is associated with urine s.g. in the 1.007–1.020 range. However, many cases of hypoadrenocorticism have urine s.g in the 1.015–1.030 range and in these patients this range combined with azotemia makes the distinction confusing because it suggests primary renal failure. The reduced ability to concentrate urine is due to chronic hyponatremia and medullary washout. If the serum UN is disproportionately increased compared to serum creatinine, a UN:Ct ratio >25, it suggests prerenal azotemia rather than renal azotemia. Lastly, if azotemia is corrected rapidly by fluid therapy this indicates prerenal azotemia.

Hypercalcemia is seen in approximately one-third of dogs with hypoadrenocorticism. When present with azotemia and a decreased Na:K ratio the hypercalcemia helps favor the diagnosis of hypoadrenocorticism over renal failure. The pathogenesis of hypercalcemia is not clear, it is considered multifactorial due to increased calcium absorption from the GI tract and urine filtrate in the absence of glucorticoids (cortisol promotes calciuria), increased serum citrate permits more calcium to be complexed in the serum, and the cortisol effect of inhibition of osteoclastic bone resorption is not present. Ionized calcium was increased in five of seven dogs that had increased total serum calcium and there were no consistent increases in PTH, PTH rp, or 1,25 dihydroxyvitamin D concentrations to explain the hypercalcemia. The hypercalcemia seen with hypoadrenocorticism is moderate, 12–15 mg/dL as compared with the more marked hypercalcemia characteristic of hyperparathyroidism or hypercalcemia of malignancy. Hypoglycemia is present in about 10–30% of the cases and is due to decreased glucocorticoids. Hypoalbuminemia or hyperalbuminemia can be seen. Hyperalbuminemia is due to dehydration; hypoalbuminemia is difficult to explain in light of hypovolemia and hemoconcentration, but is reported to be present in 10–40% of the cases. Possible causes of hypoalbuminemia may include intestinal hemorrhage, concurrent protein losing enteropathy, or hepatopathy. Mild to moderate increases in liver enzymes in 30–50% of dogs with primary is also difficult to explain and is probably nonspecific. They resolve post treatment of the hypoadrenocorticism. Total carbon dioxide and bicarbonate concentrations are decreased due to decreased tissue perfusion and decreased tubular excretion of hydrogen ions secondary to decreased aldosterone. The metabolic acidosis contributes to hyperkalemia because potassium shifts out of cells in exchange for movement of hydrogen ions into cells in an attempt to buffer acidosis.

Addison's disease is nicknamed the great pretender because it mimics so many other diseases. These include GI disturbances, liver disease, and in particular renal failure. As mentioned, over 90% of dogs with Addison's have azotemia and some have a concurrent urine s.g. suggestive of renal failure. However, if fluid therapy corrects the azotemia within hours or a day then it was not renal failure. Another "clue" to support hypoadrenocorticism rather than renal failure is hypercalcemia which is present in about one-third of the dogs with Addison's. Hyperkalemia can occur with acute renal failure, but concurrent hyponatremia is seen much more frequently with hypoadrenocorticism than acute renal failure. Urethral obstruction in male cats will produce azotemia and a low Na:K ratio that is due to marked hyperkalemia, but these cats are not hyponatremic and the diagnosis of a urethral obstruction is obvious from physical examination. Uroabdomen will produce hyponatremia and hyperkalemia, Na:K ratios <23, and azotemia. Identification of uroabdomen is done by concurrent measurement of creatinine in peritoneal fluid and blood with peritoneal fluid concentrations being greater than blood. Differential diagnoses for these electrolyte abnormalities and tests to help differentiate diagnoses are presented in Table 32.10.

Screening tests—basal cortisol and ACTH stimulation

ACTH stimulation flat line, <2 µg/dL rule in; basal cortisol decreased, <1 µg/dL 100% sensitivity 98% specificity, < 2 µg/dL 100% sensitivity and 78% specificity; basal cortisol >2 µg/dL rule out.

Basal cortisol is decreased and is an adequate screening test for primary and secondary hypoadrenocorticism due to a pituitary lesion. However, if the cause of secondary hypoadrenocorticism is exogenous steroids then basal cortisol may be increased if the steroid crossreacts with the assay (hydrocortisone, prednisone, and prednisolone) or decreased if the steroid used does not crossreact with the assay (dexamethasone). If glucocorticoids are being given to treat a suspected patient they should be stopped for at least 24–48 hours before cortisol is measured. If a steroid is used select dexamethasone as it does not crossreact with assays for cortisol and one dose of dexamethasone at 5 mg/kg causes only mild decreases in cortisol post ACTH stimulation. Basal cortisol will be less than 1 µg/dL in 85% of canine cases and less than 2 µg/dL in 90+% of cases. A basal cortisol <1 µg/dL has 100% sensitivity and a specificity of 98% in dogs, excellent. A basal cortisol <2 µg/dL still has a 100% sensitivity but the specificity is reduced to 78% indicating there will be some dogs that have serum cortisol <2 µg/dL that do not have hypoadrenocorticism and therefore are false positives, about 22%. If the basal cortisol is >2 µg/dL it is highly unlikely the dog has hypoadrenocorticism and the differential can be ruled out. The gray zone for basal cortisol is 1–2 µg/dL and when values fall in this range ACTH stimulation is needed. In cats the pre and post cortisol concentrations should be

Table 32.10 Differential diagnosis (DDx) for Na:K less than 25:1.

Hypoadrenocorticism
Uroabdomen
Urethral obstruction with intact bladder
Renal failure—acute, chronic
GI disease—whip worms, salmonella; calves and foals with diarrhea
Spurious—due to failure to separate serum and cells

Practical DDx are above this line and a more complete list is below; most of these cause the ratio shift by inducing hyperkalemia:
Severe acidosis
Chlyothorax—especially with repeated drainage
"Third space" expansion by any cause: pregnancy, pleuritis, ascites (with or without drainage)

Spurious—from RBCs and/or platelets and leukocytes
- Unique breeds and species have potassium-rich RBCs
- Dogs—Akita, Sheba, and others; potassium rich RBCs
- Horses—Young RBCs; certain breeds
- Sheep—certain breeds; potassium rich RBCs
- If there is hemolysis or if the RBCs are not separated from the plasma, then K will leach out of the RBCs increasing the concentration of K in the plasma/serum. Separate serum from RBCs to prevent this event

Leukocytosis >100,000/µL
Thrombocytosis >1,000,000/µL
Phosphofructokinase Deficiency—Springer spaniels with respiratory alkalosis
Variety of GI diseases—diarrhea; gastric dilation volvulus
Release of potassium: crush injury, aortic thrombosis, rhabdomyolysis, heat stroke
Diabetes mellitus

Tests to perform to rule in or rule out the common differentials:

Differential Dx	Test to RI/RO
Hypoadrenocorticism	Basal cortisol; ACTH stimulation
Uroabdomen	Compare serum and abdominal creatinine
Urethral obstruction	Male, cat, history, anuria, palpation
Renal failure	All data, urinalysis and response to fluids
GI disease	Fecal for parasites and culture
Spurious	Separate serum from blood

Table 32.11 ACTH response test: baseline measurement of cortisol, inject ACTH then measure cortisol again; Addisonian patients fail to increase cortisol in response to exogenous ACTH.

Reference interval (RI) cortisol µg/dL (or nmol/L) Dogs

Basal normal (RR) 0.5–6 µg/dL (10–160 nmol/L)

Basal cortisol >2.0 rule out hypoadrenocorticism

Post ACTH stimulation:

Normal post stim 6–18 µg/dL; 2–3 × increase in dogs is a normal response

Hypoadrenocorticism <2.0 flat line

ACTH stim does not differentiate primary from secondary Addisonian and endogenous ACTH will aid this differentiation:
- Adrenal dependent (common) = Increased ACTH
- Pituitary dependent (rare) = Decreased ACTH
- Iatrogenic = Decreased ACTH

<2.0 µg/dL to confirm hypoadrenocorticism. Only primary hypoadrenocorticism and iatrogenic hypoadrenocorticism have been observed in cats.

The gold standard to confirm diagnosis of hypoadrenocorticism is the ACTH stimulation test. Both primary and secondary Addisonian's fail to respond to exogenous ACTH by either doubling their cortisol or increasing post stimulation cortisol beyond 2 µg/dL (Tables 32.11 and 32.12). ACTH stimulation is expensive, but it is the gold standard and the case management decision at stake is lifelong therapy with mineralocorticoid and glucocorticoid medications. Furthermore the use of a low-dose ACTH stimulation test reduces cost. If there are inconsistent clinical findings or laboratory data, then ACTH stimulation should be performed. Basal cortisol is an excellent screening test to rule out hypoadrenocorticism. A basal cortisol of >2 µg/dL (>60 nmol/L) will rule out hypoadrenocorticism.

If the Na:K ratio is decreased and other clinical and lab data fit with primary hypoadrenocorticism then a basal cortisol <1 µg/dL is probably sufficient to rule in primary hypoadrenocorticism. Another important consideration is that the dog or cat was not given steroids, mitotane, ketaconazole, or other drugs that interfere with steroid production. Samples should be collected for basal cortisol and routine lab data before treatment is started and based on the results of these tests decide if ACTH stimulation is needed.

Secondary hypoadrenocorticism, glucocorticoid deficiency with normal Na:K ratio, should be confirmed with ACTH stimulation. Although basal cortisol will be decreased in cases of secondary hypoadrenocorticism caused by a pituitary lesion, the clinical signs and laboratory data are too nonspecific to rely on just a basal cortisol. Secondary hypoadrenocorticism due to iatrogenic use of steroids requires ACTH stimulation and knowledge of the type of steroid used. Depending on the steroid used, the basal cortisol measured could be decreased or increased.

Table 32.12 Expected results for primary and secondary hypoadrenocorticism.

	Na:K Ratio	ACTH Stimulation Cortisol µg/dL			Adrenal Lesion
		Basal	Post	e-ACTH	
Primary	<25:1	<1.0	<1.5	>300 (>40 pmol/L)	Adrenalitis zg, zf, zr
Secondary Pituitary lesion	>27:1	<1.0	<1.5	<20 (<2 pmol/L)	Atrophy zf
Secondary Iatrogenic	>27:1	<1.0*	<1.5	<20 (<2 pmol/L)	Atrophy zf
Reference interval	27:1 to 40:1	0.5–6	6–18	20–100 pg/mL (2.2–20 pmol/L)	Normal

Either basal cortisol less than 1.5–2.0 µg/dL and/or post stimulation cortisol of <1.5–2.0 µg/dL are findings indicative of hypoadrenocorticism. Consult with the reference lab for specific recommended cut-off values. Consult with the reference lab for reference intervals and cut-offs for e ACTH, as these vary with the methodology.

*This value could be increased if the steroid used crossreacted with the assay for cortisol, e.g., prednisone, prednisolone, hydrocortisone.

ACTH stimulation requires a basal sample and samples at 30, 60, 90 or 120 minutes post ACTH administration depending on the protocol used. Failure of ACTH stimulation to either double the basal cortisol or to increase it above 2.0 µg/dL is diagnostic of hypoadrenocorticism in dogs and cats. It is recommended to consult the reference lab used for the diagnostic guidelines they use for pre and post ACTH cortisol values for diagnostic purposes. Common protocol recommendations include the following. Collect a serum/plasma sample for prestimulation basal cortisol. For a high dose ACTH procedure, administer 0.25 mg (250 µg) of synthetic ACTH IV to dogs and cats >5 kg and 0.125 mg IV if <5 kg. Collect a second sample for cortisol in 30–60 minutes post ACTH for dogs. For cats collect two samples post ACTH, one at 60 minutes and again at 90–120 minutes. A low-dose ACTH stimulation protocol uses synthetic ACTH (cosyntropin) at 5 µg/kg IV in dogs. Collect a basal sample and the second sample 60–90 minutes later.

Protocols for intramuscular ACTH that are used to diagnose hyperadrenocorticism may not be as reliable in Addisonian suspects as ACTH absorption may be impaired due to hypovolemia and poor hydration. For intramuscular protocols collect a serum sample for prestimulation basal cortisol, administer 2.2 U/lb of ACTH IM, and two hours later collect a sample for poststimulation cortisol. Newer reagents use a 1 hour stimulation protocol; consult the reference lab and/or package insert with the ACTH. In cats inject 125 µg of cosyntropin IM and collect samples at 0, 30 and 60 minutes.

Multiple ACTH stimulation protocols are available and all work well. An advantage of low-dose ACTH stimulation protocol is reduced costs. Clinical trials have demonstrated that any dose of synthetic ACTH greater than 5 µg/kg will maximally stimulate the adrenal corticies 60 minutes post administration and therefore higher doses are not needed. Additionally, it can be repeated in 24 hours or 2 weeks with similar results. It distinguishes dogs with hypoadrenocorticism from dogs with nonadrenal illnesses that appear clinically similar to hypoadrenocorticism. If repetition of the ACTH stimulation test was needed because of sample mishandling or other causes, then the low dose ACTH or the high dose of 0.25 mg/dog can be repeated in 24 hours with reliable results.

The low dose of 5 µg/kg of cosyntropin will maximally stimulate the adrenal corticies of normal dogs and dogs with hyperadrenocorticism and can be used as a screening test for hyperadrenocorticism. False positive or increased cortisol post stimulation in dogs without hyperadrenocorticism is seen in about 15% of dogs.

Confirmatory tests

Endogenous ACTH (e ACTH) is increased in primary and decreased in secondary hypoadrenocorticism.

Basal cortisol and ACTH stimulation are used to diagnose hypoadrenocorticism, but they do not distinguish primary from secondary hypoadrenocorticism. Measuring the endogenous concentration of ACTH is an easy way to distinguish these two diseases. Dogs with primary hypoadrenocorticism have e ACTH > reference interval (typically 40–1250 pmol/L) and dogs with secondary primary hypoadrenocorticism have e ACTH < reference interval (typically 1–2 pmol/L) (Table 32.12). Dogs with primary hypoadrenocorticism have markedly increased concentrations of e ACTH, dogs with atypical primary hypoadrenocorticism have increased

concentrations and dogs with secondary hypoadrenocorticism have decreased or undetectable concentrations of e ACTH. Absolute statements such as this are dependent on the disease being fully developed. As the lesions develop, the concentrations of basal cortisol and e ACTH are in transition. The earlier diagnostic tests are used during the progression of a disease, the more likely that the results may be equivocal. The stages of the patient's disease development will greatly influence the clinical signs and laboratory results that are being expressed at that moment. Primary hypoadrenocorticism has increased e ACTH because the entire adrenal cortex is being destroyed and serum cortisol is decreasing. Eventually there is no negative feedback to the pars distalis and coticotrophs will secrete ACTH in attempt to stimulate cortisol production. This cycle continues unabated until treatment is started. Serum concentration of e ACTH can be marked in these cases, >300 pg/mL to >500 pmol/L depending on the laboratory. Although helpful in the distinction of primary and secondary hypoadrenocorticism the collection, shipment and measurement of e ACTH is delicate. It is recommended that the laboratory be consulted regarding protocol and interpretation.

If there are the characteristic electrolyte abnormalities, primary hypoadrenocorticism is most likely. Greater than 90% of the cases are primary and when the Na:K ratio is <23:1 and basal cortisol and/or ACTH stimulation results indicate hypoadrenocorticism, measurement of e ACTH may be optional with this set of findings. Secondary hypoadrenocorticism will have decreased to undetectable e ACTH. The pituitary lesion destroys corticotrophs and therefore ACTH production is decreased. Alternatively, the steroids being administered cause negative feedback to corticotrophs that then decrease the production and secretion of e ACTH.

Other species

Hypoadrenocorticism occurs rarely in cats and the information provided above is essentially the same for cats. An exception is that only primary hypoadrenocorticism and iatrogenic hypoadrenocorticism have been observed in cats.

It is rare in all other domestic animals and when present in calves and foals it is usually due to diarrhea or septicemia with an embolic shower to the adrenal gland, as well as other organs. Calves and foals develop hyponatremia and hyperkalemia with hypoadrenocorticism similar to dogs, but these electrolyte abnormalities are much more likely to occur with infectious diarrhea or sepsis than primary hypoadrenocorticism. Calves with *E. coli* septicemia are an animal model for the Waterhouse Freidrichson syndrome—endotoxin-induced hypoadrenocorticism. Herpes virus will produce adrenal cortical necrosis in fetuses and neonates such as piglets, calves, and foals.

There are reports that weak and/or septic foals may have a relative adrenal insufficiency (RAI) syndrome, as reported in people. The concept is there is inadequate production of cortisol during critical illnesses, especially with sepsis. One report indicated that the mean ACTH to cortisol ratio was significantly higher in septic foals that did not survive than in septic foals that did survive. In a more recent report the authors did not confirm this correlation and also found that the majority of ill foals had adequate responses to cosyntropin administration and only a small group had low cortisol, low ACTH concentrations and low responses to cosyntropin indicating a dysfunctional hypothalamic-pituitary-adrenal axis. However, the authors did not conclude this subgroup actually had RAI or that the endocrine status contributed to the illness in these foals. In fact, some foals with septicemia had markedly increased concentrations of ACTH. Endotoxin and the cytokines interleukin-1 and tumor necrosis factor alpha have been shown to cause an increase in ACTH in various species. They also reported that foals that survived had a higher concentration of cortisol in response to low dose cosyntropin stimulation than did foals that did not survive. This finding suggested that an ACTH stimulation test may be useful to establish a prognosis in these cases. Approximately 50% of critically ill foals will have decreased basal cortisol and an inadequate response to ACTH. It would be beneficial to resolve these conflicting data in foals with sepsis and formulate a consensus to know if steroids should be administered to ill foals and which endocrine tests may be of use in case management.

Paired low-dose (10 µg) and high-dose (100 µg) protocols have been developed to evaluate normal and critically ill foals. Basal samples for cortisol are collected, 10 µg of cosyntopin is given IV as a bolus, and a sample is collected 30 minutes later to assess peak response. Ninety minutes after the 10 µg dose a basal sample is collected and 100 µg of cosyntropin is given IV as a bolus and samples are collected 30 and 90 minutes later for peak cortisol response. Other protocols inject cosyntropin intravenously at 0.1 µg/kg, and measure plasma cortisol concentrations before (baseline), and at 30 and 60 minutes after cosyntropin. Plasma ACTH concentration can be determined via an automated analyzer by enzyme based immunometric assay with chemiluminescent detection. This has been validated for use with equine samples.

Other tests

Plasma to aldosterone, aldosterone to renin, and cortisol to ACTH ratios have been used to help diagnose hypoadrenocorticism in a limited number of dogs. As clinical studies progress these tests may help differentiate primary, atypical, and secondary Addisonian's and replace or supplement ACTH stimulation testing.

Primary hyperaldosteronism

Primary hyperaldosteronism (Conn's syndrome) is a rare disease in veterinary medicine, but has been reported in dogs, cats, and ferrets. The lesion is hyperplasia or neoplasia of the adrenal cortex that involves the zona glomerulosa. Cells in the zona glomerulosa produce excess aldosterone

which causes hypokalemia and hypernatremia along with increased blood pressure and other effects. Aldosterone binds to mineralocorticoid receptors on cells in the distal convoluted tubules and collecting ducts stimulating increased production of Na-K ATPase and an increased number of sodium pumps in the nephron resulting in potassium excretion and sodium reabsorption. Confirmation requires measurement of serum aldosterone and renin combined with physical examination to rule out congestive heart failure and other possible causes of increased blood pressure. Secondary hyperaldosteronism is a normal reaction to decreased blood pressure and activation of the renin-angiotensin-aldosterone system to retain sodium and increase blood pressure. The laboratory differentiation of primary and secondary hyperaldosteronism requires concurrent measurement of aldosterone and renin.

Primary hyperaldosteronism has increased serum concentrations of aldosterone and decreased renin.

Secondary hyperaldosteronism has increased concentrations of aldosterone and renin.

Reference intervals for aldosterone are best obtained from the laboratory analyzing the samples. A guideline is 14–957 pmol/L for dogs and 194–388 pmol/L for cats.

Hyperadrenocorticism—HAC—Cushing's disease, syndrome

There are several causes of hyperadrenocorticism that are described below (Fig. 32.5). All will have similar clinical signs and basic laboratory data. Special endocrine tests are useful in distinguishing the cause and this is useful for targeted therapy in case management.

Pituitary dependent hyperadrenocorticism (PDH)

The primary lesion is a pituitary tumor that secretes ACTH autonomously and stimulates bilateral adrenal gland hypertrophy and cortisol secretion. Serum ACTH is increased. PDH is the cause of HAC in over 80% of dogs and 100% of cats. It is also the cause in 100% of horses with a tumor being in the pars intermedia that secretes various intermediary substances.

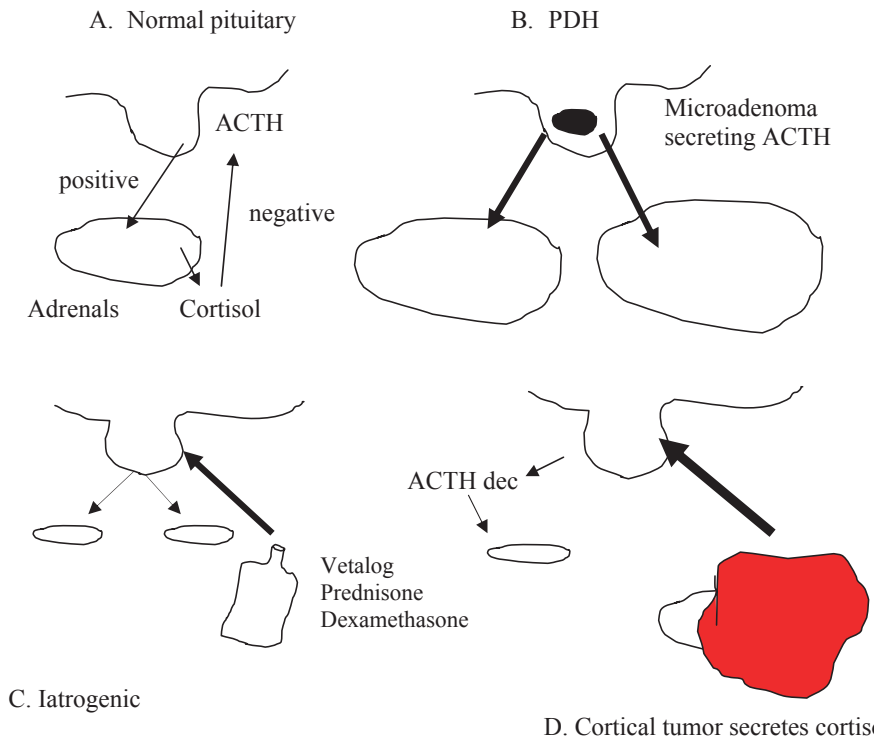

Figure 32.5 Mechanisms of hyperadrenocorticism (HAC): Arrows represent relative amounts of hormones being secreted with a normal pituitary-adrenal axis and in three disease states. Adrenal gland sizes are relative for normal (A), pituitary dependent HAC (B), iatrogenic HAC (C), and functional adrenal tumor HAC (D). Secondary HAC is due to a functional pituitary tumor (B) producing ACTH which stimulates bilateral adrenocortical hypertrophy (pituitary dependent hyperadrenocorticism [PDH]). Primary HAC is due to a functional adrenocortical tumor (D) that secretes cortisol that causes negative feedback to the pituitary resulting in decreased ACTH and atrophy of opposite adrenal gland. Iatrogenic HAC is due to an exogenous steroid (C) that causes negative feedback to the pituitary that results in decreased ACTH and bilateral adrenocortical atrophy. The common feature of all three disease states is increased glucocorticoids that produce the physical and clinical pathologic features of HAC.

Adrenal dependent hyperadrenocorticism (AT or ADH)

The primary lesion is an adrenal cortical tumor that autonomously secretes cortisol. Serum ACTH is decreased. This cause is responsible for 10–15% of HAC cases in dogs. It is rare in most other species. About half of functional adrenal cortical tumors are benign. In ferrets adrenal cortical tumors are the most common cause of hyperadrenocorticism and these tumors secrete both sex hormones and cortisol.

Iatrogenic

Exogenous steroids produce the same clinical signs and routine laboratory abnormalities seen in spontaneous cases. Basal concentrations of cortisol will vary with the type of steroid used. It can be increased or decreased depending on crossreactivity with cortisol assay. Serum ACTH is decreased. ACTH stimulation is the confirmatory test of choice.

Others

There are a few reports of ACTH ectopically produced by nonadrenal tumors and one report due to food-dependent hypercortisolemia in the dog. These mechanisms are very rare. Ectopically produced ACTH is much more common in human beings because they have a neuroendocrine lung tumor, oat cell tumor, that produces this syndrome. Perhaps because this tumor is rare in veterinary medicine we do not see this paraneoplastic syndrome.

General considerations

This is primarily a disease of dogs and ferrets, but it also occurs less frequently in horses and rarely in cats. In dogs, the distinction of primary (adrenal dependent 10–15%) from secondary (pituitary dependent 80–85%) is important because primary hyperadrenocorticism is usually treated by surgical removal of the adrenal tumor and pituitary dependent hyperadrenocorticism is treated chemotherapeutically or by surgical removal of the pituitary. Mitotane (Lysodren, op'DDD) is capable of selective cytotoxicity of the adrenal cortex. If chemotherapeutic adrenalectomy is the preferred treatment option, it is still important to differentiate the cause of HAC because the dose of mitotane is increased when used on an adrenal tumor. The cause also has bearing on discussion of the prognosis related to treatment. First, the fact that about half of the adrenal tumors are malignant affects prognosis. Second, efficacy of chemotherapy is more variable in AT than in PDH. Furthermore drugs that inhibit cortisol synthesis do not work as well if the cause is an AT.

Approximately 80% of the dogs with pituitary dependent HAC disease have a microadenoma in the pituitary that autonomously produces excess ACTH. The increased ACTH stimulates bilateral adrenal gland hypertrophy and hyperplasia and increased secretion of cortisol. Only about 10–20% of PDH cases are due to a macroadenoma or a pituitary carcinoma. The tumor in these dogs is larger, invasive, and, therefore, may cause visual defects (pressure or invasion into optic chiasm) or other endocrinopathies due to compression of trophic cells in the pituitary (secondary hypothyroidism) and possibly central nervous system signs if the tumor invades the brain. If some of these problems are detected in a Cushingoid dog then a malignant pituitary tumor is more likely. These cases can be challenging to diagnose as the suppressive tests can respond similar to the results obtained in dogs with adrenal tumors.

Another 10–15% of hyperadrenocorticism (HAC) cases in dogs are due to adrenal cortical tumors that secrete cortisol autonomously. Approximately half of the tumors are benign the other half may metastasize into the vena cava, liver, regional lymph nodes, and lung. Bilateral tumors are present in 10% or less of the dogs with HAC; they may be cortical or medullary in origin. Not all adrenal cortical tumors secrete cortisol; some tumors are nonfunctional as is possible for any endocrine tumor. Adrenal cortical tumors do not respond as well to the suppressive and stimulatory tests used to diagnose HAC and these differences can be used to help distinguish PDH from AT.

The distinction of pituitary versus adrenal dependent HAC in cats is not as critical because both forms of the disease are treated by surgical adrenalectomy. Feline adrenal glands are less affected by chemotherapy. In cats the pituitary tumor is proportionally much larger than in dogs, however, they are benign. Most cats, 90–100%, with hyperadrenocorticism will have concurrent diabetes mellitus. Concurrent diabetes mellitus in dogs with Cushing's disease is variably reported as 10–33%. However, if resistance to insulin is discovered in either species then investigations for concurrent HAC is a recommended case management consideration.

In horses, Cushing's disease or *pituitary pars intermedia dysfunction* (PPID) is caused by a pituitary tumor or hyperplasia present in the pars intermedia. The pathogenesis and the clinical problems in horses are different from dogs and are discussed later in this chapter under pituitary disease.

About 80–85% of Cushingoid dogs, 90% of cats and nearly 100% of horses should have a pituitary tumor. These expected results should be considered when trying to interpret the results of endocrine tests. Before the least common cause of HAC is ruled in, an adrenal tumor in a dog and especially an AT in a cat, the test results should be unequivocal. Any equivocal test results should be reconciled by performing multiple tests. Imaging studies to visualize the adrenal tumor and possibly the pituitary tumor have become viable options and should be considered in cases that have confounding endocrine data.

Clinical problems

Almost all the clinical signs and lesions in dogs and cats are due to the increased concentration of glucocorticoids. Therefore iatrogenic Cushingoid dogs look identical to spontaneous Cushingoid dogs. There are numerous abnormalities in

Table 32.13 Clinical signs of HAC and approximate percentage of dogs and cats with these abnormalities.

	Dogs	Cats
Polyuria and polydipsia	80–90	90
Alopecia	60–75	60
Fragile skin		50
Polyphagia	50–60	70
Pot-belly	70	85
Hepatomegaly	50–70	35
Lethargy	80	50
Anestrus	55	
Calcinosis cutis	10, but considered pathognomonic	
Pyoderma		40
Hyperpigmentation	33	
Comedomes	33	
Concurrent diabetes mellitus depending on report	10–33	90

Others: Muscle wasting, prominent cutaneous blood vessels, testicular atrophy, decreased libido, bronchopneumonia, pulmonary mineralization, cystitis, facial nerve paralysis, and pulmonary thromboembolism.

Table 32.14 Laboratory abnormalities seen with HAC and approximate percentage of dogs and cats with these abnormalities.

	Dogs	Cats
Alkaline phosphatase increased	85–95	15
Alanine aminotransferase increased	50–80	40
Hyperglycemia, fasting	30–40	95
Urea nitrogen decreased	30–50	
Hypophosphatemia	20–40	
Hyperlipidemia	50–80	
Cholesterol increased	50	40
Urine s.g. <1.020	80	Infrequent, usually conc
Urinary infection*	50	Infrequent
Proteinuria	75	Common
Glucosuria	10	90
Stress leukogram	Common	Infrequent
Nucleated RBCs	Common	Infrequent
Thrombocytosis	85	
Decreased thyroxine	50	
Decreased free T4	25	
Alkaline phosphatase steroid isoenzyme	85–90	Does not exist

*Some canine cases will have bacteriuria, dilute urine and no or few white blood cells.

dogs and cats with HAC: alopecia, polyphagia, polyuria and polydipsia, pot-belly, muscle wasting, prominent cutaneous blood vessels, comedones, calcinosis cutis, anestrus, decreased libido, bronchopneumonia, cystitis, and possible pulmonary thromboembolism (Table 32.13). If the cortisol concentration can be decreased, the clinical problems recede, even if a pituitary tumor remains. If blindness or other signs referable to the central nervous system are present, then a large pituitary tumor compressing the optic chiasm and the brain should be suspected. Cats and ferrets have many of the clinical problems seen in dogs with HAC.

Routine laboratory data

Complete blood count (CBC)

A stress leukogram is expected in dogs and is characterized by leukocytosis, mature neutrophilia, lymphopenia, eosinopenia, and monocytosis. Lymphopenia and eosinopenia are the most constant of these. This is present frequently in Cushingoid dogs, but is obviously not specific. An increased PCV is seen in a small percentage of cases and some dogs' exhibit nucleated red blood cells without regeneration, which is inappropriate and is suggestive of HAC or microangiopathies. Thrombocytosis is often present.

Cats receiving steroids will have a stress leukogram, but cats with naturally occurring HAC do not consistently exhibit a stress leukogram. Erythroid and platelet determinations are normal.

Ferrets may have anemia and/or leukopenia and thrombocytopenia late in the disease due to secretion of estradiol. The estrogen induced bone marrow toxicity may cause pancytopenia similar to that induced by persistent estrus in intact female ferrets.

Clinical chemistry *(Table 32.14)*

Alkaline phosphatase (ALP) is increased in over 90% of dogs with HAC (sensitive), but it is also increased with many other diseases (not specific). If serum ALP is not increased

it is a good indicator that HAC is not present. The increase in ALP is mild, moderate, or marked. Mild and moderate increases are not very helpful to the diagnosis, but marked increases, e.g., 2,000–10,000 IU/L, are very suggestive of HAC. However, there is no correlation between the magnitude of the increase and severity of the disease or predicting response to treatments. The increase in ALP in dogs is due to hepatic and a species-unique steroid induced isoenzyme. Although the corticosteroid induced isoenzyme increases in almost all dogs with HAC (sensitivity 95%) it is also increased in the serum of many dogs with nonadrenal illnesses. Therefore the low specificity (about 18%) limits the diagnostic value of the steroid isoenzyme determination. Even small increases in serum cortisol, such as those occurring with exogenous steroid administration in ocular preparations, can induce the steroid isoenzyme. GGT parallels the increase in ALP in dogs. ALP has a short half-life in cats and therefore does not increase frequently or to the same magnitude as it does in dogs with HAC. Any increase in ALP is significant in cats and should be investigated. Cats and horses do not have a steroid isoenzyme.

Other liver enzymes may also be increased due to hepatomegaly caused by glycogen accumulation that produces hepatocellular vacuolation (not lipid). Glycogen accumulation in hepatocytes is characteristic of HAC, and lipid accumulation is characteristic of diabetes mellitus. Cats therefore will have a fatty liver or a mixture of fat and glycogen. The increases in ALT and AST are mild to moderate and are seen in the majority of dogs. Serum bile acids are increased in about one-third of the dogs and are a nonspecific change that is likely due to hepatomegaly caused by the glycogen hepatopathy. Hyperglycemia is due to the effects of cortisol. If serum glucose is greater than 300 mg/dL in a dog or a cat with HAC consider concurrent diabetes mellitus. Consider HAC in any dog or cat in which it is difficult to regulate serum glucose with insulin as steroids are an insulin antagonist. The majority of feline cases are diagnosed after discovery of insulin resistance. Serum fructosamine may be increased due to chronic hyperglycemia in dogs and an increase is expected in cats. Cholesterol is increased in about half of the dogs and cats. Triglycerides are increased in nearly 90% of dogs, but are not increased in cats.

Serum phosphorus is decreased in dogs. The mechanism is not clear, but is attributed to the phosphaturic stimulus of glucocorticoids. Urea nitrogen and creatinine may be low due to diuresis in approximately 33–50% of the dogs.

Thyroid status

Approximately 50% of dogs with HAC have decreased total thyroxine (TT4) and 15–50% will have decreased free thyroxine (fT4). Endogenous TSH is normal or decreased in most of these dogs, which means they do not have primary hypothyroidism. If TT4, fT4, and endogenous TSH are decreased they should have secondary hypothyroidism due to the ACTH secreting pituitary tumor that is compressing thyrotrophs; this is a rare situation. Increased fT4 is seen in about one-third of the dogs and may be similar to the increase seen in cats with nonthyroidal illness. Glucocorticoids may also suppress the release of TSH from the pituitary. If endogenous TSH is in reference interval then the decrease in TT4 is attributed to drug interference. Similar abnormalities in thyroid hormones are seen with exogenous steroids and iatrogenic Cushing's. Steroids should be stopped for at least 4 weeks before thyroid profiles are performed.

Dogs with hyperadrenocorticism or hypothyroidism share many clinical problems such as alopecia, obesity, lethargy, and enlarged liver. They also share similar clinical chemistry abnormalities such as increased hepatic leakage enzymes, cholesterol, and triglycerides. Furthermore, TT4 is often included in geriatric panels and is therefore observed early in the evaluation of these cases. When these two diseases are differential diagnoses testing to rule in or rule out HAC should be done prior to thyroid profiles. Thyroid hormone therapy is not needed in dogs that are successfully treated for HAC as thyroid values return to reference intervals.

Urinalysis

Dilute urine, 1.004–1.020; cystitis, bacteriuria with or without inflammation, proteinuria with or without inflammation.

Dilute urine is due to interference with ADH and/or its receptors by the increased cortisol, resulting in a "biochemical nephrogenic diabetes insipidus." Urine specific gravity <1.020 is seen in 85% of dogs with HAC. Polyuria and polydipsia are compensatory responses and are seen in over 85% of the dogs. If water is withheld these dogs can usually concentrate into the 1.025 range. Glucosuria is uncommon in dogs, present in 10% of the canine HAC cases, but is present in 90%+ of feline cases. Ketonuria does not occur in HAC. If ketonuria is present it should alert one to the possibility of concurrent diabetes mellitus. Persistent glucosuria and/or moderate to marked hyperglycemia are more consistent with diabetes mellitus. Proteinuria is seen in up to 75% of the dogs and in many dogs this is due to cystitis. Proteinuria is also seen in the absence of cystitis and in these cases it may be due to concurrent glomerulonephritis which is common in dogs over 9 years old. It could also be due to steroid induced glomerular lesions. Urine protein : creatinine ratios range from 1 to 6 (normal <1.0), and successful treatment of HAC does not always reverse proteinuria; therefore concurrent glomerulonephritis seems likely in some dogs with HAC. Typically there is not concurrent hypoalbuminemia or other features of nephrotic syndrome if the dogs only have HAC.

Bacterial urinary tract infections are present in half of the dogs with HAC. Bacterial infections are attributed to the compromised immune state due to the corticosteroids. Some dogs will have bacteria in their urine without inflammatory

cells. Dilute urine and bacteria without inflammation is an unusual combination and when observed is suggestive for Cushing's disease. Consider urine culture in dogs even if no inflammation or bacteria are observed.

Screening tests

Low Dose Dexamethasome Suppression (LDDS) Urine Cortisol:Creatinine Ratio (UCCR) and ACTH Stimulation.

If the history, clinical signs, and routine laboratory data are suggestive for HAC then the diagnosis at this stage is a two-step process: first rule in or rule out HAC with screening tests and then try to differentiate PDH and AT with confirmatory tests. Differentiation is critical because they are treated differently: chemotherapeutic destruction of the hypertrophic adrenal cortex for PDH and adrenalectomy for adrenal tumors. If surgical correction is not an option and the AT will also be treated with mitotane, differentiation is still appropriate as the dose of mitotane selected is generally higher for an AT than for PDH. Since approximately half of the adrenal tumors are carcinoma, the correct identification of the cause of HAC has prognostic implications. Regardless, this first step is critical. Obtain a correct diagnosis of HAC because either treatment is rigorous.

Each of the screening tests has value. LDDS is the most popular test and is the test of choice if the dog has characteristic signs and lab data of HAC. It also has the highest false positive rate and should therefore not be used, or used cautiously, if clinical signs and lab data are not classical of HAC. If surgical adrenalectomy of an adrenal tumor is not an option then LDDS is the test of choice. ACTH stimulation has the lowest false positive rate and should be used when clinical signs and lab data are not classical of HAC. UCCR is an inexpensive and easy screening test to rule out HAC, but should not be used to rule in HAC. When these tests are used in combination very few cases are misdiagnosed. These tests must be used in conjunction with routine clinicopathologic data; they are not stand-alone diagnostic tests used in isolation.

UCCR (urine cortisol:creatinine ratio)

Depending on the study, 90–100% of dogs with HAC will have excess quantities of cortisol in the urine (high sensitivity). However, urine cortisol will also be increased in 80% of dogs with nonadrenal diseases that look like HAC (20% specific), and therefore it cannot be used to rule in HAC. Over 90% of dogs that have urine cortisol in the reference interval or below do not have HAC. Therefore, this is a very good, easy, and inexpensive screening test to rule out HAC. If a dog has an increased UCCR then LDDS or ACTH stimulation is required before HAC is diagnosed. The greatest ratios for UCCR are seen in dogs with PDH as opposed to dogs with AT. If UCCR is >100 the dog is very likely to have PDH. A UCCR is the least expensive screening test.

It is imperative to perform this test on urine collected at home in the morning when physiologic stress is at a minimum. Urine for this test should not be collected during a visit to the veterinary hospital as the stress of visitation will produce increased concentrations of serum and urine cortisol that produce false positive results. Dogs that visit a veterinary hospital, especially if an orthopedic examination is performed, will have moderate increases of UCCR that are in the range seen with Cushing's. Have the owners collect a midstream urine sample, deliver it to the office, and submit at least 1 milliliter of centrifuged urine to the laboratory. The lab measures cortisol and creatinine and reports a number without units. A UCCR >20 $\times 10^{-6}$ is consistent with a diagnosis of hyperadrenocorticism, depending on the lab and the techniques used to measure cortisol. Reference interval values vary with the laboratory. Cortisol may be present in the urine in free form or as metabolites and the assays used will vary in what they recognize, and therefore so will the reference intervals. Creatinine is used in the denominator as it is excreted at a relatively constant rate in the urine and therefore adjusts for differences in urine volume and degree of urine concentration. UCCR permits the use of spot urine or single point determinations as opposed to 24 hour collections of urine. A good way to utilize this test is the following. If HAC is a differential on initial examination or after the CBC and chemistry panel are reviewed, then rather then returning the dog to the hospital, have the owners collect a morning urine sample for the next screening test. If it is within reference interval then RO HAC and if it is increased continue to pursue HAC.

Summary—UCCR

Good estimate of cortisol production for last 24 hours

- 90% + of dogs with normal UCCR do not have Cushing's = RO disease
- 95% + of dogs with Cushing's have increased UCCR (sensitivity 95%)
- 80% of sick dogs with nonadrenal disease have increased UCCR, specificity 20%; false positives (80%) too high to rule in HAC.
- UCCR is sensitive to detect increased cortisol in dogs with Cushing's and in dogs with other nonadrenal diseases (stressed).
- If a dog with undiagnosed PU PD has a UCCR in reference interval and serum ALP is not increased then HAC is highly unlikely and can be ruled out

Reference interval

0.5–17.7 $\times 10^{-6}$ check with lab that performed assay
<15 rule out HAC
≥20 consistent with HAC
15–19 gray zone

Low dose dexamethasone supression (LDDS) test

Patients with HAC will not suppress and "non-Cushingoid" patients will suppress.

The principle of the LDDS test is that dogs or cats that have HAC (PDH or AT) will not decrease their serum cortisol in response to the administration of a low dose of dexamethasone. Dexamethasone will cause a decrease in serum cortisol in "non-Cushingoid" dogs that are normal and in about 50% of dogs with other diseases. In normal dogs dexamethasone is recognized by receptors in the pituitary/hypothalamus resulting in a decreased release of ACTH. The decrease in ACTH results in decreased release of cortisol from the adrenal cortex that is interpreted as suppression. This suppression is used to rule out HAC. In normal dogs, LDDS will decrease serum cortisol in 2–3 hours and cortisol will remain decreased for 24–48 hours. LDDS is an excellent test to distinguish normal dogs from Cushingoid dogs, but it is not entirely reliable to distinguish Cushingoid dogs from sick, stressed dogs that have a variety of other diseases.

The concentration of cortisol at 8 hours post dexamethasone administration is used to determine HAC. Dexamethasone sodium phosphate or dexamethasone in polyethylene glycol can be used. The recommended dosage is 0.01 mg/kg intravenously for the low dose protocol. Samples are collected before (basal) the administration of dexamethasone and at 4 and 8 hours post administration. Use the 8-hour sample to rule in or rule out HAC, use the 4-hour sample to differentiate PDH from AT. Reagents used to measure cortisol do not crossreact with or recognize dexamethasone, hence, there is no "false," crossreactive increase from the administration of dexamethasone. Because of crossreactivity, prednisolone or prednisone cannot be used as the suppressing steroid. Dexamethasone is approximately 40 times as potent as cortisol, hence it can suppress ACTH when the concentration of endogenous serum cortisol cannot.

The reference interval for plasma (serum) cortisol is 0.4–6.0 µg/dL (10–160 nmol/L). Reference intervals and suggested cut-offs for interpretation should be provided by the reference laboratory used. LDDS will decrease serum cortisol to <1.5 µg/dL (<30 nmol/L) at 4 and 8 hours post dexamethasone in normal dogs. If a dog has PDH or AT the 8-hour post dexamethasone sample will be >1.5 µg/dL (>40 nmol/L). Some labs use greater than or equal to 30 nmol/L or 1.4 µg/dL. If a dog has PDH, the low dose of dexamethasone will not decrease ACTH secretion from the pituitary tumor sufficiently to suppress cortisol secretion from the hyperplastic adrenal glands. If a dog has an AT the concentration of ACTH is already suppressed, the AT continues to secrete cortisol independent of ACTH. If the 8-hour cortisol is equal to or greater than 1.5 µg/dL (>40 nmol/L) then there was no suppression. The patient has HAC, either PDH or AT, or the test result is a false positive. All dogs with AT fail to suppress and 90–95% of dogs with PDH fail to suppress.

Many veterinarians prefer this test because 95% of the dogs with Cushing's disease do not suppress, which is similar to the high sensitivity of UCCR. LDDS is a test that recognizes 95% of the dogs with the disease that is being looked for, a very positive feature. However, it "recognizes" another 50% of dogs that do not have the disease, but the results of the test suggest that they do. The specificity of LDDS is between 75% and 44% depending on the study, which means the false positive rate is 25–56%. Sick, stressed dogs with a variety of illnesses may have hyperplastic adrenals that do not suppress cortisol secretion in response to LDDS and the more severe the nonadrenal illness, the more likely there will be a false positive test result.

An additional use of LDDS is to differentiate PDH from AT in a dog that has classical signs and lab data of Cushing's, see confirmatory or differentiating tests, below.

Summary LDDS

- An 8 hr cortisol <1.5 µg/dL is suppression, rule out HAC
- An 8 hr cortisol >1.5 µg/dL is no suppression, rule in HAC
- 95% of dogs with HAC do not suppress, 95% sensitivity
- 25–56% of dogs without HAC do not suppress, false positives; 44–75% specificity; therefore could rule in HAC when the dog does not have HAC
- 5% of dogs with HAC suppress; false negative; may miss the diagnosis
- Excellent test to RO Cushing's when suppression occurs; if UCCR is not increased and a dog suppresses with LDDS rule out HAC
- LDDS can also be used to differentiate PDH and AT in about 25% of dogs
- Popular test, but false positive is 25–50%; therefore signs and lab data must be characteristic of Cushing's before this test is used to rule in HAC.

ACTH stimulation test

The principle of the ACTH stimulation test (ACTH stim) is that dogs or cats with HAC will have an exaggerated increase in the concentration of serum cortisol in response to an injection of ACTH. Normal dogs will increase their serum cortisol approximately two fold; but remain at less than 15 µg/dL. Dogs with hypoadrenocorticism will not increase their serum cortisol (<2 µg/dL) and dogs with PDH or AT will have increases of serum cortisol greater than 2–5 times the basal concentration and greater than 22 µg/dL (>500 nmol/L); see Table 32.15. The protocols for this test require 1 or 2 hours depending on the product used. This test will recognize approximately 80–85% of dogs with PDH, but only 60% of dogs with AT. Dogs with PDH have hyperplastic adrenal cortices that are primed to respond to exogenous ACTH. The AT dogs have neoplastic cells that are functioning independent of endogenous ACTH and therefore may not respond to exogenous ACTH. However, only 10% of dogs with HAC have AT and AT is rare in cats. So although this test only recognizes 60% of dogs with AT, this is an uncommon cause of HAC and therefore few HAC cases are missed. This negative fact is offset by one its best features: it has the lowest false positive rate of any of the screening tests, only 15%. A

Table 32.15 Expected responses to ACTH stimulation test in dogs.

	Cortisol µg/dL
Normal basal	0.5–6 µg/dL; 10–160 nmol/L
Normal post stim	6–18 µg/dL; >220–560 nmol/L; 2–5 times basal
HAC diagnostic	>22 µg/dL
Gray zone	18–22 µg/dL
Iatrogenic HAC	<5 if product does not cross react
Basal Addison's	<1.5
Post stim Addison's	<1.5 flat line response
ACTH Stimulation Protocols:	
Easy, done in 1–2 hours; several types of products	
2.2 U/kg IM ACTH aqueous porcine gel	
Basal sample and 2 hours post ACTH dog or cat and 8 hours horse	
or	
Cosyntropin IM or IV 250 µg (1 vial)	
5 µg/kg will maximally stimulate adrenal cortex	
Basal sample and 60 minutes post ACTH for dog; two post ACTH samples for cats, at 60 and 90 min	
Store reconstituted ACTH in plastic syringes at –20°C for 6 months	
Results: Cortisol µg/dL reference interval (RI)	

Cortisol	Dog	Cat	Horse
Basal normal (RI)	0.5–6 µg/dL	0.5–4	3–6

False positive, or increased cortisol poststimulation in dogs without HAC is seen in about 15% of dogs.

false positive test result for HAC has serious case management consequences. Therefore this test may be the test of choice in a dog that only has a few of the problems associated with HAC and the clinician is searching for a diagnosis. A reasonable first step in cases that have limited features of HAC is to perform UCCR as it is inexpensive, easy, and does not require a new office visit. If UCCR is in reference interval, and especially if serum ALP is not increased, then rule out HAC. If UCCR and ALP are increased, then follow up with an ACTH stimulation test. ACTH stim is a relatively expensive diagnostic test due to the cost of ACTH.

There are other uses for the ACTH stimulation test. It recognizes iatrogenic HAC. It is the test of choice to monitor response to therapy and provides a baseline response for monitoring therapy. Lastly, it may identify rare and unusual cases of HAC that have increases in precursors to cortisol (17-hydroxyprogesterone), but normal concentrations of the end product, cortisol.

Patients that look Cushingoid, but have low UCCR, low basal cortisol, and a flat line response to ACTH stimulation may have iatrogenic HAC when the steroid being used does not crossreact with the cortisol assay, e.g., dexamethasone. Patients that look Cushingoid and have a high UCCR, high basal cortisol that remains high, and in a flat line in response to ACTH stimulation have iatrogenic HAC and the steroid being used crossreacts with the cortisol assay, e.g., prednisone. If a LDDS was performed on this latter group, they would react like an AT with high basal cortisol that does not suppress at 4 or 8 hours post dexamethasone.

When both ACTH stim and LDDS were used on a group of approximately 65 dogs with HAC, no dog had normal results for both tests. Results of any test are not always clear-cut and therefore tests may need to be repeated at different times or used in combination, especially when investigating endocrine diseases. Multiple ACTH stimulation protocols are available for dogs, cats, and horses and all work well (Table 32.15). The protocols are presented in this chapter under discussion of hypoadrenocorticism. An advantage of the low-dose ACTH stimulation protocol is reduced costs, at least for smaller dogs. The low dose of 5 µg/kg of cosyntropin will maximally stimulate the adrenal cortices of normal dogs and dogs with hyperadrenocorticism and can be used as a screening test for both hyperadrenocorticism and hypoadrenocorticism.

Summary ACTH STIM

- Approximately 80–85% of dogs with PDH will stimulate abnormally high
- Highest specificity, 85%, and therefore lowest false positive results of the screening tests
- It is affected less by nonadrenal illnesses than the other screening tests
- Recognizes iatrogenic HAC and atypical HAC, and is used to monitor treatment

Various attributes and comparisons of screening tests are presented in Tables 32.16, 32.17, and 32.18.

Confirmatory-differentiating tests: endogenous ACTH, HDDS, LDDS, oral dexamethasone, UCCR, and ultrasonography

These tests are designed to distinguish AT from PDH. This will aid in the selection of treatment and providing a prognosis (Table 32.19).

Endogenous ACTH (e ACTH)

Pituitary dependent = increased e ACTH

Adrenal dependent = decreased e ACTH

Iatrogenic = decreased e ACTH

Pituitary tumors that cause HAC synthesize and secrete ACTH and therefore plasma from these dogs will have high concentration of e ACTH. AT secrete cortisol which suppresses ACTH synthesis and release and therefore these dogs will have decreased or undetectable plasma e ACTH. Like all tests this works well on advanced cases or cases that are classical, but there will be cases that fall into the gray zone

Table 32.16 Screening tests for hyperadrenocorticism in dogs.

Test	Use	Interpretation		Cortisol µg/dL	Comment
UCCR	RO HAC	RO HAC if $<15 \times 10^{-6}$			
			Basal	*Post-stim*	1 and 2 hr protocols
ACTH stim	Dx HAC	RO HAC	2–10	8–19	
		RI HAC	2–10	>24	15% false positive
		Favor HAC	2–10	18–24	
		Iatrogenic	<8	<8	
			4 hr	*8 hr*	
LDDS	Dx HAC	RO HAC	–	<1.5 µg/dL	
		RI HAC	–	>1.5 µg/dL	25–50% false positive

Consult reference laboratory for reference intervals and interpretation.
RO = rule out, RI = rule in, HAC = hyperadrenocorticism.

Table 32.17 Comparison of screening tests for HAC.

Screening test	Positive (Sensitivity)	False positive	False negative
UCCR	95%	75–85%	0–5%
LDDS	95% at 8 h	55% at 8 h	0–5%
ACTH (when PDH)	80–85%	15%	10–25%
ACTH (when AT)	60%	–	40%
Basal cortisol	Do not use	35%	25–35%

Some dogs with nonadrenal disease will have false positive test results. Therefore it is essential to correlate results with clinical signs, lab data, and when more than one screening test when needed. The percentages summarized above change depending on the cut-off values used for abnormal test results.

Table 32.18 Comparison of LDDS and ACTH stimulation.

ACTH stimulation	LDDS
	Good features
Fewest false positive	Popular
Easy, 1–2 hours	95% sensitivity
Recognize iatrogenic	May differentiate AT vs. PDH
Used for atypical HAC	Rules out HAC
Provides baseline for treatment	
	Not so good features
60% sensitive for AT	High false positive 25–55%
Expensive	Eight plus hours

of equivocal results. Reference intervals should be generated by the lab performing the assay and the assay must be validated for dogs. Reference interval for e ACTH via radioimmunoassay is approximately 20–100 pg/mL; values <20 indicate AT, values in the 20–45 range are nondiagnostic or equivocal, and values >45 are consistent with PDH. Some cases of PDH may have values greater than 200 µg/dL. Approximately 90% of dogs with PDH will have e ACTH >45 pg/mL, 70% of dogs with AT will be <20 pg/mL. There is some overlap of each category with each other and with reference intervals. These latter cases require another differentiating test or resubmission for e ACTH at a latter time. Approximately 80% of samples tested from 245 dogs had concentrations of e ACTH that were diagnostic for PDH or AT. When new samples were analyzed from dogs in which the initial result was equivocal, 235 out of the 245 dogs, or 96%, had diagnostic results. Although there may be overlap with reference intervals, e ACTH is being used to distinguish PDH from AT after HAC has been ruled in. It is not used to distinguish normal dogs from dogs with HAC. Endogenous ACTH is a differentiation test and is not to be used as a screening test to rule in HAC.

Endogenous ACTH concentration measured via a two-site solid-phase chemiluminescent immunometric assay (immunoluminometric assay) was very discriminating in 109 dogs with HAC separated into two groups, 91 with PDH and 18 with AT. The reference interval was determined to be 6–58 pg/mL and a threshold for diagnosis was set at 5 pg/dL: e.g., adrenal dependent hyperadrenocorticism <5 pg/mL> pituitary dependent hyperadrenocorticism. The limit of detectable ACTH is 5 pg/dL, and the working range of the

Table 32.19 Differentiating tests for hyperadrenocorticism in dogs.

Test	Interpretation	Cortisol µg/dL	ACTH pmol/mL
e-ACTH	AT		<20
via RIA	PDH		>100
	Gray zone		20–100
e-ACTH via immunomedtric	AT <5 µg/dL> PDH		
		4 hr	*8 hr*
LDDS	RO HAC	–	<1.5
	PDH	<1.5	>1.5
	PDH	<50% basal	>1.5
	PDH	–	>1.5 & <50% basal
	PDH or AT	>1.5	>1.5
HDDS		*8 hr*	
	PDH	<1.5	Suppression
	PDH	<50% basal	Suppression
	PDH 25% of cases	>1.5 or >50% basal	No suppression
	AT	>1.5 or >50% basal no suppression	
		UCCR post dexamethasone	
Oral DS	PDH	<50% basal	
	PDH or AT	≥50% basal	

Consult reference laboratory for reference intervals and interpretation.

assay is 5–1250 pg/mL. All 18 dogs with adrenal dependent HAC had concentrations of ACTH below the limit of detectability and all dogs with PDH had detectable ACTH that ranged from 6–1250 pg/mL with a median concentration of 30 pg/mL. Using a cut-off of 5 pg/mL there was no overlap between dogs with AT and dogs with PDH. However, there is considerable overlap of e ACTH between dogs with PDH and normal dogs. The reference interval is 6–58 pg/mL and therefore many dogs with PDH had concentrations of ACTH within reference interval.

This is perhaps the easiest and most straightforward way to differentiate PDH and AT; however, sample collection requires attention to details. It is recommended that samples be collected in the morning, between 8 and 9 AM, but different studies reporting on e ACTH have not adhered to this time frame and achieved diagnostic results. ACTH is best measured in plasma, heparin or EDTA as anticoagulant, and the sample should never be in contact with regular glass tubes as this will bind the ACTH and artifactually decrease ACTH. Use only plastic syringes and tubes for the collection and the storage of samples that are used to measure e ACTH. Silicon-coated EDTA tubes can also be used to collect blood. Then use plastic tubes for all other steps to harvest plasma. Most EDTA tubes are silicone coated. Try to collect in chilled tubes. Centrifuge chilled right after collection and transfer plasma to plastic tubes for freezing at –20 to –70°C. Pack samples on dry ice for over night shipping to the lab. ACTH is very unstable at room temperature. The most critical event to avoid is freeze thawing, as this will degrade almost all proteins, especially hormones. Aprotinin (Trasylol) is a proteinase inhibitor that blocks trypsin, plasmin, and kallikrein and greatly prolongs activity of ACTH. It is used as a preservative that can be added to the blood as soon as it is collected and it will help prevent in vitro decay from time and temperature. It is available from diagnostic labs or the manufacturer. Follow directions supplied; add 500 units per mL of blood collected. With this preservative the activity of ACTH is preserved at 4 or –20°C for 4 days. Samples are acceptable if shipped in a container with frozen packs for 4 days. Loss is approximately 10% activity at 22°C in 4 days. Avoid freeze–thaw cycles. However, do not use aprotinin if the assay for ACTH is immunoluminometric as aprotinin causes a negative bias that may result in inability to quantitate ACTH. It is best to follow all instructions provided by the laboratory where the samples are sent.

ACTH can be measured via radioimmunoassay, immunoradiometric and immunoluminometric assays. Reference intervals and units will vary with the assay used therefore use a reference lab that has validated the assay for animals and that provides reference intervals and expected values for AT versus PDH for their methodology.

	eACTH*
Reference interval	20–100 pg/mL
Adrenal tumor	<20 pg/mL; seen in approximately 75% of cases
Gray zone	20–45 pg/mL repeat in a few weeks or perform another test
Pituitary tumor	>45 pg/mL; seen in approximately 90% of PDH; and often markedly increased, e.g., >200

*e ACTH measured via radioimmunoassay; consult reference lab that performs the assay for their recommended values to interpret results.

	eACTH*
Reference interval	6–58 pg/mL
Adrenal tumor	<5 pg/mL
Pituitary tumor	>5 pg/mL

*e ACTH measured via immunoluminometric assay; consult reference lab that performs the assay for their recommended values to interpret results and where they set the threshold for diagnosis: e.g., adrenal dependent hyperadrenocorticism <5 pg/mL> pituitary dependent hyperadrenocorticism. Do not use aprotinin for ACTH measured via immunoluminometric assay.

High-dose dexamethasone suppression test

75% of dogs with PDH suppress; 100% of dogs with AT and 25% of dogs with PDH do not suppress; dogs that suppress have PDH and dogs that do not suppress have either AT or PDH; this latter group is small overall and will require another test to differentiate: e ACTH and/or abdominal ultrasound.

A high-dose dexamethasone suppression test (HDDS) can identify approximately 75% of the dogs that have PDH, but it cannot definitively identify an AT. The principle of HDDS is that when the concentration of dexamethasone gets high enough, it will decrease the release of ACTH from pituitary microadenomas and the decrease in ACTH will cause a decrease in serum cortisol that is interpreted as suppression. This decrease in cortisol post HDDS also occurs if the dog is normal, or if the dog is sick and stressed by a nonadrenal disease. This has important diagnostic implications because if a HDDS is performed on a dog with a false positive LDDS result, this dog would now suppress with HDDS and lead to an erroneous diagnosis of PDH. HDDS should not be performed on dogs in which the clinical diagnosis is uncertain and it should never be used as a screening test. Dogs with an adrenal tumor secrete cortisol independent of ACTH. These dogs already have low concentrations of ACTH (decreased by the negative feedback of cortisol secreted from the adrenal tumor), therefore, the administration of dexamethasone has no observable effect on ACTH and cortisol secretion continues from the adrenal tumor, hence, there is no suppression in the serum concentration of cortisol. However, approximately 25% of dogs with PDH also do not suppress. This latter group may have pituitary macroadenomas or carcinomas or the tumor may be arising from the pars intermedia which does not respond to negative feedback from corticosteroids. Differentiation of the dogs that are resistant to dexamethasone suppression requires e ACTH and/or ultrasonography. Dexamethasone resistance means the dog has either PDH or AT and the odds are about 50:50. There is also some data to suggest that a HDDS only provides a clearer interpretation of the 4- and 8-hour LDDS in about 10% of the cases.

This test is performed similar to the LDDS except the dose of IV or IM dexamethasone is now 0.1–1.0 mg/kg or 10 times greater than LDDS. Basal, 4-, and 8-hour samples are collected. An alternate protocol omits the 4-hour sample. Suppression indicates PDH and is defined as:

- cortisol less than 1.4 µg/dL (40 nmol/L) at 4 or 8 hours
- cortisol <50% of basal cortisol at 4 or 8 hours

If a portion of the IV injection becomes extravascular cancel and repeat the procedure after 72 hours or longer.

LDDS as a differentiating test

An additional use of LDDS is to differentiate PDH from AT in a dog that has classical signs and lab data of Cushing's. If suppression is detected, the diagnosis is PDH. If there is no suppression, the diagnosis may be either AT or PDH. Suppression is defined as any of the following in a dog that has an 8-hour cortisol >1.4 µg/dL

- 4 hr cortisol <1.4 µg/dL (consult reference lab for cutoff value they use)
- 4 hr cortisol <50% of basal cortisol (4 hr could still be >1.4 µg/dL)
- 8 hr cortisol <50% of basal, but >1.4 µg/dL

Critical to correct interpretation is that there is no suppression at 8 hours, they "all" have a value of cortisol >1.4 µg/dL, consistent with HAC. Overall, approximately 60–80% of dogs with PDH meets one or more of the above criteria and therefore demonstrates a type of suppression with LDDS. These results make the LDDS a useful adjunct to distinguish PDH and AT. However, approximately 20–40% of dogs with PDH do not demonstrate suppression via these criteria. Similarly, 25% of dogs with PDH also do not suppress with HDDS. There are data to suggest that the dogs with PDH that do not suppress, those that are resistant to dexamethasone, have larger pituitary tumors, macroadenomas or carcinomas. It is logical that the pituitary tumor could be so large that a low dose of dexamethasone would not totally suppress the secretion of ACTH or that the tumor lacks receptors for

cortisol or is located in the pars intermedia. One hundred percent of dogs with AT do not suppress with LDDS, therefore when no suppression is observed with a LDDS the dog has either an AT or PDH, perhaps a large pituitary tumor. Abdominal ultrasonography or e ACTH may help differentiate these. The pattern of suppression at 4 hours followed by an increase at 8 hours is called a "rebound" response and is considered diagnostic of PDH. Critical to the correct interpretation of all the confirmatory tests is that the patient meets clinical and laboratory criteria for a diagnosis of HAC.

Oral dexamethasone at high dose and UCCR

Urine is collected at home in the morning for two consecutive days and stored individually in the refrigerator in closed containers. After urine is collected on day two, dexamethasone is given orally by the owner at 0.1 mg/kg every 8 hours (three doses). Urine is collected on the third morning and all urine samples are analyzed for cortisol and creatinine and UCCR determined. The UCCR on the first two samples is averaged for a baseline value. A baseline value in reference interval will rule out HAC. If the baseline value is increased, then compare this value with the post dexamethasone ratio. If the UCCR post dexamethasone is <50% of baseline it indicates suppression occurred and the diagnosis is consistent with PDH. If the post dexamethasone sample is >50% of basal it indicates suppression did not occur and this is consistent with an AT or PDH, similar to the HDDS test when suppression does not occur. Since there is a high false positive rate with spot UCCR, it is recommended that a positive test result be correlated with a poster card ideal case of Cushing's and that one other screening test confirm HAC is present.

Diagnostic imaging: US, CT, MRI

These are obviously not clinical pathology tests, but they are procedures used in conjunction with laboratory data. Abdominal ultrasonography can differentiate AT from PDH. However, it is not sensitive enough to differentiate hyperplastic adrenal glands secondary to PDH from other causes of adrenal hyperplasia or even some normal adrenal glands. Therefore, it is not a screening test to diagnose HAC. Furthermore, not all adrenal tumors can be found. Some are missed because the tumor is too small to be visualized or the affected adrenal gland could not be located. If present, adrenal mineralization is a strong indicator of HAC and is seen in hyperplastic and neoplastic adrenal glands in plain radiographs as well as other imaging techniques. Approximately half of the adrenal tumors have mineralization. Ultrasonography may identify invasion of blood vessels or masses in the liver, but it cannot differentiate adenomas from carcinomas unless there is evidence of metastasis such as vascular invasion, masses in the liver, or enlarged regional lymph nodes. The larger the adrenal mass the more likely it is malignant. Diagnostic accuracy of abdominal ultrasonography is dependent on the experience of the ultrasonographer and confounders in the patient such as abdominal fat, large body size, hepatomegaly, gastrointestinal distension, renal mineralization, nodular adrenocortical hyperplasia of old dogs, small size of normal adrenals, and their position in the abdominal cavity. Similar to endocrine testing abdominal ultrasonography is used to distinguish PDH from AT after there is a diagnosis of Cushing's disease. Also similar is that there are false positive and false negative results.

Computed tomography (CT) can be used to look for characteristic abdominal lesions or visualize the pituitary. It is more discriminating than abdominal radiographs or ultrasonography, but it also has limitations and conflicting reports as to its discriminating capabilities. CT and magnetic resonance imaging (MRI) are used to visualize a pituitary tumor, especially if a large tumor is suspected. They are more useful in the cat due to the larger size of pituitary neoplasms in cats with HAC or acromegaly. They do not replace endocrine testing as they can only visualize about half of the pituitary tumors in dogs because the tumors are so small. Imaging cannot differentiate a functional versus nonfunctional pituitary mass. However, if the endocrine data suggests a large pituitary tumor versus an AT, then CT or MRI are useful techniques to separate these differentials. If neurologic signs exist then CT or MRI may identify a large pituitary tumor.

Newer technology will continue to improve diagnostic imaging and eventually they may be sensitive enough to accurately distinguish PDH from other forms of adrenal hyperplasia and to visualize pituitary microadenomas. Just like the suppression and stimulatory tests, there will be false positive and false negative test results with imaging studies. Clinical pathology and especially clinical endocrinology has done a good job reporting false negatives, positives, sensitivities, and specificities.

Summary of endocrine testing

Dogs and cats that have classical signs for HAC are relatively easy to identify based on clinical signs and laboratory results. However, it can be a diagnostic challenge to recognize HAC in animals that only have some of the signs of HAC or are early in the development of the disease. These are the cases that laboratory testing is needed the most. Each test has its advantages and disadvantages. UCCR is a good first screening test to rule out the differential of HAC as it is easy; the sample can be collected at home, it does not require another office visit, and it is relatively inexpensive. The test's high sensitivity and low specificity mean that most of the time the result is increased (positive). So if it is normal or decreased, it is very unlikely the patient has HAC. A negative UCCR combined with a reference interval ALP is sufficient evidence to rule out HAC. LDDS is the test of choice when the patient looks Cushingoid because of its high sensitivity of 95%. Like the UCCR there are numerous false positives (up to 55%), so avoid LDDS if the pet does not have multiple

clinical signs or lab data characteristic of HAC. Because an animal may be stressed by its primary disease, its LDDS can be falsely "positive"; that is, it does not suppress, but it does not have HAC. If HDDS is used in this situation, the results will show suppression that indicates PDH; this is a false positive diagnosis with serious consequences. Never use HDDS as a screening test. In patients that do not look typical for HAC and may only have a few of the lab data characteristic of HAC, then ACTH stimulation is preferred as it has the fewest false positives, 15%. In patients that look typical for HAC and that have multiple laboratory results characteristic of HAC, then LDDS is preferred. Phenobarbital therapy infrequently produces abnormal results for LDDS, but it does not induce abnormalities for ACTH stimulation tests or e ACTH.

Clinical pathology does a good job of telling its users what the sensitivity and specificity are of lab tests. For endocrine testing that has led to clarification and some confusion because the ranges provided and cut-off values for diagnoses may vary due to different methodologies. If the reference intervals are wider, the lab tests are less discriminating. Where the cut-off is set for diagnostic values changes sensitivity and specificity. Therefore use reference intervals and cut-off values from the lab providing results. No test can be 100% positive or negative, or 100% sensitive and specific because there are too many biologic and methodologic variables.

Summary ideas

- If serum ALP is not increased, HAC is very unlikely.
- If UCCR is not increased, rule out HAC. If both ALP and UCCR are not increased rule out HAC. Consider retesting in future if HAC remains a differential.
- UCCR is a good test to rule out HAC; never use to rule in HAC.
- UCCR avoids a visit, easy first step.
- If characteristic signs and lab data are present, use LDDS.
- If limited signs and lab data are present = UCCR to rule out, follow with ACTH stim if UCCR increased; avoid LDDS as false positive rate is too high.
- If a nonadrenal illness is more likely than HAC: use UCCR, ACTH stim if needed.
- PU PD with characteristic other signs: use LDDS.
- PU PD with few signs of HAC: Use UCCR to rule out and follow with ACTH stim.
- Suspect an adrenal tumor: LDDS is preferred; ACTH stim has lower sensitivity for AT; consider imaging to search for AT.
- Suspect iatrogenic: Use ACTH stim and evaluate history.
- Suspect hypoadrenocorticism: measure basal cortisol and ACTH stim if needed.
- How long to withdraw steroids before retesting: It is difficult to be certain when the pituitary adrenal axis will return to normal. It ranges from 2 to 8 weeks depending on dosages and duration of steroid administration.
- Unit conversion: to convert cortisol to µg/dL (mcg/dL) from nmol/L divide cortisol reported in nmol/L by 27.6.

Differentiating tests

- e ACTH: Easy, just submit one sample; differentiates PDH and AT.
- LDDS: Can rule in HAC and identify 60–80% of dogs with PDH.
- HDDS: Traditional test to differentiate AT and PDH; dogs that suppress have PDH and dogs that do not suppress have either AT or PDH.
- HDDS: Only 10% of the cases that LDDS did not distinguish, a HDDS may successfully identify.
- 100% of AT do not suppress with HDDS or LDDS.
- If resistant to dexamethasone consider imaging to look for AT and/or measure e ACTH
- If suspecting a large pituitary tumor, consider imaging by CT or MRI.

Unusual cases

Most dogs that look Cushingoid but that do not test positive with ACTH stim and LDDS do not have HAC. However, some of these dogs could have "atypical or occult hyperadrenocorticism." This form of HAC may have increased concentrations of one or more precursors of cortisol. The most diagnostically important precursor is 17-hydroxyprogesterone (17-OHP). If dogs with this atypical form have a deficiency in one of the enzymes needed to convert precursor molecules into cortisol, then the molecule before this enzyme deficiency will increase. For example, 17-OHP will increase if the enzyme 21 beta hydroxylase is deficient or 11-deoxycortisol will increase if the enzyme 11 beta hydroxylase is deficient. If atypical hyperadrenocorticism is suspected then the test of choice is ACTH stimulation. The protocol is modified to measure both cortisol and 17-OHP pre and post stimulation and comparing both concentrations to reference intervals provided by the laboratory. Post stimulation cortisol that is not out of the reference interval combined with an increased concentration of 17-OHP is consistent with what is called atypical or occult hyperadrenocorticism.

Although increases in 17-OHP can be measured in dogs, it is not clear if this hormone causes lesions or if the "syndrome" actually causes clinical disease. Dogs without adrenal disease can have increased serum 17-OHP just as they have increased serum cortisol due to stress of a concurrent disease. There also are reports of cases treated with trilostane that improved despite continued increase in serum 17-OHP. Furthermore, others have observed that as adrenal function is stimulated by a true adrenal disease or nonspecifically by stress that the production of all hormones increase, cortisol and sex hormones. Other sex hormones that can be measured are progesterone, estradiol, testosterone, and androstenedione, basal and post ACTH stimulation. This has led to "steroid hormone profiles" or measurement of the parent

products, cortisol and estradiol, as well as their various precursors. In ferrets these profiles are diagnostically helpful. If measured, estradiol is often increased in dogs being investigated for HAC. However studies that correlate these hormone profiles with clinical disease in dogs and cats are needed, as well as the determination of false positives and negatives. If 17-OHP can increase post ACTH in dogs that do not have adrenal disease it seems reasonable that other sex hormones would increase as well, further weakening the concept that occult hyperadrenocorticism causes clinical disease. Additional studies are needed before the syndrome of occult HAC is recognized widely.

21-Hydroxylase deficiency does cause prolonged gestation in Holstein cattle because the adrenal gland cannot produce sufficient cortisol to stimulate delivery. The result is a fetal giant.

Ferrets

Hyperadrenocorticism is a common disease in ferrets and produces many of the clinical signs and lab abnormalities described for dogs and cats, with the most striking physical abnormality being baldness. The adrenal lesion and the hormones that cause the disease, however, are very different. The lesion is in the adrenal gland. Approximately half of the lesions are hyperplasia and half are neoplastic. About 80% of the lesions involve the left adrenal gland and about 15% are bilateral. Unilateral adrenalectomy provides successful treatment for those cases with proliferations in the left adrenal. The principle hormone that causes the clinical signs is estrogen. Serum cortisol is rarely increased. If the disease exists for a long enough period the increased estrogens will cause bone marrow suppression leading to anemia and thrombocytopenia. Diagnosis is confirmed by measurement of estradiol or estradiol precursors (17-hydroxyprogesterone and/or androstenedione). The precursors to estradiol contribute to the disease and if estradiol is not increased, then the precursors should be measured. Commercial assays for these hormone profiles are available.

Most of the adrenal lesions classified as neoplastic are considered carcinomas via histologic features. However, very few metastasize and therefore their biologic behavior is similar to an adenoma. Some cases have life-threatening hemorrhage from necrosis within the tumor.

Pheochromocytoma—tumor of adrenal medulla, increased epinephrine and norepinephrine

Pheochromocytoma is the most common tumor of the adrenal medulla, but it is rare. It occurs in all species, probably most frequently in cattle, dogs and rats, and in a few horses. In bulls and people it is associated with concurrent tumors of C-cells and is part of multiple endocrine neoplasia (MEN). A concurrent tumor in other adrenal locations is seen in about half of the dogs with a pheochromocytoma. Clinical signs are attributed to the increased catecholamines in the blood, but they may also be due to direct effects of the tumor or concurrent neoplasia. Signs reported in dogs and horses include tachycardia, arrhythmias, tachypnea, panting, weakness, collapse and seizures. Polyuria and polydipsia are reported frequently in cats with pheochromocytomas. Routine clinical pathology is usually of little help. Nonspecific observations include increased liver enzymes in 10–50% of dogs, mild proteinuria in 50% perhaps secondary to increased blood pressure induced glomerular leakage, and dilute urine due to inhibition of vasopressin by the increased catecholamines. Approximately half of the dogs will have a stress leukogram and 75% will have increased ALP. A few of these dogs have concurrent HAC. Sometimes the first time a pheochromocytoma is considered is when abdominal ultrasonography reveals a mass in the adrenal region and/or masses in the vena cava. Metastases in dogs are reported in 20–50% of the cases.

Norepinephrine is the principal catecholamine secreted from pheochromocytomas in dogs. Urinary vanillylmandelic acid and free unconjugated catecholamines are increased in bulls with pheochromocytoma. Stimulatory and inhibitory tests are used, but have side effects and the measurement of catecholamines in these tests should be coordinated with a lab that will perform assays and provide reference intervals. Cytology of the suspected tumor reveals "naked nuclei" or nuclei with very little cytoplasm.

Pituitary disorders

The pituitary gland is composed of the adenohypophysis (pars distalis or anterior lobe), neurohypophysis (pars nervosa or posterior lobe), pars intermedia (intermediate lobe), and pars tuberalis (infundibular stalk). The adenohypophysis is formed by the differentiation of embryonic oral ectoderm, Rathke's pouch, into trophic secretory cells producing growth hormone (GH) or somatotropin, prolactin, thyroid stimulating hormone (TSH), follicle-stimulating hormone (FSH), luteinizing hormone (LH), and adrenocorticotrophic hormone (ACTH). The most common disease and lesion from the pars distalis is hyperadrenocorticism due to an adenoma secreting ACTH (Table 32.20). Neoplasms that produce other trophic hormones and that cause disease are rare. An example is a growth hormone producing adenoma that causes acromegaly. Nonsecretory neoplasms, inflammation, and embryonic cysts that destroy the pituitary gland are uncommon and result in panhypopituitarism, dwarfism, or selective decrease in specific trophic hormones. Hypoadrenocorticism and hypothyroidism are the most common diseases due to pituitary destruction. These diseases are described in the adrenal and thyroid sections of this chapter.

The neurohypophysis is composed of axons that originate within the supraoptic and paraventricular nuclei of the

Table 32.20 Regions in the pituitary gland and associated lesions and the most common diseases.

Pars distalis	Neoplasia	Hyperadrenocorticism; acromegaly—cat
Pars distalis	Cyst	Dwarfism
Neurohypophysis	Varied	Diabetes insipidus
Pars intermedia	Neoplasia	Pituitary pars intermedia dysfunction—horse
Pars tuberalis	Obstruction	Diabetes insipidus

hypothalamus. Separate neurons within both nuclei synthesize vasopressin (antidiuretic hormone, ADH) and oxytocin. These hormones migrate through the infundibular stalk in axons as precursor proteins to be stored in secretory granules in the neurohypophysis, pars nervosa, and are released under appropriate stimuli. Oxytocin stimulates uterine contraction and milk secretion. ADH stimulates water reabsorption in the distal and collecting tubules.

Various pituitary disorders are described below.

Equine Cushing's like syndromes

Pituitary pars intermedia dysfunction (PPID)

Equine Cushing's disease is more appropriately called pituitary pars intermedia dysfunction (PPID). This term is deemed more appropriate because the pituitary lesion does not have to be neoplastic. It is located in the pars intermedia. There are dysfunctional biochemical events and it stimulates a unique pathogenesis with a different profile of pituitary hormones that do not result in marked adrenal cortical hypertrophy. The pituitary lesions range from hyperplasia to large tumors. Clinical disease, hormone profiles, and biochemical changes may occur without overt pituitary adenoma formation. Rare causes of Cushing's in the horse is a neoplasm in the pars distalis or in the adrenal cortex. Nodular adrenocortical hyperplasia does not cause clinical disease and is similar to the lesion in older dogs.

Classical signs such as an aged horse (mean age 24 years, range 15–32) with swayback, hirsutism, laminitis, muscle atrophy, lethargy, abnormal fat distribution, hyperhidrosis, polyphagia, intermittent fever, and secondary infections are adequate to diagnose many cases and start treatment with pergolide. Owners often complain that each year the horse sheds hair later or not at all and the excessive sweating leads to the characteristic long curly coat that mats and persists in warm months. Hirsutism is a unique abnormality present in 80–100% of horses with PPID and it is considered pathognomonic. It probably is a manifestation that occurs late in the course of the syndrome. PUPD is reported, but owners do not consistently observe it. Secondary skin infections and poor wound healing are observed in 25–50% of horses. Up to 80% of horses with PPID develop laminitis, apparently from carbohydrate intolerance. The hypothalamus is the primary control center for cyclic shedding of hair, appetite control, and temperature regulation. One proposal is that pressure on the hypothalmus from the expanding tumor in the pituitary is responsible for the clinical signs of polyphagia, hypertrichosis, and fever. Tumors may compress the hypothalamus due to their relatively large size and an incomplete sella turcica in the horse. Pressure on the hypothalamus and increased concentrations of intermediary substances and cortisol produce the characteristic physical abnormalities that are diagnostic of this syndrome. Despite large pituitary tumors in some horses, neurologic or visual signs are infrequent and variably reported at 5–50%. PPID is also seen in horses with small pituitary lesions that are hyperplastic. Therefore size is not the major reason for the disease, but larger lesions correlate with older age of affected horses and more fully developed clinical signs that have characteristic endocrine profiles. PPID is a progressive disease and identification of the starting point is vague. Early disease recognition is difficult because the lab tests and physical characteristics are not definitive in the early stages.

The pars distalis processes the precursor peptide pro-opiomelanocortin into ACTH. Melanotrophs in the pars intermedia synthesize pro-opiomelanocortin and cleave it into ACTH and further processes it into α-and β-melanocyte stimulating hormone (MSH), β-endorphins, and corticotrophin-like intermediate lobe peptide (CLIP). Plasma and tumor concentrations of ACTH are only mildly increased in horses with this syndrome, but the tumor and plasma concentrations of the intermediary peptides listed above are markedly increased. These data help explain the mild to moderate increases in ACTH and cortisol combined with modest adrenocortical hyperplasia versus the marked increases in plasma concentrations of MSH, CLIP, and beta endorphin seen in horses with PPID. Measurement of these peptides is more useful in the diagnosis of PPID in horses than is the measurement of basal cortisol or ACTH. Cortisol strongly inhibits ACTH secretion from the pars distalis, but has little effect on peptides secreted by the pars intermedia because melanotrophs do not express glucocorticoid receptors. Hence the dexamethasone suppression tests used in dogs are less useful in horses and require modifications.

Physical and historical data are more helpful to the diagnosis than are routine clinical pathology abnormalities that are common in small animals. Urine specific gravity ranges from 1.022 to 1.047, which helps explain why PUPD is infrequently reported in horses. This seems paradoxical

given that cortisol can be increased and large pituitary tumors could interfere with function of the pars nervosa and ADH release.

When PPID is fully developed, the horse has marked hypertrichosis and the diagnosis is easy to confirm with endocrine tests. It is the early cases that are problematic to diagnose. When confirmation is desired in a horse with hypertrichosis the standard method of testing is the 19-hour (overnight) dexamethasone suppression test (DST). Normal horses should suppress and failure to suppress is diagnostic for PPID. Inject dexamethasone IM 40 µg/kg and serum cortisol should be <1 µg/dL (27.6 nmol/L) 19 hours later in 97% of normal horses. Horses with PPID do not suppress, cortisol is >1.0 µg/dL, at least when the disease is fully developed. Confounding factors of the DST include concurrent laminitis, time of the year, and stage of the disease. Therefore test results should be correlated with all the other clinical and laboratory information. DST may fail to suppress normal horses and ponies in the fall (false positives). Steroids may induce laminitis and early on in the disease horses with PPID may suppress with DST, false negative. A concern of DST is that it may exacerbate laminitis in horses with a history of current or past laminitis. However, in a study of 43 PPID horses in which DST was used, this was not observed. There are no other diseases that look similar to PPID in its late stages and therefore the main distinction is to separate horses with PPID from horses with normal aging changes. DST is successful in distinguishing these two groups. However, recognizing PPID early in its development, when the pituitary lesion is small and the clinical signs mild, requires tests other than DST.

Other tests that have value are endogenous ACTH, α-MSH, TRH (thyrotropin-releasing hormone response test), combined hormone response tests, and measurement of ACTH after oral administration of domperidone. These tests may prove helpful in recognizing PPID in its early stages. DST is useful for more advanced cases and it is easy to perform. Basal cortisol is not diagnostic and loss of diurnal secretion of cortisol is controversial. ACTH stimulation is not a useful test as it recognizes <20% of the cases. This lack of stimulation is likely because adrenocortical hyperplasia is not a prominent feature of PPID. Endogenous ACTH is also used to diagnose PPID. Using a 10–50 pg/mL reference interval and a cutoff of >55 pg/mL indicates PPID with a disease range of 104–1000 pg/mL. Variables include where the cutoff value is set (critical), time of day, and different reference intervals for ponies versus horses. It is critical to use values set by the reference lab that analyzes the samples. Although this is a simple one-collection procedure it does not recognize horses in the early stages of PPID and misses some horses in the late stages of the disease. Therefore basal ACTH is not ideal and provocative testing is recommended. Assays for ACTH should be validated for equine and collection procedures for ACTH outlined for dogs should be followed.

Measurement of α-MSH may be better than ACTH in horses because MSH is produced primarily in pars intermedia and ACTH is primarily secreted from pars distalis. Plasma α–MSH hormone concentration >91 pmol/L is considered diagnostic of PPID. However, there is seasonal variation in mean concentrations and ranges: fall = 50–60 pmol/L + 65 pmol/L, spring, summer, winter = 11 pmol/L + 4 pmol/L. Therefore additional guidelines considered diagnostic for PPID are if either plasma α-MSH is >19 pmol/L of mean concentration in spring, summer, winter or if plasma α–MSH is >148 pmol/L of mean concentration in the fall. Plasma α-MSH and ACTH concentrations increase as daylight decreases, from maximum daylight hours to 12 hours of daylight (September), but serum insulin does not fluctuate. This occurs in normal horses and ponies and in horses and ponies with PPID, hence the season of the year should be considered when interpreting results. Ambiguous results can be repeated at a later time, even in a different season. Horses and ponies receiving pergolide, dopamine antagonists have a significantly less increase in α-MSH and lower plasma ACTH. Another diagnostic approach is to measure α-MSH after a DST. A α-MSH >90 pmol/L post DST is the cutoff value between normal horses and horses with PPID.

The pars intermedia is partially regulated by dopaminergic input from neurons in the hypothalamus and loss of dopaminergic inhibition is hypothesized to stimulate the pars intermedia causing the hyperplastic lesions that lead to neoplasia and PPID syndrome. Domperidone is a synthetic benzimidazole used to treat fescue endophyte agalactia in mares and it blocks dopamine receptors. Therefore the correct dose of domperidone should block dopamine receptors thereby permitting melanotrophs to release the pars intermedia peptides α-MSH, β-endorphin, CLIP, and ACTH and the concentrations of these substances should be greater in horses with PPID than in normal horses. A recent study tested this theory on 33 horses and discovered that horses with histologic lesions in the pars intermedia characteristic of PPID had increased concentrations of ACTH in response to domperidone that distinguished them from aged horses without pituitary lesions. Samples were collected before oral administration of domperidone, 3.3 mg/kg and at 4 and 8 hours post administration (see below):

Pituitary grade	ACTH pg/mL	Lesion, n; mean age (yr); 33 total horses
Grade 1	20.0	Normal 3, 7.5 y
Grade 2	27.1	Focal lesion of hyperplasia 9, 14.5 y
Grade 3	64.4	Diffuse hyperplasia 5, 21.0 y
Grade 4	128.0	Microadenoma 12, 23.3 y
Grade 5	720.5	Adenoma 4, 25 y
Reference interval	10–59	

This study used horses without hirsutism or obvious signs of advanced PPID. It found that although basal ACTH was not consistently increased in horses with pitutitary lesions, ACTH post domperidone was increased consistently in horses with pituitary lesions characteristic of PPID. Horses categorized as grade 3 and above had pituitary lesions and mean ACTH concentrations greater than twofold above horses without significant pituitary lesions and were consistent with a diagnosis of PPID. However, approximately 25% of the horses in grade 3 and 4 did not increase ACTH post domperidone beyond the upper limit of the reference interval. Although horses were tested in all seasons of the year, the authors suggested the need to perform these evocative tests on a greater number of normal and affected horses to determine if there is any seasonal effect or any effect from repeated testing. It is known that season of the year influences concentrations of α-MSH, although it seems unlikely that repeat testing could interfere with the utility of evocative tests. Blood for ACTH is collected into silicon-coated EDTA tubes or plastic tubes with EDTA, plasma harvested, and kept frozen until assayed; chemiluminescent immunoassay, immulite ACTH has been validated for the horse.

Horses with PPID have increased basal concentrations of insulin: 35–260 μU/mL, reference interval 27–53 μU/mL. This is not a diagnostic test for PPID as insulin will be increased for other reasons, but it may help explain carbohydrate intolerance, obesity, and propensity for laminitis. The increase in insulin may be due to a combination of the antagonistic effects of cortisol and increased concentrations of CLIP that can stimulate release of insulin. UCCR reference interval is reported as $4.7–16 \times 10^{-6}$ and a cut-off of $>20 \times 10^{-6}$ is consistent with PPID; however, additional studies are needed.

Horses that have classical signs for PPID are easy to identify from the results of physical examination and laboratory test results. However, it can be a diagnostic challenge to recognize PPID in older horses that only have some of the signs of PPID. These are the cases that laboratory testing is most needed. Increased awareness of this disease and medications to treat the disease has resulted in increased testing for PPID when the disease is still developing. Horses can be treated medically with low doses of pergolide (0.0017 mg/kg/day divided in two oral doses or 0.75 mg/day for a 450 kg horse, cyproheptadiine (antiserotonergic) and bromocriptine (dopaminergic agonist).

Peripheral cushing's syndrome or equine metabolic syndrome (EMS)

This is a metabolic disorder of older horses (8–18 years) that have hyperinsulinism, activation of cortisol in peripheral tissues, obesity, and laminitis. The disease develops in genetically susceptible horses (Morgans and Spanish breeds) that are overfed and under-exercised. These horses will be overweight, have excess fat in the rump region, "cresty necks," and laminitis that is often attributed to hypothyroidism or PPID. However, DST is negative for PPID and thyroid stimulation tests are negative for hypothyroidism even though there may be a low TT4 concentration on single point determinations. Owners report the horses are "easy keepers" and they maintain their weight despite dietary attempts to reduce it. Fat cells in the abdominal region are important in the pathogenesis of obesity as apparently adipocytes in the abdomen are hormonally different from fat cells in other locations and compound the effects of obesity.

Horses with EMS have hyperinsulinemia and a resistance to the action of insulin that results in increased serum glucose and glucose intolerance or delayed reduction of glucose similar to type 2 diabetes. The mechanism of the insulin resistance is unknown. Substances released or produced in fat cells that lead to insulin resistance are free fatty acids, tumor necrosis factor, leptin, cortisol, and resistin. Resistin is an adipocyte hormone that may be pivotal in this syndrome. Apparently there is conversion of inactive cortisone to cortisol in peripheral tissues with excess cortisol activity in skin, fat and laminar tissue of horses with EMS. The enzyme 11 beta-hydroxysteroid dehydrogenase-1 converts inactive cortisone to active cortisol in adipocytes and other tissues. Equine diets rich in grains are referred to as having a high glycemic index because they stimulate increased serum glucose and insulin for sustained periods. Feeding adult horses excess grain for the amount of exercise performed leads to obesity and may start the cascade of events in susceptible individuals. Typically, fasting blood glucose in the horse is 60–90 mg/dL and insulin is <5–20 uIU/mL. Glucose >250 mg/dL and insulin >200 uIU/mL is clearly abnormal.

Diabetes insipidus

Central diabetes insipidus (CDI) may manifest as partial or complete forms. They are characterized by decreased concentration of ADH, PUPD, and low urine specific gravity ranging from 1.002 to 1.012. They may be dehydrated in the face of dilute urine. They have few to no abnormalities in the CBC or chemistry panel. Typically there is no renal disease and the patient responds to exogenous ADH by gradually increasing urine specific gravity. The main differentials are nephrogenic diabetes insipidus and psychogenic polydipsia. Polydipsia is usually water consumption of >100 mL/kg/day and polyuria is urine production >50 mL/kg/day in dogs and cats.

General

ADH is released or retained under appropriate stimuli from osmoreceptor neurons located in the hypothalamus that sense changes in plasma osmolality. As plasma osmolality increases beyond 310 mOsmo/kg these osmoreceptors stimulate the release of ADH that binds to specific V2 receptor

sites and stimulate cellular events leading to the creation of channels in the distal tubular and collecting duct epithelial cells to transport water from the glomerular filtrate into the medulla and then into blood vessels. Failure to produce or release ADH results in excretion of dilute urine with a specific gravity (s.g.) less than 1.012, but typically much lower.

Central diabetes insipidus (CDI) is due to inadequate release and/or production of ADH (vasopressin). Idiopathic is the most common "cause," either because a structural lesion cannot be found or one is not looked for. Known causes include tumors, inflammation, head trauma, parasitic, cysts, and pituitary surgery. CDI secondary to surgery on the pituitary may be transient or permanent. Tumors are usually of pituitary origin, but can be neural in origin. In dogs and cats there are two forms of CDI, partial and complete. Complete is essentially no ADH and is associated with little to no increase in urine osmolality with increasing plasma osmolality. Animals with complete CDI will have persistent hyposthenuria, severe urine diruesis, and urine specific gravities of 1.005 or less, even if dehydrated. Partial diabetes insipidus is associated with a small, but insufficient increase in urine osmolality with increasing plasma osmolality. These animals can concentrate urine into the isosthenuric range of 1.008–1.015, but cannot increase the specific gravity beyond 1.020 even if dehydrated.

Inhibition of adequate quantities of ADH at the level of renal tubules is referred to as nephrogenic diabetes insipidus (NDI) and can be due to decreased tubular cells or compounds in the blood that interfere with the action of ADH. These include corticosteroids, hypercalcemia, *E. coli* toxin in pyometra, and hypokalemia. A rare congenital form occurs in Siberian huskies and cats and may be due to a receptor defect.

Measurement of ADH in serum has not been applied to these diseases because assays are not readily available or used. As assays become available and affordable they may be used to define the syndromes and aid diagnoses. Until then the diagnoses will be established via formulation of differential diagnoses, rule in/rule out tests for each, and use of water deprivation studies to distinguish CDI, NDI, and psychogenic polydipsia. The other causes of PU PD are identified without water deprivation studies (Table 32.21).

Table 32.21 Causes of polyuria (polydipsia) and dilute urine via associated mechanisms.

Decreased ADH—Central diabetes insipidus (DI)
Pituitary (hypothalamic rare) tumor, abscess, idiopathic, congenital

Inadequate response of tubular cells to adequate ADH—Nephrogenic DI
Hypercalcemia, steroids, hypokalemia, pyometra *E. coli* endotoxin, congenital lack of response of tubular cells to ADH

Decreased renal mass = lesions in kidneys, loss of tubular cells
With azotemia = >75% involvement; especially if lesions in medulla and pelvis
Without azotemia = 66% to 75% involvement of total renal mass

Excess fluid intake
Psychogenic polydipsia
Fluid overload diuresis

Medullary washout—medullary interstitium not saturated with sodium and urea
Addison's—prolonged hyponatremia
Liver failure—decreased urea nitrogen (other lab data also); congenital and acquired shunts; end stage liver disease
Psychogenic polydipsia
Fluid overload diuresis

Solute overload
Diabetes mellitus, Acromegaly, Fanconi's syndrome, salt toxicity

Diuretics—many; they act on different regions of the tubules

Others, poorly understood mechanism
Hypoparathyroidism, hyperthyroidism, polycythemia, myeloma without hypercalcemia

History, signalment, and routine laboratory data

There is no age, breed, or sex predilection for CDI, but young adults are most commonly affected. The disease has been recognized in dogs ranging range 8 weeks to 14 years, with a mean of 5 years. In cats the observed age range is 8 weeks to 6 years, with a mean of 1.5 years. The major clinical signs of DI are moderate to marked polyuria (up to 100 mL/kg/day) and a nearly constant demand for drinking water, polydipsia. Neurologic signs are infrequent and if present are associated with neoplasms of the CNS or pituitary. The severity of clinical signs varies if CDI is partial or complete. In severe cases nocturia may be hourly and incontinence may be of sudden onset or of several months duration. Weight loss occurs because drinking is so excessive it interferes with eating. The Brattleboro rat has a hereditary form of complete DI such that it will exceed 70% of its body in water consumption and urine output daily.

Routine complete blood count and serum biochemical panel are normal in animals with CDI. Persistent low urine specific gravity, <1.012 is often the only abnormality detected on routine clinical pathology data. If plasma osmolality is measured it is often high (>310 mOsm/L) due to mild dehydration; they cannot drink sufficient water to keep up with urinary water loss. Mild hypernatremia may be observed in a chemistry panel and is secondary to the dehydration. If water is withheld from dogs with the complete form of CDI they can develop marked hypernatremia, >170 mEq/L, marked hyperosmolality, >380 mOsmol/kg, and lethal hypertonic encephalopathy within hours of water restriction. The combination of hyposthenuria and hypernatremia favors DI. When abnormalities such as slightly increased

hematocrit or hypernatremia are present at initial evaluation, they usually are secondary to dehydration from water restriction by the owner. Animals with primary polydipsia have low plasma osmolality (<290 mOsm/L) as a result of overhydration. The reference interval for plasma osmolality in normal dogs and cats is 280–310 mOsm/L.

At the time of initial presentation the majority of dogs with DI will have urine s.g. <1.006 and many will be 1.001–1.003 if unlimited water is available. If the urine specific gravity can go below 1.005 then occult renal disease is unlikely because a specific gravity this low implies the kidneys functioned sufficiently to remove solutes, diluted the glomerular filtrate to the 1.005 range, but then had inadequate ADH to remove water in the collecting tubules. Urine specific gravity 1.007–1.013, proteinuria, and white blood cells in the urinalysis favor renal failure, often chronic pyelonephritis in which there is fibrosis in the renal medulla.

Examples of urinalysis findings useful for disease differentiation are:

Patient	A	B	C	D	E
Urine s.g.	1.003	1.016	1.011	1.002	1.010
Proteinuria	Neg	++	Neg	Neg	+
Glucosuria	Neg	+	Neg	Neg	Neg
Cells	Neg	++	Neg	Neg	Neg
Interpretation	CDI	Renal	Renal	CDI	Renal

A and D: Severe hyposthenuria with no abnormalities in urinalysis favor complete CDI; urine s.g. lower than expected for renal; other differentials are: partial CDI, psychogenic polydipsia, and NDI caused by interfering substances such as hypercalcemia or steroids.
B: Urine s.g. higher than expected for CDI, in combination with proteinuria, glucosuria and cells favor nephritis, structural lesion, possible pyelonephritis, recommend culture of urine; do not perform water deprivation study. Partial CDI can concentrate into this range if water is restricted. This type of renal failure patient may not be azotemic (<75% nephron loss), but may be polyuric (>66% nephron loss).
C: If urine s.g. is persistent in this range it is isosthenuric. With no other abnormalities in UA, then chronic renal is most likely, with or without azotemia; partial CDI can concentrate into this range if water is restricted.
E: If the urine s.g. is persistent in this range then the patient has isosthenuria. Proteinuria at one plus in dilute urine is abnormal. Recommend protein:creatinine ratio, microalbuminuria determination, and periodic UA to confirm urine s.g. range and persistence of proteinuria. If cellular abnormalities develop consider urine culture. This pattern favors chronic renal insufficiency; therefore consider fractional excretion of sodium, creatinine clearance and/or palpation, visualization studies of kidneys before a water deprivation study is recommended.

Diagnostic tests

Table 32.21 lists the causes of PUPD, and most of these are diagnosed from history, physical examination, routine lab data, and appropriate diagnostic tests. The first step is to determine urine s.g. at several times during the day; one of these must be a morning collection when concentration is usually at its maximum. The main differentials that have few to no helpful diagnostic results other than dilute urine are central DI, nephrogenic DI, and psychogenic polydipsia. The test used to differentiate these is one of the water-deprivation tests or response to ADH supplementation. The modified water-deprivation test is indirect evidence of ADH responsiveness designed to determine whether endogenous ADH is released in response to dehydration and whether the kidneys respond by concentrating the urine. The more common causes of polyuria and polydipsia, however, should be ruled out before this procedure is performed. Failure to recognize azotemia before water is restricted is a major clinical error. Nephrogenic diabetes insipidus (NDI) is due to a structural lesion that decreases renal tubular cells or a biochemical lesion, substances in the blood that interfere with the action of ADH. These include corticosteroids, hypercalcemia, *E. coli* toxin in pyometra, and severe hypokalemia. These inhibitory factors are ruled out by diagnostic procedures for each substance or the disease that causes them. That leaves the possibility of an occult renal lesion that has resulted in loss of greater than 66% of the renal mass, hence polyuria and dilute urine before enough mass is lost to result in appreciable azotemia. This situation is easier to diagnose or at least suspect if the history indicates the patient survived a previous bout of renal azotemia, but PU PD have persisted or are now present. If the patient had a prior diagnosis of pyelonephritis, then this scenario is even more likely. An inherent risk of a water deprivation study is the possibility of exacerbating renal problems in these patients. They have renal failure (polyuria, dilute urine) and a structural renal lesion, but they are not yet azotemic. The diagnosis of occult renal lesions should be suspected if there is proteinuria (increased protein:creatinine ratio), isosthenuria, and/or white blood cells in the urine. If these are present avoid a water deprivation study and perform fractional excretion of sodium or creatinine clearance study to rule in/rule out structural renal lesions. If the urine specific gravity can go below 1.005 then occult renal disease is unlikely. If the urine specific gravity can increase beyond 1.020 then occult renal disease and CDI are unlikely and psychogenic polydipsia is more likely. Another risk of a water deprivation study is induction of marked hypernatremia, hyperosmolality, and hypertonic encephalopathy within hours of water restriction in patients with complete CDI. For these reasons patients must be evaluated frequently during water deprivation studies.

The water deprivation principle is to stimulate endogenous ADH production and release by withholding water and

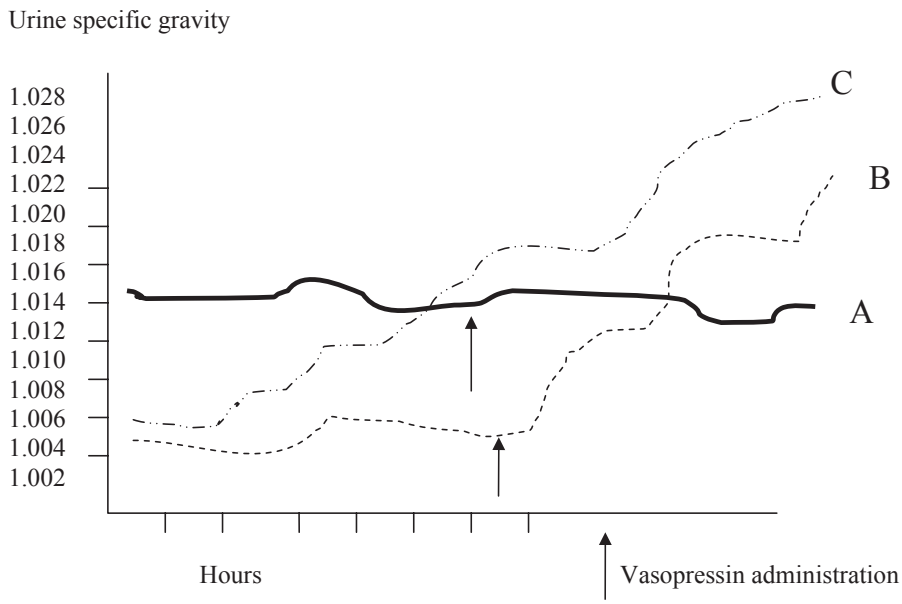

Figure 32.6 Water deprivation study results for dogs with renal insufficiency (A), central diabetes insipidus (B), and psychogenic polydipsia (C). All three dogs are not azotemic. The dog with renal insufficiency has urine specific gravity (s.g.) that starts and remains in the isosthenuric range, even after administration of vasopressin (ADH). The dog with CDI has more dilute urine than the dog with renal insufficiency because the tubules can function and therefore have diluted the glomerular filtrate. This dog has urine s.g. that starts in the hyposthenuric range and starts to concentrate only after the administration of ADH. A dog with psychogenic polydipsia (C) should have a urine s.g. in the hyposthenuric range that starts to concentrate after water is withheld and before it is necessary to give ADH. The dogs with CDI and psychogenic polydipsia increased urine s.g. in a gradual or stepwise fashion because the concentration gradient in the medulla had been "washed out" and needed to reestablish.

inducing mild dehydration of less than 5%. If an animal can start to concentrate urine with water withdrawal then psychogenic polydipsia is the diagnosis. If the patient does not concentrate until exogenous ADH is administered, then central DI is diagnosed and if water withdrawal and exogenous ADH administration do not stimulate urine concentration, then renal disease is diagnosed. Never perform this test on an azotemic patient. Before the study is started the bladder is emptied and baseline data is gathered. Urine is then evaluated and recorded every 1–3 hours, depending on the severity of the PU PD until the study is stopped. The bladder is emptied at each collection period and body weight, skin turgor, PCV, plasma protein, UN, urine s.g., and osmolality of plasma and urine are optimal parameters to monitor. Osmolality does not have to be measured, but it is ideal if immediately available. Urine s.g. and body weight are usually more practical substitutes. Three to five percent weight loss is maximum stimulus to release endogenous ADH. If the urine is not concentrating at this point in the study, then it is time to administer exogenous ADH. Another means to determine it is time to give ADH is if urine s.g. has increased less than 10% for three consecutive collections. If plasma osomolality is known during the study then administer ADH when plasma osomolaltiy is >310 mOsm/kg because this is adequate stimulus to release endogenous ADH. When urine s.g. is in the 1.025–1.035 range it indicates the kidneys can concentrate urine and the study can be stopped. The urine s.g. may not increase rapidly in a water deprivation study. The urine s.g. may sometimes increase in a stepwise fashion due to medullary washout (Fig. 32.6). The prolonged polyuria and rapid transit of the tubular filtrate has resulted in dilution of the medullary interstitium sodium and urea concentrations. Until the interstitium is re-saturated with solutes, the kidneys can only concentrate the glomerular filtrate a limited amount. Depending on the severity of the medullary washout it may take up to 24 hours or longer of complete or partial water restriction for dogs with primary polydipsia to concentrate into the 1.030 range. Monitor the UN and if azotemia develops stop the study. Serum UN and/or creatinine should not increase in a water deprivation study. The renal failure patient has between 2/3 and 3/4 decreased renal mass and the closer the reduced mass is to the 3/4 mark the easier it will be to induce azotemia via dehydration.

If the urine s.g. increases beyond 1.025 with just water restriction, then primary polydipsia is the diagnosis. If urine remains nonconcentrated after several hours with concurrent mild dehydration then the disease present is either CDI or NDI (Fig. 32.6). The NDI patient will not concentrate urine in response to ADH. The CDI patient that has a

complete form of the disease will increase urine s.g. by approximately 50% and the partial form will increase s.g. by 15–20% in response to exogenous ADH. The time it takes to lose 3–5% of the body weight (dehydration) is also a clue to the diagnosis. CDI patients often attain this weight loss in less than 6 hours while partial CDI and psychogenic polydipsia dogs will take 8–10 hours or longer.

Pituitary dwarfism and acromegaly

General

Growth hormone (GH), or somatotropin, is a single-chain protein that is species-specific in its activity. It is produced by acidophilic somatotrophs. Hypothalmic GH-releasing hormone stimulates production and release of GH, while somatostatin is inhibits release. Further inhibition is caused by insulin-like growth factors (IGFs) that stimulate somatostatin release from the hypothalmus and directly inhibit GH in the adenohypophysis. Canine mammary gland epithelial cells produce GH that is identical to pituitary GH. Administered progestins stimulate production of mammary GH, increase plasma GH concentrations, are not inhibited by somatostatin, and can cause acromegaly. The luteal phase of the estrous cycle of healthy bitches has high serum progesterone concentrations that also increase serum concentrations of GH. This progesterone-induced production of mammary GH is important in stimulating lactational mammary tissue and in the development and progression of mammary tumors in the bitch. GH concentration in colostrum is hundreds of times greater than in plasma and may stimulate neonatal gastrointestinal development.

GH stimulates the production of IGFs (IGF-1 or somatomedin) from the liver that in turn stimulates protein synthesis, chondrogenesis, and longitudinal and appositional bone growth. GH may also stimulate these events directly. Absence of GH during the period of growth results in pituitary dwarfism and excessive amounts of GH in adults cause acromegaly. GH also inhibits the action of insulin and therefore increases the serum glucose concentration via reduced glucose transportation into cells, increased gluconeogenesis, and lipolysis leading to insulin resistant diabetes. This form of diabetes mellitus is transitory if associated with the estral cycle or pregnancy. However, diabetes can be permanent if a pituitary tumor secretes GH and these antagonistic effects on insulin are prolonged. Beta cells will increase insulin production in an attempt to balance the hyperglycemia. If transitory, there is glucose stabilization, but if prolonged this can result in beta cell exhaustion, vacuolation, degeneration, and permanent diabetes mellitus.

Diseases caused by increased concentrations of GH are acromegaly, diabetes mellitus, and mammary neoplasms. Dwarfism is caused by decreased concentrations of GH.

Acromegaly (hypersomatotropism)

Increased serum GH may be due to a GH secreting pituitary tumor in cats or endogenous or exogenous progesterone that stimulates GH production from the mammary gland in dogs. Measurement of increased IGF-1 is a screening test. Clinical features include soft tissue proliferation, thick coarse facial features, hyperglycemia, and insulin-resistant diabetes mellitus.

Chronic excessive GH causes insulin resistance and acromegaly in adult dogs and cats. Canine acromegaly is due to increased progesterone from the luteal phase of the estrous cycle or the administration of progestational compounds for suppression of estrus in intact female dogs. Both of these cause excessive secretion of GH from mammary epithelial cells. The GH producing pituitary tumor in cats is rare or at least infrequently recognized. They are of acidophilic origin, large, grow slowly, and may be present for a long period of time before the onset of clinical signs. Feline acromegaly occurs in older male cats (8–14 years of age).

Initial signs in dogs are usually changes in their physical appearance. This may include soft tissue proliferation of the neck, head, tongue, mouth, gingiva, and pharynx, with abnormal respiratory noises. Characteristic enlargement of extremities, body size, jaw, tongue, gingival hyperplasia, widened interdental spaces, thick folds of facial skin-subcutis, and inspiratory stridor from proliferation of laryngeal soft tissue (50% of cats) are characteristics of the disease. Visceromegaly, including enlarged kidneys, liver, and endocrine organs, may result in abdominal enlargement. Cardiomegaly, hypertrophic cardiomyopathy, murmurs, and congestive heart failure develop late in the course of disease in cats. Neurologic signs in cats, if present, are due to the large pituitary macroadenoma. Physical changes are not as dramatic in cats and therefore most of the easily recognized signs are referable to diabetes mellitus. PU PD and polyphagia are common problems in cats with acromegaly and these will also occur in some dogs. Acromegaly is most often tested for in cats when insulin resistance cannot be explained via other mechanisms.

Routine clinical pathology

Laboratory tests may reveal hyperglycemia and increased liver enzymes, but there are no specific abnormalities. The more common causes of hyperglycemia and increased liver enzymes must be ruled out before acromegaly is considered. Dogs tend to have hyperglycemia without glucosuria. Cats have hyperglycemia with glucosuria, but are not usually ketotic. Hypercholesterolemia and mild increases in serum liver enzymes activities are attributed to hepatic lipidosis from the diabetic state. Alkaline phosphatase may be increased due to bone or liver production. Hyperphosphatemia without azotemia is reported, but was absent in another study. Increased serum phosphorus may be due to GH-stimulated bone growth. Urinalysis is unremarkable, except

for glucosuria and persistent proteinuria. There is usually increased serum protein in the range of 8–9.5 g/dL. Azotemia develops late in the course of disease in approximately 50% of acromegalic cats. Azotemia may be caused by chronic interstitial nephritis, a common disease of old cats, concurrent dehydration, or unregulated diabetes. Measurement of endogenous insulin reveals increased serum insulin concentrations. Resistance to insulin (insulin requirement >2–3 U/kg/BID; or >20 U/day) is common in diabetic cats and consideration of a concurrent pituitary tumor inducing hyperadrenocorticism or acromegaly is done after more common causes of insulin resistance are ruled out.

Establishing a definitive diagnosis of acromegaly in an animal with characteristic signs is accomplished by one or more of the following: cessation of exogenous progestogen in dogs, staging the estral cycle, response to ovariohysterectomy in dogs, measurement of increased plasma GH, and measurement of increased IGF-1. All of the endocrine assays are infrequently performed and some may not be commercially available. The reference laboratory used should be consulted for protocol. Basal GH concentration greater than 6 µg/L is consistent with acromegaly if appropriate signs are present. Concentrations of GH greater than the upper limit of reference interval is considered diagnostic, >5 µg/L dogs, >7 µg/L cats, especially if other data is corroborative. Like any endocrine disease concentrations of GH may not be increased early in the progression of the disease. The diagnosis of acromegaly in dogs is centered on response to treatment rather than hormone assays. Physical examination abnormalities may be a clue to consider acromegaly. Diabetes mellitus in dogs is usually transitory and therefore hyperglycemia and glucosuria are reversed and can be used to monitor response.

The diagnosis in cats uses a combination of insulin resistance increased IGF-1, physical characteristics of acromegaly, and if available imaging of the pituitary region and GH values. It is very difficult to regulate insulin and hyperglycemia in acromegalic cats. Insulin rates >2–3 U/kg/BID or >20 U/day should prompt consideration of concurrent acromegaly. Often the main reason these cats are seen is resistance to insulin and the only clinical pathology abnormalities are referable to diabetes mellitus. Imaging studies of the pituitary are not 100% accurate, but are very useful. Cats with hyperadrenocorticism or acromegaly have insulin resistance and both have macroadenomas of the acidophils and therefore imaging studies of the pituitary do not distinguish these differentials. Serum IGF-1 combined with stimulatory and inhibitory tests of the pituitary adrenal axis (LDDS, ACTH stimulation) should be used to screen for these differentials. Serum IGF-1 or somatomedin C is increased by GH and can be used as a screening test for acromegaly in cats. It has a sensitivity of 84% and a specificity of 92%. IGF-1 is not a species-specific assay therefore assays designed for humans can be used. The reference interval for cats is 208–443 ng/mL. Concentrations >1000 ng/mL are suggestive of acromegaly and can be used to screen for this disease. However, increased IGF-1 has been reported in diabetic cats unrelated to acromegaly and the concentrations of IGF-1 can vary widely. Administration of large doses of insulin, particularly in poorly perfused sites such as the back of the neck, can cause a crossreaction with the IGF-1 assay. Additionally, cats with hyperadrenocorticism have weight loss as opposed to weight gain in acromegalics and may have fragile skin that tears easily as opposed to thick, coarse soft tissue proliferation and skeletal abnormalities of acromegaly. Some individuals consider acromegaly more common than previously thought and is an underdiagnosed endocrinopathy in cats.

Pituitary dwarfism

Major features include decreased GH, cystic Rathke's pouch, proportionate dwarfism, and panhypopituitarism.

Pituitary dwarfism results from failure of the embryonic ectoderm to differentiate into trophic secretory cells of the adenohypophysis. There is a cyst of Rathke's pouch that is primary or secondary to the failed differentiation. It is seen most frequently in German shepherd littermates or related litters and has a simple autosomal recessive mode of inheritance. Affected German shepherds have a deficiency of GH, TSH, and prolactin, but adequate ACTH secretion. There is a panhypopituitarism in Carnelian bear dogs associated with decreased production of TSH, ACTH, LH, FSH, and GH. Other affected breeds include Spitz, miniature pinschers, Labrador retrievers, and Weimaraners. It may also occur in cats. Pups with pituitary dwarfism are indistinguishable from littermates until 2–4 months of age when reduced growth rate is noticed and mental retardation is manifested as difficulty in house-training. Two diagnostic physical examination findings are proportionate dwarfism and retained puppy hair coat. The hair coat is wooly due to retention of puppy or lanugo hairs which epilate easily and therefore a gradual alopecia is seen that may involve the total body that sometimes spares the limbs. Other clinical problems are delayed dental eruption, alopecia, cutaneous hyperpigmentation, skin infections, infantile genitalia, cryptorchidism, and anestrus. Craniopharyngioma is a very rare tumor of remnants of craniopharyngeal duct that also can produce dwarfism in dogs.

Results of CBC, serum biochemistry panel, and urinalysis are usually normal. Eosinophilia, lymphocytosis, mild normocyctic normochromic anemia, and occasionally hypoglycemia are seen if ACTH is deficient. GH deficiency is associated with abnormal development of glomeruli, decreased glomerular filtration rate, and azotemia. Secondary hypothyroidism occurs in most forms of pituitary dwarfism, therefore TT4, fT4, and TSH are decreased. Response tests for TSH, ACTH, and GH are decreased and can be used to establish a diagnosis. Basal GH and IGF-1 (somatomedin)

are decreased. In pituitary dwarfs, GH fails to increase 2–4 fold after administration of intravenous stimulants such as GH-releasing hormone (1 µg/kg), clonidine (10 µg/kg), or xylazine (100 µg/kg). Samples are collected basal and at 20 or 30 minutes after the stimulant is given. A GH value <10 µg/L is an inadequate response.

Other diseases of the pituitary

Other diseases of the pituitary gland are prolonged gestation in Jersey and Guernsey cattle due to inherited aplasia of the adenohypophysis in the fetus. Gestation may be prolonged by >200 days; the fetus is underdeveloped, often hairless, and looks like development stopped at 6–7 months. It may have alopecia and short legs, and it will have hypoplasia of all endocrine organs that are dependent on the pituitary for trophic stimulation. Holstein and Ayrshire cattle have prolonged gestation due to an inherited autosomal recessive enzyme deficiency in the adrenal cortex of the fetus such that cortisol is not produced. In the absence of cortisol, there is stimulation to secrete ACTH, which in turn causes marked bilateral adrenal cortical hypertrophy with fetal giants of up to 225 lbs with long shaggy hair. The cycle of decreased cortisol, increased ACTH and adrenal cortical hypertrophy continues because of the inherited enzyme deficiency. Sheep grazing the teratogenic plant *Veratrum californicum* at 9–14 days of gestation will have fetuses that have an abnormal pituitary gland or a pituitary that functions autonomously from the hypothalamus. The result is prolonged gestation, cyclopia, facial abnormalities, and other musculoskeletal malformations.

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Parathyroid Glands and Calcium and Phosphorus Metabolic Pathology

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General introduction

A change in the serum calcium concentration of as little as 5–10% can stimulate or inhibit the secretion of parathyroid hormone. Hypoparathyroidism caused by lymphocytic parathyroiditis and hyperparathyroidism due to a parathyroid adenoma are the two primary diseases of the parathyroid gland.

The parathyroid gland is a simple endocrine organ with intricate cellular events that closely regulate the serum concentration of calcium. The parathyroid or chief cells primarily respond to calcium. Low calcium stimulates the parathyroid cells to undergo hypertrophy and hyperplasia, as well as increasing the production and release of parathyroid hormone (PTH). High calcium inhibits these events resulting in decreased secretion of (PTH) and eventually, atrophy of the glands. A change in the serum calcium concentration of as little as 5–10% (total calcium 0.25–0.5 mg/dL, or ionized calcium 0.1 mmol/L) can stimulate or inhibit the secretion of parathyroid hormone within minutes. Therefore calcium does not need to be out of reference interval before it changes the production and secretion of PTH. This concept is important when interpreting serum concentrations of PTH and calcium. Calcium-sensing receptors (CaSR) in parathyroid chief cells and other CaSR-expressing cells detect changes in the concentration of ionized calcium and make adjustments to PTH secretion that rapidly normalizes serum calcium. CaSR in the kidney stimulates cellular events that influence calcium and water homeostasis and these pathways may play a pivotal role in the development of hypercalcemia seen in some animals with renal failure.

Parathyroid hormone rarely needs to be measured. The only time it is "required" is to confirm primary hypoparathyroidism and in some cases of primary hyperparathyroidism. In these diseases it seems logical the concentration of serum PTH would be decreased or increased respectively. However PTH is often within reference intervals in each of these diseases which seems paradoxical, but the PTH value is *inappropriate* for the serum concentration of calcium. These situations happen with regularity because of the close regulation of PTH, calcium, and CaSR. A serum calcium concentration of 15 mg/dL (increased) should trigger the parathyroid gland to decrease and or stop the secretion of PTH. Therefore, if the concentration of PTH is within reference interval it is "inappropriately high" and diagnostic of hyperparathyroidism. In fact 75% of dogs with hypercalcemia due to primary hyperparathyroidism have serum concentrations of PTH within reference interval.

What is equally or more important to parathyroid chief cells than the absolute concentration of serum calcium is the delta in calcium as the CaSR and parathyroid chief cells can reset their signals based on the change of calcium in the blood or at the receptor. For example, in nutritional hyperparathyroidism the serum concentration of calcium is often in the reference interval, which also seems paradoxical given the pathogenesis of the disease and the marked bone resorption (fibrous osteodystrophy or "rubber jaw"). Even though a dietary imbalance of calcium and phosphorus are constantly lowering the serum and cellular concentration of calcium, the compensatory parathyroid hyperplasia and secretion of parathyroid hormone are normalizing the serum concentration of calcium, at the expense of decreasing bone mass.

This chapter will discuss the laboratory tests related to basic blood calcium and phosphorus concentrations as well as the less frequently measured parathyroid regulatory peptides. This is followed by discussion of primary parathyroid disorders that affect calcium metabolism. Lastly, numerous causes of calcium and phosphorus metabolic pathology are outlined. Because of the frequency of calcium metabolic pathology, serum calcium and phosphorus are measured in

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all routine chemistry panels. This practice is established convention because hypocalcemia and hypercalcemia usually provide clues to diagnosis of underlying disorders that affect calcium metabolism.

Background on relevant laboratory tests

Summary

Total serum calcium

Excellent screening test, included on chemistry panels.

Ionized calcium

Biologically active fraction; if available, measure in critical care patients no matter what their primary disease or total calcium concentration is; it is excellent for STAT needs. It is more useful in hypocalcemic than hypercalcemic patients. Special sample handling is required (see Chapter 1).

Adjusted calcium

Use if hypoalbuminemic; it explains the most common cause of hypocalcemia. This is not needed for hypercalcemia. It cannot be used to estimate ionized calcium.

- Calcium disorders are usually discovered from a routine chemistry panel. There are very few clinical signs referable to hypercalcemia. Tetany may be seen with hypocalcemia in dogs and paresis is seen in cows.
- Differential diagnoses for hyper- and hypocalcemia are the same if total or ionized calcium is used.
- Examine Ca and P together, then albumin, azotemia, and lipase. Determine the Ca × P product to predict risk of soft tissue mineralization.
- Hypercalcemia and hypophosphatemia—two differential diagnoses are hyperparathyroidism, which is rare, and hypercalcemia of malignancy, which is common. This finding occurs with renal failure in some horses.
- PTH needed rarely, measure in a panel with PTH rp and total or ionized calcium
- Veterinarians can now select total serum calcium, ionized calcium, and they can use an adjustment formula for hypoalbuminemia if total serum calcium is low. The determination of total serum calcium will remain the screening test on routine chemistry panels to detect abnormalities in serum calcium, predict soft tissue mineralization, and to formulate differential diagnoses. Measurement of ionized calcium is now available in practice. It can be used to formulate differential diagnoses just as well as using total calcium. It is the test of choice for monitoring hypocalcemia in critically ill patients.
- Adjustment formulae for calcium is used to help determine if a decrease in total serum calcium is likely due to hypoalbuminemia, which is the most common cause of hypocalcemia. Only use a formula if the serum concentration of albumin is low, it is not needed if the serum albumin is normal or increased or if hypercalcemia is detected. The adjusted calcium is not a real number; the real concentration of calcium in the blood is the measured total and or ionized.

Calcium and phosphorus metabolism measurements

Total serum calcium

Of the body's calcium 99% is in bones, 1% is intracellular, and only 0.1% is in extracellular fluid. It is this small latter fraction that is measured in the blood. Positively charged calcium ions in the blood are bound to anionic sites on proteins (primarily albumin) or nonprotein anions (citrate, phosphate, lactate) and as an unbound free ionic form. These are listed as protein-bound calcium, approximately 40%, ionized calcium 50%, and complexed calcium (citrate, phosphate etc.) approximately 5–10%. The latter two fractions are freely diffusible, stimulate cellular events, and the ionized fraction is the most prevalent of the two and biologically active. Each fraction can be measured individually; however, the only fraction that needs to be measured routinely in serum of animals is the total calcium concentration. Total serum calcium is stable in vitro. It can be determined in chemistry panels by various methodologies and reference intervals from different labs are fairly similar. Interfering substances depend on the methodology. For example hemolysis may artifactually increase total serum calcium by some methods, but not others. Always consult with the lab or procedure documentation about possible interference or sample handling concerns. If an abnormality in total calcium is detected, the concentrations of calcium and phosphorus should be compared to help formulate differential diagnoses. The total serum calcium value multiplied by the phosphorus value should be calculated to predict the risk of soft tissue mineralization, especially when there is hyperphosphatemia. Then examine albumin, urea nitrogen, and lipase and make a decision if ionized calcium is needed. Ionized calcium is of value if hypocalcemia is detected and is also more useful in critically ill patients. The list of differential diagnoses for hyper- and hypocalcemia are the same if either the total or ionized calcium is used. The magnitude of hypercalcemia for ionized or total serum calcium does not predict a disease, but in general hypercalcemia of malignancy and hyperparathyroidism have the highest concentrations of either total or ionized serum calcium. Hypoparathyroidism and lactational tetany are associated with the lowest calcium concentrations.

Interpretive discrepancies between abnormal total and abnormal ionized calcium concentrations may occasionally occur. Notable discrepancies are that some dogs and cats with renal failure may have increased total serum calcium, but the ionized calcium is in reference interval or mildly decreased. However, this is not absolute as some dogs and cats in renal failure will have both increased ionized calcium and total calcium. In fact, if ionized calcium is used to rank

diseases that cause hypercalcemia, then renal failure is the second most common cause in dogs. The total serum calcium is increased in 100% of dogs with primary hyperparathyroidism, and ionized calcium is increased in 90–95%. If primary hyperparathyroidism is a differential diagnosis, then measure total serum calcium. Some hyperthyroid cats have a mild decrease in ionized calcium and normal total calcium. Urinary obstructed cats may have a more pronounced decrease in ionized calcium as compared to the decrease in total calcium. If hypocalcemia is marked or there are clinical signs of tetany then measure ionized calcium. If it is not available then treatment decisions are made from total serum calcium and or tetany. Ionized calcium is preferable to total calcium in critically ill patients, hypocalcemic patients, and animals with sepsis. These differences point out some of the situations when having both the total and ionized calcium values are beneficial. Perhaps if total and ionized were measured in the same sample, or on the same day, there would be better interpretive concordance.

Ionized and total serum calcium measurements result in the same list of differential diagnoses for hypocalcemia or hypercalcemia (Tables 33.1 and 33.2).

Ionized calcium

Ionized calcium and other electrolytes are now easily measured in practice (Chapter 1). Ionized calcium is not measured in serum chemistry profiles, it requires a second sample. For routine screening the total serum calcium will remain the sample of choice. It is important to follow specific sample handling instructions when measuring ionized calcium. Ionized calcium is reported in two different units (Table 33.3). Interpret the values compared to appropriate reference intervals exactly as total serum calcium is interpreted.

Ionized calcium is most valuable if hypocalcemia is detected, especially if total serum calcium is less than 7.0 mg/dL. Decreased ionized calcium is important in the care of critically ill patients. Ionized hypocalcemia has been shown to be a predictor of longer duration of care in ICU and hospital stay of critically ill dogs, but is not associated with decreased survival. This study did not examine the same variables for total serum calcium to determine if it was predictive. Septic foals and cattle appear to have calcium aberrations as well. Determining the serum concentration of ionized calcium could be very beneficial during and post surgery of thyroid glands for hyperthyroidism or parathyroid adenomas. However, even knowing the ionized calcium does not predict clinical signs, for instance within hours post treatment of hypocalcemia in lactational paresis of cattle that the concentrations of ionized calcium have returned to pre-treatment concentrations, yet no clinical signs are observed. Similarly some of the indicators used to determine if calcium and vitamin D supplementation is needed postthyroidectomy or postparathyroidectomy in cats and dogs is not only

Table 33.1 Differential diagnoses for hypocalcemia.

Ca decreased, P variable	Ca decreased, P increased
Hypoalbuminemia intestinal, liver, renal	Azotemia—pre-renal, renal, or post-renal
	Hypoparathyroidism—P mild increase
Renal failure—any cause	Secondary hyperparathyroidism renal or nutritional
Ethylene glycol—severe decrease in Ca	
Pancreatitis; diabetic ketoacidosis	Phosphate enemas—P marked increase
Lactation—eclampsia, milk fever	Tumor lysis syndrome
Critically ill / sepsis	

Hypomagnesemia—dogs; cattle—grass tetany; horses—Blister beetle

Cantharadin toxicity—horse; Blister beetle

Thyroidectomy—damage and or removal of parathyroid glands

Hyperthyroidism—ionized decrease, unknown mechanism

Parathyroid atrophy secondary to hypercalcemia

Nutritional hyperparathyroidism—dietary imbalances Ca:P

Oxalate rich plants

Endurance racing—horses

Myopathies—exertional, hyperthermia

Sick patients—all species; critically ill, sepsis, unspecified illness

Urinary tract obstruction—azotemia

Preparturient hypocalcemia—cats, severe hypocalcemia

Hypovitaminosis D—rickets; GI disease, exocrine pancreatic deficiency

Hyperadrenocorticism—uncommon, unknown mechanism

Metabloic alkalosis

Tumor lysis syndrome

Soft tissue trauma

Healing long bone fractures—mild, first 20 days of callus formation

Medullary carcinoma C-cell of thyroid—rare

Pseudohypoparathyroidism—not reported in animals

Chelation

- EDTA, Oxalate—if in vitro = zero calcium
- Citrate—anticoagulant used in blood transfusions

Iatrogenic: anticonvulsants, furosemide diuretics, IV phosphate solutions; calcium free IV solutions; transfusions with citrate as anticoagulant; excess bicarbonate infusions, bolus infusion tetracycline

Diseases above the line are common and account for the majority of cases of hypocalcemia. Whenever phosphorus is increased, there is potential for calcium to be decreased.

Table 33.2 Differential Diagnoses for Hypercalcemia.

↑ Ca and N or ↑ P	↑ Ca ↓ P
Hypercalcemia of malignancy	Hypercalcemia of malignancy
Primary hyperparathyroidism	Primary hyperparathyroidism
Idiopathic—cats	Renal failure- only horses
Hypoadrenocorticism	
Renal failure—chronic more common than acute	
acute—grapes and currant toxicities	
Vitamin D toxicosis—rodenticides, plants, iatrogenic	
Granulomatous diseases—blastomycosis, other fungi, FIP, schistosomiasis, mycobacterium, toxoplasmosis	
Iatrogenic—calcium supplements	
Young, rapid growing, esp. large breed dogs and horses	
Xylitol toxicity—concurrent hypoglycemia can be severe	
Hyperthyroidism—uncommon in dogs vs. decreased calcium in cats	
Hypothermia—rare, may not be cause and effect	
Spurious—lipemia, hemolysis, type of heparin, methodology dependent	
Acidosis—ionized calcium increased	
Osteolytic bone lesions—doubtful; cases of hypertrophic osetodystrophy are probably due to young age of dogs	
Bone metastases—probably humoral and not just the osteolysis from local tumor	

With the exception of primary hyperparathyroidism, entities listed above the solid line are common and entities listed below the dashed line are physiologic, spurious, or the hypercalcemia may not be caused by the entity listed.

the concentration of total or ionized calcium, but if clinical signs are present. Like any laboratory test, correlation of calcium and phosphorus with clinical signs is vital.

Shifts in ionized calcium happen with shifts in acid/base balance. Acidosis shifts calcium to the ionized compartment and alkalosis decreases ionized calcium. Alkalosis will increase the available negatively charged binding sites on albumin and lead to increased calcium bound to albumin. These compartmental shifts in calcium may have clinical importance. Acidotic animals with hypocalcemia may not show clinical signs because more calcium is in the ionized compartment, whereas alkalotic animals with the same serum calcium theoretically could have clinical signs of hypocalcemia. Correcting acidosis rapidly in a patient with hypocalcemia could produce tetany. This would warrant calcium supplementation in IV fluids. This could happen when correcting the acidosis seen in ethylene glycol toxicity or neonatal diarrhea in calves, both of which may have severe acidosis with total and ionized hypocalcemia. Because large acid-base changes may occur during critical care, it is prudent to monitor both ionized calcium and blood gases if any clinical signs of tetany are present.

Protein adjusted calcium

The majority of the calcium bound to serum proteins is bound to albumin. As the concentration of albumin decreases, the protein-bound fraction of calcium as well as the total calcium decreases. Therefore, it is recommended to "adjust" a low total serum calcium concentration if hypoalbuminemia is present. If the adjusted calcium concentration is within the reference interval, then the cause of hypocalcemia is hypoalbuminemia. There is a limit to this relationship; if serum calcium is <7.0 mg/dL in a hypoalbuminemic animal there probably is a second cause of hypocalcemia. Depending on the species, approximately 20–30% of the decrease in total calcium is due to changes in serum albumin. If ionized calcium is desired it must be directly measured. The correction formula does not predict ionized calcium. The real calcium in the patient is the value that is measured, ionized or total. The adjusted calcium is not a real number. It simply helps the clinician determine that a decrease in total serum calcium is due to hypoalbuminemia. If serum albumin is normal or increased do not use any correction and investigate the cause of hypocalcemia; it is not due to a protein abnormality.

The most common cause of hypocalcemia in animals is hypoalbuminemia. This cause of hypocalcemia is asymptomatic. Hyperalbuminemia does not cause hypercalcemia. The relationship between albumin, total protein and calcium has resulted in "correction formulas" that adjust the measured total serum calcium concentration for the degree of hypoalbuminemia, or hypoproteinemia. These formulas are most reliable in dogs are somewhat helpful in cats and are less reliable in horses and cows. While other formulae exist, the recommended, commonly used correction formula is:

$$\text{Adj. calcium} = \text{measured calcium} + (3.5 - \text{measured albumin})$$

Example:

Measured Ca = 7.6 and measured Alb = 2.0

Adj. calcium = 7.6 + (3.5– 2.0)

Adj. calcium = 9.1

In the example above the hypocalcemia was corrected for by the hypoalbuminemia and the adjusted calcium is now in reference interval (8.8–11.2 mg/dL) therefore the cause of the hypocalcemia in this case has been determined. It is important to now determine the cause of the hypoalbuminemia.

Calcium—phosphorus product (Ca × P)

Calcium and phosphorus should be interpreted together in a chemistry panel as the list of differential diagnoses will

Table 33.3 Example reference intervals and conversion factors for calcium measurements.

To convert from mmol/L to mg/dL multiply by 4; convert from mEq/L to mg/dL multiple by 2.
To convert from mg/dL to mmol/L multiply by 0.25; convert from mg/dL to mEq/L multiply by 0.5; convert from mEq/L to mmol/L multiple by 0.5

Reference intervals (RI) of serum calcium in dogs and cats and expected problem ranges; consult with reference lab performing determination.

		Total serum calcium	Ionized calcium	Ionized calcium
		mg/dL	mmol/L	mEq/L
Dogs	RI	9–11	1.2–1.5	2–3
Dogs	RI	8.7–11.2	1.12–1.40	2.5–3.0
Dogs	RI		1.25–1.45	2.3–2.8
Dogs	RI	9.2–11.3	1.12–1.32	
Clinical problems may be detected:				
Hypocalcemia		<6.5	<1.0	<2
Hypercalcemia		>12	>2	>4
Cats	RI	8.3–10.5	1.15–1.35	2.2–2.6
Cats	RI	9.2–10.3	1.15–1.40	2.1–2.7
Clinical problems may be detected:				
Hypercalcemia		>11	>1.5	
Horses	RI	11.5–13.5	1.45–1.75	
Horses	RI		1.53–1.61	
Horses	RI		1.61–1.85	

Young dogs: 0.2–1.0 mg/dL higher in total calcium, with concurrent mild hyperphosphatemia; horses and rabbits have the highest reference intervals for total and ionized calcium of any species, up to 13 mg/dL total serum calcium.

vary depending on their concurrent values. Additionally, the product of these two electrolytes is predictive of soft tissue mineralization. A product of Ca × P > 70 indicates soft tissue mineralization is likely and a product >90 indicates mineralization is occurring. Phosphorus and vitamin D are more important in the process of mineralization than is calcium. If phosphorus is increased, the likelihood of mineralization is greater than if calcium is increased. If they are both increased and or if the product is greater than 90 mineral is being deposited in characteristic locations such as blood vessels, kidney, stomach, lung, heart, intercostals, and intestinal submucosa. If mineralization is severe it can be seen in radiographs and induce or amplify renal failure via nephrocalcinosis. Use total serum calcium in the product formula.

Examples: TCa × P

1. Ca 10.2 mg/dL, P 14 mg/dL = 143
2. Ca 8.1 mg/dL, P 21 mg/dL = 170
3. Ca 15.5 mg/dL, P 1.8 mg/dL = 28

1. Normocalcemia and hyperphosphatemia with an increased product is common in renal failure. Soft tissue mineralization is occurring and making the renal failure worse as kidneys are one of the most common tissues to become mineralized. The mineralization starts in basement membranes and mitochondria of tubular cells and progresses to glomeruli and interstitium.
2. Hypocalcemia and marked hyperphosphatemia with a product of 170. Despite hypocalcemia, the Ca × P product is markedly increased and soft tissue mineralization is predicted. These values can occur with renal failure and the uremia will hasten soft tissue mineralization.
3. Hypercalcemia and hypophosphatemia, and a normal product; this is seen in dogs with primary hyperparathyroidism and hypercalcemia of malignancy. Despite hypercalcemia soft tissue mineralization is not occurring at this time. However, there can still be side affects from hypercalcemia (dilute urine, paresis, blood pressure changes).

Clinical signs

The most critical signs of imbalances in calcium relate to synaptic neural signal transmission, skeletal muscle contraction, and cardiovascular muscle function. Ionized and complexed calcium are critical to the development of clinical signs. Tetany and seizures are classical signs of hypocalcemia in small animals and horses, but paresis is the predominant sign in cattle. The signs referable to hypocalcemia are distinctive, but are generally only seen with severe hypocalcemia. Therefore, signs are usually only seen with lactational hypocalcemia or primary hypoparathyroidism. Clinical signs and history are so obvious with lactational tetany or paresis (milk fever) that measurement of serum calcium is seldom

performed. How rapidly there is a shift in calcium and the acid base status at that moment will influence development of impending clinical signs. Rapid onset hypocalcemia, especially with alkalosis, may produce tetany or paresis, while a comparable concentration of total or ionized would not if the onset was slower and or the blood gas status was acidotic.

The clinical signs referable to hypercalcemia are paresis and PU PD. Both are mild and often unapparent to owners. In many cases there are no detectable clinical problems. Therefore with both hypo- and hypercalcemia there are relatively few clinical signs that cause suspicion of calcium abnormalities. In the majority of cases, the abnormality in calcium homeostasis is found from routine chemistry panels. Primary hypoparathyroidism and primary hyperparathyroidism are two diseases in which the results from a chemistry panel are the clue to the diagnosis in the absence of physical examination findings.

Parathyroid hormone (PTH)

Ionized calcium is the key signal to calcium receptors on chief cells of the parathyroid gland. Minor contributions come from other factors such as the following. Calcitriol decreases production and secretion of PTH. Hypomagnesemia decreases production and secretion of PTH. Epinephrine has a minor influence on PTH secretion. Phosphorus directly or indirectly stimulates the opposite effects of calcium. PTH is a peptide hormone; it has a half-life that is so short it is measured in minutes and PTH reacts within minutes to changes in the serum concentration of calcium. The chief cells use a calcium-sensing receptor (CaSR) to recognize the serum concentration of ionized calcium and adjust the secretion and production of PTH. This relationship is sigmoid such that at high and low concentrations of calcium the secretion of PTH flattens. A continued increase or decrease in calcium does little to change the amount of PTH secreted. However, in the linear part of the curve a small change in the serum concentration of calcium will change the secretion of PTH in minutes so that physiologic adjustments can be made to keep the serum calcium in a close reference interval. This is referred to as the calcium set point and the set point of ionized calcium is approximately 1.2 mmol/L for dogs, 1.37 mmol/L for horses and 1.0 mmol/L for humans. Acquired and familial diseases may change this set point and or CaSR. PTH stimulates an immediate rise in serum calcium via multiple steps, one of which is the osteocytic osteolysis pump. PTH also stimulates a sustained increase in serum calcium by stimulating osteoblasts and osteocytes to secrete cytokines that in turn stimulate osteoclastic osteolysis for long-term calcium homeostasis. It is these latter steps that lead to lesions in bones associated with renal and nutritional hyperparathyroidism and hypercalcemia of malignancy. PTH exerts its effect primarily through the binding and activation of PTH receptors, PTH1R.

Receptors for PTH reside on osteoblasts and osteocytes and activation triggers an interaction between these cells and development/activation of osteoclasts. PTH causes the release of calcium and phosphorus from bone, increasing both into the serum. In the kidney, PTH increases the reabsorption of calcium and decreases the maximum renal reabsorption of phosphorus, thereby promoting urinary phosphorus excretion. Ionized and complexed calcium, but not albumin bound calcium passes freely into the glomerular filtrate. 99% of the calcium that enters the urinary filtrate is reabsorbed from various locations within the tubules. Hence hyperparathyroidism is associated with hypercalcemia because of bone and renal factors (indirectly through the GI tract and calcitrol). Hypophosphatemia occurs because of the strong phosphaturic stimulus of PTH that exceeds phosphorus resorption from bone. Parathyroid hormone related protein (PTH rp), operative in hypercalcemia of malignancy, uses the same PTH1R receptors. Therefore, it acts identically to native PTH and produces hypercalcemia and hypophosphatemia as seen in hypercalcemia of malignancy. Primary hyperparathyroidism and hypercalcemia of malignancy are the only two diseases that cause hypercalcemia and hypophosphatemia in dogs and cats. Due to the phosphaturic effect of PTH the fractional excretion of phosphorus in the urine can be used to estimate an increased serum PTH. If the fractional excretion of phosphorus is greater than reference interval in a nonazotemic animal then PTH (or PTH rp) is increased. This can be determined on the day serum and urine are sampled, while waiting for the return of PTH measurements. This is clinically useful when distinguishing between primary or nutritional secondary hyperparathyroidism. Serum PTH measurement should compliment fractional excretion studies.

PTH also stimulates 1-alpha hydroxylase located in renal proximal tubular epithelium. This enzyme is the rate limiting step in the synthesis of the most potent form of vitamin D, calcitriol or 1,25-dihydroxycholecalciferol. Calcitriol stimulates the production of calcium and phosphorus binding proteins in the intestinal tract to increase the absorption of calcium and phosphorus. Calcitriol also inhibits the production of PTH and has a minor effect on the kidney to increase calcium and phosphorus reabsorption. It stimulates osteoclastic osteolysis releasing calcium and phosphorus into the serum. Only PTH and PTH rp have phosphorus lowering effects hence only primary hyperparathyroidism and hypercalcemia of malignancy are associated with hypercalcemia and hypophosphatemia. Vitamin D toxicity is associated with hypercalcemia and hyperphosphatemia. Calcitonin is secreted from C cells in the thyroid gland and acts to decrease the serum concentration of calcium. Calcitonin may prevent postprandial hypercalcemia, but its effect otherwise is relatively weak compared with PTH. C cells can form medullary carcinomas, but reports of tumor induced hypocalcemia in animals are rare.

The only other cells in the parathyroid gland that can produce lesions are remnants of the duct that connected the thymus and the thyroid. These cells and the ductular remnants can become cystic, referred to as a "Kursteiner cyst." They are of no clinical significance. The only time they may cause problems is during exploratory neck surgery, or imaging of this area, if they were confused with a parathyroid adenoma. These cysts will be fluctuant and light gray in color. A tumor of parathyroid cells will be solid and tan-red in color. It is much more likely that the cysts would be identified microscopically; in fact, they are present in approximately 75% of histological sections of the thyroid and parathyroid glands of dogs. They can be located in the parathyroid gland, thyroid gland, or in the adjacent tissues. Occasionally they even have remnants of thymus with them.

PTH and PTH rp assays

Reference labs have assays for PTH and PTH rp that can be used on plasma or serum from dogs, cats and horses. Because of peptide stability factors, it is critical to consult the reference lab for specific sampling procedures and interpretive guidelines.

The most diagnostic information is gained when PTH, PTH rp, and calcium are measured in the same sample or at the same time. Usually the final diagnosis is not established when these substances are being measured so request all three as a mini-panel. This will avoid resubmission and delays if the primary differential is not confirmed. Furthermore clinical interpretation depends on the relative concentration of each compared to the others. Plasma is preferred for PTH and PTH rp, but serum can be used.

Assays for PTH can measure the amino-terminus (biologically active fragment), mid-region, carboxy-terminus (biologically inactive but immunologically detectable), the entire 1–84 amino acid sequence of PTH, or large fragments of PTH. Studies have not been done in animals to compare which assay is the most diagnostically useful. It would seem the biologically active terminus, amino fragment, would be best, but studies in humans have shown the carboxy-terminal assays have equal or better diagnostic relevance. A two-site assay is available and has been validated to measure serum PTH in dogs, cats, and horses. This is useful when the cause of hypercalcemia or hypocalcemia cannot be determined by other diagnostic aids. The principle of this assay is to use two antibodies; one is specific for the carboxy-terminal region and the other for the amino-terminal region. The assay requires that both bind and it is designed such that the final assay recognizes only intact PTH or at least only large fragments of PTH. The sample can be measured via immunoradiometric (IRIA) technique which is what most commercial labs use or via a chemiluminescent technique (Immulite) that is a rapid assay and results are completed in 20 minutes. This latter technique can be used to measure PTH during surgery at a veterinary center to determine when all of the hyperactive parathyroid tumor(s) has been removed. The antibody reagents developed to date are not species specific, but they crossreact sufficiently to make meaningful assays. This may explain some of the ambiguous or gray zone results obtained when these assays are used in animals.

If the patient is azotemic, PTH should not be measured, as it will be increased. PTH increases in renal failure due to hypocalcemic induced parathyroid hyperplasia, hyperphosphatemia, decreased degradation of PTH, and decreased renal clearance of PTH fragments.

Assays designed for humans to measure the complete molecule of PTH rp use an immunoradiometric technique and have been used in dogs, cats, and horses. PTH rp shares a nearly identical amino acid sequence at its amino terminal end as native parathyroid hormone. Since the amino terminal end is the biologically active fragment, both molecules are recognized by and stimulate the same receptors (PTH1R) and therefore produce the same biological responses. The midvalent and carboxy regions of native PTH and PTH rp molecules are sufficiently different that antibodies directed at each molecule will recognize one, but not the other, and therefore can be used in diagnostic assays. The ranges for interpretation should be obtained from the laboratory. Example guideline interpretations for PTH and hypercalcemia are shown below. It is important to correlate the PTH value with the total or ionized calcium and PTH rp value.

	PTH independent	Equivocal	PTH-dependent
Cat hypercalcemic	<2.3	2.3–4.6	>4.6 pmol/L
Dog hypercalcemic	<2.0	2.1–8.0	>8 pmol/L

The coefficient of variation for a measured substance is a number that indicates the expected variability with repeat measurements. For hormone assays the coefficient of variation can be as high as 20%. A much lower percent is preferred, <2% is the approximate coefficient of variation for a hematocrit. This indicates there is considerable variability for hormone assays independent of any real change in the concentration of the hormone measured in the patient. Assays for PTH and PTH rp report coefficient of variations <10% and for hormone assays this is good. The practical application is that the value reported actually has a range; do not take it as an absolute number as there can be considerable variability within assays runs and especially between assay runs. When performing mini-panels of hormones from one patient, whether it is cortisol, thyroid hormones or PTH and PTH rp request they be placed in the same assay run. If

comparisons are to be done try to send all samples together and request all the samples from one animal be analyzed in the same assay run to decrease interassay variability. Stand-alone absolute values of hormones are rarely diagnostic. They need to be correlated with other laboratory and clinical findings.

Primary parathyroid disorders

Hypoparathyroidism

Lesions and pathogenesis

Spontaneous hypoparathyroidism is due to lymphocytic plasmacytic destruction of parathyroid tissue which eventually leads to fibrosis, absence of inflammatory cells, and few if any parathyroid cells (sometimes called idiopathic atrophy). The damage is permanent and requires life-long treatment with calcium and or vitamin D. Do not try to biopsy as very little of the glands remain and they are extremely difficult to visualize during surgery. The histologic lesion is similar to immune mediated destruction of the thyroid or adrenal glands. The disease is uncommon to rare in dogs, extremely rare in cats, and not reported in other animals.

Iatrogenic hypoparathyroidism is due to thyroidectomy in cats with hyperthyroidism. The inadvertent removal or damage of parathyroid tissue results in surgical hypoparathyroidism. If this is recognized during surgery the excised parathyroid tissue can be reinserted in adjacent muscles and it may establish blood supply and function. Despite the removal of the parathyroid glands, these cats rarely require calcium and/or vitamin D supplementation beyond the first month after surgery. Transitory hypocalcemia is expected post operatively, but most cats stabilize long term and do not require life long calcium supplementation. Regeneration of damaged parathyroid tissue may explain this. The mechanism for compensation in others is not known. Studies to investigate the possible role of ectopic parathyroid tissue producing PTH or the production of PTH rp by other tissues are inconclusive. Since most animals eventually normalize their serum calcium it is important not to "overtreat" with calcium and vitamin D. If these supplements are used in excess they may induce hypercalcemia that will prevent damaged parathyroid glands from regenerating. Many animals with total serum calcium and ionized calcium that are decreased do not exhibit clinical signs. It is nice but not necessary to have calcium in reference interval; much like regulating serum glucose in a diabetic. If there are no signs of hypocalcemia try not to over treat because the hypocalcemia is a stimulus for parathyroid hypertrophy.

Animals with lactational tetany or milk fever do not have hypoparathyroidism. If PTH is measured in cattle with milk fever it is secreted and responding to the stimulus of hypocalcemia; this is discussed under differentials for hypocalcemia.

Signalment

Lymphocytic parathyroiditis is seen most frequently in young to middle aged, spayed female dogs; a few sporadic cases are reported in cats.

Signs

Tetany, seizures, fever (due to seizure activity), cataracts, stilted gait, vomiting, diarrhea, panting, and facial pruritus that results in chewing, rubbing, and licking are reported. However, in most cases hypocalcemia is not suspected on initial examination and the hypocalcemia discovered in a routine chemistry panel is often the first clue to the diagnosis. The only hypocalcemic disease that is consistently associated with clinical signs is lactation hypocalcemia, tetany in bitches and mares and paresis in cattle. If the hypocalcemia goes on long enough even dogs will become paretic.

Routine clinical pathology abnormalities

Moderate to severe hypocalcemia and mild hyperphosphatemia with normal albumin, urea nitrogen, creatinine and lipase is essentially diagnostic. If the serum calcium is less then 6 mg/dL and the patient is not azotemic and not lactating then primary hypoparathyroidism is the most likely diagnosis. Ionized calcium can be measured to confirm hypocalcemia (<1.0 mmol/L; <2.5 mEq/L) and monitor treatment. Total serum calcium can be <6.5 mg/dL in animals with ethylene glycol toxicity but these patients are very sick and are azotemic or will develop azotemia shortly and phosphorus is markedly increased. Of all the causes of hypocalcemia lymphocytic parathyroiditis, parathyroidectomy and lactational tetany produce the lowest concentrations of calcium. Serum phosphorus is decreased in cattle with milk fever.

Serum phosphorus is greater then the serum total calcium in some animals with hypoparathyroidism. Hyperphosphatemia is expected due to decreased PTH, however, the magnitude of the increased phosphorus is usually mild and if phosphorus is not increased do not rule out hypoparathyroidism. Renal failure causes hypocalcemia and hyperphosphatemia, but these patients will be azotemic, have dilute urine and the hyperphosphatemia is much greater than the mild hyperphosphatemia of hypoparathyroidism. The distinction of these two diseases is clear. Pancreatitis is associated with hypocalcemia and hyperphosphatemia, but lipase will be increased 3 to 5 fold and the patient may be icteric, have increased liver enzymes and the clinical signs are very different.

Any cause of hypocalcemia if severe enough can be associated with hyperglycemia. In addition to the stress induced by the primary disease, calcium is required for microfilament and microtubular contraction which is needed for the intracellular transportation and secretion of neurosecretory granules containing insulin. Inability to secrete insulin results in hypoinsulinemia and hyperglycemia.

Confirmatory test

Concurrent PTH and ionized or total calcium is measured on the same sample or collected at the same time.

Serum PTH will be decreased or undetectable depending on the stage of the disease. When all the parathyroid tissue is destroyed PTH will be undetectable. However, depending when the disease process is recognized, there may be viable foci of chief cells, in which case, if the concentration of parathyroid hormone (PTH) was measured in the serum, it may be detectable. However, if PTH is in reference interval and especially at the low end of reference interval while there is concurrent and severe hypocalcemia this is an inappropriate response and is still diagnostic of primary hypoparathyroidism.

Examples that are all consistent with primary hypoparathyroidism:

Case	Total Ca	P	PTH
1	5.5	6.6	2.6
2	6.1	7.4	0
3	4.4	6.8	1.3
4	5.8	8.1	0.8
5	5.2	4.7	3.9
Reference interval	9–11 mg/dL	3–5 mg/dL	2–13 pmol/L

Eventually all of the parathyroid glands will be destroyed, in which case, the concentration of parathyroid hormone in the serum will be undetectable (case 2). In each case above, hypocalcemia is severe and the concentration of PTH is "inappropriately" low for the serum concentration of calcium and therefore each case is diagnostic for hypoparathyroidism even though some PTH was detected in cases 1, 3, 4 and 5. Hypocalcemia of these magnitudes should be associated with increased concentrations of PTH in an attempt to increase serum calcium. Concentrations of PTH below the lower limit of reference interval should be regarded as undetectable (zero). When the serum concentration of PTH is very low the assay may not be measuring true PTH. Every test has a limit of detectability. When a sample contains so little of the substance being measured that the value is below the level of detectability or linearity then the lab should report, "undetectable."

Primary hyperparathyroidism—primary HPTH—parathyroid adenoma (hyperplasia, carcinoma)

HPTH is due to a functional, autonomous secretion of parathyroid hormone resulting in persistent hypercalcemia and hypophosphatemia. Parathyroid adenomas account for >90% of canine cases of HPTH, carcinoma for less than 5%, and the remainder are considered hyperplasia because a nodule is in more than one gland. These latter cases may represent multiple adenomas, especially if only two glands contain nodules, rarely are three or more parathyroid glands enlarged in dogs. Histologic and molecular distinction of adenoma versus hyperplasia is not always easy. The tumors can be found via ultrasonography, but they are small, 4–10 mm in diameter, and are unilateral in most cases. If a large neoplastic mass is found it is more likely of thyroid origin. Kursteiner cysts are present in or near the parathyroid thyroid glands microscopically in over 75% of dogs and occasionally are macroscopic and could be confused with an adenoma. These cysts will be fluctuant and light tan to gray in color and contain fluid. A tumor of parathyroid cells will be solid, tan-red in color and do not contain fluid. The prognosis for full recovery following surgical removal is excellent with a recurrence rate of less than 10%. Postsurgical hypocalcemia is expected because the adjacent nonneoplastic parathyroid glands are atrophic.

The disease is much less common in cats. Most lesions in cats are benign adenomas, but hyperplasia of all four glands may occur. Most tumors are small, 3–5 mm, but they can be >4 cm, and may be localized with ultrasonography and less frequently via palpation.

Clinical signs are generally vague, mild, and nonspecific. They may include weakness, polyuria/polydipsia (PUPD—50–80%), hematuria, stranguria, crystalluria, urolithiasis, urinary tract infections, lethargy, inappetence, and signs referable to the nervous system. Weakness and PU PD are due to hypercalcemia and are seen with regularity in dogs. PUPD is seen less frequently in cats with hypercalcemia. However, many dogs and cats are asymptomatic and the diagnosis is first considered when hypercalcemia and hypophosphatemia are observed in a routine chemistry panel submitted during an annual wellness exam. Clinical signs such as lethargy may be so gradual and mild that owners do not recognize them.

Most dogs, 75%, will have no abnormalities on physical examination. Dogs are older, mean 11 years, with no sex or breed predilection. It also occurs in the cat. The disease is inherited as an autosomal dominant trait in Keeshonds and genetic testing is available. There is one report of a hereditary form in German shepherd pups. Most cases do not have detectable bone lesions clinically or radiographically. If the mandible or maxillas are enlarged, have osteolysis and new bone formation, then the diagnosis is more likely to be secondary hyperparathyroidism. Urinary calculi, crystalluria and hematuria are reported in approximately 30% of cases. If urinary calculi or crystals are identified as calcium oxalate or calcium phosphate then consider a diagnosis of primary HPTH or idiopathic hypercalcemia.

Laboratory abnormalities are fairly characteristic. Total serum calcium will be increased in all dogs with primary HPTH. Serum phosphorus is decreased in 90% of dogs and

is expected based on the inhibition of phosphorus reabsorption by PTH in the kidneys. There are only two differentials for a dog or cat with hypercalcemia and hypophosphatemia: primary hyperparathyroidism and hypercalcemia of malignancy (HCM) Table 33.2. There are many causes of hypercalcemia and the concentration of serum phosphorus is usually increased or within the reference interval in other causes. The serum concentration of calcium and phosphorus should always be compared and not interpreted individually. Horses can have hypercalcemia and hypophosphatemia with renal failure, but dogs and cats with renal failure induced hypercalcemia have concurrent hyperphosphatemia. Hypophosphatemia is expected in over 90% of the cases of primary hyperparathyroidism; however, if the animal develops azotemia, the serum phosphorus may be within the reference interval because of reduced glomerular filtration and retention of phosphorus. Hypercalcemic renal injury and hyperphosphatemia is much more likely with HCM than primary HPTH.

Hypercalcemia is constant and is of variable magnitude. Most cases in dogs will be in the 12–16 mg/dL range, but some will exceed 18 mg/dL and infrequently greater than 20 mg/dL. Of all the differentials for hypercalcemia, the highest concentrations of serum calcium are seen with primary HPTH and HCM. Total and ionized calcium concentrations are increased and are due to PTH stimulated bone resorption, renal reabsorption of calcium, and indirectly from calcitrol stimulated intestinal absorption of calcium. The total serum calcium is increased in 100% of dogs with primary hyperparathyroidism, yet ionized calcium is increased in 90–95% of these dogs. If primary hyperparathyroidism is a differential diagnosis, but ionized calcium is in reference interval then measure total serum calcium.

Despite marked hypercalcemia, less than 5% of primary HPTH cases have azotemia. They rarely develop soft tissue mineralization because of concurrent hypophosphatemia. The Ca × P product is typically less than 70. The concept that hypercalcemia is a medical emergency should probably be modified based on the Ca × P product as chronic hypercalcemia with mild or no symptoms is typical of primary HPTH in dogs, cats and people. Azotemia is seen with more regularity in dogs with hypercalcemia of malignancy.

Animals with primary HPTH often have dilute urine and PU PD due to the inhibitory effect of calcium on ADH. The urine specific gravity will be less than 1.020 in 95% of the cases and the average urine specific gravity is 1.012. Calcium oxalate crystaluria is a diagnostic tip to consider hypercalcemia. Approximately one-third will have urolithiasis and a similar percentage will have urinary tract infections. The calculi are usually calcium oxalate or calcium phosphate. Approximately 20% of the cases will require surgical removal of calculi.

The approach to the diagnosis involves use of screening and confirmatory tests. By way of screening tests, it is recommended to repeat the serum calcium and phosphorus to be certain there is hypercalcemia and hypophosphatemia. If they persist, then there are only two diagnoses, primary hyperparathyroidism (relatively rare) and hypercalcemia of malignancy (HCM) (relatively common). The easiest way to distinguish these two differentials is to find the tumor in dogs with HCM. Lymphoma is the most common tumor and adenocarcinoma of the anal sacs is next most likely, although any tumor can produce this syndrome. In cats the cancer is usually a carcinoma followed by lymphoma, myeloma, and others. However, the HCM syndrome is uncommon in cats. If a nonparathyroid tumor cannot be localized, then consider confirmatory tests such as measuring PTH, PTH rP, and calcium and/or performing ultrasonography of the neck to search for the parathyroid tumor. PTH, PTH rP, and calcium should be measured in the same sample or samples collected concurrently. It is important to measure both PTH and PTHrp and compare results because there can be overlap of the values for both hormones in dogs with primary HPTH and HCM.

Expected findings

Ca	P	PTH	PTH rp	Diagnosis
Inc	Dec	RI to inc	Dec	Primary HPTH
Inc	RI	RI	Dec	Primary HPTH
Inc	Dec to RI	Dec to RI	Inc	HCM
RI	Inc	Inc	—	Secondary HPTH
Inc	Inc	Dec	Dec	Vitamin D toxicity

RI = reference interval; see discussion of hypercalcemia for results seen with granulomatous disease, Addison's etc.

If PTH and serum calcium are increased and the patient is not azotemic then the diagnosis is easy, primary hyperparathyroidism. However, increased PTH is only present in about 30% of dogs and the majority of dogs (75%) with primary HPTH will have a concentration of PTH within reference interval: mean = 11.3 pmol/L; RI 2.0–13.0, 210 dogs. In fact 45% of the cases had serum PTH concentrations in the low to middle range, 2.3–7.9 pmol/L. Increased PTH is more typical in humans with primary HPTH probably because of better crossreactivity of the antibody used to measure PTH. Increased concentrations of PTH are the exception in dogs, but if PTH is detectable in an animal that is hypercalcemic and not azotemic then this combination is inappropriately abnormal because PTH should be decreased or undetectable in response to nonparathyroid induced hypercalcemia. If PTH is within the reference interval, it is inappropriately high in the face of hypercalcemia, and therefore diagnostic for primary HPTH. It indicates the parathyroid gland is

secreting PTH at a time when secretion should be suppressed. It is critical to measure PTHrp concurrently as many dogs with HCM will have measureable PTH. Measure PTH, PTHrp, and calcium as a mini-panel and compare results of each for the best interpretation. It is not recommended to measure PTH or PTH rP if the patient is azotemic because PTH is degraded and excreted via the urinary system and may be increased due to delayed clearance. Increases in PTH can be enormous in dogs with chronic renal failure, >10,000 pg/dL, especially in assays that measure the carboxy-terminus of PTH but even midvalent and amino terminal assays will measure an increased PTH and confuse interpretation. These marked increases are due to concurrent secondary HPTH and decreased clearance of PTH.

Cats with primary HPTH usually have concentrations of PTH within reference interval, which is inappropriate for their hypercalcemia. Consult the reference laboratory about validity of the assay for cats and reference intervals which are usually considerably lower than dogs, 0–4.6 pmol/L; PTH rp <1.5 pmol/L. Assays for PTH and PTH rp have been validated for cats.

Primary HPTH is rare in horses, as is HCM. However, PTH and PTH rp can be used to establish a diagnosis in horses, but only if hypercalcemia and hypophosphatemia are not due to renal failure.

Ultrasonography of the neck region may clarify ambiguous hormone data and localize the site of the tumor. If HCM is ruled out in a dog with hypercalcemia and hypophosphatemia then ultrasonographic identification of a mass in the neck region is equally as diagnostic as or better than serum assays for PTH. Based on positive imaging findings, treatment can be started before results of hormone assays are returned. Furthermore this technique localizes the site for surgery or ethanol or heat ablation. Of dogs with primary HPTH in which ultrasonography was performed, 129 of 130 had one or more masses detected in the parathyroid region.

Hyperparathyroidism: summary

- Hypercalcemia—100% Hypophosphatemia—90%
- 100% if total serum calcium is measured; 90% if ionized calcium is used
- Azotemia occurs in less then 5% of the canine cases.

The total serum calcium is increased in 100% of dogs with primary hyperparathyroidism yet ionized calcium is increased in 90–95% of these dogs. If primary hyperparathyroidism is a differential diagnosis then measure total serum calcium.

Laboratory tests are helpful during and following treatment. Measurement of PTH during surgery can now be performed via a rapid assay that helps determine when all neoplastic, hyperfunctioning parathyroid tissue is removed. The half-life of PTH in a nonazotemic animal is only 5–10 minutes. Therefore, serum or plasma PTH will decline within minutes of removal of a secreting parathyroid lesion. The remaining atrophic parathyroid glands will not start secreting PTH for hours to days depending on the severity of their atrophy. If plasma PTH does not decrease within minutes of parathyroidectomy, then continued surgical exploration for additional hyperfunctioning parathyroid tissue is indicated. Ideally a new baseline plasma PTH is measured just prior to the start of surgery, during anesthesia and without palpating the thyroid-parathyroid area (avoids spiking secretion of PTH). After the tumor is removed, 5–10 minutes are lapsed and postremoval PTH is measured to determine if there is a >50% decrease in PTH indicating successful removal of the offending lesion. While waiting the 10–20 minutes for the assay results exploration should be performed to search for other enlarged parathyroid tissues. This is essential in Keeshonds in which more than one enlarged parathyroid gland is expected and in cats. Excision is considered successful when the plasma concentration of PTH decreases by >50%. However, if a second enlarged gland was found while waiting for the results of PTH it should be removed even if PTH decreases. This latter situation was found in 3 of 5 dogs with multiglandular parathyroid disease indicating the necessity to continue surgical exploration and not rely entirely on the change in PTH to determine successful removal of all hyperactive tissue. Whether these tissues would have caused subsequent hyperparathyroidism is not known. If a third sample is required, wait 5–10 minutes from the last sample. This technique is used in our university teaching hospital approximately six times a year. Measurement of PTH is via the chemiluminescent rapid assay, Immulite.

Calcium is monitored postoperatively. If serum calcium does not return to the reference interval following parathyroidectomy, then there was a second parathyroid tumor, or excessive vitamin D was used preoperatively, or there is a different diagnosis than primary HPTH. Measurement of PTH, PTH rp, calcium, and phosphorus should clarify these possibilities. Recurrence of primary HPTH happens in 5–10% of dogs and takes months to a year.

Postoperative hypocalcemia occurs in about one-third of the dogs following parathyroidectomy, it is usually asymptomatic and may happen immediately or take up to one week. Ionized calcium is the ideal fraction to monitor because it can be determined rapidly and is biologically active, but total calcium can be used as well. The atrophic parathyroid glands cannot produce and secrete sufficient PTH rapidly enough in some patients to prevent hypocalcemia after the parathyroid tumor is removed. Hypocalcemia may be dependent on the severity and chronicity of the hypercalcemia and therefore the severity of parathyroid gland atrophy. If the remaining parathyroid glands are not too atrophic they will start to secrete PTH in response to the declining calcium and prevent postoperative hypocalcemia. The higher the serum calcium prior to parathyroid surgery the more likely there will be postoperative hypocalcemia;

duration of hypercalcemia, if known, would probably be predictive as well.

Therapy for hypocalcemia postsurgery can induce hypercalcemia and delay the return of the normal Ca–PTH axis. Vitamin D requires several days before it exerts its effects on calcium and if the dose of oral calcium is increased during this time or additional vitamin D is given then these will work in concert and may increase the serum calcium into the hypercalcemic range. Overzealous treatment with calcium and vitamin D is the correct interpretation for hypercalcemia that occurs in the first week postsurgery rather than recurrence of tumor if normocalcemia was confirmed after surgery. Treating the symptoms of endocrine diseases may be more important than treating the absolute values of hormones or their end products. If clinical signs referable to hypocalcemia are not present, supplementation should be avoided.

There is a report of concurrent hyperparathyroidism in dogs with hyperadrenocorticism. Individual data is not provided, but observations from averaging values from these dogs are the following. There is increased PTH in 92%, three fold increase in PTH in 34%, no differences or consistent pattern in ionized and total calcium. Serum phosphorus is higher than controls, but the actual incidence of hyperphosphatemia was not reported. Dogs with renal failure were not included in data sets. The authors concluded there is unexplained hyperparathyroidism in dogs with adrenal and pituitary dependent hyperadrenocorticism. Measuring PTH in dogs with hyperadrenocorticism did not change clinical decisions and that the status of adrenal function should be considered when interpreting increased concentrations of PTH in dogs.

Disorders of calcium and phosphorus metabolism

There are a number of diseases that disturb calcium metabolism to a degree that cannot be managed by functional parathyroid gland regulation (Tables 33.1 and 33.2). These diseases result in either hypocalcemia or hypercalcemia that occasionally is recognized by clinical signs, but is more often detected by routine biochemical test results.

Hypocalcemia

Hypocalcemia—differential diagnoses, summary

Causes of hypocalcemia that are more much common than primary hypoparathyroidism include: hypoalbuminemia, hypoproteinemia, renal disease, ethylene glycol toxicity, pancreatitis, and eclampsia/"milk fever." Most of these differential diagnoses are easy to establish without knowledge of serum calcium. Serum calcium is rarely measured for a diagnosis of lactational tetany, and if measured is usually done after treatment, as confirmation. A complete list of differentials is provided in Table 33.1. The list of differential diagnoses for hypocalcemia is much shorter if hyperphosphatemia is present. Most cases of hypocalcemia are easy to diagnose. Some situations are diagnosed from signalment and presentation without measurement of serum calcium or any other analytes: lactation in any species, endurance horses, and grass tetany. If a pet had neck surgery and it was not hypocalcemic prior to surgery and it is postsurgery, then trauma to the parathyroid glands is the cause.

If the signalment and history are not diagnostic and a chemistry panel reveals hypocalcemia, then examine albumin and urea nitrogen (or creatinine). If albumin is decreased apply the correction formula. If the patient is azotemic that is the most likely explanation for hypocalcemia. If serum calcium is <7 mg/dL and the patient has acute renal failure, test for ethylene glycol toxicity. If these do not identify the cause of hypocalcemia then examine lipase to rule in or rule out pancreatitis. The only hypocalcemic disease that serum PTH may be required to confirm is primary hypoparathyroidism. Ruling out other causes of hypocalcemia usually rules in this diagnosis, but it can be confirmed by measuring PTH which should be undetectable or markedly low and therefore is inappropriate for the degree of hypocalcemia. The determination of ionized calcium is of value in critically ill patients no matter what their primary disease or total calcium concentration is.

Artifactual hypocalcemia due to use of calcium binding anticoagulants such as EDTA or oxalate is often listed. Although this is possible, the serum concentration of calcium is usually "zero." Nothing else will do this and therefore there is no confusion as to the diagnosis. If in doubt repeat the measurement and be sure to use a serum sample. The purpose of anticoagulants such as EDTA (purple top tubes) or oxalate (black top) is to chelate calcium as the means of anticoagulation.

Hypocalcemia disorders

The following is a more in-depth discussion of various causes of hypocalcemia.

Hypoalbuminemia

This is the most common cause of hypocalcemia by a large margin in all species. Because hypoalbuminemia is associated with a decrease in the protein-bound fraction of calcium, there are no clinical signs associated with this hypocalcemia. "Adjust" the total serum concentration of calcium for the hypoalbuminemia and if the adjusted-calcium concentration is within reference interval, then other causes of hypocalcemia do not need to be pursued. There is a limit to this relationship and if serum calcium is <6.5 mg/dL in a hypoalbuminemic animal there may be a second cause of hypocalcemia and measurement of ionized calcium is recommended. If ionized calcium is desired it must be measured, the correction formulas do not predict ionized calcium

they simply provide an index of how much the decrease in total calcium is due to hypoalbuminemia. The real calcium in the patient is the value that is measured, ionized, or total. The adjusted calcium is not a real number it simply helps the veterinarian determine that the decrease in total serum calcium is due to hypoalbuminemia. Diagnostic efforts are now directed at the cause of hypoalbuminemia. The adjustment formula for total protein can also be used but is not necessary, just use albumin. If serum albumin is normal or increased do not use any correction and investigate the cause of hypocalcemia; it is not due to a protein abnormality. If gastrointestinal disease is the cause of the hypocalcemia there may be multiple mechanisms involved such as hypoalbuminemia, malabsorption, hypomagnesemia, and/or abnormalities of vitamin D absorption and metabolism. Similarly if there is pancreatic atrophy or pancreatitis there may be multiple mechanisms working in concert to produce hypocalcemia: maldigestion, malabsoprtion, hypomagnesium, decreased vitamin D, and hypoalbuminemia.

Renal disease

The second most common cause of hypocalcemia. Renal failure may be associated with hyper, normo, or hypocalcemia. Most animals with renal failure are normocalcemic. Most cases follow the same pattern for total and ionized calcium, but there are examples where total and ionized concentrations disagree. The stage of renal failure is important in predicting the serum calcium. In compensated renal failure (early or mild) the serum concentrations of total or ionized calcium are usually in the reference interval. However, as renal failure advances into the uncompensated stages and finally end stage renal failure, hypocalcemia is expected except in some horses where hypercalcemia may be seen.

The diagnosis of renal failure is obvious when there is concurrent hyperphosphatemia (often marked), azotemia, and dilute urine. With chronic renal failure there will be marked parathyroid gland hyperplasia and hypertrophy, accompanied by marked increases in the serum concentration PTH. This is a result of the parathyroid response to a decreasing serum calcium that may still be in the reference interval as well as decreased renal excretion of PTH due to the renal failure. The decrease in calcium happens first and this induces parathyroid hypertrophy, hyperplasia, and secretion of parathyroid hormone in attempt to compensate for the urinary loss of calcium and raise the serum calcium. In end stage renal failure there are radiographic and microscopic bone lesions (osteoclastic osteolysis), but only a small percent of chronic renal failure animals have bone lesions severe enough to produce clinically detectable disease (fibrous osteodystrophy = "big head," rubber jaw). Bone lesions are more common in dogs than cats, horses, or ruminants. The most sensitive areas to detect these bony changes in radiographs are teeth, calvarium and "flat" bones that are more metabolically active in the adult than the long bones. Cases of "rubber jaw" or fibrous osteodystrophy still occur in all species, but only with chronic renal failure, not acute.

The hypocalcemia of renal failure is due to: (1) decreased reabsorption of calcium from tubules due to loss of tubular cells, (2) decreased concentration of vitamin D due to destruction of renal cells that produce vitamin D, (3) increased concentration of phosphorus that reciprocally decreases calcium, (4) soft tissue deposition of Ca × P (mineralization), (5) hypoalbuminemia, if present, and (6) if the cause of renal failure is ethylene glycol toxicity the calcium will be "complexed" with oxalate. Renal failure induced hypocalcemia is accompanied by moderate (9 mg/dL) to marked (>15 mg/dL) hyperphosphatemia. When the Ca × P product is greater than 70 the possibility of soft tissue mineralization is likely and when it is greater than 100 it is occurring, even if hypocalcemia is present. Phosphorus and vitamin D are major precipitators of mineralization and are more important for mineralization than calcium. Mineralization is enhanced with renal failure due to uremic induced vasculitis and tissue damage (dystrophic mineralization).

The following are examples of TCa × P in dogs with renal failure and azotemia:

Case	Total Ca	P	Ca × P
1	8	10	80
2	7	15	105
3	9	18	162
4	7.4	16.6	123
Reference interval	9–11 mg/dL	3–5 mg/dL	

Soft tissue mineralization is occurring in all of these examples even though serum calcium is decreased or within the reference interval. There are no studies that predict soft tissue mineralization using ionized calcium times phosphorus.

Although it is easy to explain the hypocalcemia associated with renal failure, most cases of renal failure are normocalcemic and some are hypercalcemic. This is true if the calcium status is determined via total or ionized calcium. Emphasis is placed on examples of increased total serum calcium while the ionized calcium is normal or decreased in dogs and cats with renal failure. Ionized calcium can also increase with renal failure and if ionized calcium is used to classify hypercalcemia in dogs then renal failure is the number two cause. The diagnosis of renal failure is usually straightforward. What is clinically more important than the serum calcium is determination of why the patient is in renal failure and what the Ca × P product is. However, dogs in renal failure that have a total serum calcium <8.6 mg/dL have a poorer prognosis for survival and discharge from a hospital than do dogs with higher serum calcium concentrations. This prob-

ably reflects the severity of the renal failure and associated magnitude of hyperphosphatemia.

Hypocalcemia is also seen with prerenal and postrenal azotemia, probably secondary to the increase in serum phosphorus. Anytime serum phosphorus is increased there is potential for hypocalcemia due to the physiologic balance of calcium phosphorus homeostasis, soft tissue mineralization, increased renal excretion of phosphorus anion and therefore calcium cation, and phosphorus inhibition of vitamin D production.

Obstructed tom cats have hyperphosphatemia, hyperkalemia, and azotemia that is associated with hypocalcemia. The decrease in ionized calcium may be disproportionately greater than the decrease in total calcium. For example, more obstructed cats have a decrease in ionized calcium as compared to total calcium. Tetany is not typically observed even when ionized calcium is as low as 1.10 mEq/L or total calcium less than 5 mg/dL. Fluid therapy that rapidly corrects acidosis, thereby decreasing the ionized fraction, may precipitate tetany.

Secondary hyperparathyroidism

This is a chronic parathyroid response secondary to one of two disorders of calcium–phosphorus metabolism. In secondary hyperparathyroidism, either renal disease or a calcium-phosphorus imbalance nutritional problem initiates absolute or relative hypocalcemia and hyperphosphatemia. Parathyroid hypertrophy and hyperplasia occurs in all the glands as a secondary response to the hypocalcemia caused by the underlying disease. Serum calcium does not need to decrease below the reference interval to produce this syndrome. The decreasing serum calcium is recognized by calcium sensing receptors that trigger parathyroid secretory and cellular responses in an attempt to raise the serum calcium via usual actions in bone, intestines, and kidneys. Until the primary renal or nutritional problem is corrected, these cyclic events continue and eventually produce clinical disease. Both nutritional and renal secondary HPTH will produce bone lesions that range from mild osteolysis only detected radiographically to fractured bones to enlarged bones from excess fibrous tissue deposition.

Examples of expected calcium and phosphorus values are shown below.

Serum	Ca	P	PTH
1° hyperparathyroidism	↑	↓	↑ or N
2° hyperparathyroidism	↓ N	↑	↑
Renal	↓ N	↑↑↑	↑↑↑
Nutritional	N ↓	↑	↑↑↑

N = normal value, within reference interval.

Renal secondary hyperparathyroidism is usually easy to diagnose. It is associated with hypocalcemia, hyperphosphatemia, and severe chronic renal disease with marked azotemia, inability to concentrate urine, and nonregenerative anemia. Measurement of ionized calcium and or repeat measurements of total and ionized calcium may detect a nadir of hypocalcemia, but there will be wide fluctuations if calcium is measured frequently. Younger animals are more likely to develop bony abnormalities that are associated with soft flexible "rubber" bones that result in lameness, bowing of the limbs, and mild to marked facial swellings and distortions. The increase in size of bones is due to excessive fibrous tissue deposits in response to the bone resorption and probable cytokine driven fibroplasia. If radiographs are taken, especially of the head, severe loss of bone can be seen around the teeth and in the calvarium. Radiographically detectable bony lesions are observed rarely with primary hyperparathyroidism or hypercalcemia of malignancy although, osteolysis is present microscopically in both diseases. The most significant clinical problem is the renal failure which is always chronic and usually severe.

Nutritional secondary hyperparathyroidism is a disease of carnivores, exotics (iguanas, etc.), and horses. Ruminants are more likely to have osteoporosis or rickets (vitamin D or phosphorus deficiency). In the majority of cases, the serum concentration of calcium is within the reference interval and there is mild to moderate hyperphosphatemia. It is associated with diets that either have insufficient calcium or too much phosphorus, or that have a calcium : phosphorus imbalance, such that the ratio of calcium : phosphorus in the diet is no longer 2:1. Diets associated with this disease include all meat diets for carnivores and excessive grain diets (high phosphorus, low calcium) in horses and high grains/nuts diets for reptiles (high phosphorus, low calcium). Horses and reptiles need less grain/nuts and more hay, legumes, or green leafy vegetables to balance dietary Ca : P. It can also be seen in herbivores that graze pastures with oxalate containing plants that chelate and lower serum calcium. The low dietary intake of calcium and/or the reciprocal lowering of calcium by the high dietary phosphorus intake lower the serum calcium concentration resulting in parathyroid hormone production and release and parathyroid hypertrophy and hyperplasia. To stimulate these events, the ionized serum calcium need only change by 0.1 mmol/L or the total calcium by 0.25–0.5 mg/dL; it is not necessary for the serum calcium concentration to be reduced below the reference interval to trigger parathyroid hormone production and release; e.g., serum calcium decreases from 9.7 mg/dL to 9.2 mg/dL and the 0.5 mg/dL decrease in serum calcium will stimulate PTH production and secretion. Ionized calcium is the biologically active trigger and it is decreasing as the total serum calcium decreases. The release of parathyroid hormone stimulates the mechanisms to normalize serum calcium by increasing calcium absorption and resorption. In nutritional

HPTH this will continue until the dietary imbalance is corrected. Therefore when total serum calcium is measured diagnostically it is usually "normal," but at the lower end of reference interval. Phosphorus, on the other hand, is not regulated as well as calcium. It may be high in the diet and it is also being released from bone, therefore serum phosphorus is usually increased and this is a key to the diagnosis. As always examine serum Ca and P concurrently. The increased PTH will stimulate compensatory increased P excretion in the urine. This compensatory mechanism is usually not enough to prevent hyperphosphatemia, but can be used to help establish the diagnosis by measuring the fractional excretion of phosphorus, an indirect indicator of PTH activity.

Fractional excretion of electrolytes, measurements of parathyroid hormone, and dietary evaluation with measurements of calcium and phosphorus in feed are usually the best ways to screen and confirm the diagnosis of nutritional secondary hyperparathyroidism.

Fractional excretion (Fx Exc) of electrolytes:

$$\frac{\text{Serum creatinine}}{\text{Urine creatinine}} \times \frac{\text{Urine electrolyte}}{\text{Serum electrolyte}} \times 100$$

A key to the diagnosis of nutritional secondary hyperparathyroidism is an increased serum PTH that results in an increased Fx Exc of phosphorus.

Dietary analysis should demonstrate a calcium to phosphorus imbalance, with increased phosphorus or decreased calcium. Often the history of the diet is helpful to establish the final diagnosis. As described above, the diagnosis of renal secondary hyperparathyroidism is easy because of chronic azotemia. A Fx Exc Na of >1% indicates renal impairment. Fractional excretion studies of calcium or phosphorus are not indicated when there is azotemia.

Ethylene glycol toxicity

This can result in marked hypocalcemia, <7.0 mg/dL, because of the mechanisms associated with renal failure. Severe hypocalcemia is due to the chelation of calcium with oxalate. However, these dogs and cats rarely have tetany, probably because they are severely acidotic and sick (uremic). The acidosis will preferentially shift calcium to the ionized compartment and therefore make more "biologically active" calcium available, thus preventing tetany. The possibility exists that if the acidosis was corrected rapidly the calcium would shift away from the ionized compartment and tetany could be induced, especially if calcium was not added to the IV fluids. Diagnosis of ethylene glycol toxicity is described in the urinary chapter of this book.

Pancreatitis

This is associated with mild hypocalcemia, often in the 8 mg/dL range. All the clinical problems are referable to pancreatitis and not hypocalcemia. The mechanism of the low serum calcium is unknown; investigations that evaluated ionized calcium, parathyroid hormone concentrations, and precipitation of calcium in the areas of fat necrosis, have not established a definitive pathogenesis. Correction of the pancreatitis results in normalization of the serum calcium concentration. The diagnosis is usually obvious because the hypocalcemia is associated with history, clinical signs, and other laboratory abnormalities that are consistent with pancreatitis. If hyperphosphatemia is present it is due to concurrent prerenal azotemia. Ionized calcium is also decreased. Ionized hypocalcemia is also present in about half of the dogs with diabetic ketoacidosis and is associated with a higher likelihood of death.

Lactation tetany

This is the most common cause of hypocalcemia that has associated clinical signs.

Eclampsia, or puerperal tetany, is most commonly recognized in the dog. The hypocalcemia associated with eclampsia is not a diagnostic mystery; the patient is usually a bitch that is approaching peak lactation, approximately 3 weeks postparturient. It is more common in smaller breeds of dogs and seen less frequently in large dogs, cats, ewes, and horses. The hypocalcemia is often severe, <6.0 mg/dL, and is one of the few hypocalcemic disorders associated with tetany. If the condition remains untreated the tetany will progress to paresis. If examined the pupils are often dilated and respond slowly to light. Other signs include tachycardia, fever, salivation, restlessness, and muscle spasms that may progress to seizures. Treatment is usually started before results of serum calcium are known. An ionized calcium measurement is ideal to rapidly confirm the diagnosis and monitor treatment. Symptomatic treatment of the hypocalcemia coupled with reduced lactation and/or supplementation (calcium + vitamin D) to the bitch usually corrects the problem.

Preparturient hypocalcemia has been reported in queens. The condition appears to be rare. The hypocalcemia can be severe, total calcium <5.0 mg/dL and similar to cows with milk fever, the cats tend to be hypothermic. Ewes may also develop signs prior to parturition, usually in the last month of gestation. This may be part of the pregnancy toxemia syndrome of sheep and is associated with stress and decreased food intake.

Milk fever is the common name for lactation hypocalcemia in cattle. The clinical diagnosis is easy. It is a cow, 1–4 days postparturient. A Channel Island breed is perfect (Jersey or Guernsey, but it can occur in any breed). It is much less frequent in beef cows. The affected cow is recumbent, head and neck often folded toward flank, and has bradycardia with possible arrhythmias. Fever is a misnomer as affected cows have a normal or subnormal temperature. Serum calcium is rarely measured and treatment is started based on

classical presentation. If serum is sampled total and ionized calcium are markedly decreased, phosphorus is mildly decreased, and magnesium and glucose will be increased. Total serum calcium can be <4.0 mg/dL and ionized calcium <2.0 mEq/L, <1 mmol/L. The relatively high calcium in the diet during the dry period (not lactating) has resulted in suppression of the parathyroid gland which in turn has decreased the osteoclastic pool. At and shortly after parturition there is a combination of events that result in the syndrome. These include anorexia, estrogen surge, and increased milk production and secretion. As a result serum calcium rapidly decreases. The parathyroid gland recognizes the decreasing serum calcium and secretes an adequate amount of PTH. However, the bone pool of osteoclasts is so suppressed that they cannot respond to the PTH rapidly enough to mobilize sufficient calcium to prevent paresis. Calcium continues to exit into milk and there is inadequate influx of Ca from bone, renal, and intestinal sources so hypocalcemia progresses to the point the cow collapses with paresis. If serum total or ionized calcium is measured at this time they will be markedly decreased. However, if measured several hours after successful treatment the serum total and ionized calcium has often returned to pretreatment levels, but there are no signs of paresis. There is more to this disease than simply the serum concentration of total or ionized calcium and acid-base status. Hyperglycemia is common and is due to stress and decreased insulin due to the hypocalcemia.

Ionized and complexed calcium, but not albumin bound calcium passes freely into the glomerular filtrate, CSF, and aqueous humor. Therefore the concentration of total calcium in these fluids is normally approximately half of the serum concentration, 4–5 mg/dL. If CSF or aqueous are used post mortem to confirm hypocalcemia, the concentration of total calcium must be less than 4 mg/dL to be compatible with milk fever.

Iatrogenic—thyroidectomy or removal of a parathyroid adenoma

Removal of the thyroid glands to treat hyperthyroidism in cats often results in complete or partial parathyroidectomy. Therefore, hypocalcemia and hyperphosphatemia may occur within 24–48 hours of the surgery, but can be delayed for up to one week. This also happens in dogs when a parathyroid tumor is excised. In dogs there is usually not damage to the other parathyroid glands, but they are atrophic due to chronic hypercalcemia induced by the parathyroid adenoma. After removal of the parathyroid secreting adenoma the remaining atrophic glands cannot resume synthesis of PTH rapidly enough to prevent postsurgical hypocalcemia. The degree of atrophy is a combination of the severity and duration of the hypercalcemia. Interestingly, this does not occur following surgical or chemical removal of anal sac tumors or with chemotherapy of lymphomas and other tumors associated with hypercalcemia of malignancy.

Cats and dogs with iatrogenic hypocalcemia usually only require treatment with intravenous or oral calcium and/or vitamin D if clinical signs occur or if the total calcium is <7 mg/dL, ionized <3 mEq/L, <1 mmol/L. In dogs the treatment period is short, days to a week and even in cats oral treatment is usually only necessary for one to two months post surgery. If cats are weaned off the calcium and vitamin D therapy, their serum calcium concentrations usually remain within the reference interval. The exact mechanism for normalization of the serum calcium is not known. Investigations that tried to determine if there was ectopic parathyroid tissue that hypertrophied and produced parathyroid hormone in response to postoperative hypocalcemia indicated this is not the case. Even dogs with experimental complete surgical parathyroidectomy will eventually stabilize their serum calcium and not require calcium or vitamin D supplementation. Parathyroid hormone-related protein (PTH rp) could have a role in the normalization of serum calcium in these patients as the hormone is produced by many tissues in adults and fetuses.

Dogs or cats with hypercalcemia due to a parathyroid tumor often develop hypocalcemia postexcision of the tumor. One dilemma of treatment is to provide just enough oral calcium and vitamin D to prevent symptoms, but not enough to induce hypercalcemia and continued suppression of the atrophic glands. It is preferable to keep the serum calcium slightly below the reference interval to stimulate the atrophic parathyroid glands to undergo hypertrophy. If possible only treat with calcium as it is easier to monitor and change the dose of calcium than it is to regulate serum calcium with vitamin D. Vitamin D requires 3–7 days to reach peak effect. Therefore it is more difficult to adjust the dose of vitamin D since there is a delayed response before an effect is observed. It is relatively easy to increase the dose of calcium and vitamin D such that therapy induces postoperative hypercalcemia. Treatment related hypercalcemia persists until the vitamin D is metabolized (up to 1 week).

Endurance racing—horses

Hypocalcemia is due to loss of calcium, along with other electrolytes in sweat and insufficient replacement during 50–100 mile races. Contributing to the hypocalcemia may be an alkalosis induced by hypochloremia and rapid respirations. Equine sweat is hypertonic and rich in calcium, potassium, sodium, and chloride. There is greater loss of electrolytes than water in the sweat of horses and therefore electrolytes must be supplemented during races especially in hot and humid weather conditions. Human sweat is isotonic and electrolyte replacement is not as critical as is water replacement. The hypocalcemia seen in endurance horses can be symptomatic and cause tetany, "thumps" or diaphragmatic flutter (tetany in muscles of diaphragm), weakness, cramping, and a variety of neuromuscular dysfunctions including ileus and colic. This can usually be prevented by

adequate supplementation with forced electrolytes during the event. High calcium in the prerace diet such as alfalfa hay (regular feed program) could precipitate this condition similar to milk fever in cattle. The most common electrolyte disturbance in endurance horses is hypochloremia.

Cantharidin toxicity

The hypocalcemia of cantharidin toxicity can be severe and is an important clue to the diagnosis of blister beetle toxicosis in the horse. The mechanism of the hypocalcemia is not clear, but is possibly related to concurrent hypomagnesemia. Hematuria, hypocalcemia and hypomagnesemia in a colicky horse eating alfalfa is sufficient to diagnose this toxicity. Toxicosis is confirmed via high pressure liquid chromatography that quantifies cantharidin in the urine (20 mL of urine), in gastric contents (one pint of stomach contents), liver, or kidneys of dead horses. Some horses will have increased muscle enzymes and myoglobinuria. Tetany, diaphragmatic flutter, paresis, and facial muscle spasms can be seen. The toxin causes acantholysis of esophageal and gastrointestinal mucosa, myocardial necrosis and renal tubular necrosis.

The blister beetles are found in alfalfa hay near the time of bloom. If hay is cut before the bloom phase it reduces the likelihood of the beetles being present and if crimping is not done it gives the beetles a chance to leave the cut plants. The crimping process crushes the alfalfa stems and any beetle present, leaving them in the hay to be ingested. Crimping is done to crush the stems, squeeze out water and speed drying of hay in eastern U.S. states. Alfalfa hay produced in the arid West is not crimped and the beetles simply walk out of the cut hay. The toxin is secreted by males, given to the female during mating and the female covers her eggs with it as a defense against predators. Horses are highly sensitive to cantharidin; the lethal dose-50 for horses is approximately 1 mg/kg body weight.

Hypomagnesium

Hypomagnesemia results in impaired PTH release and calcitriol resistance leading to secondary hypocalcemia. This is an uncommon or uncommonly recognized problem. Hypomagnesemia and hypocalcemia are associated with protein-losing enteropathy (PLE) in small animals, grass tetany in cattle, and cantharidin toxicity in horses. If PLE is present then the most likely cause of hypocalcemia is hypoalbuminemia. Contributing factors may be intestinal loss, malabsorption, and/or abnormalities of vitamin D and parathyroid hormone metabolism. If the concentration of ionized calcium is decreased there are other factors involved than just hypoalbuminemia, such as hypomagnesemia and decreased PTH. Electrolyte replacement may be required to avoid neurologic and metabolic problems. It is reported to be more common in Yorkshire terriers.

Hypomagnesemia causes grass tetany in beef and dairy cattle when they graze lush grass pastures. Animals are usually recumbent and generalized tetany is present or is especially obvious in cervical muscles. Tetany is preceded by an uncoordinated gait, "grass staggers," and an agitated behavior. Lush grass predominant pastures tend to be deficient in magnesium. Adequate serum concentrations of magnesium are dependent on adequate dietary intake. Cattle, tend to be hypocalcemic and treatment includes calcium and magnesium preparations. Tetany versus paresis in cattle with hypocalcemia is most easily explained by examination of serum magnesium. If magnesium is decreased as in grass tetany they will be tetanic and if increased as in milk fever they will be paretic. If hypocalcemia goes untreated long enough even dogs with lactational tetany may become paretic. The serum glucose may be increased due to stress and hypocalcemia induced hypoinsulinemia.

Oxalate

Plants rich in oxalates may lower serum calcium in herbivores. In chronic cases this has been associated with nutritional secondary hyperparathyroidism and fibrous osteodystrophy. Example plants with excess oxalates are halogeton, dock, rhubarb, greasewood, and soursob.

Critically ill animals—measure ionized calcium, total and ionized hypocalcemia

Measurement of ionized calcium in critically ill patients, regardless of the primary disease may influence therapy and prognosis. A mild decrease may not be important, but moderate to marked decreases could influence treatments, especially those aimed at stabilizing cardiovascular deterioration. Serum ionized hypocalcemia in critically ill dogs is associated with a longer duration in ICU and total days in the hospital, but is not associated with decreased survival. Critically ill dogs with renal failure, diabetic ketoacidosis, or pancreatitis were more likely to have ionized hypocalcemia, but diseases associated with hypocalcemia were not ranked. The study in dogs did not look at total serum calcium to determine if it was associated with predictive outcomes. Previously it has been shown that dogs in acute renal failure with total serum calcium <8.6 mg/dL have a poorer prognosis for survival and discharge from a hospital than do dogs with higher serum total calcium concentrations.

Critically ill adult horses with endotoxemia and gastrointestinal disease may have hypocalcemia, hypomagnesemia and parathyroid gland dysfunction. Decreased total and ionized calcium can cause or be associated with ileus and colic. Nearly 90% of horses with colic had decreased ionized calcium at the time of admission. Horses with very low ionized calcium were 12 times more likely to develop ileus. A fatal outcome was nine times more likely for horses in the very hypocalcemic group. Ionized calcium and response to calcium replacement was used to predict prognosis. Endur-

ance horses with marked hypocalcemia and hypochloremia can have an ileus that is so severe that it is erroneously diagnosed as a surgical colic. Septic foals were reported to have ionized hypocalcemia, hyperphosphatemia, and increased serum PTH, but no differences in magnesium, PTH rp, or calcitonin. However, in this study it is impossible to determine if these changes were related to renal function because creatinine (or urea nitrogen) and PTH concentrations were not reported for individual animals. Therefore comparisons of Ca to PTH, or PTH to azotemia cannot be made. Calves with severe neonatal diarrhea will have hypocalcemia, but due to concurrent acidosis the ionized hypocalcemia may not be as severe. With fluid therapy and correction of the acidosis both total and ionized calcium will decrease further. Tetany may develop during therapy as alkalinization shifts calcium from ionized to protein bound.

Sick patients

Hypocalcemia is seen in a variety of illnesses in small and large animals in which the pathogenesis is not known. It becomes difficult to know if there is cause and effect or simply an association. Pancreatitis, ketoacidosis, critical illness, sepsis, colic, endotoxemia, inflammatory diseases, protein abnormalities, cantharidin toxicity, and others could fit in this category of associated problems. There may be a mechanism that is reasonable, such as hypomagnesemia, cytokines or it may represent overlap of common problems. Hypocalcemia is seen in sick cattle with a wide variety of conditions including retained placenta, rumen overload, lymphoma, neonatal calf diarrhea, foot diseases, and abomasal ulcers, to list a few. There may be specific diseases to be aware of or it may be that the severity of the disease/illness in animal and human patients correlates better with the degree of hypocalcemia than the specific disease.

Feline hyperthyroidism

There is a calcium, phosphorus, and parathyroid imbalance in some cats with hyperthyroidism that is not understood. Hyperphosphatemia without azotemia is seen in 25–40% of hyperthyroid cats and total serum calcium is usually in the reference interval. However, a mild decrease in ionized serum calcium, without clinical signs is observed in approximately 30% (4 of 15) but the mechanisms are not known and the numbers of cats studied is small. Increased concentration of parathyroid hormone is reported in up to 77% of hyperthyroid cats studied (small numbers) and hyperparathyroidism and hyperthyroidism are listed as coexistent problems in cats. These abnormalities could be due to concurrent renal disease, which is common in geriatric cats. However, urea nitrogen and creatinine are usually in the reference interval. Another possibility is the growing association of hypocalcemia with or without an increase in PTH in a variety of concurrent illnesses. The parathyroid hyperplasia may help explain why some cats do not develop postsurgical hypocalcemia and may help explain why postsurgical hypocalcemia is usually not permanent. Humans with hyperthyroidism tend to have the opposite pattern: increased bone turnover, hypercalcemia, and decreased PTH. Hypercalcemia has been reported for dogs with hyperthyroidism, it is mild, the mechanism is not known, and it may not be related to hyperthyroidism. Although the phosphorus, ionized calcium, alkaline phosphatase (ALP), and bone relationship is an interesting paradox in hyperthyroid cats, it is clinically insignificant.

Iatrogenic

Various treatments such as anticonvulsants, intravenous phosphate solutions, calcium free IV solutions, transfusions with citrate as anticoagulant, excess bicarbonate therapy, tetracycline, and furosemide diuretics can be associated with hypocalcemia. Furosemide inhibits sodium and chloride reabsorption in the loop of Henle and secondarily inhibits calcium reabsorption that can lead to hypocalcemia. Furosemide can be used to lower serum calcium in hypercalcemic patients with adequate hydration. Tetracycline will chelate calcium and rapid intravenous boluses may decrease calcium. Hypocalcemia induced by transfusions is only associated with massive transfusions; e.g., open heart surgery, transfusion equal to 50% of blood volume in a 3-hour period, or transfusion equal to total blood volume over 24 hours can decrease ionized calcium by 1.2 mg/dL (0.3 mmol/L).

Hypercalcemia

Causes of hypercalcemia are outlined in Table 33.2. If ionized calcium is used to classify and rank the frequency of causes of hypercalcemia in dogs they are: HCM (58%), renal failure (17%), hyperparathyroidism (13%), hypoadrenocorticism (5%), miscellaneous diagnoses (4%), and vitamin D toxicity (3%). Cats and horses have the same differentials. Following is discussion of various causes of hypercalcemia that are not related to autonomous parathyroid hormone hypersecretion. A general diagnostic approach for evaluation of the hypercalcemic patient is outlined in Table 33.4.

Hypercalcemia of malignancy (HCM) is the term used to describe a common syndrome in which a nonparathyroid tumor produces a substance that acts like PTH and causes hypercalcemia and hypophophatemia. There are a variety of substances produced by tumors and the one that is most important from a clinical diagnostic perspective is parathyroid hormone related peptide (PTH rp). Total and ionized calcium are increased but concurrent hypophosphatemia, when present, is the major diagnostic clue as the only two diseases of dogs and cats that do this are HCM and primary HPTH. Historically, HCM was called pseudohyperparathyroidism because the disease resembled primary HPTH. The reason for this is that many tumors produce the protein PTH rp which shares a nearly identical amino acid

Table 33.4 Evaluation of a hypercalcemic patient.

1. Consider age of patient first and compare calcium and P, examine for azotemia.
2. If increased calcium is not due to young age then consider repeating serum calcium to confirm hypercalcemia; consider ionized calcium if available; if P is decreased there is no need to repeat serum calcium.

If **hypercalcemic and hypophosphatemic: HCM vs. Primary hyperparathyroidism**

1. If a horse and they are azotemic, done, renal failure is the cause of hypercalcemia, pursue causes of renal failure; more likely to be chronic and have a poor prognosis
2. If dog or cat pursue HCM first followed by primary HPTH
3. Hypercalcemia of malignancy (number one cause of persistent hypercalcemia in dogs and cats); perform a cancer search, do not measure PTH or PTH rp initially.
   Lymphoma most likely; #1 tumor associated with HCM
   Evaluation of lymph nodes, anterior mediastinum, bone marrow, FeLv; 50% of dogs have an anterior mediastinal mass; <10% leukemic; determination of hepatosplenomegaly; aspiratiional cytology of enlarged lymph nodes and or organs
   Adenocarcinoma apocrine glands of anal sac, #2 tumor associated with HCM in dogs
   Thorough rectal-perirectal examination; metastases in pelvic canal and or sublumbar present at time of initial diagnosis; 50% do not protrude caudally and therefore are not visible, but can be palpated
   Myeloma
   Markedly increased total protein, monoclonal gammopathy; multiple lytic bone lesions; aspirational cytology; Bence Jones proteinuria
   Other tumors
   Mammary, pulmonary, malignant histiocytosis, squamous cell carcinoma any tumor possible, perform search
   Horse—squamous cell carcinoma of stomach; endoscopy; paracentesis; lymphoma search
   If one of the tumors above is found that is the cause of hypercalcemia; if further confirmation desired then measure PTH and PTH rp concurrently
4. If a cancer cannot be found or only a benign skin cancer is found then consider primary hyperparathyroidism: ultrasound the thyroid-parathyroid complex and or measure PTH and PTH rp concurrently. Small mass in thyroid region with hypercalcemia and hypophosphatemia is usually sufficient evidence for diagnosis and localizes the tumor to left or right; if further confirmation desired measure PTH and PTH rp concurrently.

Hypercalcemia and hyperphosphatemia:

1. Rule in or out malignancy first as outlined above; HCM much more likely to be hyperphosphatemic than patients with primary HPTH; <5% of dogs with primary hyperparathyroidism are hyperphosphatemic; it is "practical" to rule out hyperparathyroidism if hyperphosphatemic; exceptions always exist.
2. Examine for azotemia which is very likely, and assess degree of azotemia; the higher the azotemia the greater likelihood of primary renal; attempt to rule in rule out acute vs. chronic renal failure; if a young dog, especially purebred then progressive familial renal nephropathy is most likely; if acute renal failure consider grape toxicity.
   If azotemia is mild to moderate then the order of DDx are Addison's, renal disease and vitamin D toxicity; all are likely to have dilute urine: <1.025 while azotemic.
   90% of Addison's are azotemic, rule in with basal cortisol <2 µg/dL and Na : K <23, decide if ACTH stimulation needed; rule out with cortisol >2 µg/dL.
   Rule in/out renal failure: response to fluids; acute vs. chronic; imaging of kidneys; Fx Exc Na; etc.
   Differentiation of renal causing vs caused by hypercalcemia: >P more likely primary renal and >calcium more likely hypercalcemia came first; if total calcium increased and ionized normal or decreased renal more likely; ionized can be increased in renal failure in dogs and cats.
   Consider vitamin D toxicity from history, possible exposure, soft tissue mineralization, assay for vitamin D; plants unlikely in carnivores, more likely in herbivores.
3. If all above ruled out pursue idiopathic in cats; granulomatous diseases: blastomycosis, other fungi, FIP, schistosomiasis, mycobacterium, toxoplasmosis; iatrogenic.

Hypercalcemia and normophosphatemia—consider all of above

- Patients with HCM—cancer search more practical than PTH PTH rp determinations. Lymphoma first, followed by anal sac, lung, mammary, malignant histiocytosis
- Hypoadrenocorticism: Na : K <23:1; basal cortisol; ACTH stimulation test
- Renal disease—azotemia, very rare to have normophosphatemia if they are also hypercalcemic; evaluate renal size; acute vs. chronic
- Primary hyperparathyroidism: Serum PTH PTH rp; ultrasound, exploratory surgery of cervical region
- Idiopathic—cat; rule out others, calcium crystalluria; steroid responsive
- Bone lesion—other mechanisms besides direct osteolyis are involved
- Granulomatous—aspirational cytology is test of choice, find etiologic agent
- Ionized calcium—if desired measure; same list of differential diagnoses and diagnostic approaches; ionized calcium more important to measure in hypocalcemic than hypercalcemic situations
- Do not use adjustment formulae for protein or albumin if hypercalcemia is present

sequence at the amino terminal end of the molecule to native parathyroid hormone. Since the amino terminal end is the biologically active fragment, both molecules are recognized by and stimulate the same receptors (PTH1R) and therefore produce the same biological responses. Many cells throughout the body produce PTH rp, but the only disease associated with this molecule in veterinary medicine is HCM. PTH rp is important for fetal calcium regulation, transplacental calcium transportation, calcium homeostasis of the fetus, important in milk, and no doubt will be linked to other physiological functions and diseases. It can be identified via immunohistochemistry in tumors that cause hypercalcemia and in tumors from normocalcemic animals. In addition to PTH rp, a group of less commonly recognized substances can be produced by tumors and act individually or in concert to amplify this syndrome. These include interleukin-1, interleukin-6, tumor necrosis factor, prostaglandin E2 (PGE_2), fibroblast stimulating factor, epidermal growth factor, transforming growth factor, PTH, and vitamin D derivatives. Some of these factors are important in the hypercalcemia associated with lymphomas and may act synergistically. The only two substances on this list that are diagnostically practical in veterinary medicine are PTH and PTH rP. However, if a malignant tumor was present and both PTH and PTH rp were decreased, then one of these other humoral/local osteolytic substances may be causing the hypercalcemia.

HCM is the most common cause of hypercalcemia in dogs and it has been associated with numerous tumors. The two most common tumors are lymphoma (by a wide margin) and apocrine gland adenocarcinoma of the anal sac. Lymphoma associated with hypercalcemia in dogs is usually a T-cell lymphoma, as it is in people. Approximately 50% of the dogs will have a mass in the anterior mediastinum. Only 6% are leukemic and osteolytic bone lesions are seen rarely in conventional radiographs. If neither of these tumors are present search for multiple myeloma, mammary carcinoma, pulmonary tumor, and histiocytic sarcomas in that order. If a tumor cannot be found then measuring PTH and PTH rp are helpful diagnostic tests as is ultrasonography of the thyroid region and possibly the abdominal cavity to search for less physically obvious neoplasia.

HCM is not as common in cats when compared with dogs, but HCM is the number one or two cause of hypercalcemia in cats. It is associated with carcinomas, especially squamous cell carcinoma (SCC), lymphoma, feline leukemia virus with or without lymphoma, myeloma, and rarely with a variety of other malignant tumors. Anal sac tumors are rare in cats and only a few are associated with hypercalcemia. Most cats with this tumor are normocalcemic. Ferrets also have this anal sac tumor, but hypercalcemia is not seen. HCM is rare in horses and has been associated with lymphoma, myeloma, ameloblastoma, and gastric squamous cell carcinoma. Although lymphoma is common in cattle, there have been no reported cases associated with hypercalcemia. Interstitial cell tumors of the testes are associated with HCM in rats and an integumentary carcinoma will produce the disease in rabbits. There are multiple animal models in rabbits, rats and mice.

In most cases of HCM, the diagnosis is easy and is based on physical examination and/or laboratory findings of neoplasia in the categories described above. Essentially any tumor could produce this syndrome, most are malignant but some are benign. Aspirational cytology of enlarged lymph nodes or the perineal mass or another tumor can easily confirm the diagnosis. Measurement of PTH and PTH rp is not needed if a tumor can be identified. Glucocorticoids are useful in lowering the serum concentration of calcium, but avoid their use until a diagnosis is established. Steroids will cause lymphocytolysis which will greatly interfere with interpretation of any lymph node aspirates examined via cytology. If an injection of steroids corrected hypercalcemia in a dog with enlarged lymph nodes, then the diagnosis was very likely lymphoma. Aspiration of lymph nodes at this time will probably be nondiagnostic, but when hypercalcemia returns cytologic confirmation of lymphoma is indicated.

Most clinical signs are referable to the cancer, but some are due to hypercalcemia. These include weakness and PU PD. Animals with HCM have more dramatic clinical signs than animals with primary HPTH due to the malignancy such as cachexia, mass lesions, pulmonary metastases, generalized ill health, and azotemia. Hypercalcemia and hypophosphatemia are the key diagnostic laboratory abnormalities. Apocrine adenocarcinomas are one of the best examples to demonstrate how the tumor induces these electrolyte abnormalities. In the following example of an 8-year-old female spayed German shepherd dog there is hypercalcemia and hypophosphatemia at the time of diagnosis that is rapidly corrected following the first surgical removal of the malignant tumor. When the tumor recurred, so did hypercalcemia and hypophosphatemia and this cycle repeated itself until the dog's death.

Serum	Total calcium	Phosphorus
Initial diagnosis	21.2	2.4
24 hr post surgical removal	10.4	3.9
Recurrence 13 months later	16.8	1.8
24 hr post surgical removal	9.6	4.2
Recurrence 13 months later	18.1	3.4
Reference interval	9–11 mg/dL	3-5 mg/dL

Hypercalcemia can be used as a marker of tumor recurrence and/or metastases. These sequential changes in serum calcium and phosphorus associated with surgical removal of

tumors and their recurrence was part of the initial evidence that tumors were producing humoral factors that acted like PTH. In the example above, the serum concentration of PTH was appropriately undetectable prior to surgical removal of the tumor and increased rapidly after surgery, preventing postoperative hypocalcemia. Despite the malignant behavior of anal sac tumors, they spread slowly and surgical resection or chemotherapy often gives affected patients months or even years of life postdiagnosis. Only about half of the tumors protrude caudally to be visible in the perineum, but all can be detected on rectal palpation. They tend to enter the pelvic vault and sublumbar lymph nodes before spreading to liver and or lungs. This tumor is distinct from benign circumanal or perianal gland adenomas that are common in male dogs and are visible in the perineum.

The hypophosphatemia in dogs, cats, horses, lab animals, and people with HCM is due to the phosphaturic effect of PTH rp on the renal tubules. Causes of hypercalcemia other than HCM and primary HPTH do not stimulate renal phosphorus excretion; hence the serum P is normal or increased in other diseases. Given time, dogs with HCM often develop normophosphatemia or hyperphosphatemia. This is likely due to concurrent dehydration and prerenal azotemia. Other contributing factors include lymphoma involvement of the kidneys or renal mineralizaton after the phosphorus increases. Animals with HCM are much more likely to be hyperphosphatemic or normophosphatemic and azotemic than are animals with primary HPTH. This is probably due to the more frequent development of renal complications and subsequent phosphorus retention. Once the Ca × P product is over 90 soft tissue mineralization is likely and one of the common tissues that is predisposed to this is the kidney. Hence, nephrocalcinosis is a frequent complication, which further contributes to renal problems.

Urine specific gravity is dilute due to hypercalcemia inhibiting the action of ADH. The triad of azotemia, dilute urine, and hypercalcemia produces the diagnostic challenge of what came first, renal failure or hypercalcemia and what is causing the hypercalcemia? There are multiple ways to solve this. First, it is much more likely that the hypercalcemia is due to a tumor than renal failure. Second, the physical examination finding of a malignant tumor (particularly lymphoma or anal sac adenocarcinoma) establishes a cause for the hypercalcemia. Next, the greater the serum calcium the more likely it is HCM and not primary renal failure. The lower the serum P, the more likely it is HCM, while the higher the phosphorus, the more it favors primary renal disease. Lastly, if a primary renal disease can be diagnosed then that it is the cause. Since both diseases tend to be in geriatric patients, they may have both HCM and chronic renal failure.

Following the use of screening tests such as physical findings, calcium, and phosphorus, confirmatory tests may be used. Measurement of PTH and PTH rp are the best laboratory methods for confirmation. Use these if an obvious cancer cannot be found. Request PTH, PTH rp, and calcium on the same sample or samples drawn concurrently in a nonazotemic patient. Ideally PTH will be decreased or undetectable and PTH rp will be increased in patients with HCM. The increase in PTH rp is usually diagnostic, but other substances may cause HCM. Therefore a decreased value of PTH rp does not rule out HCM. The PTH concentration could be within the reference interval making interpretation difficult because PTH is often in the reference interval in primary hyperparathyroidism. However, PTH rp will be decreased or undetectable in patients with primary hyperparathyroidism while PTH is normal or increased. There is overlap of the absolute values for each disease and that is why both hormones should be measured concurrently and the results compared to each other and the serum calcium concentration. If just one hormone is measured, interpretation is difficult and may necessitate repeat sampling.

The easiest and more practical way to differentiate primary HPTH from HCM, however, is simply to find the cancer associated with HCM and then perform aspirational cytology. If a cancer cannot be located then consider performing ultrasonography of the thyroid region to search for a parathyroid adenoma. The measurement of serum/plasma PTH and PTH rp may be useful adjuncts when physical examination and ultrasonography are not conclusive. Identification of the other humoral factors associated with HCM is difficult and is a research and or investigative situation.

Examples of interpretation of PTH and PTH rp results for hypercalcemia include:

PTH	PTH rp	Diagnosis
Inc	Dec	Primary HPTH
RI	Dec	Primary HPTH
RI	Inc	HCM
Dec	Inc	HCM
Inc	Inc	Primary HPTH and HCM
Inc	Inc	Renal
Dec	Dec	Other causes of hypercalcemia
Dec	Dec	An ectopic substance other than PTH rp
Dec	Dec	In vitro decay

RI = reference interval.

If both PTH and PTH rp are increased check the serum UN, creatinine, and phosphorus as a likely explanation is decreased clearance due to renal failure.

Interestingly, hypocalcemia does not happen after surgical resection of a tumor associated with HCM or after chemotherapy for tumors causing HCM, which has significant clinical application. Parathyroid hormone secretion is suppressed for several days following the removal of a parathy-

roid adenoma in dogs with primary HPTH and hypocalcemia develops in some of these dogs. However, parathyroid hormone is secreted rapidly following surgical removal of anal sac gland adenocarcinomas, preventing postsurgical hypocalcemia. Following removal of anal sac tumors the concentration of serum calcium returns to normocalcemic ranges in less than 24 hours as serum PTH, which was decreased or undetectable before surgery, now increases. Apparently the suppression and atrophy of parathyroid glands is greater in primary hyperparathyroidism than in HCM. An explanation for this observation is not clear.

Renal disease with hypercalcemia

In cats this is the number one or number two cause of hypercalcemia, in horses it is the number one cause, and it is a relatively common cause in dogs. If ionized calcium is used to classify causes of hypercalcemia in dogs, renal failure is the number two cause. Dogs with renal failure are usually normocalcemic, but hypocalcemia is common and hypercalcemia may be present in about 10%. Chronic renal failure is more commonly associated with hypercalcemia than acute renal failure, although grape and currant toxicities are examples of acute renal failure that may be associated with hypercalcemia. The cause of hypercalcemia with renal failure is not known, it is referred to as tertiary hyperparathyroidism. Increases in total and ionized calcium are usually mild compared to the hypercalcemia seen in HCM or primary HPTH, e.g., total calcium 11.5–13 mg/dL for renal failure versus >16 mg/dL for HCM or primary HPTH. Examine the phosphorus and determine the Ca × P product. The product is often increased with renal failure.

The cause of hypercalcemia with renal failure is not known; it is referred to as tertiary hyperparathyroidism. The pathogenesis probably involves an abnormality with the calcium receptor for PTH located on the parathyroid chief cells and other cells. Calcium-sensing receptor (CaSR) controls PTH secretion and arms parathyroid chief cells and other CaSR-expressing cells to detect changes in the concentration of calcium and to make adjustments that normalize serum calcium. Several disorders in humans are due to inherited or acquired abnormalities of these CaSR pathways such that the receptor is reset at a serum calcium concentration that disrupts normal regulation and there is resulting hypercalcemia or hypocalcemia. These pathways may play a pivotal role in the development of hypercalcemia seen in some animals with renal failure. Acquired abnormalities in this receptor may result in a failure of an increasing concentration of calcium to decrease the production and release of parathyroid hormone. It is as if the thermostat was turned up and it no longer shuts off production of PTH at a high concentration of calcium. Therefore, parathyroid hormone continues to be produced and secreted even though the present concentration of calcium (high) would normally inhibit the release of parathyroid hormone. Parathyroid hormone concentrations are increased in patients with renal failure due to hypocalcemia (relative or absolute), hyperphosphatemia, parathyroid hyperplasia, and decreased clearance and degradation of PTH as kidneys excrete PTH. Parathyroid hormone is considered one of the "uremic toxins."

When hypercalcemia is present in dogs with renal disease, it is usually seen in younger dogs that have progressive familial renal dysplasia (breeds such as Lhasa Apso, Elkhound, Doberman, Wheaton terrier). Nearly all of these patients will also have hyperphosphatemia and be azotemic. Patients with hypercalcemia and hyperphosphatemia are very prone to the formation of soft tissue mineralization. Attempts to lower the serum phosphorus and azotemia should be vigorous because renal mineralization will compound the renal failure and may make it irreversible. The differentiation of hypercalcemia due to renal failure from azotemia caused by another hypercalcemic disease is problematic. A generalization is the higher the serum phosphorus, the more likely the cause is renal disease, and the higher the serum calcium, the more likely the cause is not renal. If renal failure is causing the hypercalcemia, the serum phosphorus concentration is usually in double figures in dogs and cats, e.g., 10–25 mg/dL. The lower the concentration of serum phosphorus, the more likely the cause of the hypercalcemia is something other than renal failure. For example, there is a substance in circulation that is both increasing calcium and decreasing phosphorus, such as PTH or PTH rp. These substances have a phosphaturic effect and if phosphorus is decreased or even if in reference intervals while a patient is azotemic that is strong evidence that the patient has increased PTH or PTH rp. If the total serum calcium is increased, but ionized calcium is in reference interval or decreased this pattern is more characteristic of primary renal failure.

Patients with hypercalcemia and renal failure are expected to be hyposthenuric—from the renal disease or the action of calcium blocking the function of ADH on the collecting ducts. Approximately one-third of horses in chronic renal failure develop the unusual combination of hypercalcemia and hypophosphatemia. This is unique to the horse. Other horses have the traditional hyperphosphatemia expected with azotemia and either hypocalcemia or normocalcemia. The mechanism for hypercalcemia and hypophosphatemia in these horses is unknown, but there are several ideas.

Addison's disease

Approximately one-third of dogs with hypoadrenocorticism will have hypercalcemia. The mechanism is not entirely known, but postulated components are increased complexed calcium to citrate, an absence of glucocorticoids and therefore absence of corticoid calciureic effects. Steroids promote calciuria and block osteoclastic osteolysis. In the absence of steroids, these two physiologic events may contribute to hypercalcemia. Ionized calcium was increased in five of seven Addisonian dogs that all had increased total serum

calcium, but there were no consistent increases in PTH, PTH rp, or 1,25 dihydroxyvitamin D concentrations to explain the hypercalcemia. Almost all dogs with hypoadrenocorticism are azotemic, many do not concentrate their urine beyond 1.020, and most are hyperphosphatemic. Because renal failure and Addison's disease can have similar electrolyte abnormalities, these diseases can appear similar. If hypercalcemia is present with the above laboratory abnormalities it favors hypoadrenocorticism. If the Na:K ratio is <23 in an azotemic patient it favors hypoadrenocorticism, although this lowered ratio may be seen in renal failure and uroabdomen.

Vitamin D toxicity

This usually produces hypercalcemia and normo- or hyperphosphatemia since vitamin D stimulates both calcium and phosphorus absorption from the GI tract and resorption from bone, without a direct phosphorus lowering effect on the kidneys. The combination of hypercalcemia and hyperphosphatemia can produce lethal soft tissue mineralization. Mineralization of muscles, tendons, heart, lungs, gastrointestinal tract, and blood vessels is expected and is the cause of death. Sources of vitamin D include dietary supplements, rat poisons, and plants (*Cestrum diurnum* or day blooming jasmine, *Solanum malacoxylon, Trisetum flavescens*). Plant toxicities are more common in herbivores. Rodenticides are more common in dogs and cats. Day blooming jasmine is a house plant and has been reported to cause hypercalcemia in pets that eat it. Overzealous treatment of hypocalcemia with vitamin D and calcium products can cause hypercalcemia. This happens with some regularity in the postoperative treatment of hypocalcemia following removal of a parathyroid tumor. Production of one or more metabolites of vitamin D is involved in the pathogenesis of some cases of HCM and granulomatous diseases (fungi, parasitic). Ingestion of a topical cream used to treat psoriasis in people, calcipotriene, tacalcitol, or Dovonex is another source of vitamin D that has caused hypervitaminosis D in dogs. Calcipotriene is a synthetic derivative of calcitrol that was used to treat osteoporosis in people and it is not detected by assays for 25-hydroxyvitamin D. Assays for vitamin D are available and include precursors or 1,25 di-hydroxycholecalciferol. Most assays detect 25-hydroxylated forms of vitamin D2 or D3; consult the reference lab for sample handling, reference intervals, and interpretation guidelines.

Granulomatous diseases

Hypercalcemia has been associated with granulomatous diseases caused by a variety of organisms in dogs and cats. Blastomycosis is a fairly well known cause of hypercalcemia, but very few dogs with blastomycosis are hypercalcemic. Blastomycosis may involve bones. However, increased production of vitamin D by macrophages in the granulomas is a more likely mechanism than direct osteolysis from granulomas in bone. Macrophages and some cancer cells can convert vitamin D precursors into calcitrol. Eleven of 22 dogs infected with the flatworm *Heterobilharzia americana* that causes canine schistosomiasis were hypercalcemic. Resolution of hypercalcemia required treatment with praziquantel. Increased serum PTH rp was demonstrated in two dogs with schistosomiasis. Feline infectious peritonitis, tuberculosis, toxoplasmosis, cryptococcosis, and actinomyces are other granulomatous diseases associated with hypercalcemia infrequently in cats. These "causes" should be viewed as "associated with" until studies confirm a mechanism.

Diagnosis is established by ruling out other causes of hypercalcemia, by confirming the etiologic agent by serology and or cytology, and by response to treatment.

Young animals

Young, rapidly growing dogs may have mild asymptomatic hypercalcemia and hyperphosphatemia. The increase is seen more frequently in giant breeds, but may occur in any breed. The total serum calcium is seldom over 12 mg/dL in young dogs and by 6 months of age the total serum calcium should be in the reference intervals for adult dogs. Serum concentrations of calcium and phosphorus that are slightly greater than reference intervals in young growing animals should be interpreted as normal for their age. This pattern is not seen in kittens or foals.

Idiopathic

Idiopathic hypercalcemia is one of the more common causes of hypercalcemia in cats and it may be on the rise. This disorder is unique to cats. It has been suggested that the increased use of acidifying diets for the control of struvite urolithiasis, since the early 1990s, is a contributing factor. Fractional excretion of calcium is increased and urolithiasis (calcium oxalate or struvite) is expected in 50–75% of these cats. Taken together, the data suggests that some susceptible cats develop hypercalcemia, hypercalciuria, and calcium oxalate urolithiasis while on acidifying diets. However, many cats on acidifying diets do not develop this syndrome, or at least it is not recognized. Presence of calcium crystals in the urine should prompt consideration to measure serum calcium. Diagnosis is established by ruling out other causes of hypercalcemia and a test trial of steroids.

Cats with this syndrome are normophosphatemic and hypercalcemic (total and ionized). Treatment with prednisone (5–12 mg/cat/day) seems to reverse the hypercalcemia. Hypercalcemia may be increased for years in some cats with or without clinical signs. FeLV and FIV are not causative. Individuals do not believe it is caused by hyperparathyroidism, because the concentrations of PTH are not increased and because subtotal parathyroidectomy only corrects the hypercalcemia temporarily. However, others could interpret the normal concentration of PTH as inappropriately high for the degree of hypercalcemia. Increased and decreased con-

centrations of PTH rp are reported, but neither PTH rp nor vitamin D is believed to be causative. Another possible undocumented cause could be a congenital or acquired defect in the calcium-regulating receptor.

Xylitol toxicity

This is reported naturally and experimentally in dogs, most of these describe severe hypoglycemia and or liver failure. Hypercalcemia is reported as are increases in liver enzymes, hypokalemia, and hyper- and hypophosphatemia. Xylitol is a sugar substitute found naturally in trees, vegetables, and fruits and is used as a sweetener in sugar free gum, candy, baked goods, desserts, oral care products, and in granulated forms for baking. It is lower in calories than sugar and has low impact on serum glucose, but it stimulates rapid insulin release and causes severe hypoglycemia in dogs. Xylitol's effects on glucose are clinically more important than hypercalcemia.

Bone lesions

Osteolytic bone lesions caused by bone metastases, hypertrophic osteodystrophy, or osteomyelitis have been associated with hypercalcemia, but it is unlikely these are a true cause of hypercalcemia. Or at least the mechanism is more complicated then direct osteolysis by a focal lesion and instead involves one or more humoral components. Any increased release of calcium into the blood from bone resorption around a localized lesion will be normalized by renal excretion. Bone metastases associated with hypercalcemia are more likely due to production of tumor factors that act locally to resorb bone and also stimulate renal calcium reabsorption and phosphorus excretion. Dogs with hypertrophic osteodystrophy may have increased serum calcium. It is likely due to their young age and not the infection in the bones, unless cytokines work locally and in the kidneys. These "causes" of hypercalcemia should be viewed as "associated with" until additional studies confirm a mechanism.

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V

Clinical Chemistry of Common Nondomestic Mammals, Birds, Reptiles, Fish, and Amphibians

34 Chemical Chemistry of Mammals: Laboratory Animals and Miscellaneous Species

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Blood biochemistry profiles are commonly used to assess the health of nondomestic, mammalian patients. Biomedical research involves use of laboratory animals such as mice, rats, and rabbits, resulting in a large amount of information concerning the interpretation of biochemical profiles in these species. Fewer clinical chemistry studies, however, have been performed on other nondomestic mammals, such as ferrets, sugar gliders, and hedgehogs. In general, interpretation of clinical chemistry results in non domestic mammals is the same as that described for domestic species.

Many variables, such as age, gender, hydration, and nutritional status, affect biochemical test results. Environmental conditions such as photoperiod, temperature, and husbandry as well as the sampling and analytic methods and the instrumentation used are other sources of variation. Sampling variables include restraint methods, type of anesthetic used, time of day when sampled, anticoagulant used, site of sample collection, and sample processing and storage. A 16–18-hour fast is required in rats to obtain nonlipemic plasma samples, whereas a 16-hour fast in rabbits results in decreased plasma glucose and insulin concentrations but increased glucagon and fatty acid concentrations.[16,23] Release of epinephrine related to excitement of transportation and blood collection in rabbits results in increased plasma glucose and free fatty acid concentrations. Blood collected by cardiocentesis may be contaminated with muscle enzymes such as creatine kinase (CK), aspartate aminotransferase (AST), lactate dehydrogenase (LD), and alanine aminotransferase (ALT), which are found at high concentrations in cardiac muscle. In rodents, plasma biochemistry results tend to vary in samples obtained from the orbital sinus compared with those obtained by cardiocentesis.[7,9,20] Results of clinical chemistry analyses performed on identical serum or plasma samples often vary significantly among laboratories; thus, published reference values exhibit considerable variation for many analytes (Tables 34.1, 34.3, 34.5, and 34.6).

Sample collection and handling

Blood samples for biochemical studies can be collected using the same techniques as those described for hematologic studies (see Chapter 18, Mammalian Hematology: Laboratory Animals and Miscellaneous Species). Many modern analyzers can perform as many as 20 tests on as little as 50 µL of serum or plasma. Heparinized plasma is routinely used for clinical chemistry evaluations in small rodents such as mice, hamsters, and gerbils; because collection of serum commonly results in hemolysis and a larger sample volume can be obtained with plasma than with serum. The aqueous form of lithium heparin is the preferred anticoagulant for plasma biochemical analysis. As a general guideline, a blood sample volume comprising 10% or less of the total-body blood volume (or 1% of the body weight) can be safely taken from a healthy mammal.

Hemolysis or prolonged contact between serum and blood cells produces changes in the analyte concentrations. Increases in the potassium, phosphorus, LD, and bilirubin concentrations as well as decreases in the glucose concentration may be observed. Serum samples from guinea pigs have greater LD and g-glutamyltransferase (GGT) activity compared with that in plasma samples produced by leakage of these enzymes from erythrocytes during the clotting process.[22,24] In mice, serum CK activity decreases with freezing.[5] Because of the cryoprecipitation of some proteins in serum or plasma samples from rats, protein concentrations may decrease during freezing.[5]

Plasma biochemical analyte reference intervals for common small mammals are provided in Tables 34.1, 34.3, 34.5, and 34.6. Tables 34.2 and 34.4 compare serum or plasma hormone concentrations of rodents and rabbits, respectively.

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Table 34.1 Plasma biochemical values in rodents.

	Mice	Rat	Hamster	Gerbil	Guinea pig	Chinchilla
Glucose [mg/dL]	196–278	114–143	65–144	—	89–95	—
	73–183	74–163	60–160	47–137	60–125	60–120
Urea nitrogen [mg/dL]	21–26	16–19	14–30	—	22–25	—
	18–31	12–22	14–27	17–30	9.0–31.5	10–25
Creatinine [mg/dL]	0.5	0.5–1.4	0.5–0.6	—	1.4	—
	0.48–1.1	0.38–0.8	0.4–1.0	—	0.6–2.2	—
Uric acid [mg/dL]	—	1.3–2.8	1.3–5.1	—	—	—
	—	—	—	—	—	—
Total protein [g/dL]	5.0–7.0	6.4–8.5	1.3–5.1	—	4.8–5.6	—
	5.9–10.3	5.9–7.8	5.5–7.2	4.6–14.7	4.2–6.8	5–6
Albumin [g/dL]	3.0–4.0	4.1–5.4	3.2–4.3	—	2.4–2.7	—
	2.5–4.8	3.3–4.6	2.0–4.2	1.8–5.8	2.1–3.9	2.5–4.2
Calcium [mg/dL]	7.9–10.5	10.5–13.0	10.4–12.4	—	9.6–10.7	—
	4.6–9.6	7.6–12.6	8.4–12.3	3.7–6.1	8.2–12.0	10–15
Phosphorus [mg/dL]	5.6–9.2	5.0–13.0	5.0–8.0	—	5.0	—
	5.2–9.4	5.3–8.4	4.0–8.2	3.7–11.2	3.0–7.6	4–8
Sodium [mEq/L]	138–186	143–150	128–145	—	122–125	—
	143–164	142–150	124–147	143–147	120–152	130–155
Potassium [mEq/L]	5.3–6.3	5.3–7.5	4.7–5.3	—	4.9–5.1	—
	6.3–8.0	4.3–6.3	3.9–6.8	3.6–5.9	3.8–7.9	5.0–6.5
Chloride [mEq/L]	99–108	85–102	94–99	—	92–97	—
	105–118	100–109	92–103	93–118	90–115	105–115
Cholesterol [mg/dL]	—	36–100	94–237	—	—	—
	59–103	44–138	65–148	90–141	16–43	40–100
Total bilirubin [mg/dL]	—	0–0.6	0.2–0.5	—	0–0.9	—
	0.3–0.8	0.2–0.5	0.2–0.7	0.8–1.6	0–0.9	—
Alkaline phosphatase [IU/L]	66–262	70–132	8–202	—	66–74	—
	43–71	40–191	6–14.2	—	55–108	3–12
Alanine aminotransferase [IU/L]	40–189	26–37	28–107	—	39–45	—
	44–87	52–144	22–63	—	25–59	10–35
Aspartate aminotransferase [IU/L]	77–383	40–53	53–202	—	46–48	—
	101–214	54–192	43–134	—	26–68	15–45
Lactate dehydrogenase [IU/L]	—	63–573	94–237	—	—	—
	366	225–275	134–360	—	—	—
Creatine kinase [IU/L]	— 155	6–309	469–1553	—	—	—
	155	111–334	366–776	—	—	—

Top row of data in each column is compiled from the ranges of mean values without consideration of stain, age, gender, and method of blood collection as published in Loeb WF, Quimby FW (eds.) (1989) *The Clinical Chemistry of Laboratory Animals*. New York, Pergamon Press, pp. 417–509. Second row of data in each column is obtained from Quesenberry KE, Carpenter JW (eds.) (2003) *Ferrets, Rabbits, and Rodents Clinical Medicine and Surgery*, 2nd ed. St. Louis, Saunders, pp. 243 and 290.

Rodents

Laboratory evaluation of the kidneys

Laboratory evaluation of rodent kidneys is the same as that for domestic mammals, and it involves evaluation of blood parameters, such as urea nitrogen, creatinine, and electrolytes, and urinalysis. The plasma urea nitrogen is influenced by diet, liver function, gastrointestinal absorption, and hydration. Increases in plasma urea nitrogen and creatinine concentrations only occur when more than 75% of renal function is compromised; therefore, these tests lack sensitivity for renal disease. Common causes of renal azotemia in rodents, especially mice, include amyloidosis, immune complex diseases, and polycystic disease. Serum or plasma urea nitrogen concentrations increase with high protein diets because of increased nitrogen metabolism rather than renal disease. Age should be considered when evaluating

Table 34.2 Plasma concentrations of the major hormones in rodents.

	Rats	Mice	Hamsters	Guinea pigs
Triiodothyronine [ng/dL]	30–100	30–100	30–80	20–60
Free triiodothyronine [ng/dL]	—	—	—	0.20–0.32
Thyroxine [µg/dL]	3–7	3–7	3–7	2–4
Free thyroxine [µg/dL]	—	—	—	0.9–2.0
Thyroid-stimulating hormone [ng/mL]	400–600	300	300	40–100
Adrenocorticotropic hormone [pg/dL]	30–100	2.6–5.5	40[a]	23[a]
Corticosterone [µg/dL]	15–23[d] 1–6[e]	9[a,b] [males] 40[a,b][females] 5[a,c][males] 13.5[a,c][females]	2.75[c][males] 0.33[c][females]	—
Cortisol [µg/dL]	—	—	—	5–30
Free cortisol [µg/dL]	—	—	—	0.6–5.8
Parathormone [pg/mL]	70–700[males] 0–400[females]	—	—	—
Calcitonin [pg/mL]	200–500 [6–8 mo-old males] 450–1100[6–8 mo-old females] 400–900 [12–14 mo-old males] 700–1800[12–14 mo-old females]			
1,25-dihydroxy-vitamine D [pg/mL]	72–86[males] 79–113[females]			

[a]Average concentration.
[b]Start of dark period.
[c]End of dark period.
[d]Mean maximum value.
[e]Mean minimum value.

plasma urea nitrogen in rodents; aged hamsters demonstrate increased plasma urea nitrogen concentrations. Other laboratory abnormalities that may be associated with renal disease are hyperphosphatemia, resulting from decreased glomerular filtration, and hypoproteinemia, resulting from glomerular disease and urinary protein loss.

γ-Glutamyltransferase, N-acetyl-β-D-glucosaminidase, and alkaline phosphatase (AP) have high tissue activity in the kidney, and measurement of these enzymes in urine may improve the sensitivity of clinical chemical testing for renal disease in rodents. Testing of endogenous creatinine clearance may provide a specific and sensitive test for decreased glomerular filtration before plasma urea nitrogen and creatinine concentrations are increased.

Urine may contain artifacts if proper attention is not paid to the collection technique. The urine should be collected on a clean, dry surface. Without use of commercially available metabolism cages, urine commonly is contaminated with feces, food, hair, bedding, or drinking water. Rodents often spontaneously urinate when handled, thereby providing a clean sample for those who are prepared to collect this urine. Cystocentesis eliminates much of the artifact associated with voided urine but may result in blood contamination. Urinalysis should be performed within 2 hours of collection; otherwise, urine may be refrigerated at 4°C for as long as 48 hours. Refrigerated urine should be warmed to room temperature before testing.

The urine of normal rodents usually is yellow, but it may vary in both shade and transparency depending on the hydration status of the animal. Urinary pH is influenced by diet. Diets that are high in animal proteins contain high concentrations of sulfates and phosphate precursors, which produce more acid urine; cereal protein–based diets tend to produce a neutral to slightly alkaline urine. Rodents tend to have alkaline urine because of the bacterial conversion of urea to ammonia. The urine pH is helpful in determining the acid-base status of the animal. Rodents suffering from catabolic conditions such as starvation, ketosis, or fever commonly have acidic urine.

Table 34.3 Plasma biochemical values in rabbits.

	a	b
Glucose [mg/dL]	89–144	75–155
Urea nitrogen [mg/dL]	14–23	13–29
Creatinine [mg/dL]	0.8–2.9	0.5–2.5
Uric acid [mg/dL]	1.1–1.2	—
Total protein [g/dL]	5.0–8.5	5.4–8.3
Albumin [g/dL]	3.0–3.4	2.4–4.6
Calcium [mg/dL]	13.0–15.0	5.6–12.5
Phosphorus [mg/dL]	5.6–9.2	4.0–6.9
Sodium [mEq/L]	114–156	131–155
Potassium [mEq/L]	4.4–7.4	3.6–6.9
Chloride [mEq/L]	89–120	92–112
Cholesterol [mg/dL]	22–69	10–80
Total bilirubin [mg/dL]	0–0.7	0–0.7
Alkaline phosphatase [IU/L]	<120	4–16
Alanine aminotransferase [IU/L]	<100	48–80
Aspartate aminotransferase [IU/L]	<100	14–113
Lactate dehydrogenase [IU/L]	<200	34–129
Creatine kinase [IU/L]	<275	—

[a]Data compiled from the ranges of mean values without consideration of stain, age, gender, and method of blood collection as published in Loeb WF, Quimby FW (eds.) (1989) *The Clinical Chemistry of Laboratory Animals*. New York, Pergamon Press, pp. 417–509.
[b]Data from Quesenberry KE, Carpenter JW (eds.) (2003) *Ferrets, Rabbits, and Rodents Clinical Medicine and Surgery*, 2nd ed. St. Louis, Saunders, p. 151.

Table 34.4 Plasma concentrations of the major hormones in rabbits.

Triiodothyronine [ng/dL]	130–143
Thyroxine [μg/dL]	1.7–2.4
Thyroid-stimulating hormone [μU/mL]	40–100
Protein-bound iodine [nmol/L]	400 [Adults]
Adrenocorticotropic hormone [pg/dL]	25[a]
Cortisol [μg/dL]	2.6–3.8[early morning]
Aldosterone [ng/dL]	20[a] [early morning] 50[a] [late afternoon]
Calcitonin [pg/mL]	1125–1200
1,25-dihydroxy-vitamine D [pg/mL]	27–47

[a]Average concentration.

Table 34.5 Plasma biochemical values in ferrets.

	All ferrets[a]	Albino[b]	Fitch[b]
Glucose [mg/dL]	67–124	94–207	63–134
Urea nitrogen [mg/dL]	17–32	10–45	12–43
Creatinine [mg/dL]	0.2–0.6	0.4–0.9	0.2–0.6
Total protein [g/dL]	5.3–7.2	5.1–7.4	5.3–7.2
Albumin [g/dL]	3.3–4.1	2.6–3.8	3.3–4.1
Calcium [mg/dL]	8.5–11	8.0–11.8	8.6–10.5
Phosphorus [mg/dL]	3.3–7.8	4.0–9.1	5.6–8.7
Sodium [mEq/L]	146–160	137–162	146–160
Potassium [mEq/L]	3.7–5.4	4.5–7.7	4.3–5.3
Chloride [mEq/L]	112–129	106–125	102–121
Cholesterol [mg/dL]	60–220	64–296	119–209
Total bilirubin [mg/dL]	0.0–0.3	<1.0	0–0.1
Total Co_2 [mmol/L]	17–23	16.5–28	16–28
Alkaline phosphatase [IU/L]	30–120	9–84	30–120
Alanine aminotransferase [IU/L]	30–100	82–287	78–149
Aspartate aminotransferase [IU/L]	15–40	28–120	57–248
Creatine kinase [IU/L]	60–300	—	—

[a]Data from Thrall MA, *et al.* (eds.) (2004) *Veterinary Hematology and Clinical Chemistry*. Philadelphia, Lippincott Williams & Wilkins, p. 471.
[b]Data from Quesenberry KE, Carpenter JW (eds.) (2003) Ferrets, Rabbits, and Rodents Clinical Medicine and Surgery, 2nd ed. St. Louis, Saunders, p. 20.

Urine specific gravity and osmolality are used to evaluate the ability of the kidneys to concentrate or dilute urine. A water-deprivation test for detecting renal disease in rodents can be conducted by withholding water for 24 hours, after which the urinary specific gravity is determined. Those animals that are unable to concentrate their urine to a specific gravity greater than 1.030 either have significant renal disease with the inability to concentrate their urine or suffer from diabetes insipidus. The urine specific gravity value obtained from a refractometer will be erroneous if the urine contains significant quantities of glucose, protein, or other metabolites that normally are not found in urine. Urine osmolality is the definitive method for measuring the concentrating ability of the kidneys; it depends on the number of particles in solution and is not affected by the degree of ionization or the mass of molecules and ions that are present. The normal urine osmolality of rats and hamsters ranges from 331 to 445 and 307 to 355 mOsm/Kg, respectively.[1,6,11]

The urine of normal rodents may contain a trace amount of glucose. Large amounts of ascorbic acid normally are found in mouse urine and may interfere with urine chemical

Table 34.6 Plasma biochemical values in sugar gliders and hedgehogs.

	Sugar gliders	Hedgehogs
Glucose [mg/dL]	130–183	89+/– 30
Urea nitrogen [mg/dL]	18–24	13–54
Creatinine [mg/dL]	0.3–0.5	0–0.8
Total protein [g/dL]	5.1–6.1	4.0–7.7
Albumin [g/dL]	3.5–4.3	1.8–4.2
Calcium [mg/dL]	6.9–8.4	5.2–11.3
Phosphorus [mg/dL]	3.8–4.4	2.4–12.0
Sodium [mEq/L]	135–145	120–165
Potassium [mEq/L]	3.3–5.9	3.2–7.2
Chloride [mEq/L]	—	92–128
Cholesterol [mg/dL]	—	86–189
Total bilirubin [mg/dL]	0.4–0.8	0–1.3
Alkaline phosphatase [IU/L]	—	8–92
Alanine aminotransferase [IU/L]	50–106	16–134
Aspartate aminotransferase [IU/L]	46–179	8–137
Lactate dehydrogenase [IU/L]	—	57–820
Creatine kinase [IU/L]	210–589	333–1964
γ-Glutamyl transferase [IU/L]	—	0–12

Data from Quesenberry KE, Carpenter JW (eds.) (2003) *Ferrets, Rabbits, and Rodents Clinical Medicine and Surgery*, 2nd ed. St. Louis, Saunders, pp. 335 and 345.

strips that use glucose oxidase, thus resulting in a false-negative glucose determination.

Proteinuria is common in normal mice and rats. The semi quantitative urine chemical strips detect large-molecular-weight proteins such as albumin, but not the low-molecular-weight glycoproteins of renal origin that are found in the urine of rodents. The normal proteinuria of rodents is associated with a variety of urinary proteins, which include a- and b-globulins, uromucoid protein, and prealbumin. The degree of proteinuria increases with age, and male mice tend to be more proteinuric than female mice.

Rodent urine sediment normally contains fewer than five erythrocytes and leukocytes per high-power field. Increases in the concentration of these cells are suggestive of urinary tract inflammation, calculi, or neoplasia. If urinary casts containing erythrocytes and leukocytes are concurrently present, cells are likely of renal origin, whereas increased numbers of cells without casts are suggestive of lower urinary tract inflammation, such as cystitis and urethritis. Interpretation of rodent urine sediment findings is the same as that described for domestic mammals.

Electrolytes and acid-base

Interpretation of serum or plasma electrolyte and acid-base changes in rodents is the same as that described in domestic mammals. Normal serum and plasma sodium concentrations in mice (174 ± 23 mEq/L or mmol/L) tend to be slightly greater than those reported for other mammals. Hypernatremia resulting from neurogenic diabetes insipidus occurs as a hereditary disorder in some strains of rat. Nephrogenic diabetes insipidus, which usually is associated with renal amyloidosis, frequently occurs in certain strains of mice and aged Syrian hamsters. Chronic nephropathies causing abnormal retention of sodium in rats may cause hypernatremia, which in turn results in myocarditis. Renal amyloidosis alters the renal tubular permeability to water, thereby resulting in hyperchloremia. Increased serum and plasma phosphorus concentrations occur in younger rodents compared with concentrations in adults. Serum or plasma magnesium concentrations increase in hamsters during hibernation.

Laboratory evaluation of the liver

Serum or plasma enzymes commonly used to detect liver disease in rodents include AP, GGT, AST, ALT, LD, and sorbitol dehydrogenase. Serum or plasma concentrations of these enzymes increase with increased production, increased release, or decreased clearance. Other biochemical tests to detect liver disease in rodents include serum or plasma total bilirubin, bile acid, and cholesterol concentrations.

Alkaline phosphatase is a membrane-bound enzyme with highest activity in osteoblasts, biliary epithelium, and epithelial cells of the kidneys and intestines. Young rodents have higher plasma AP activity than adults because of osteoblastic activity, and male rats tend to have higher plasma AP activities than female rats. Hepatic AP of rodents is heat labile at 56°C and sensitive to levamisole inhibition.[9] Significant increases in serum or plasma AP activity occur in rodents with hepatic cholestasis. Ligation of the bile duct in rats produces elevation of both hepatic and intestinal AP isoenzymes. Plasma or serum AP activity is a more sensitive test than bilirubin or ALT for detection of hepatic disease in hamsters.[11,12] Drugs that increase AP synthesis and plasma activity in rats include cortisol, phenobarbital, and theophylline.[19] Increased plasma AP activity occurs in zinc- and manganese-deficient guinea pigs.[24]

Plasma GGT activity is significantly increased in hamsters and rats with experimentally induced hepatic injury resulting in cholestasis. Guinea pigs have higher hepatic GGT activity than rats and demonstrate higher plasma GGT activities with cholestasis. Serum GGT activity is increased in guinea pigs after in vitro blood clot formation, which can be avoided with use of plasma for enzyme testing. The kidneys of rodents have the highest GGT activity, but the enzyme is nondetectable in the plasma or serum of most rodents. The kidneys of rats have 200–300-fold the GGT activity of the liver.

Aspartate aminotransferase is a mitochondrial and cytosolic enzyme with high activity in the liver, heart, skeletal

muscle, and kidney and low activity in the intestines, brain, lung, and testes. Increases in plasma or serum AST activity usually are associated with hepatic, cardiac muscle, or skeletal muscle injury.

In rats and mice, the activity of ALT, which is a cytosolic and mitochondrial isoenzyme, is highest in the liver. The ratio of the cytosolic to mitochondrial ALT isoenzymes in the liver and heart muscle of rats is 5:1 and 50:1, respectively. In rodents, the intestines, kidneys, heart, skeletal muscle, brain, skin, and pancreas also have ALT activity. In guinea pigs, ALT activity in the heart is almost equal to that in the liver. Plasma and serum ALT activity increases with hepatocellular damage in most rodents, and the enzyme appears to be liver specific in rats and mice. Plasma ALT, however, does not appear to have diagnostic value for hepatic disease in guinea pigs, which have only half the hepatic ALT activity of rats and mice. Increases in serum ALT activity correlate with the degree of hepatic necrosis in rats. A threefold increase in plasma ALT activity occurs in mice that are restrained by holding the body compared with those that are restrained by the tail.

Lactate dehydrogenase is a cytosolic enzyme with the highest activity in skeletal muscle, followed by cardiac muscle, liver, kidney, and intestines, respectively. In the mouse, LD is characterized by five isoenzymes: LD-1 and LD-2 are found in cardiac muscle, LD-5 in the liver and skeletal muscle, and LD-3 in most other tissues. Serum or plasma LD activity elevates with hepatocellular disease in rodents; however, normal values are highly variable and depend on the analytic method used.

Sorbitol dehydrogenase is a cytosolic enzyme that is found in the liver, kidney, and seminal vesicles of mice but is liver specific in rats. Increases in serum or plasma sorbitol dehydrogenase activity occurs with hepatic disease in rodents and is a more sensitive test than ALT for detection of hepatocellular disease in rats. Sorbitol dehydrogenase assays usually are not performed by veterinary laboratories.

Serum and plasma total bilirubin concentration increases in rodents with primary hepatobiliary disease, extra hepatic biliary obstruction, or hemolysis. Increases in plasma or serum total bilirubin concentration should be evaluated by determining the erythrocyte mass and performing other tests that evaluate the liver or biliary system.

The total serum and plasma bile acid concentration is a sensitive and specific test for hepatobiliary disease and disorders of the enterohepatic circulation. Plasma bile acid concentration has an excellent potential for detecting hepatobiliary disease in rodents, especially rats with a high concentration of circulating bile acids.

The plasma cholesterol concentration may increase in rodents with extrahepatic biliary obstruction. Normal plasma cholesterol concentration varies between strains of mice. Hypercholesterolemia often is associated with fatty infiltration of many tissues. In guinea pigs, the intestine, rather than the liver, is the primary site of cholesterol production.[22] Normal plasma cholesterol concentration (112–210 mg/dL or 2.90–5.43 mmol/L) of hamsters is higher than that of other rodents and decreases during short photoperiods but increases with cold temperatures.[6,11]

Laboratory evaluation of proteins

The normal plasma protein concentration in mice varies among strains. In mice, hyperproteinemia often is associated with severe dehydration and often occurs with loss of urinary protein from renal disease. The major classes of serum or plasma proteins in rodents are evaluated using electrophoresis. The major globulins of rats are α_1- and β-globulins, with lower concentrations of α_2- and γ-globulins. In hamsters, albumin concentrations decrease during the first year of life, α_2-globulins increase during the first 6 months of age, and β-globulins decrease at 8 weeks of age.[3,6,11] Fibrinogen migrates into the γ-globulin peaks in hamster protein electrophoretic scans. Amyloidosis is a common disease of hamsters older than 18 months and results in hypoalbuminemia and hyperglobulinemia.

Laboratory evaluation of glucose metabolism

Cells must be quickly separated from the serum or plasma of rodents, or fluoride added to the collection tube, to prevent decreased glucose concentration because of in vitro glycolysis. The plasma glucose concentration in rats and mice decreases with age, with an average decrease of 2 mg/dL per month in the latter.

Many strains of mice are used as animal models for diabetes mellitus; therefore, glucose tolerance tests have been developed for mice.[2] A 1-hour glucose tolerance test compares the preinjection plasma glucose concentration to the glucose concentration obtained 1 hour after an intra peritoneal injection of glucose at a dose of 2 mg/g body weight. A 4-hour oral glucose tolerance test compares the baseline plasma glucose concentration with a plasma glucose concentration obtained 4 hours after the oral administration of a 10% glucose solution at a dose of 10 mL/kg. Certain strains of rodents, such as ob/ob obese mice, Zucker fatty rat (fa/fa), and the LA/N corpulent rat, are used as animal models for noninsulin-dependent diabetes mellitus. The Chinese hamster and Wistar BB rat are animal models for insulin-dependent diabetes mellitus. Insulin-dependent diabetes may result in guinea pigs from an infectious agent that causes fatty degeneration of the pancreas and affects both exocrine and endocrine pancreatic functions; affected guinea pigs have hyperglycemia, glucosuria, ketonuria, and beta-cell hypoplasia. Immunoassays for the determination of insulin in rats can be calibrated to measure plasma insulin in mice, but guinea pig insulin is immunologically different and cannot be determined using rat antibodies. Rat glucagon is measured using human immunoassay techniques; however, guinea pig glucagon, like insulin, is immunologi-

cally different and cannot be determined with human antibodies.

Laboratory detection of muscle injury

Creatine kinase is a dimeric cytosolic enzyme that is composed of M and B subunits. Skeletal muscle contains MM subunits, and cardiac muscle contains MM, MB, and BB subunits. Brain contains BB subunits. As in domestic mammals, plasma CK activity is a useful marker for muscle injury in rodents. Nutritional myopathies, such as those resulting from hypovitaminosis E and selenium deficiency, cause increased plasma CK activity in rats and mice.

Laboratory evaluation of endocrine disorders

The major hormones of rodents are secreted into the peripheral blood in a circadian rhythm that may vary among species. Hormonal secretion also is influenced by environmental factors, such as light-dark cycle. An ultradian rhythm, in which hormones are secreted in an episodic or pulsatile manner with a periodicity of less than 24 hours, can be superimposed on the normal circadian secretion of a hormone. Suggested ranges for the major plasma hormones in rodents are provided in Table 34.2.

Normal male rats have higher plasma thyroid-stimulating hormone (TSH) concentrations with use of reference preparation-1 standard from the National Hormone and Pituitary Program compared with normal female rats. Plasma TSH concentrations of normal female rats peak at the onset of the light cycle. Mice and hamsters have lower normal plasma TSH concentrations compared with rats (according to the same assay method used for rats). A bioassay method using radio-labeled iodine also can be used to obtain plasma TSH concentrations in rodents.

Plasma or serum thyroxine (T_4) and triiodothyronine (T_3) concentrations in rodents can be measured by radio immunoassay. Transport proteins and binding affinity for T_4 and T_3 vary among species. In rats and mice, approximately 80% of the bound T_3 and T_4 are bound to albumin and 20% to T_4-binding prealbumin. Approximately 0.05% of plasma T_4 and 0.25% of T_3 in rats is the free, physiologically active form. Normal plasma total T_4 and T_3 concentrations in rats and mice vary between different strains but generally range between 3 and 7 µg/dL and 30 and 100 ng/dL, respectively. Plasma T_4 and T_3 concentrations exhibit a diurnal rhythm, in which peak concentrations occur during the light phase and minimum concentrations during the dark phase.

Normal plasma adrenocorticotropic hormone (ACTH) concentrations in rodents have been determined using either radioimmunoassay or bioassay techniques. The plasma ACTH concentration in normal mice exhibits a normal circadian rhythm, in which minimal concentrations occur during the morning and peak concentrations during the afternoon.

Corticosterone, which is the primary glucocorticoid in the plasma of mice and rats, exhibits a marked diurnal variation that is affected by the light cycle. In mice, maximum plasma corticosterone concentrations occur at the start of the dark period and minimum concentrations at the end of the dark period. Male mice have lower plasma corticosterone concentrations compared with female mice. Maximum plasma corticosterone concentrations occur late during the light period in rats, and minimum concentrations occur during the end of the dark period. In rats, approximately 80% of plasma corticosterone is bound to transcortin and 10% to albumin, thereby leaving 10% or less in the free, unbound state. Both corticosterone and cortisol are found in the plasma of normal hamsters. The total plasma glucocorticoid concentration 5.5 hours after onset of the light period in hamsters averages 1.8 µg/dL, with an average corticosterone : cortisol ratio of 3.5. The plasma corticosterone concentration is greater in male hamsters than in female hamsters. Cortisol is the primary glucocorticoid in the plasma of normal guinea pigs. Guinea pigs demonstrate maximum plasma cortisol concentrations late in the light period and, again, late in the dark period. Minimum concentrations occur early during the light period and, again, during the middle of the dark period. The stress of restraint or removal of a cagemate significantly increases plasma glucocorticoid concentrations. A twofold increase in plasma corticosterone concentration occurs in rats with 2 minutes of restraint, and a 12-fold increase results after 20 minutes of restraint.

In rodents, plasma concentrations of the calcium-regulating hormones parathormone, calcitonin, and 1,25-dihydroxyvitamin D_3 are influenced by dietary calcium, age, gender, photoperiod, and strain. Using radio immunoassay techniques, the normal plasma parathormone concentration in male rats tends to be greater than that in female rats.

Normal plasma calcitonin concentrations of rats are extremely variable because of age, stage of light cycle, strain, and gender. Plasma calcitonin concentrations also are influenced by the stage of estrus in females, in which maximum concentrations occur during proestrus. Six- to eight-month-old male Wistar rats have lower plasma calcitonin concentrations compared with 12–14-month-old Wistar rats. Male Wistar rats also have lower plasma calcitonin concentrations than female rats. Plasma concentrations of 1,25-dihydroxyvitamin D vary in rats with strain, gender, and dietary calcium intake. Normal male Wistar rats have lower plasma 1,25-dihydroxyvitamin D concentrations than females.

Rabbits (*Oryctolagus cuniculus*)

Laboratory evaluation of the kidneys

Laboratory evaluation of the kidneys in rabbits is the same as that for rodents and domestic mammals. Plasma urea

nitrogen and creatinine commonly are used as markers for renal function in rabbits. The normal plasma urea nitrogen of rabbits is influenced by breed, strain, and gender. Protein catabolism associated with high dietary protein intake, vigorous exercise, or disease increases plasma urea nitrogen concentration. The time of day when the blood sample is taken also influences the plasma urea nitrogen concentration in rabbits, in which peak concentrations occur between 4.00 and 8.00 pm. Plasma urea nitrogen and creatinine are insensitive tests for renal disease in rabbits, however, requiring a 50–75% loss of function before plasma concentrations increase. Renal failure in rabbits is often associated with increased plasma BUN, creatinine, calcium, phosphorus, and potassium concentrations. Renal failure rabbits may also exhibit isosthenuria and depending upon the cause (i.e. nephritis) may exhibit proteinuria, ketonuria, pyuria, and urinary cast formation.

Electrolytes and acid-base

The normal plasma calcium concentration of 13–15 mg/dL (3.24–3.74 mmol/L) of rabbits is higher than that of most other mammals. The mean urinary fractional calcium excretion of rabbits is approximately 45%, compared with less than 2% in other mammals. Normal plasma electrolyte concentrations of rabbits vary with the breed and strain. Normal plasma magnesium concentrations of most rabbits are between 2.0 and 4.5 mg/dL. The serum iron- and total iron-binding capacity of normal rabbits vary with the time of day when the blood was collected, with the lowest concentrations occurring at 8:00 AM and the highest at 8:00 PM. Serum iron concentrations of normal rabbits range between 165 and 250 µg/dL (29.6–44.8 µmol/L).[16]

Laboratory evaluation of the liver

Plasma enzymes used to detect liver disease in rabbits include ALT, AST, LD, glutamate dehydrogenase, AP, and GGT. In rabbits, ALT activity is equal in the liver and cardiac muscle; however, increased plasma ALT activity is considered to be a specific indicator of liver disease in rabbits. The degree of hepatic necrosis correlates positively with the increase in plasma ALT activity. Interestingly, the rabbit liver ALT activity is less than half that of the dog. Significant AST activity occurs in the liver, heart, skeletal muscle, kidney, and pancreas of rabbits. Therefore, increases in plasma AST activity are suggestive of injury to one or more of these tissues. Increases in plasma AST activity may be associated with cardiac or skeletal muscle injury during blood collection by cardiocentesis or use of restraint methods that cause exertion. Lactate dehydrogenase activity is present in a wide variety of tissues, with each demonstrating a different isoenzyme composition that corresponds with isoenzymes 1 through 5 in humans. Isoenzyme LD-1 and LD-2 predominate in the liver and skeletal muscle. Because erythrocytes have high LD activity, hemolysis may result in high plasma LD activity. The plasma LD activity can be used to detect liver disease in rabbits, but because of its wide tissue distribution and the effect of handling and hemolysis on plasma activity, it is not commonly used. Plasma glutamate dehydrogenase activity (range, 5.5–7.0 IU/L, mmol/L), although not commonly measured in veterinary laboratories, may be useful in the evaluation of hepatocellular injury in rabbits. Normal plasma AP activity of rabbits varies with age, breed, and strain.

Rabbits are unique in having three AP isoenzymes. Rabbits have an intestinal and two liver/kidney forms, compared with the intestinal and liver/kidney/bone forms found in mammals other than primates. The predominant liver/kidney isoenzyme of rabbits is similar to the intestinal form and the minor liver/kidney isoenzyme to the liver/kidney/bone isoenzyme of other mammals. The predominant liver AP isoenzyme is not inhibited by levamisole or heating to 56°F, as the hepatic AP isoenzyme of other mammals is. The plasma GGT activity of normal rabbits is less than 8 IU/L (mmol/L), is derived primarily from bile duct epithelial cells, and increases significantly in rabbits with hepatobiliary obstruction.

Rabbit bile contains approximately 70% biliverdin and 30% bilirubin, of which 90% is conjugated as a mono conjugate. Normal rabbit plasma lacks biliverdin, however, and the normal bilirubin concentration is low. A marked increase in plasma bilirubin concentration is expected in rabbits with biliary obstruction.

The normal plasma cholesterol concentration of rabbits varies with age, breed, strain, and gender. At birth, the plasma cholesterol concentration is approximately that of adults, increases by 25 days of age, and then returns to the adult concentrations by 60–80 days of age. Normal adult male rabbits have twice the plasma cholesterol concentration of adult female rabbits. A diurnal variation in plasma cholesterol occurs as well, with peak concentrations being seen between 4:00 and 8:00 PM. The plasma cholesterol concentration may increase in rabbits with extrahepatic biliary obstruction. Rabbits are used extensively as animal models for cholesterol metabolism studies because of their ability to rapidly develop cholesterolemia with high-cholesterol diets. Daily feeding of 1 g of cholesterol increases the serum cholesterol concentration to greater than 1000 mg/dL. The Watanabe heritable hyperlipemic rabbit, which primarily exhibits low-density lipoprotein (LDL) cholesterol, is an animal model for familial hypercholesterolemia in humans. The normal serum lipoprotein distributions of adult female rabbits are 46–58% high-density lipoprotein, which transports approximately two-thirds of the total cholesterol; 9–15% pre- or intermediate-density lipoprotein or very LDL (VLDL); and 30–42% LDL. High-cholesterol diets fed to rabbits lead to a 20–40-fold increase in VLDL and a 4–5-fold increase in LDL.

Liver function tests that evaluate plasma disappearance and biliary excretion of dyes, such as sulfobromophthalein (BSP) and indocyanine green (ICG), have been characterized for rabbits. The overall rate of BSP clearance for rabbits has been reported as 1.8 mg/min per kg, in which 75 % of the BSP is excreted in the conjugated form. Intravenous BSP dosages of 30, 60, and 120 mg/kg result in 32-minute plasma concentrations of 1, 2, and 20 mg/dL, respectively.[14] Indocyanine green is excreted in the bile in the unconjugated form. Rabbits have a curvilinear plasma ICG clearance curve, with a greater capacity to remove ICG from the circulation than either dogs or rats. Rabbits that are given intravenous ICG dosages of 8, 16, and 32 mg/kg demonstrate disappearance rates of 46%, 20%, and 10% per minute, respectively.[16]

Laboratory evaluation of proteins

The normal plasma total protein concentration in rabbits varies slightly with breed, strain, and gender. Approximately 40–60% of the total plasma protein is albumin. The normal protein electrophoretic components of rabbit serum also include 5–10% α_1-globulin, 5–10% α_2-globulin, 5–15% β-globulin, and 5–15% γ-globulin. The normal albumin to globulin ratio ranges between 0.5 and 1.2. Female rabbits tend to have higher plasma albumin concentrations than male rabbits. Severe renal and hepatic diseases are responsible for most disorders that result in hypoproteinemia and hypoalbuminemia in rabbits. Hyperproteinemia commonly occurs with dehydration, shock, and hyperthermia.

Laboratory evaluation of glucose metabolism

The normal plasma glucose concentration of rabbits is influenced by genetics, age, and diet. Pre- and postprandial plasma glucose variation occurs, in which the lowest plasma glucose concentrations are found 1 hour before feeding and the highest 3 hours after a meal. Healthy rabbits can maintain normal plasma glucose concentrations during short periods of fasting (e.g., <16 hours). Extreme hyperglycemia occurs with diabetes mellitus. Hyperglycemia and increased plasma urea nitrogen concentration occur with the increased protein catabolism associated with hyperthermia. Hyperglycemia resulting from glycogenolysis because of stress occurs early in the course of mucoid enteropathy. This common digestive tract disorder of the rabbit causes anorexia, and when glycogen stores become depleted, the rabbit develops hypoglycemia.

Laboratory detection of muscle injury

Laboratory detection of muscle injury in rabbits follows the same methods as that in rodents and domestic mammals, in which plasma CK, AST, and LD activities are sensitive to muscle injury. Blood collected by cardiocentesis contains CK-MB isoenzyme activity that is not found in serum collected from the ear vein. Blood collected by jugular venipuncture also contains CK-MB activity. Plasma CK activity, primarily the CK-MM isoenzyme, is a rapid, sensitive, and specific indicator of muscle disease in rabbits, and it increases more rapidly than AST and LD activities after muscle injury. Nutritional-related myopathies, such as those caused by hypovitaminosis E and selenium deficiency, result in increased plasma CK activity.

Laboratory evaluation of endocrine disorders

Laboratory evaluation of endocrine disorders in rabbits follows the same methods as that in rodents and domestic mammals. The TSH concentration in rabbit serum can be obtained using a bioassay method that measures the percentage increase in blood levels of radiolabeled iodine. Serum T_4 and T_3 concentrations from normal rabbits are listed in Table 34.4. The serum protein-bound iodine concentration, as an indicator of thyroid function in rabbits, increases by 20 days of age before decreasing to adult concentrations by 60 days of age. The serum protein-bound iodine concentration varies with strain, gender, and time of day. The plasma ACTH concentration of rabbits as determined by bioassay is subject to circadian variation. The major plasma glucocorticoid of rabbits is cortisol. Evidence is suggestive that genetics and circadian rhythms influence the plasma aldosterone concentration of rabbits, but little information is available regarding the plasma concentration of parathyroid hormone in rabbits.

Ferrets (*Mustelus putorius furo*)

Laboratory evaluation of the kidneys

Laboratory evaluation of renal function in ferrets involves blood biochemical tests, such as plasma urea nitrogen, creatinine, protein, bicarbonate, and electrolyte concentrations, and urinalysis. Interpretive considerations for the biochemical tests used to evaluate the kidneys are the same as those in domestic carnivorous mammals such as cats and dogs. In normal and azotemic ferrets, the plasma creatinine concentration is lower than that in dogs and cats. The mean plasma creatine concentration of healthy ferrets is 0.4–0.6 mg/dL (35.4–53.0 µmol/L) with a range of 0.2–0.9 mg/dL (17.7–79.6 µmol/L).[4] As a result, a moderate increase in the plasma creatinine concentration (i.e. 1–2 mg/dL or 88.4–176.8 µmol/L) in a ferret is significant and suggestive of renal disease.[13] Insulin and exogenous creatinine clearance are sensitive tests for measuring glomerular filtration in ferrets; however, delayed clearance may occur before significant increases in plasma urea nitrogen or creatinine concentrations.

Electrolytes and acid-base

Interpretations of plasma electrolyte and acid-base disturbances in ferrets are the same as those in dogs and cats.

Disorders that commonly result in electrolyte disturbances in dogs and cats, such as hypoadrenocorticism, hyperaldosteronism, primary hyperparathyroidism, pseudohyperparathyroidism, hypoparathyroidism, and hypercalcitonism, have been poorly documented in ferrets.

Laboratory evaluation of the liver

Evaluation of the livers in ferrets by laboratory testing is the same as that for those in dogs and cats. The ferret liver has 3–10-fold more ALT activity than any other tissue, and the plasma ALT activity is a sensitive and specific test for hepatocellular disease in ferrets. Ferrets with hepatocellular disease commonly have increased AST and LD activities as well. Those with cholestasis likely have increased plasma AP and GGT activities. Ferrets rarely become icteric or have plasma bilirubin concentrations greater than 2.0 mg/dL, even when hepatobiliary disease is severe.[8,13]

Laboratory evaluation of proteins

The causes of hypoproteinemia and hyperproteinemia in ferrets are the same as those in dogs and cats. Ferrets with Aleutian disease typically demonstrate hypoalbuminemia and hyperglobulinemia, in which more than 20% of the total protein is γ-globulins.[13]

Laboratory evaluation of glucose metabolism

A high incidence of insulin-secreting pancreatic neoplasms (i.e., insulinomas) resulting in hypoglycemia occurs among domestic ferrets in North America. The normal plasma glucose concentration of ferrets varies with the genetic type. A 4–5-hour fasting plasma glucose level often is used to screen ferrets for insulinomas. Fasting plasma glucose concentrations less than 60 mg/dL (3.33mmol/L) are supportive of a presumptive diagnosis of insulinoma, whereas concentrations between 60 and 90 mg/dL (3.33–5.0 mmol/L) merely are suggestive of an insulinoma. Concentrations greater than 90 mg/dL (5.0 mmol/L) usually are considered to be normal. Normal serum reference intervals for serum immunoreactive insulin and the insulin:glucose ratio have been reported as being 4.6–43.3 μU/mL (SI units, 33–311 pmol/L) and 3.6–34.1 μUmg (SI units, 4.6–44.2 pmol/mmol), respectively.[19] To compare immunoreactive insulin and the insulin:glucose ratio results from other laboratories using different radioimmunoassay kits to these reference intervals, however, one must validate the results by demonstrating a high correlation between the two assay methods. Although rarely utilized because measurement of a fasting plasma glucose concentration provides the most reliable method for establishing a strong presumptive diagnosis for insulinoma in the ferret, calculation of an amended insulin:glucose ratio (AIGR) may aid in establishing the diagnosis of hyperinsulinism where

$$\text{AIGR} = \text{insulin}\ (\mu\text{U/mL}) \times 100/\text{fasting glucose}\ (\text{mg/dL}) - 30$$

in which AIGR values greater than 30 are suggestive of hyperinsulinism.[15] Other occasional causes for hypoglycemia in ferrets include delayed separation of plasma from erythrocytes, starvation, chronic hepatic disease, septicemia, and endotoxemia.

Other than a postprandial increase in plasma glucose concentration, hyperglycemia in ferrets may result from glucocorticoid excess (e.g., stress induced, exogenous corticoids, and hyperadrenocorticism), epinephrine release related to exertion, and diabetes mellitus. Diabetes mellitus in ferrets usually is iatrogenic and associated with surgical removal of pancreatic insulin-secreting neoplasms or with drugs, such as megestrol acetate, that affect insulin production and secretion.

Laboratory detection of muscle injury

Detection of muscle injury in ferrets follows the same methods as those described in dogs and cats. Increases in the nonspecific plasma enzymes AST and LD and in the specific muscle enzyme CK are to be expected with muscle injury.

Laboratory evaluation of endocrine disorders

The mean basal plasma T_4 concentration as reported for ferrets ranges between 0.99 and 2.63 μg/dL (12.7–33.8 nmol/L).[10] A thyroid function test using 1 IU of TSH given intravenously to a ferret and measuring changes in the plasma T_4 concentration is preferable to use of thyrotropin-releasing hormone and measuring changes in the plasma T_3 concentration. The plasma T_4 concentration increases significantly as early as 2 hours after TSH stimulation in normal ferrets, whereas no increase in the plasma T_3 concentration is observed. Plasma T_4 concentration should at least double after TSH stimulation; failure to do so is suggestive of hypothyroidism.

Cortisol is the predominant circulating glucocorticoid in ferrets. The mean basal plasma cortisol concentration as reported for ferrets ranges between 0.45 and 2.13 μg/dL (12.4–58.8 nmol/L).[18] Intravenous or intramuscular injection of ACTH at a dose of 0.5–1.0 mg/kg to a normal ferret results in a 3–4-fold increase in plasma cortisol by 30 minutes that persists for as long as 1 hour. A threefold decrease in plasma cortisol concentration occurs in normal ferrets after intravenous injection of 0.2 mg of dexamethasone. This dexamethasone suppression continues even after 5 hours, when plasma cortisol concentrations demonstrate a 4–5-fold decrease.

Domestic ferrets in North America experience a high incidence of adrenal gland neoplasms that produce a number of hormones. Excessive production of estradiol is a common occurrence with adrenal gland neoplasms of ferrets, but excessive cortisol production also can occur. The ACTH stimulation and dexamethasone suppression tests have not been useful in establishing the diagnosis of hyperadrenocorticism associated with adrenal neoplasia in domestic ferrets.

Less common small mammal pets

African hedgehogs (*Atelerix albiventris*) and sugar gliders (*Petaurus breviceps*) are often presented for veterinary care and blood is often sampled for biochemical testing.[12,17] Little is known about interpretation of plasma biochemical testing in these omnivorous animals; therefore, results are interpreted in the same manner as domestic nonherbivorous mammals.

Blood for plasma biochemical evaluation can be collected from hedgehogs and sugar gliders by puncture of the jugular vein or cranial vena cava. Both structures cannot be visualized; however, knowledge of their anatomical location should guide one's approach. Jugular venipuncture is performed by inserting a small gauge needle (i.e., 25 gauge) on the ventral aspect of the neck midway between the point of the shoulder and the ramus of the mandible avoiding the trachea and esophagus. Puncture of the cranial vena cava is performed by inserting the needle at the thoracic inlet (sternal notch) using a 30 degree angle and pointing the needle in the direction of the opposite hip.

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35 Clinical Chemistry of Birds

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Sample collection and handling

A blood sample representing 1% of the bird's body weight is generally accepted to be a safe volume of blood that can be obtained from nonanemic and dehydrated birds. Avian veterinarians often collect the blood sample into a syringe that contains sodium heparin; however, this is not necessary as a quality sample can be collected in a nonheparinized syringe as long as the blood is immediately transferred into a microcollection tube containing lithium heparin, which must be filled to the appropriate volume. Adding heparin to the syringe has the risk of causing sample dilution and interference with analytical testing of sodium and protein. Once the sample has been collected into a syringe, it should not be passed through the needle a second time, especially small bore needles (25 gauge or smaller); the needle should be removed before blood is dispensed into collection tube to minimize hemolysis of the sample.

Most chemical analyses are conducted on plasma, although testing could also be performed on a serum sample. The reason for this is that collection of serum from birds frequently yields a very small sample size compared to what can be obtained from plasma. This is especially important when collecting blood from very small birds. Also, avian serum often clots once it has been harvested and the gelled sample is difficult to analyze; therefore, plasma is preferred for routine blood biochemical evaluations of birds.

The method by which blood samples are collected and handled during processing has a significant effect on the test results. Blood should be collected rapidly, immediately transferred into the lithium heparin collection tube, and mixed well with the anticoagulant. Hemolysis of the sample during collection and handling should be avoided. Hemolysis results if blood is placed into tubes too quickly, agitated too vigorously while mixing with an anticoagulant, or improperly stored. Blood that is stored at room temperature for a period of greater than 1 hour, kept at too high a temperature, or frozen will hemolyze.

Fasting samples are not often obtained from birds, because withholding food from birds that are sick is not advisable. Also, considering the nature of their digestive physiology and anatomy, a fasting state may be difficult to achieve safely. As a result, plasma samples obtained from birds are often lipemic, which can interfere with many biochemical tests. In some avian species, it is normal for the plasma to be yellow because of carotenoid pigments from the diet. It is important to note that such samples do not represent icterus and the pigment does not interfere with biochemical testing.

Reference intervals and decision levels

Published reference intervals for biochemical tests for a few of the common species of birds seen in veterinary practices traditionally have been established to produce a 95% confidence interval for each analyte.[10–12,25,26,40,43] Establishment of normal reference intervals for a given species of bird depends on many factors, including age, state of health, and nutrition. Because of the avian patient's ability to mask illness, it is often difficult to guarantee that a given bird is free of disease for inclusion into a population of normal healthy birds. Likewise, because the nutritional requirements for most birds are unknown, it is difficult to determine if their nutritional needs are being met. Both environmental factors and the physiologic status of the birds should be considered when establishing reference values. Factors such as gender, age, temperature, humidity, photo period, season of the year, and time of day may influence the results of a particular analyte.[46] Also, methodology often varies among veterinary laboratories, which can create dif-

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ficulty when comparing laboratory data obtained from one center with reference values provided by another. For these reasons, reference values should be established for an individual bird during health, and the same laboratory should be used so that subtle changes in the blood biochemistries can be detected.

Because of the difficulty in obtaining meaningful reference intervals for each species of bird that may be presented to a veterinary hospital, many avian clinicians use decision levels when assessing avian biochemical profiles. Although variations associated with factors such as seasonal variability, circadian rhythm, and gender may be significant, they may be too small to influence the clinical decision making process. Decision levels are threshold values above or below which a decision is made to respond to the abnormality. The response may vary from repeating the test, ordering additional tests, or treatment of the patient. Decision levels may be obtained by using published reference intervals and by applying these values to those obtained by the laboratory. Decision levels may vary among avian clinicians depending on their experience and the laboratory results. Values suggested in this text for each analyte in the avian blood profile are simply guidelines that can be used as decision levels. Obtaining a set of normal values from the healthy individual bird housed in a stable environment with a consistent husbandry protocol can refine the process of evaluating that avian patient later when it becomes ill. Therefore, when the bird becomes ill, it has its own set of normal reference values for comparison.

Laboratory evaluation of the avian kidney

Normal anatomy and physiology of the avian kidney

The avian urinary system consists of paired kidneys that are located in the renal fossa of the synsacrum. Each kidney is composed of three divisions: cranial, middle, and caudal. In turn, each division is composed of lobules that contain poorly demarcated, large cortical areas and smaller medullary areas. A ureter transports urine from each kidney to the urodeum of the cloaca. Unlike mammals, birds lack a renal pelvis and a urinary bladder.

Birds have two types of nephrons.[18,19,50,53] The superficial cortical or reptilian-type nephron has a glomerulus with a tubular system that is devoid of loops of Henle and is located entirely in the cortex. Cortical nephrons radiate around the central efferent veins to form lobules, and they empty at right angles to the collecting ducts. The deeper medullary or mammalian-type nephron has a glomerulus with a tubular system that contains loops of Henle. Therefore, medullary nephrons are involved in the countercurrent multiplier and osmotic gradient process to form urine as in mammalian kidneys. The glomeruli of birds are smaller with lower filtration rates (GFR) compared to those of mammals; however, they more numerous and as a result the overall GFR of birds is similar to that of mammals.[19] The loops of Henle and the collecting ducts that drain both types of nephrons are bound by connective tissue to form a medullary cone, and each cone ends as a branch of the ureter.

Birds have a juxtaglomerular apparatus but only a rudimentary macula densa.[50] The juxtaglomerular apparatus consists of an afferent arteriole, secretory juxtaglomerular cells that produce renin, and extaglomerular mesangial cells. Renin leads to the formation of angiotensin I and II, which are vasoconstrictors that stimulate the release of aldosterone. In turn, aldosterone stimulates NaCl and water reabsorption by the distal convoluted tubules and collecting ducts.

Blood is supplied to the kidney by the renal arteries, which eventually supply the afferent glomerular arterioles. Avian kidneys also receive blood from a renal portal system, in which the renal veins behave as arteries and supply blood to the kidney tubules; however, the amount of blood supplied in this manner varies with species, stress, and temperature.[19,53] The cranial and caudal portal veins, which receive blood from the pelvic limbs, intestines, and oviduct, form a vascular ring around the kidney. Valves at the junction of the bifurcation of the external iliac veins control the renal portal blood supply; these valves are controlled by both adrenergic and cholinergic nerves. The renal portal system facilitates tubular secretion by the cortical nephrons by supplying blood to the peritubular capillary plexus, which supplies the cortical nephron and the proximal and distal tubules of the medullary nephron.

The avian kidney plays a major role in osmoregulation by maintaining water homeostasis and electrolyte balance. The avian kidney filters large volumes of water that is later resorbed by the tubules. The medullary nephron concentrates urine by the counter current multiplier mechanism; however, it is less efficient than mammalian kidneys, perhaps because urea does not play a role in medullary hypertonicity among birds. Filtration occurs in the glomeruli, in which crystalloids and substances of small and medium molecular size pass into the glomerular filtrate. Electrolytes, glucose, uric acid, urea, and creatinine are a few of the substances that are removed from blood by glomerular filtration. Some filtrates (e.g., glucose) are reabsorbed by the tubules. The glomerular filtration rate (GFR) of birds is more variable than that of mammals because of intermittent filtration by avian glomeruli. The GFR, as measured by inulin clearance, varies between 1.2 and 4.6 mL/kg per minute and is affected by the state of hydration.[50] Arginine vasotocin, like mammalian vasopressin, decreases the GFR and increases water reabsorption in response to dehydration or increases in plasma osmolality.[6,22] The avian kidneys respond to a decrease in the GFR as occurs with dehydration by shunting blood from the reptilian-type nephrons to the mammalian-type

nephrons because of the latter's ability to concentrate urine. This urine concentration ability varies between species and appears to be related to the size of the bird; smaller birds have a greater ability to concentrate urine than do large birds.[18]

The intestinal tract and salt glands of birds also aid the kidneys in their osmoregulatory function. Urine that makes its way to the urodeum of the cloaca can retropulse into the rectum via the coprodeum where water can be resorbed. This retropulsion can have a profound effect on the urinalysis.

Other important functions of the avian kidneys include excretion of metabolic wastes and toxins, metabolism of vitamin D, and production of erythropoietin.

Blood chemistry evaluation

Uric acid, produced by the liver and kidneys, is the major end product of nitrogen metabolism in birds. Uric acid is excreted primarily by renal tubular secretion and is largely independent of tubular water resorption; however, decreased GFR from severe dehydration may result in stasis of uric acid movement through the tubules.[22] The principal site of uric acid secretion appears to be in the proximal tubules of the cortical nephrons. Approximately 90% of blood uric acid is removed by the kidneys.[19,53] Therefore, evaluation of the serum or plasma uric acid concentration has been widely used in the detection of kidney disease in birds. In general, a blood uric acid concentration greater than 13 mg/dL (750 Umol/L) is suggestive of impaired renal function from a variety of causes, including nephrotoxins such as lead or aminoglycoside antibiotics, urinary obstruction, nephritis, nephrocalcinosis, and nephropathy associated with hypovitaminosis A. The blood uric acid concentration is influenced by species, age, and diet. Juvenile birds tend to have lower blood uric acid values than adults, and carnivorous birds tend to have higher concentrations than granivorous birds. Increases in blood uric acid may be observed in birds shortly after consumption of a high-protein meal.[41,48] This is especially apparent among raptors, in which 24-hour fasting is required to avoid postprandial increases in plasma uric acid concentration. The uric acid concentration may also increase with severe tissue necrosis or starvation because of increased catabolism of nitrogenous compounds such as proteins and nucleic acids.

When the plasma uric acid concentration exceeds the solubility of sodium urate, uric acid (in the form of monosodium urate monohydrate crystals) precipitates in tissues, which is a condition known as gout. Birds with gout exhibit precipitation of urate crystals especially in the synovial joints and on the visceral surfaces. Blood uric acid concentrations are extremely increased (e.g., fivefold greater than normal) in birds with gout and result from severe renal dysfunction.

Uric acid is not a sensitive test for renal disease in birds, because a significant loss (approximately 75%) of renal function is required to increase the blood concentrations of this analyte. Uric acid is also not a specific test for renal disease, because increases can occur after ingestion of a high-protein meal, during starvation, or with severe tissue necrosis. Therefore, whereas the blood uric acid can be used as an indicator of renal function in birds, it does not provide a diagnosis, nor do normal values guarantee an absence of renal disease. Blood uric acid concentration when used as a sequential evaluation can be useful in monitoring treatment or progress of disease.

Because birds are uricotelic, they possess only very small quantities of urea in plasma. Urea is formed in the liver as a product of protein catabolism, and higher concentrations are present in carnivorous than in granivorous birds because of differences in dietary protein intake. The normal blood urea nitrogen (BUN) concentration of normal, noncarnivorous birds ranges between 0 and 5 mg/dL (0–1.8 mmol/L). Urea is generally considered to have limited diagnostic value in the detection of renal disease in birds compared with that of uric acid. Unlike uric acid, which is generally excreted independently of hydration, BUN may be a sensitive test for prerenal azotemia in some avian species because it is eliminated by glomerular filtration, which depends on the hydration status of the bird. Therefore, an increased BUN concentration may be useful in the detection of reduced renal arterial perfusion in some birds. Like the uric acid concentration, the plasma urea nitrogen concentration increases in birds, especially raptors, after ingestion of a high-protein meal.[41] Plasma BUN and uric acid values are often evaluated together in an effort to differentiate prerenal azotemia, renal pathology, and postprandial effects.

Potassium is filtered and actively excreted by the kidneys. Birds with severe renal disease may retain potassium and develop hyperkalemia.

Sodium is filtered by the glomerulus and, depending on the osmotic needs, may be resorbed into the plasma or secreted by the kidney tubules for elimination. Birds with chronic renal disease may lose the ability to retain sodium, thereby resulting in hyponatremia.

Hyperphosphatemia can occur in birds with severe renal disease. However, it is not a consistent finding with decreased GFR.

Severe renal disease may result in an increased plasma creatinine concentration. Creatinine is usually considered to have poor diagnostic value, however, because in birds, creatine is excreted by the kidney before it is converted to creatinine. Therefore, the plasma creatine rather than the creatinine concentration may better detect a decreased GFR in birds. Unfortunately, veterinary laboratories do not routinely provide creatine analysis.

The presence of upper gastrointestinal hemorrhage in mammals leads to a disproportionate increase in BUN concentration relative to reductions in the GFR resulting in a greater BUN: creatinine ratio. This does not appear to occur

in birds where it has been demonstrated that plasma BUN, creatinine, and uric acid concentrations are unaffected by the presence of blood in the digestive tract.

Urinalysis

A urinalysis, which routinely is applied to mammalian urine, also can be performed on avian urine. Indications for performing a urinalysis on an avian patient include polyuria, azotemia, and abnormal appearance of the urinary component of the dropping. The urinalysis includes notation of the gross appearance, measurement of the specific gravity or osmolality, chemical evaluation, and microscopic examination.

Urine is collected by aspirating the liquid part of the dropping into a pipette or syringe once the dropping has been deposited onto a nonabsorbable surface (e.g., aluminum foil or wax paper). Aspiration of fecal material or urates along with the liquid urine should be avoided. Ureteral urine enters the cloaca and is forced into the colorectum by antiperistaltic activity, thereby allowing reabsorption of water and electrolytes to occur. For this reason, exposure of urine to cloacal membranes and the large intestine cannot be prevented; however, this exposure is presumed to be minimal when urine is produced at moderate to high rates. Catheterization of the ureter is possible but not routinely performed because it requires general anesthesia and is technically difficult.

Normal avian urine is the clear fluid component of a bird's droppings. The amount of urine produced varies with species, diet, and environmental factors, such as temperature and humidity. Generally, avian urine is hyperosmotic to plasma (362–2000 mOsmol/L), especially in birds that have adapted to arid environments; however, this is affected by the hydration status of the bird.[50] The normal specific gravity of avian urine ranges from 1.005 to 1.020 depending on the species, hydration status, and osmolality.[22] Osmolality is a direct measure of the number of solute particles in the urine, whereas the specific gravity is a crude index of renal tubule function and is affected by the number, size, and weight of solute particles in the urine. The two determinations are related, however, and both can be used to determine the loss of concentrating ability in birds with renal disease.[1] A urine osmolality of 450 mOsmol/kg represents the normal renal concentrating ability in the pigeon (Columbia livia) and can be used as a guide for water-deprivation studies in that species.[34]

The color of avian urine can be helpful in detection of certain diseases. For example, a biliverdinuria represented by green urine is suggestive of severe liver disease or hemolysis in birds. Yellow urine is observed in some species such as macaws with liver disease, and this most likely represents bilirubinuria. Hematuria or hemoglobinuria is represented by red urine that changes to brown on standing. Polyuric birds produce liquid urine that may appear to be cloudy if contaminated with urates or containing large concentrations of cells, mucus, fat, or bacteria. Microscopic examination can determine the cause of the cloudy appearance. Green bile pigment from the feces and dietary pigments can stain the urine especially if the dropping is held in the cloaca for a prolonged period of time. A positive urobilinogen on the test strip is supportive of fecal contamination of the urine sample.

The principle difference in the nitrogenous components of avian urine compared with those of mammalian urine is the large amount of uric acid and creatine. Uric acid in avian urine typically occurs as a thick, mucoid, white to cream-colored colloidal suspension containing the small spherical conglomerates of insoluble sodium and potassium urates and protein. This semisolid material is not part of the specific gravity measurement of the urine supernatant.

Commercially available test strips for biochemical examination of mammalian urine can be used for avian urine as well.[51] These test strips usually indicate a negative to trace amount of protein in the urine of normal birds. Protein not reabsorbed from the proximal tubules becomes part of the urate conglomerate, which is not measured in the supernatant. For this reason, renal loss of protein may be difficult to detect by urinalysis; however, detection of significant proteinuria in the absence of hematuria, hemoglobinuria, and fecal contamination of the sample is suggestive of the renal proteinuria that occurs with abnormal glomerular permeability, such as that which occurs with glomerulonephritis. Alkaline urine (pH >8) can produce a false-positive reading in the protein portion of the test strip. Therefore, other methods for testing urine protein should be employed with alkaline urine. Postrenal proteinuria is associated with inflammation of the lower urinary tract and cloaca.

The pH of avian urine varies from 4.7 to 8.0 and depends primarily on the diet. Carnivorous birds that ingest large amounts of animal protein have acidic urine, whereas granivorous birds have more alkaline urine. Increased urine acidity (pH <5.0) may result from acidosis or increased protein catabolism, such as that which occurs during starvation. Increased urine alkalinity (pH >8.0) may be associated with alkalosis. The urine pH also can vary with physiologic state. For example, in poultry, acidic urine is observed in laying hens that are depositing calcium into developing egg shells.[50]

Urine from normal birds contains no glucose, because glucose is completely reabsorbed by the tubules after glomerular filtration. Glucosuria occurs when the renal threshold for glucose is exceeded. In most birds, this threshold is approximately 600 mg/dL. Birds with diabetes mellitus, however, often exhibit blood a glucose concentration of greater than 800 mg/dL and significant glucosuria.

Normal avian urine is devoid of ketones. Excessive ketone formation and ketonuria occurs with increased oxidation of fatty acids as an energy source. This may explain the ketonuria found in migratory birds following migration.

Ketonuria may be expected with severe malnutrition or diabetes mellitus, but this has been poorly documented in birds. One explanation for negative ketones in avian urine may be a result of the urinalysis test strips that do not test for the major ketones produced by birds.

Biliverdin is the major bile pigment of birds; therefore, bilirubin is not normally present in the urine. Biliverdin does not react with the bilirubin portion of the urine test strip. Biliverdinuria is indicated by a green coloration of the urine and is suggestive of hepatobiliary disease or hemolysis in birds. The normal urinary urobilinogen concentration in birds ranges from 0 to 0.1 mg/dL. In general, this test has limited diagnostic value in birds and when positive suggests fecal contamination of the sample.

Occult blood in the urine of a normal bird measures negative to trace on the urinary test strips. A positive occult blood reaction is suggestive of erythrocytes, free hemoglobin, or myoglobin in the urine. Microscopic examination of the urine sediment can determine the presence of erythrocytes. A positive test of the supernatant after centrifugation is suggestive of hemoglobinuria, and increased plasma creatine kinase (CK) values may be supportive of myoglobinuria. Hematuria is indicative of hemorrhage originating from either the urinary, reproductive, or gastrointestinal tracts or the cloaca. Hemoglobinuria is suggestive of the intravascular destruction of erythrocytes or, perhaps, the lysis of erythrocytes in hypotonic urine. Hemoglobinuria has been reported in psittacine birds with heavy metal toxicity, especially lead poisoning.

Urinary nitrite tests are designed to detect bacteriuria. This test has limited diagnostic value, however, in the urine of birds.

Microscopic examination of urine is an important part of urinalysis. Whereas 5 mL of urine is suggested to provide a uniform semiquantitation in mammalian urine, this amount rarely is achieved in most avian samples. Microscopic examination, however, can still provide valuable information.

Normal avian urine contains few cells. As many as three erythrocytes and three leukocytes per high-power field (40×) are considered to be normal in direct smears of the urine. Epithelial cells are rare in normal avian urine samples and can originate from the urinary tract, gastrointestinal tract, reproductive tract, or cloaca. Increased cell numbers are a cause for concern.

Casts in the urine of birds are indicative of renal disease, because casts are formed within the renal tubules. Granular casts are the most common and are suggestive of renal tubular epithelial cell degeneration (i.e., tubular nephrosis). Cellular casts also may be present, and the types of cells that are observed reflect the renal pathology. Casts containing epithelial cells are suggestive of acute tubular damage that results in sloughing of the cells that line the tubules. Leukocytes in the casts are indicative of renal inflammation (nephritis). Erythrocytes within the casts are indicative of renal hemorrhage and, typically, occur after trauma to the kidneys.

Crystals in avian urine sediment are primarily sodium and potassium urates. These are round crystals with a spoke-like appearance that are refractile under polarized light. The needle-shaped uric acid crystals may also be present in avian urine. A few drops of sodium hydroxide can be added to the urine sample to dissolve the uric acid crystals to facilitate the examination of the cellular components in the urine.

Microorganisms in urine sediments usually originate in the intestinal tract or cloaca, and represent contamination of the sample. Large numbers of bacteria, however, may be indicative of a urinary tract infection, especially when they occur as a uniform population of a single morphologic type and when casts are present. Because bacteria frequently contaminate avian urine samples and multiply during storage, resulting in artificially high bacterial numbers, fresh samples should be examined.

Electrolytes and acid/base balance

Water consumption in birds is influenced by species, age, size, environmental temperatures, and both the type and amount of food that is consumed. Water intake often relates inversely to body size and ranges between 5% and 50% of body weight per day.[19] Young birds tend to consume more water than adults. Carnivorous birds as well as those that have evolved in arid environments normally drink little water.

Water deprivation, hemorrhage, or administration of hypertonic saline produces thirst in birds, which is caused by the release of angiotensin II.[19] Angiotensin II induces the release of arginine vasotocin (i.e., antidiuretic hormone [ADH]), aldosterone, and corticosterone. Arginine vasotocin increases the reabsorption of water in the renal tubules and collecting ducts; other neurohormonal factors also play a role in the hypothalamic regulation of water intake. Disorders of the hypothalamus and deficiency of ADH from the posterior pituitary result in diabetes insipidus, thereby causing polydipsia and polyuria. These conditions have been reported in chickens and a few other species. A water-deprivation test or administration of exogenous ADH can be performed to differentiate polyuric disorders of birds.[1] In chickens, water deprivation increases the plasma osmolality from 315 to 325 mOsm/L after 24 hours and to 340 mOsm/L after 72 hours.[50] During dehydration and 24-hour water deprivation, the urine osmolality of normal birds is greater than 450 mOsm/L.

The ability of the avian kidney to conserve and excrete water has a wider range than that of mammals. The fractional excretion of water in birds can be as high as 33% during hydration and as low as 1% during dehydration.[50] Cessation of renal water loss during dehydration results

partially from shutdown of the cortical nephrons; it is not strictly a function of the tubular resorption of water.

Electrolyte metabolism in birds is similar to that of mammals. Therefore, predominate extracellular and intracellular anions and cations are similar in both types of animals.

Sodium

Sodium is the primary osmotically active electrolyte in the plasma and urine of birds. Dietary sodium is absorbed in the intestines and carried to the kidneys, where it is excreted by glomerular filtration. Depending on the need for sodium by the bird, sodium may be resorbed into the plasma or secreted by the renal tubules and then excreted.

Birds having salt (i.e., nasal) glands can excrete large amounts of sodium by an extrarenal route. The paired salt glands are located just above the orbits in most marine birds. Ducts from these glands deliver secretions into the nasal cavity, which flow through the nares and drip off the tip of the rhinotheca (i.e., beak). Nasal secretion of sodium occurs not only in marine species but also in at least two orders of terrestrial birds (falconiforms and cuculiformes).[19] Salt glands also are found in ducks and geese. The typical concentration of sodium in the salt-gland secretions of most species that have been studied ranges between 500 and 1000 mEq/L (mmol/L).[19] The rate of sodium secretion by the salt glands varies among species, however, as do the degrees of hydration and salt loading. The primary stimulus for salt-gland secretion is plasma osmolality, but hormonal influences also affect nasal secretion, which is increased by adrenal corticosteroids and aldosterone.

Hyponatremia in most species occurs when the plasma sodium concentration is less than 130 mEq/L (mmol/L). Diseases affecting the kidneys, gastrointestinal tract, or perhaps, the salt gland can be associated with excessive sodium loss. Excessive hydration because of polydipsia or iatrogenic delivery of low-sodium intravenous fluids (e.g., 5% dextrose in water) also can result in hyponatremia. Hyponatremia can be corrected by addressing the cause of the sodium loss, control of overhydration, or use of fluid therapy with an appropriate electrolyte balance.

Hypernatremia occurs when the plasma sodium concentration exceeds 160 mEq/L (mmol/L) and may be due to excessive dietary salt intake, decreased water intake, or increased water loss. After salt loading, hypernatremia occurs more rapidly in birds without functional salt glands. Marine birds that are given freshwater over a period of time exhibit atrophy of the salt glands, thereby resulting in hypernatremia after the ingestion of salt water. Hypernatremia associated with salt loading can be associated with excessive dietary sodium or administration of intravenous fluids containing excessive sodium. Hypernatremia associated with excessive free-water loss occurs with diarrhea, renal failure, or rarely, diabetes insipidus.

Chloride

Chloride is the anion of highest concentration in the extracellular fluid. Chloride and sodium represent the primary osmotically active components of plasma. For most species, hypochloridemia is indicated by a plasma chloride concentration of less than 100 mEq/L (mmol/L), whereas hyperchloridemia is indicated by a plasma chloride concentration greater than 120 mEq/L (mmol/L). These conditions rarely are reported in birds. Hyperchloridemia can be associated with dehydration and salt loading.

Potassium

Potassium is the major intracellular cation. Therefore, an artifactual increase in the serum or plasma potassium concentration occurs with hemolysis. Artifactual hyperkalemia or hypokalemia may occur with delayed separation of the cells in the sample, and these changes are species specific. For example, a 30% increase in plasma potassium occurs in macaws (*Anodorhynchus* sp.) following a 4-hour delay in plasma separation, whereas a 30% decrease was found in chickens (*Gallus gallus*) following a 2-hour delay in plasma separation.[22] True hyperkalemia in most species is indicated by a plasma potassium concentration of greater than 4.0 mEq/L (mmol/L). Hyperkalemia results from renal failure with decreased secretion of potassium, acidosis, and severe tissue necrosis. Hypokalemia in most species of birds is indicated by a plasma potassium concentration of less than 2.0 mEq/L (mmol/L). Hypokalemia can be associated with chronic diarrhea, prolonged anorexia, and alkalosis. Hypokalemia has also been reported in birds with renal disease, which may be associated with chronic loss of potassium in the urine. Use of potassium-poor fluids during fluid therapy in chronically anorectic birds may dilute the plasma potassium to a hypokalemic level, which may enhance the renal loss of potassium. Diuretic therapy rarely is used in birds, but it may enhance renal potassium loss as well. Imbalances of plasma potassium may result in muscle weakness, serious cardiac disturbances (e.g., sinus bradycardia and arrest), or both. Hypokalemia can be corrected with the addition of potassium to supportive fluids.

Calcium

Control of calcium metabolism is mediated by parathormone (PTH), calcitonin, and vitamin D_3 (i.e., 1,25-dihydrocholecalciferol, calciferol). Other hormones, such as estrogens, corticosteroids, thyroxine (T_4), and glucagon, also influence calcium metabolism. The primary function of PTH is to maintain normal plasma calcium concentrations by its action on bone, kidney, and intestinal mucosa. When plasma concentrations of ionized calcium decrease, the parathyroid glands are stimulated to release PTH. The primary effect of this is to mobilize calcium from bone; however, increased

calcium absorption by intestinal mucosa and calcium reabsorption by renal tubules also aid in the restoration of normal plasma ionized calcium concentration. Parathormone also enhances the renal excretion of phosphorus to maintain a normal calcium:phosphorus ratio.

Calcitonin is produced by the ultimobranchial glands of birds. Avian C cells, which are the calcitonin-secreting cells, migrate from the sixth pharyngeal pouch during embryonic development to form the ultimobranchial gland. In some species of birds, C cells also can be found in parathyroid or thyroid tissue. Calcitonin has the opposite action from that of PTH. Therefore, as the plasma calcium concentration increases, calcitonin is released to inhibit calcium reabsorption from bones.

Calciferol, which stimulates calcium and phosphorus absorption by the intestinal mucosa, increases the sensitivity of bone to the effects of PTH and is important for bone mineralization. The kidney is involved with the conversion of vitamin D_3 to its hormonally active state, 1,25-dihydroxycholecalciferol (calciferol). Renal synthesis of 1,25-dihydroxycholecalciferol is regulated, at least partially, by PTH.

Birds differ from mammals by the increased development of medullary bone in the long bones (of hens) before egg laying, hypercalcemia in response to estrogen (and reproductive activity) in females, and the ability of hens to use 10% of their total body calcium stores for egg production on a daily basis for extended periods without detrimental physiologic consequences.[28] Calcium deposition occurs in the medullary spaces of the femur, tibiotarsus, and other nonpneumatic long bones in female birds during the first 10 days before oviposition (egg-laying). This is referred to as medullary bone formation, and it is under the influence of the ovarian hormones, estrogen and testosterone. Medullary bone formation occurs 1 to 2 weeks before the increase in total plasma calcium concentration and renal hydroxylase activity that increases formation of the hormonally active vitamin D_3. During the ovulation-oviposition cycle, periods of medullary bone formation alternate with periods of medullary bone depletion.

Prolactin and sex hormones influence the 1-hydroxylation of 25-hydroxyvitamin D_3 in the kidney, which in turn plays an important role in calcium metabolism.[28] This activity increases just before egg laying and corresponds to the increase in total blood calcium concentration. Therefore, the renal vitamin D endocrine system is involved in the increased intestinal calcium absorption during the ovulation-oviposition cycle of laying hens.

The total blood calcium concentration of a laying hen ranges from 20 to 30 mg/dL (5.0–7.5 mmol/L). Total calcium consists of ionized calcium, which is the biologically active form, and calcium bound to anionic proteins and nonprotein anions. Estrogen stimulates the production of calcium-binding proteins such as vitellogenin and albumin; therefore, the total plasma calcium concentration increases because of an increase in protein-bound calcium. This occurs several weeks before oviposition in chickens. The ionized calcium level remains unchanged.

Calcium for egg formation is derived from intestinal absorption and bone mobilization. If the dietary calcium is adequate, then most of the eggshell calcium is derived from intestinal absorption. Bone is an important source of eggshell calcium during the night, when food is not being consumed, or if the dietary calcium intake is inadequate.

The normal plasma concentration of calcium in most nonlaying birds ranges from 8.0 to 11.0 mg/dL (2.0–2.8 mmol/L). Approximately one-third to one-half of the plasma calcium is bound to albumin. Therefore, the total plasma calcium concentration is affected by the plasma albumin or total protein concentration; however, this may vary with species. In general, the total plasma calcium concentration usually decreases with hypoalbuminemia and increases with hyperalbuminemia. A significant correlation between total calcium and albumin has been determined for African Grey parrots (*Psittacus erithacus*) with the following correlation formula:

$$\text{Adjusted Ca (mmol/L)} = \text{Ca (mmol/L)} - 0.015 \times \text{Albumin (g/L)} + 0.4$$

A significant correlation was found between total calcium and total protein in the ostrich and Peregrine falcon (Hierofalco peregrinus) with correlation formulae for the ostrich:

$$\text{Adjusted Ca (mmol/L)} = \text{Ca (mmol/L)} - 0.09 \times \text{Total protein (g/L)} + 4.4$$

And for the Peregrine falcon:

$$\text{Adjusted Ca (mmol/L)} = \text{Ca (mmol/L)} - 0.02 \times \text{Total protein (g/L)} + 0.67$$

These examples indicate that the total calcium, albumin, and total protein concentrations have a linear relationship that varies between species of birds.[22] These formulae may not be clinically useful when dealing with other avian species.

Evaluation of ionized calcium would be more clinically useful because it is the most physiological active fraction of the total calcium concentration; however, there have been few studies evaluating ionized calcium to disease states in birds and reference values have not been established. Ionized calcium concentration of birds that have been studied suggest that normal concentrations range between 1.0 and 1.6 mmol/L.[23] The concentration of ionized calcium is affected by the acid-base balance. The ionized calcium level will increase during acidosis and decrease during alkalosis.

Most species of birds are considered to be hypocalcemic when the plasma total calcium concentration becomes less than 8.0 mg/dL (2.0 mmol/L), and hypocalcemia has been associated with dietary calcium and vitamin D_3 deficiency, excessive dietary phosphorus, alkalosis, and hypoalbuminemia. African Grey parrots often exhibit a hypocalcemic syndrome with a plasma calcium concentration of less than 6.0 mg/dL (1.5 mmol/L) that results in seizure disorders. The pathophysiology of this condition is unknown, but it has been considered to be a form of nutritional hypoparathyroidism or, possibly, a result of hypovitaminosis D_3. Secondary nutritional hyperparathyroidism commonly is observed in birds that are fed calcium-poor diets (e.g., all-seed or all-meat diets). Affected birds have a decreased plasma calcium concentration, normal plasma phosphorus concentration, and increased plasma alkaline phosphatase (AP) activity.

Hypercalcemia in most species of birds is indicated by a plasma calcium concentration of greater than 11 mg/dL (2.7 mmol/L). Hypercalcemia has been associated with hypervitaminosis D_3, osteolytic bone lesions secondary to neoplasms, and hyperalbuminemia. Causes of hypercalcemia in mammals, such as primary and pseudohyperparathyroidism, certain plant toxicities, and hypoadrenocorticism, have not been documented in birds, but should also be considered as possible causes of this condition.

Phosphorus

Plasma phosphorus is primarily regulated via renal excretion stimulated by PTH. Young, growing birds tend to have higher plasma phosphorus concentrations compared with adult birds.

Hypophosphatemia in birds is indicated by plasma phosphorus concentrations of less than 5 mg/dL (1.6 mmol/L). This may occur with hypovitaminosis D_3 (hypocalcemia also occurs), malabsorption, or starvation. Long-term corticosteroid therapy also may result in hypophosphatemia in birds; other disorders in mammals that result in hypophosphatemia have not been reported in birds, but should also be considered as possible causes.

Hyperphosphatemia in birds is indicated by plasma phosphorus concentrations of greater than 7.0 mg/dL (2.3 mmol/L), and may occur with severe renal disease because of reduced glomerular filtration, hypervitaminosis D_3 resulting in increased intestinal phosphorus absorption, and excessive dietary phosphorus. Hypoparathyroidism also may be considered in some cases of hyperphosphatemia in birds. Improper sample handling can cause artifactual hyperphosphatemia because erythrocytes contain a higher concentration of phosphorus than is present in plasma. Therefore, hemolysis or delayed separation of plasma from erythrocytes, which allows intracellular phosphorus to leak out, will increase the plasma phosphorus concentration.

Acid-base balance

The normal pH of birds is maintained between 7.33 and 7.45, and the buffering systems that regulate blood pH in mammals appear to be present in birds. The bicarbonate/carbonic acid buffer system is the most important because of the rapid rate of CO_2 elimination by the lungs after conversion from H_2CO_3. Therefore, alterations in plasma bicarbonate and CO_2 content are useful in the detection of acid-base disturbances in birds. Because most CO_2 in plasma is derived from bicarbonate, the clinical interpretation of the total CO_2 concentration is the same as that of the bicarbonate concentration. The total CO_2 concentration rarely is reported, but concentrations between 20 and 30 mmol/L are considered to be normal for most species. Increases in the total CO_2 concentration are suggestive of a metabolic alkalosis or a compensation for a respiratory acidosis, whereas decreases are suggestive of a metabolic acidosis or compensation for a respiratory alkalosis, as occurs with excessive ventilation. During active shell formation in laying hens, the plasma bicarbonate concentration decreases, thereby resulting in a metabolic acidosis.

Blood gases rarely are determined in birds. Avian erythrocytes continue to be metabolically active after blood collection, and alterations in blood gas values in vitro can occur quickly. These alterations are influenced by temperature; therefore, blood gas analysis should be performed as quickly as possible after sample collection. Portable blood gas analyzers that are designed for bedside monitoring of human patients may provide a quick, reliable method for monitoring blood gases in birds.

Laboratory evaluation of the avian liver

Liver enzymes

Interpretation of liver enzyme activity, as commonly used in mammalian medicine, has been applied to birds. However, experimental studies evaluating the sensitivity and specificity of these enzymes for detecting liver disease have been limited to only a few avian species. Because the specificity and sensitivity of these enzymes may vary with the species and the type of hepatic disease, only general statements regarding alterations in enzyme activity can be made. Alterations in the plasma activity of enzymes used to detect hepatic disease in birds can reflect either hepatocellular injury or increased enzyme production.

Aspartate aminotransferase

High aspartate aminotransferase (AST) activity has been reported in the liver of birds. High AST activity has also been found in skeletal muscle, heart muscle, brain, and kidney.[17,34,35] The distribution of AST among tissues varies

with the species, thereby making interpretation of increased plasma AST activity challenging. In general, increases of plasma AST activity in birds are suggested when such activity is greater than 275 IU/L. Increases result from either hepatic or muscle injury, which allow the leakage of intracellular AST into the blood. Plasma AST activity is considered to be markedly increased when the activity is greater than 800 IU/L. Activity of this magnitude is suggestive of severe hepatic insult, especially in the presence of biliverdinuria or biliverdinemia. However, increased AST activity does not provide information about hepatic function. It is useful to evaluate CK activity (see Laboratory Detection of Muscle Injury) in conjunction with AST activity to differentiate between muscle and liver injury.

Alanine aminotransferase

Alanine aminotransferase (ALT) activity has been reported in the liver, skeletal muscle, and many other tissues of birds, and leaks into blood when such tissues are injured.[17,34,35] Plasma ALT activity is considered to be neither a specific nor a sensitive test for hepatocellular injury in birds. Plasma ALT activity in most species of normal birds ranges from 19 to 50 IU/L and may be more useful for the detection of hepatic disease in carnivorous birds. Plasma ALT activity increases with significant liver or muscle injury in birds (especially carnivores) and has no advantage compared with AST as a test for hepatocellular disease.

Lactate dehydrogenase

Plasma lactate dehydrogenase (LD) activity is nonspecific for hepatocellular disease in birds. Lactate dehydrogenase isoenzymes are found in nearly all avian tissues.[17,34,35] Specific LD isoenzymes could be helpful in determining the source of increased plasma LD activity; however, verification studies evaluating LD isoenzymes in various avian tissues would be required for a large number of avian species.

Plasma LD activity is less than 1000 IU/L in normal birds, and increased activity has been associated with hepatocellular disease. Compared with plasma AST and ALT activity, plasma LD activity increases and declines more rapidly after injury to liver or muscle. The short mean elimination half life of LD (0.48 hr) compared to that of CK (3.07 hr) makes LD a valuable test in differentiating between muscle and liver disease in the pigeon.[34,38] Determination of the plasma LD activity has no diagnostic advantage compared with plasma AST activity, especially because the former typically has a wide normal reference interval in birds and its low specificity for liver disease in birds.[30,37,39] In addition, avian erythrocytes have high LD activity; therefore, hemolysis results in increased plasma LD activity.

Glutamate dehydrogenase

Although not commonly offered by most veterinary laboratories, plasma glutamate dehydrogenase (GLDH) activity appears to be a specific test for hepatocellular disease in birds; however, sensitivity is low.[4,37] Because GLDH is a mitochondria-bound enzyme, it is released when severe cell injury has occurred. Significant GLDH activity has been found in the liver, kidney, and brain of pigeons, chickens, ducks, turkeys, and budgerigars. In general, plasma GLDH activity of greater than 10 IU/L is considered to be increased and indicative of hepatic necrosis. The degree of increase in the plasma GLDH activity reflects the severity of the hepatocellular injury. Plasma GLDH activity does not appear to increase with muscle injury, as do the activities of AST, ALT, and LD, thereby making GLDH the most liver-specific plasma enzyme among those species of birds that have been evaluated. Plasma GLDH appears to have a shorter mean elimination half-life (0.68 hr) than does AST (7.66 hr) and ALT (5.69 hr), and it can be used to evaluate not only the severity of the hepatocellular injury but also the duration (if the insult is not ongoing).[34,38]

Sorbitol dehydrogenase

Sorbitol dehydrogenase (SD) appears to be a liver-specific cytosolic enzyme, and it may be useful in establishing the diagnosis of hepatocellular injury in birds. Plasma SD has a short half-life, and its activity may remain increased for shorter periods of time than those of AST and other enzymes.[25] Plasma SD assays are usually unavailable at most veterinary laboratories. Plasma SD appears to have no diagnostic advantage compared with plasma GLDH.

Alkaline phosphatase

Alkaline phosphatase activity occurs in multiple tissues including bone and intestine, and increased plasma AP activity results not from leakage of the enzyme but from increased cellular production. Plasma AP activity in birds results primarily from osteoblastic activity. Therefore, increases in the plasma AP activity are suggestive of skeletal growth, nutritional secondary hyperparathyroidism, healing fractures, and the preovulation condition of medullary calcification in hens. Plasma AP activity is not useful in the detection of hepatobiliary disease in birds. Aflatoxin B_1-induced liver necrosis and bile duct hyperplasia have not significantly increased the serum AP activity in the pigeon, cockatiel (*Nymphicus hollandicus*), great horned owl (*Bubo virginianus*), and red-tailed hawk (*Buteo jamaicensis*). Plasma AP activity appears to be a sensitive indicator of intestinal diseases, such as coccidial infections in the duodenum, jejunum, and cecum.[29]

Gamma glutamyltransferase

Plasma γ-glutamyltransferase (GGT) activity does not predictably increase in birds with hepatobiliary disease. Similar to AP, increased plasma activity of GGT is due to increased cellular production rather than leakage. Measurable GGT activity occurs in the kidney, brain, and intestines of birds;

however, disorders of these tissues do not increase the plasma GGT activity. The highest GGT activity is found in the kidney of birds. The plasma activity does not increase with renal disease, however, because the enzyme is excreted in the urine.[25] Increased serum GGT activity has increased in some cases of birds with liver disease, but not others, suggesting that the plasma GGT activity may increase in some species of birds depending on the nature of the hepatic insult.[25,45] Plasma GGT activity is not routinely measured in birds with hepatobiliary disease; therefore, the usefulness of this enzyme in detection of avian liver diseases has not been fully determined.

Biliverdin and bilirubin

Because the avian liver generally lacks the enzyme biliverdin reductase, which is required to convert biliverdin to bilirubin, the primary bile pigment in birds is biliverdin, which is a green pigment. In spite of the fact that birds produce little to no bilirubin, clinical icterus with an increased plasma bilirubin concentration has been reported in ducks and macaws (*Ara* sp.). Presumably, some biliverdin may be reduced to bilirubin by nonspecific extrahepatic enzymes or bacteria; however, bilirubin is considered to be a poor indicator for hepatobiliary disease in most birds. The healthy avian kidney is efficient in clearing bile pigments from the blood; therefore, green-colored urine and urates are suggestive of biliverdinuria and significant liver disease in birds. The presence of biliverdinemia is indicated by green sera or plasma, which reflects severe hepatobiliary disease in birds, and is associated with a poor prognosis for survival. Most veterinary laboratories do not offer biliverdin testing. Biliverdin is an unstable bile pigment that is sensitive to light degradation. The yellow color of the plasma in many avian species may be associated with carotenoid pigments from the diet and should not be misinterpreted as bilirubinemia.

Bile acids

Because plasma enzymes are neither sensitive nor specific for the detection of liver disease in birds and also do not reflect the degree of liver disease, other blood biochemical tests are necessary to evaluate avian liver metabolism and excretion. Moreover, biliverdin and bilirubin concentrations in the blood are either not available or applicable to the detection of liver disease. Bile acid determination, however, is a sensitive test for liver function in some species of birds.[15] Bile acids are produced in the liver, excreted in the bile, reabsorbed by the intestines into the portal circulation, and removed from the blood by the hepatocytes. This process is referred to as the enterohepatic circulation.

Bile acids normally occur in very small amounts in the peripheral blood of healthy birds. The primary bile acids in birds vary among species and may not be the same as those found in dogs, cats, and humans.[7,9,16,22] Fasting plasma bile acid concentrations are lower than postprandial concentrations. The length of required fasting varies between species. For example, plasma bile acid concentrations demonstrate a postprandial peak at 2 hours in Goffin cockatoos (*Cacatua goffini*), 4 hours in African Grey parrots (*Psittacus erithacus*), and over 8 hours in Amazon parrots (*Amazona sp*).[27]

Increases in fasting plasma bile acid concentrations are suggestive of abnormal hepatic function, and may be due to failure of the liver to uptake bile acids from the portal blood, abnormal bile acid excretion caused by blockage or leakage, or portal circulation abnormalities. When measuring the bile acid concentration, a 12-hour fast is recommended because of the digestive physiology of birds. The emptying time of the ingluvies or crop varies both with diet and among species of birds; thus, the timing of postprandial sampling for bile acid testing is difficult.[27,36] Moreover, ill birds often have a slow gastrointestinal transit time—or even stasis. Conversely, increased gastrointestinal motility may interfere with bile acid release from the liver and absorption from the intestines. Because fasting is better tolerated in carnivorous birds, such as raptors, and postprandial concentrations remain higher for longer periods of time, a 24-hour fast is recommended for bile acid testing for those species.[42] Both the enzymatic and the radioimmunoassay (RIA) methods have been used for determination of bile acids in birds.[13] Results of the two methods correlate well in plasma samples obtained from six species of birds. The RIA method is linear to 50 µmol/L, whereas the enzymatic method is linear to 200 µmol/L. Lipemia and hemolysis interfere with bile acid testing using the enzymatic method, but does not interfere with the RIA method. Variable results have been reported using the RIA method, which is designed for use with human serum samples. Potential explanations include differences in the binding of antibodies to different binding sites in avian bile acids; the tendency of avian samples to have higher bile acid concentrations than are found in humans; and the different predominating bile acids in various species of birds. Others have reported that both methods have adequate precision and accuracy.

Reference intervals for fasting plasma bile acid concentrations have not been determined for many species of birds. Overall, the plasma bile acid concentration of normal birds is greater than that of mammals; therefore avian samples often require dilutions, especially with RIA methods, to stay within the linear parameters of the assay. In general, bile acid concentrations determined by the enzymatic method are considered to be normal if they are less than 75 µmol/L.[22] Assay-specific and species-specific reference intervals for normal fasting bile acid concentrations are recommended.

Cholesterol

The normal plasma cholesterol concentrations of most species of birds range between 100 and 250 mg/dL. Because cholesterol is eliminated in the form of bile acids, increases in the plasma cholesterol concentration may be associated

with extrahepatic biliary obstruction, hepatic fibrosis, and bile duct hyperplasia. Hypercholesterolemia also can be associated with conditions other than liver disease, such as hypothyroidism, high-fat diets, lipemia, and during egg production. A hypercholesterolemia occurs during vitelogenesis in female birds preparing to lay eggs. Postprandial increases in cholesterol may occur as well. Hypocholesterolemia may occur with end-stage liver disease, maldigestion or malabsorption, and starvation.

Iron

Excessive iron storage (hemochromatosis) is a common hepatic disorder of ramphastids (toucans), hill mynahs (*Gracula religiosa*), and sturnids (birds of paradise) and occurs less commonly in psittacine birds and other species.[37] It has been suggested that these species of birds develop this disorder because they are more efficient in absorbing iron form their intestines compared to other birds and like humans, some birds (i.e., mynahs) may have a genetic predisposition for the disorder. Birds with this condition may exhibit increased plasma AST activity because of iron-induced hepatocellular damage. Usefulness of serum iron concentration, total iron-binding capacity, and unsaturated iron-binding capacity testing for excessive hepatic iron storage have not been fully evaluated in birds. Studies in toucans with this condition suggest that these tests do not appear to correlate with hepatic iron concentrations. The disease is currently diagnosed based upon histopathology and tissue iron levels.

Other tests

Other abnormalities that may be suggestive of hepatic insufficiency in birds include hypoalbuminemia, hypoglycemia, hyperammonemia, and decreased levels of coagulation factors. Hypoglycemia and hypoalbuminemia could result from chronic liver disease in birds but rarely are reported. An increased plasma ammonia concentration with hepatic encephalopathy resulting from severe hepatic disease has not been documented in birds. Coagulation studies are performed only rarely in birds but could be used to help establish the diagnosis of hepatic insufficiency.

Laboratory evaluation of plasma and serum proteins

The normal plasma protein concentration in birds is less than that in mammals, generally ranging from 2.5 to 4.5 g/dL (25–45 g/L). Albumin, which represents 40–50% of the total plasma protein in birds, is produced in the liver. Other plasma proteins also produced in the liver include transport proteins, proteins of coagulation, fibrinogen, enzymes, and hormones. Immunoglobulins produced by B lymphocytes and plasma cells represent a significant component of the total plasma protein concentration. The normal plasma protein concentration is essential to the maintenance of the normal colloidal osmotic pressure, which preserves normal blood volume and pH. Hens demonstrate a marked increase in plasma total protein concentration just before egg production.[28] This estrogen-induced hyperproteinemia is associated with an increase in vitellogenin and lipoproteins, which are necessary for yolk production. These proteins are produced in the liver, transported in the blood, and incorporated into the oocytes of the ovary.

The biuret method is the method of choice for determining plasma or serum total protein concentration in birds. This method provides accurate and repeatable results when the total protein concentrations fall between 1 and 10 g/dL (10–100 g/L). Because proteins in the serum are primarily responsible for changes in the refractive index, a refractometer commonly is used to obtain a total plasma or serum protein concentration in birds. Temperature-compensated refractometers, as well as those that are not temperature compensated, tend to overestimate the total protein concentration.[3] The high glucose concentration and chromogens in the plasma of birds as well as lipemia and hemolysis affect the accuracy of the refractometric method, which frequently is used for a rapid estimate of the plasma protein concentration.[22] The biuret method, however, is more accurate.

The plasma albumin concentrations generally range from 0.8 to 2.0 g/dL (8–20 g/L) in normal birds. These values may not be accurate, however, because most analyzers determine albumin spectrophotometrically using the bromcresol green (BCG) dye-binding method using human albumin standards and controls that may have different binding affinity for the dye compared to that of avian albumin. Thus, the BCG method has not been validated in avian plasma samples and may be inaccurate at the low albumin concentrations found in avian plasma.[22]

Protein electrophoresis provides a more accurate measure of the albumin concentration as well as those of other plasma proteins; however, there are species differences in albumin migration on gel electrophoresis.[49] The total protein concentration as obtained by the biuret method combined with electrophoretic separation of plasma proteins provide an accurate absolute concentration of the plasma proteins. The primary plasma protein fractions include albumin, alpha globulins (alpha-1 and alpha-2), beta globulins (beta-1 and beta-2), and gamma globulin.[20] A transthyretin (prealbumin) fraction may be present in some species (e.g., psittacines) and absent in others (e.g., waterfowl and raptors). Normal concentrations for transthyretin, albumin, alpha globulin, beta globulin, and gamma globulin as obtained by protein electrophoresis from birds vary and require species-specific reference intervals.[14,20,22]

The normal albumin: globulin (A:G) ratio for most psittacine birds is between 1.2 and 3.6 when transthyretin is included with the albumin fraction, or 0.6–2.2 if it is not. Transthyretin can be considered to be an acute-phase reac-

tant, which is reason not to include it with the albumin fraction. β-globulin predominates in the globulin electrophoretic fraction in psittacine birds; whereas α-1 globulin predominates in waterfowl and raptors. Acute-phase proteins in cases of inflammation typically result in increases of the alpha (specifically α-2 globulins) and total globulin fractions of the electrophoretic tracing. Increases in α-2 globulins may also signal hepatic disease in psittacine birds. Chronic inflammatory disorders such as aspergillosis, sarcocystosis, and chlamydophilosis are often associated with increases in β-globulins. The γ-globulin fraction is composed of immunoglobulins such as IgA, IgM, IgG, and IgE, which increase with a humoral immune response. Some of the immunoglobulins (e.g., IgM and IgA) may migrate into the beta globulin fraction. A polygammopathy is indicative of active chronic inflammatory diseases, especially those associated with infectious agents such as *Chlamydophila*, *Aspergillus*, and *Mycobacterium* spp. Decreased γ-globulin fractions may be indicative of immunodeficiency.

Hyperproteinemia in most birds is indicated by plasma total protein (biuret) concentrations of greater than 4.5 g/dL (45 g/L). Hyperproteinemia is usually the result of dehydration, acute or chronic inflammation, or a preovulatory condition in hens. Increased albumin and globulin concentrations with a normal A:G ratio are commonly associated with dehydration. Hyperproteinemia caused by hyperglobulinemia with hypoalbuminemia results in a decreased A:G ratio and is frequently associated with chronic inflammatory diseases in birds, especially with diseases such as Chlamydophilosis, aspergillosis, tuberculosis, and egg-related coelomitis. A decreased A:G ratio with a normal total plasma protein concentration may also occur with these chronic disorders. Hyperproteinemia associated with normal albumin and elevated globulin concentrations is suggestive of acute inflammatory diseases or preovulatory conditions in egg-laying hens. With acute inflammation, electrophoretic patterns typically demonstrate increased concentration of alpha and/or beta globulins; however, gamma globulin increases may also be observed. Hens undergoing active folliculogenesis will exhibit a hyperproteinemia with a decreased A:G ratio as a result of estrogen-induced production of yolk proteins that migrate with globulins on electrophoresis. Hyperproteinemia associated with hyperalbuminemia and hypoglobulinemia results in an increased A:G ratio and suggests dehydration in birds with low plasma globulin concentrations. Dehydrated birds subjected to chronic stress or other immunosuppressive conditions may demonstrate this type of plasma protein profile.

Hypoproteinemia in birds can be associated with hypoalbuminemia and hypoglobulinemia. This can occur with overhydration during fluid therapy or a proportional loss of albumin and globulins. The latter can be associated with severe blood loss, effusions (especially birds with heart failure resulting in large volumes of intracoelomic effusion), and protein-losing enteropathies (e.g., intestinal parasitism and bacterial enteritis).

Hypoproteinemia in birds associated only with a hypoalbuminemia occurs with starvation, malnutrition, intestinal malabsorption, and gastrointestinal parasites leading to amino acid deficiencies. Hypoproteinemia also occurs with liver failure resulting in a decreased production of albumin or renal disease resulting in an increased loss of albumin in the urine.

Fibrinogen concentration is often evaluated in raptors to detect the presence of inflammatory diseases, especially those associated with bacterial infections. A plasma total protein/fibrinogen ratio in raptors that is less than 1.5 is indicative of inflammation and possible bacterial infection, whereas a ratio greater than 5 indicates dehydration.[22]

Laboratory evaluation of glucose metabolism

In general, the blood glucose concentration in normal birds ranges from 200 to 500 mg/dL (11.10–27.76 mmol/L). The plasma glucose concentration varies according to a circadian rhythm; however, this variation is clinically insignificant in healthy birds. The normal blood glucose concentration is maintained by hepatic glycogenolysis during short-term fasting. Specifically, short-term fasting (e.g., 1–8 days) in birds does not decrease glucose utilization per unit body weight, as it does in fasted mammals.[24] During fasting, the greatest energy loss is associated with fat depletion and protein mobilization, thereby resulting in the loss of body weight in birds, which is seen as a reduction in the pectoral muscle mass. The blood glucose concentration remains remarkably stable during short-term fasting in birds, and it is more stable in carnivorous birds during prolonged periods of fasting compared with granivorous birds.

Blood glucose regulation varies with avian species. Whereas insulin plays a key role in mammalian and carnivorous bird glucose homeostasis, glucagon plays a major role in the maintenance of normal blood glucose concentrations in granivorous birds.[24,31] This idea is supported by the relative abundance of alpha cells in the pancreas of granivorous birds while the distribution of the pancreatic islet cells of carnivorous birds resembles that of mammals. Granivorous birds also have a lower insulin:glucagon ratio compared with that of mammals. Their pancreas insulin content is less than 20% of the insulin content of a mammalian pancreas while the glucagon content is 2 to 5 times greater. Also, pancreatectomy induces hypoglycemia in granivorous birds, but hyperglycemia in carnivorous birds.

Glucagon is produced by pancreatic alpha cells that maintain normal plasma concentrations of 1–4 ng/mL, which is 10–50-fold greater than the normal mammalian concentrations.[24] The blood glucagon concentration increases by

100–200% in birds during a 24–48-hour fast. As a result, the blood glucose concentration increases along with an increase in free fatty acids, insulin, and cholecystokinin. On the other hand, glucose decreases the release of pancreatic glucagon. A significant increase in glucose stimulates the release of insulin from pancreatic beta cells.

Hypoglycemia in most birds is indicated by blood glucose concentrations of less than 200 mg/dL (11.10 mmol/L) and results from prolonged starvation, severe liver disease (e.g., Pacheco disease), septicemia, enterotoxemia, or endocrine disorders (e.g., hypothyroidism). Delayed separation of serum or plasma from avian blood cells does not significantly decrease the glucose level in the sample, as it does in mammalian blood, because avian erythrocytes use fatty acids rather than glucose for energy.[24] Hypoglycemia-induced seizures is a common disorder of falcons in which their blood glucose can fall as low as 80 mg/dL (4.44 mmol/L) with sudden exercise following prolonged food restriction during flight training.[22]

In general, hyperglycemia in most birds is indicated by blood glucose concentrations of greater than 500 mg/dL (27.76 mmol/L). Hyperglycemia occurs with diabetes mellitus, catecholamine release, and glucocorticosteroid excess, such as occurs with stress or administration of corticosteroids. Excess glucocorticosteroids result in a mild to moderate increase in the blood glucose concentration (≤600 mg/dL (33.31 mmol/L)) in birds. Exertion, excitement, and extreme temperatures stimulate the release of catecholamines, which also results in a mild to moderate increase in the blood glucose concentration. Glucose concentrations of greater than 700 mg/dL (38.86 mmol/L) are suggestive of diabetes mellitus in most birds. The pathophysiology of diabetes mellitus in birds varies among different species and may result from increased glucagon secretion or hypoinsulinemia. Although glucagon appears to have a major role in glucose metabolism in granivorous birds, those with diabetes mellitus have been shown to respond to insulin therapy. Birds with diabetes mellitus have polyuria and urinary glucose concentrations exceeding 1 mg/dL (0.055 mmol/L). Pancreatic islet cell tumors and pancreatitis (bacterial and viral etiologies) have been suggested as being causes of diabetes mellitus in psittacine birds. Detection of pancreatitis by increased serum amylase activity has been occasionally reported in birds. In some species (e.g., toucans, Ramphastidae), diabetes mellitus occurs commonly and appears to be related to a fruit diet. Excessive iron storage, another common disorder of toucans, can also be associated with diabetes mellitus in birds.

Plasma fructosamine and β-hydroxybutyric acid concentrations appear to be useful when monitoring the avian patient being treated for diabetes mellitus. It has been demonstrated that both analytes increase in diabetic birds with prolonged hyperglycemia and decrease following the correction of the hyperglycemic state with insulin therapy. Based on euglycemic psittacine birds, reference intervals for fructosamine are 113–238 μmol/L and intervals for β-hydroxybutyric acid are 450–1422 μmol/L. It has been demonstrated that blood glycated hemoglobin (HbG) can also be an indicator of the blood glucose status of the ostrich (*Struthio camelus*) and kestrel (*Falco sparverius*).[47] The glycated hemoglobin of these birds is 1.2 ± 0.20% of the total hemoglobin compared to 3–6% in most mammals. The relatively low normal HbG of birds appears to be related to the lower permeability of the avian erythrocyte membrane to blood glucose. Blood glycated hemoglobin reflects the glucose status of the patient for a longer period of time than does fructosamine concentration because it reflects the blood glucose concentration during the lifespan of the erythrocyte. Although measurement of glycated hemoglobin may be useful in monitoring recovery from the hyperglycemic state, it takes longer to detect changes than does measurement of fructosamine because of its long life span in erythrocytes.

Laboratory detection of exocrine pancreatic insufficiency

Exocrine pancreatic insufficiency is a disorder resulting from an insufficient secretion of pancreatic digestive enzymes. Clinical signs associated with this disorder result from digestive malabsorption and include voluminous feces with a chalklike appearance, polyphagia, and weight loss. This disorder is a result of a severe progressive loss of pancreatic acinar tissue, either from atrophy or destruction associated with an inflammatory disease (pancreatitis). A presumptive diagnosis can be obtained based upon clinical signs and response of affected birds to oral pancreatic enzyme supplementation. In birds there has been limited use of plasma assays for the activities of pancreatic digestive enzymes such as amylase and lipase that may leak from injured pancreatic cells into the blood. Reference values for pigeons indicate normal plasma amylase activity to be between 382 and 556 IU/L and lipase activity between 0 and 5 IU/L.[2]

Laboratory detection of muscle injury

Creatine kinase (CK) is a muscle-specific enzyme in birds that can be used to detect muscle cell damage.[5,8] The normal plasma CK activity in most species ranges from 100 to 500 IU/L. Increased plasma CK activity can result from muscle cell injury or marked exertion and is frequently observed in birds with seizure disorders or birds that are struggling against restraint during blood collection. Muscle

tissue damage can occur with traumatic injury, intramuscular injections of irritating fluids, or systemic infections that affect the skeletal or cardiac muscle.

Skeletal muscle injury should also be considered when plasma AST activity is increased. Measurement of plasma CK activity can be useful for determining if muscle injury versus hepatocellular injury is the cause of increased plasma AST activity. Thus, an increased plasma AST activity without an increased plasma CK activity is suggestive of hepatocellular disease in birds; however, other tests should also be employed to fully evaluate the presence of hepatic disease in birds. (See discussion under Laboratory Evaluation of the Avian Liver.) Severe skeletal muscle injury often results in marked increases of the plasma CK activity and moderate increases of the plasma AST activity. Plasma AST appears to have a longer half-life (mean of 7.10 hr from muscle) than CK (mean of 3.07 hr from muscle), and after a single insult to muscle, as may occur with an intramuscular injection of an irritating drug, the CK activity may return to normal before the AST activity.[34,38] Regarding situations in which the plasma CK activity has returned to normal after muscle injury but the plasma AST activity remains increased, an erroneous diagnosis of hepatobiliary disease may be made if those two enzymes are the only analytes examined. Unlike plasma CK activity, plasma AST activity does not usually increase significantly with normal capture and restraint of struggling birds, but under these conditions, a bird with a preexisting hepatocellular injury may have increases in both the AST and the CK activity.

Increased plasma ALT activity may occur with muscle injury. Plasma ALT appears to have a longer half-life (mean of 11.99 hr from muscle) compared with plasma CK (mean of 3.07 hr from muscle); therefore, activity of ALT remains increased longer than does CK after muscle injury.[34,38]

Plasma LD activity also increases with muscle injury. Because the plasma half-life of LD (mean of 0.48 hr from muscle)is shorter than that of CK (mean of 3.07 hr from muscle), the two enzymes can be evaluated concurrently to differentiate hepatocellular damage from muscle injury.[34,38] In most birds, increased plasma LD activity with normal CK activity is suggestive of hepatocellular disease. Validation and evaluation of LD isoenzymes may be helpful in differentiating hepatic versus muscle disorders; however, most veterinary laboratories do not routinely offer LD isoenzyme determination.

Capture myopathy in Sandhill cranes (*Grus Canadensis tabida*) was associated with increased plasma activity of CK, AST, and LDH within 1 hour of capture.[8] The peak activity of these enzymes occurred 3 days following capture and by 10–17 days, CK and LDH activities had returned to normal; however, plasma AST activities were still 2–5 times higher than normal reference values.

Laboratory evaluation of endocrine disorders

Thyroid

Both thyroxine or tetraiodothyronine (T_4) and triiodothyronine (T_3) have been isolated from birds.[32,33,44,55] Most of the secreted hormone is T_4, whereas T_3 is formed peripherally by deiodination of T_4 and in comparison to mammals, birds contain less T_4, but similar T_3 concentrations. The plasma or serum T_4 concentration of adult birds of many species ranges between 5 and 15 ng/mL (6–19 pmol/mL) and the T_3 concentration ranges between 0.5 and 4 ng/mL (0.7–1.5 pmol/Ml)[44] Circulating T_4 and T_3 are bound to protein. A T_4-binding globulin is absent in birds; therefore, most of the thyroid hormones are bound to albumin. These hormones also are bound secondarily to other proteins (e.g., transthyretin (prealbumin) and alpha globulin). The binding of thyroid hormones to albumin and other blood proteins is weak, however, thereby resulting in greater free-T_4 percentages in the blood of birds compared with those in mammals.[44] Compared with T_4, T_3 is more metabolically active at the cellular level. The ratio of T_4 to T_3 in blood varies with the species, however. Thyroid hormones are excreted primarily in the bile and urine. In birds, T_3 and T_4 have relatively short half-lives compared with those in mammals, and a significant diurnal rhythm is more easily demonstrated in birds compared with mammals. In chickens, plasma T_4 concentrations decrease during the light phase and increase during the dark phase of the light cycle. Plasma T_3 concentrations behave in the opposite manner. The pattern of food intake may influence this rhythm.

Competitive protein binding and radioimmunoassay are sensitive methods used to measure the plasma or serum T_4 and T_3 concentrations in birds. Protein-bound iodine determination is not a sensitive test for iodine-containing hormones in birds, primarily because avian blood contains a large amount of nonhormonal iodo-proteins compared with mammalian blood.

Secretion of thyroid hormones by the thyroid gland is governed by the concentration of circulating thyroid hormones. A decrease in the circulating concentration of thyroid hormones stimulates the pituitary gland to release thyrotropin-releasing hormone, which stimulates the release of thyrotropin (thyroid-stimulating hormone [TSH]) via neuroendocrine-controlled mechanisms. In turn, the release of TSH stimulates the secretion of thyroid hormones.

A TSH-stimulation test has been used to evaluate thyroid function in a variety of birds.[32,33] In general, a prestimulation plasma T_4 concentration is obtained to compare with a poststimulation sample, which is obtained 4 to 6 hours after the intramuscular administration of TSH. A dosage of 1 IU of TSH per bird, regardless of the body weight, is typically used.

A normal response is indicated by a 2.5-fold or greater increase in the T_4 level after TSH stimulation. Responses of lesser magnitude are suggestive of hypothyroidism. Measurements of T_3 concentrations appear to be inconsistent and unreliable. Low baseline total T_4 concentrations are poor indicators of hypothyroidism in birds, because many healthy birds normally have low T_4 concentrations. This may, however, reflect diurnal variation. Also, other conditions (e.g., stress and systemic disease) can decrease plasma T_4 concentrations. Therefore, TSH-stimulation testing provides a more reliable method for detecting hypothyroidism in birds.[21]

Other blood biochemical abnormalities often associated with hypothyroidism in birds include increases in cholesterol, triglycerides, uric acid, AST, and LDH. Hypothyroidism in birds may also result in a mild normocytic, normochromic nonregenerative anemia.

Hyperthyroidism is rare in birds. An increased plasma concentration of T_4 and T_3 is suggestive of hyperthyroidism.

Parathyroid

The primary function of parathyroid hormone (PTH), secreted by the parathyroid gland, is maintenance of the normal plasma calcium concentration by its action on bone, kidney, and intestinal mucosa. Unfortunately, plasma PTH analysis in birds is commercially unavailable. Therefore, detection of disorders associated with abnormal blood PTH concentrations (e.g., hyper- and hypoparathyroidism) depends on the evaluation of blood calcium and phosphorus concentrations in birds. Hypoparathyroidism has not been reported in birds.

Adrenal

Corticosterone is the primary glucocorticoid that is produced by the avian adrenal gland.[23] Corticosterone secretion in birds is regulated by adrenocorticotropic hormone (ACTH), which is released from the pituitary gland in response to corticotropin-releasing factor. In birds given an intramuscular injection of ACTH, plasma corticosterone concentrations peak in 3 hours followed by a sharp decrease at 4 hours. The plasma corticosterone concentration increases during times of stress. Corticosterone also has mineralocorticoid activity. Plasma corticosterone concentrations exhibit a diurnal rhythm as well, with maximum concentrations occurring at the beginning of the day. Plasma corticosterone concentrations are also influenced by other physiologic factors, such as the ovulatory cycle of hens and changes in the seasons.

Plasma corticosterone concentrations in birds can be determined by radioimmunoassay. Single baseline corticosterone determinations may have little value in establishing the diagnosis of hyperadrenocorticism in birds. An ACTH-stimulation test may be more valuable, however, when pre- and 60- to 90-minute post–ACTH stimulation corticosterone concentrations are compared. Normal birds should demonstrate a greater than 10-fold increase in post–ACTH stimulation plasma corticosterone concentration compared with pre–ACTH stimulation concentration. Stimulation dosages of 50 and 125 mg of ACTH have been used in birds for this test.[32]

Hyperadrenocorticism in birds is usually iatrogenic, caused by excessive administration of exogenous glucocorticosteroids; excess endogenous corticosterone is rare. The effects of glucocorticosteroid administration are variable and dependent on both dose and duration. Hematologic changes include lymphopenia, leukocytosis, and heterophilia. Blood biochemical changes include hypercholesterolemia and mild hyperglycemia, with blood glucose concentrations ranging between 500 and 600 mg/dL (27.76–33.31 mmol/L). Glucosuria should also be expected.

Adrenal insufficiency (i.e., Addison disease) is also rare in birds. The decreased production of corticosterone or aldosterone (or both) in this condition results in a decreased plasma sodium:potassium (Na:K) ratio. A Na:K ratio of less than 27, hypoglycemia, hypercalcemia, and low urine specific gravity are suggestive of adrenal insufficiency. Because hyperkalemia from other causes (e.g., delayed separation of plasma from cells, acidosis, hemolysis, and renal disease) may result in a decreased Na:K ratio, an ACTH-stimulation test may be helpful in confirming the presence of adrenal insufficiency.

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36 Clinical Chemistry of Reptiles

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Normal reference values for specific blood biochemical tests have been established for only a few of the 7,500 or so reptilian species. Reference values for snakes include Australian elapid snakes, ball python (*Python regius*), common boa constrictor (*Constrictor constrictor*), common kingsnake (*Lampropeltis getulus*), diamond-backed water snake (*Nerodia rhombifera rhombifera*), Eastern massasauga rattlesnake (*Sistrurus catenatus catenatus*), emerald tree boa (*Coralius caninus*), gopher snake (*Pituophis melanoleucus*), green tree python (*Chondropython viridis*), indigo snake (*Drymarchon coralis*), jungle carpet python (*Morella spilota cheynei*), milk snake (*Lampropeltis triangulum*), rainbow boa (*Epicrates cenchiria*), red rat snake (*Elaphe guttata*), reticulated python (*Python reticulatus*), South American rattlesnake (*Crotalus durissus terrificus*), and yellow rat snake (*Elaphe obsoleta quadrivittata*).[1,9,30,32] Reference values for lizards include bearded dragons (*Pogona vitticeps*), blue-tongued skink (*Tillqua scincoldes*), Egyptian spiny-tailed lizard (*Uromastyx aegyptius*), green crested basillsk (*Basilliscus plumifrons*), green iguana (*Iguana iguana*), Nile monitor (*Varanus exanthematicus*), Savannah monitor (*Varanus niloticus*), Tegu lizard (*Tupinambus teguixin*), and Tokay gecko (*Gecko gecko*).[9,32] Reference values for chelonians include African spurred tortoise (*Geochelone sulcata*), Aldabra tortoise (*Geochelone* sp.), common box turtle (*Terrapene carolina*), desert tortoise (*Gopherus agassizii*), gopher tortoise (*Gopherus polyphemus*), green sea turtle (*Chelonia mydas*), leopard tortoise (*Geochelone* pardalis), loggerhead sea turtle (*Caretta caretta*), Mediterranean pond turtle (*Mauremys leprosa*), Mediterranean tortoise (*Testudo* spp.), ornate box turtle (*Terrapene ornata*), painted turtle (*Chrysemys picta*), radiated tortoise (*Testudo radiate*), red-eared slider (*Trachemys scripta*), red-footed tortoise (*Geochelone carbonaria*), and star tortoise (*Geochelone elegans*).[9,16,23,32,34] Reference values for crocodilians include American alligator (*Alligator mississippiensis*) and dwarf caiman (*Paleosuchus palpebrosus*).[9]

Environmental conditions, such as temperature, season, geographic area, ecological habitat, and wild versus captive status as well as physiologic factors, such as species, nutritional status, reproductive status, gender, and age affect the blood analytes of reptiles.[8,13,15,17,19,29,31,32] It is well recognized that significant metabolic disorders occur in captive reptiles exposed to unhealthy conditions, such as inadequate nutrition, improper environment, and overcrowding. These factors often have not been considered when establishing reference intervals, thereby making those intervals less meaningful. Methods of sample collection, handling, and biochemical analysis are additional sources of variation in the published reference values. Sample collection and handling are specifically problematic for blood biochemical studies of wild reptile populations. Therefore, published references are generally used as a broad guide to the interpretation of blood biochemical results in reptiles. Because of the difficulty in obtaining meaningful reference intervals for each reptilian species seen in clinical practice, most clinicians use decision levels or common ranges when assessing such patients. As discussed with the interpretation of avian blood biochemical results (see Chapter 34, Clinical Chemistry of Birds), decision levels may vary among clinicians dealing with reptiles, depending on laboratory results and experience. The values suggested in this text are general guidelines that can be used as decision levels when evaluating each analyte in the reptilian blood biochemical profile. As suggested with valued avian patients, the process of evaluating the blood chemistries of reptilian patients can be refined by obtaining a set of normal values from that patient when housed under a given set of environmental and nutritional parameters. When that patient becomes ill, a more meaningful set of reference values, which are specific for that individual patient, can be used to evaluate the chemistry results.

Blood biochemistry profiles are frequently used to assess the health of reptilian patients; however, controlled studies

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designed to clarify the meaning of changes in the blood chemistries of reptiles compared with those of domestic mammals generally are lacking. Therefore, reptilian clinical chemistry has not achieved the same critical evaluation as that in domestic mammalian medicine. In general, interpretations of reptilian blood biochemistries are considered to be the same as those for domestic mammals, with the consideration that external factors (e.g., environmental conditions) have greater influence on the normal physiology and health of ectothermic vertebrates compared with endotherms. Reptilian blood biochemistries are influenced by species, age, gender, nutritional status, season, and physiologic status, thereby making the interpretation of results challenging.

Sample collection and handling

Blood samples for biochemical studies can be collected from reptiles using a variety of methods; the choice depends on the species, needed volume, size of the reptile, physiologic condition of the patient, and preference of the collector (see Chapter 20, Hematology of Reptiles).[26] Depending on the collection site, blood samples from reptiles, especially chelonians, are often contaminated with lymphatic fluid. It has been suggested, based upon evaluation of plasma and lymph samples in red-eared sliders (*Trachemys scripta elegans*) that most of the analytes in lymph (i.e. glucose, calcium, phosphorus, sodium, urea, and enzymes) are comparable with those of plasma or serum in reptiles while a few others (i.e. total protein and potassium) have a significantly lower concentrations in lymph compared with blood.[11] The same comparisons, however, may not be true to all species of reptiles. Therefore, the amount of lymph contamination in the blood sample should be considered when interpreting the blood biochemical parameters of reptiles. The best results are obtained from blood samples that are uncontaminated with lymph.

Many clinicians prefer to collect blood using an anticoagulant (e.g., lithium heparin) for plasma biochemical testing of reptiles, primarily because a greater sample volume can be achieved for plasma compared with serum. Collection of blood into lithium heparin also allows for evaluation of both the hemogram and blood biochemistries using one sample. Plasma is preferred over serum because clot formation in reptilian blood is unpredictable and often prolonged, thereby producing significant changes in some of the chemistries (e.g., serum electrolytes). Reptilian blood clots slowly because of a low intrinsic thromboplastin activity and a strong natural, circulating antithrombin factor, which compensates for the sluggish flow of blood.

The ideal sample for biochemical testing is obtained by separating the cells by centrifugation from the plasma immediately after blood collection. This may be difficult to achieve in field studies. The reason for immediate separation is that prolonged contact between the plasma and the cells may cause an artifactual decrease in plasma glucose due to cellular metabolism and an increase in plasma potassium due to leakage from the cells. Also, as erythrocytes age, the cell membranes become increasingly porous resulting in hemolysis which affects biochemical testing of the blood. In mammals, hemolysis may, depending upon the specific assay used, result in increases in potassium, calcium, phosphorus, magnesium, glucose, aspartate aminotransferase, alanine aminotransferase, lactate dehydrogenase, creatine kinase, total bilirubin, total protein, lipase, and amylase with decreases in creatinine, alkaline phosphatase, and bile acids. In the green iguana (*Iguana iguana*); however, hemolytic plasma samples revealed no change in sodium, calcium, uric acid, or creatine kinase, but did reveal an increase in potassium, phosphorus, total protein, albumin, and aspartate aminotransferase.[4] Increased storage time or temperature resulted in a decrease in plasma sodium in two species of tortoises with variable effects on potassium. Blood from loggerhead turtles (*Caretta caretta*) stored up to 24 hours at 4°C (39°F) before the cells were separated from the plasma did not reveal significant changes in the majority of the biochemical analytes (only plasma γ-glutamyltransferase demonstrated significant decreased activity).[18] In the same study, there were also no significant differences in plasma biochemistries between blood collected in lithium heparin or sodium heparin.

Depending upon the analytic method, the sample collected from small reptiles is often of sufficient volume for only a few tests and not for a complete panel. Therefore, the clinician must decide which tests are most beneficial in the evaluation of the reptilian patient. Blood biochemical tests that appear to be most useful include total protein, glucose, uric acid, aspartate aminotransferase (AST), creatine kinase (CK), calcium, and phosphorus. Other tests that may be helpful include creatine, lactate dehydrogenase (LD), sodium, potassium, chloride, total CO_2, and protein electrophoresis. Modern blood chemistry analyzers can perform many of these tests using a small sample volume (10–30 µL). Commercial veterinary laboratories often offer chemistry profiles that require a minimal volume of serum or plasma (0.5 mL). Blood chemistry analyzers that use dry reagents and reflectance photometry for "in-house" testing may be used for reptilian samples and often require smaller sample volumes.

The plasma of most reptiles is colorless; however, it may be orange to yellow because of carotenoid pigments in the diets of herbivores such as the green iguana (*Iguana iguana*). The plasma of some snakes, such as pythons, may be a greenish yellow because of dietary carotenoids and riboflavin. Some lizards normally have green plasma because of high concentrations of biliverdin.[3]

Laboratory evaluation of reptilian kidneys

The paired kidneys of many reptilian species are located within the pelvic canal. The elongated kidneys of snakes are located in the dorsal caudal part of the coelomic cavity, with the right kidney being cranial to the left. The ureters of snakes empty into the urodeum of the cloaca, as they do in birds. Most species of lizards and chelonians (i.e., turtles, tortoises, and terrapins) have a urinary bladder; however, it differs from that of mammals in that the ureters of these reptiles do not empty directly into the bladder but empty into the urodeum of the cloaca.[25] Terrestrial chelonians and, possibly, lizards use the urinary bladder primarily for water storage.

The reptilian renal cortex contains only simple nephrons (i.e., cortical nephrons) with a tubular system devoid of loops of Henle.[13] Therefore, reptiles cannot concentrate their urine. Nitrogenous wastes excreted by the reptilian kidney include variable amounts of uric acid, urea, and ammonia, depending on the animal's natural environment. Freshwater turtles that spend much of their lives in water excrete equal amounts of ammonia and urea, whereas those with amphibious habits excrete more urea.[13] Sea turtles excrete uric acid, ammonia, and urea. Alligators excrete ammonia and uric acid. Terrestrial reptiles such as tortoises must conserve water, and ammonia, urea, and other soluble urinary nitrogenous wastes require large amounts of water for excretion. Therefore, to conserve water, terrestrial reptiles produce more insoluble nitrogenous waste in the form of uric acid and urate salts, which are eliminated in a semisolid state.

Blood biochemical detection of renal disease in reptiles is more difficult than in mammals because of the physiologic differences in their kidneys. Blood urea nitrogen (BUN) and creatinine concentrations generally are poor indicators of renal disease in reptiles; however, plasma urea nitrogen concentrations may be more useful in the evaluation of renal disease among aquatic reptiles that primarily excrete urea. Because terrestrial reptiles primarily are uricotelic, the normal urea nitrogen concentration in these species is less than 15 mg/dL (<5.36 mmol/L), with the exception of terrestrial chelonians (especially desert species), which typically have plasma urea nitrogen concentrations that vary from 30 to 100 mg/dL (10.71–35.70 mmol/L). This is considered to be a mechanism to elevate the plasma osmolarity and reduce water loss from the body.[27] The plasma osmolarity of freshwater turtles and crocodilians is approximately the same as that of common domestic mammals, but it is higher in terrestrial reptiles. An increase in plasma urea nitrogen concentration in reptiles may be suggestive of severe renal disease, prerenal azotemia, or a high dietary urea intake; however, it does not reliably increase under these conditions.

Free-ranging desert tortoises (*Gopherus agassizii*) demonstrate a "water metabolism strategy" whereby blood urea nitrogen, uric acid, and osmolality values respond to the amount of available forage and water as determined by rainfall. In a study by Dickenson *et al.* (2002), free-ranging tortoises that exhibited increased plasma uric acid, sodium, and potassium concentrations with decreased osmolality and urea nitrogen were considered to be actively consuming water along with protein and electrolyte rich forage.[16] This is in contrast to the same population of tortoises that were not consuming significant amounts of food or water as suggested by increased plasma osmolality and urea nitrogen along with decreased uric acid concentration. Increased urea nitrogen concentration was considered to be a reflection of increased protein catabolism and perhaps dehydration. Increased uric acid concentration was considered to be an indication of increased dietary protein intake. Tortoises that were feeding with restricted water intake revealed higher plasma osmolality with decreased BUN and increased uric acid concentrations. During wet seasons, plasma osmolality and uric acid, urea nitrogen, potassium, and sodium concentrations were lower than other seasons due to increased rates of water consumption and bladder emptying. Plasma concentrations of these analytes in captive tortoises having constant access to water are expected to resemble free-ranging tortoises in wet years.

Creatinine is a normal constituent of mammalian urine, but the amount formed in most reptiles is negligible (< 1mg/dL or 88.4 µmol/L).[8] The blood creatinine concentration is generally considered to be of poor diagnostic value in the detection of renal disease in reptiles. By contrast, the blood creatine concentration may have diagnostic value in the detection of renal disease in some reptilian species, but the test is unavailable from most veterinary laboratories.

Uric acid is the primary catabolic end product of protein, nonprotein nitrogen, and purines in terrestrial reptiles, and it represents 80–90% of the total nitrogen excreted by the kidneys.[19] The normal blood uric acid concentration in most reptiles is less than 10 mg/dL.

Hyperuricemia is indicated by uric acid values of greater than 15 mg/dL, and is usually associated with renal disease. Renal diseases that are associated with hyperuricemia include severe nephritis, nephrocalcinosis, and nephrotoxicity. Hyperuricemia is not sensitive or specific for renal disease in reptiles. Hyperuricemia associated with renal disease most likely reflects the loss of two-thirds (or more) of the functional renal mass. Hyperuricemia in reptiles can also be associated with gout or recent ingestion of a high-protein diet. Carnivorous reptiles tend to have higher blood uric acid concentrations than herbivorous reptiles, and their plasma uric acid concentrations generally peak the day after a meal, thereby resulting in a 1.5–2.0-fold increase in uric acid.[19] Gout can result from an overproduction of uric acid (i.e., primary gout) or from an acquired disease that interferes with the normal production and excretion of uric acid (i.e., secondary gout). Conditions that result in secondary gout among reptiles include starvation, renal disease (especially

tubular damage), severe and prolonged dehydration, and excessive dietary purines (i.e., herbivorous reptiles fed diets rich in animal proteins). Hyperuricemia associated with renal disease and gout often results in greater than twofold increases in uric acid concentrations.

The reptilian kidney has high alanine aminotransferase (ALT) and alkaline phosphatase activity. Significant increases in the plasma activities of these enzymes, however, do not occur with renal disease, because most of the enzymes released from damaged renal cells are released in urine, not in plasma.[30]

Reptiles rarely exhibit polyuria with renal disease. Therefore, urinalysis rarely is performed to assess renal disease because a lack of available urine to perform the tests.

The normal glomerular filtration rate (GFR) based upon iohexol clearance has been established for the green iguana (*Iguana iguana*) and can be used to evaluate kidney function in that species.[21,22] Reported values are 14.78–18.34 mL/kg/hr (mean and standard deviation of 16.56 ± 3.90 mL/kg/hr).

Electrolytes and acid base

Water balance

Species, diet, and environmental conditions such as temperature and humidity influence the water consumption of reptiles. Desert species require less water than temperate and tropical species. Some reptiles have developed methods for conserving water. For example, tortoises and some lizards store water in the urinary bladder. Many reptiles can achieve water uptake through the cloaca by soaking. Water also is conserved in reptiles by the elimination of nitrogenous waste in the form of uric acid and urate salts, which are excreted in a semisolid state.

Sodium and chloride

Dietary sodium is absorbed in the intestines and transported to the kidneys, where it then is excreted or resorbed, depending on the reptile's need for sodium. Some reptiles have nasal salt glands that participate in the regulation of sodium, potassium, and chloride concentrations in the blood. Therefore, disorders of the salt gland may affect the electrolyte balance.

The normal serum or plasma sodium concentration ranges between 120 and 170 mEq/L. The normal plasma sodium concentrations of tortoises and freshwater turtles range between 120 and 150 mEq/L (mmol/L). Sea turtles tend to have higher normal sodium plasma concentrations, which range between 150 and 170 mEq/L (mmol/L). The normal plasma sodium concentrations of lizards range between 140 and 170 mEq/L (mmol/L), and those of snakes, such as boas and pythons, range between 130 and 160 mEq/L (mmol/L). Hyponatremia can result from excessive loss of sodium associated with disorders of the gastrointestinal tract (i.e., diarrhea), kidneys, or possibly, the salt gland. Iatrogenic hyponatremia can occur with overhydration when administering intravenous or intracoelomic fluids that are low in sodium. Hypernatremia results from dehydration, either from excessive water loss or inadequate water intake, or from excessive dietary salt intake.

Chloride is the principle anion in the blood, and along with sodium, represents the primary osmotically active component of plasma in most reptiles. The normal serum or plasma chloride concentration of reptiles varies among species but generally ranges between 100 and 130 mEq/L (mmol/L). Plasma chloride concentrations of turtles tend to range between 100 and 110 mEq/L (mmol/L), whereas those of most lizards and snakes range between 100 and 130 mEq/L (mmol/L). The blood chloride concentration provides the least clinically useful information regarding the electrolytes. Hypochloremia in reptiles is rare and, when present, is suggestive of the excessive loss of chloride ions or of overhydration with fluids that are low in chloride ions. Hyperchloremia is associated with dehydration and, possibly, renal tubular disease or disorders of the salt glands.

Potassium

Normal serum or plasma potassium concentrations vary among reptilian species, but they generally range between 2 and 6 mEq/L (mmol/L). The normal plasma potassium concentration of most turtles, lizards, and snakes ranges between 2 and 6, 3 and 5, and 3 and 6 mEq/L (mmol/L), respectively. The amount of potassium in erythrocytes also differs among reptiles; therefore, the potential for artifactual hyperkalemia due to hemolysis varies with species.[4] Common imbalances of serum or plasma potassium include inadequate dietary potassium intake or excessive gastrointestinal potassium loss (i.e., hypokalemia) or decreased renal secretion of potassium (i.e., hyperkalemia). Hypokalemia can also be associated with severe alkalosis. Hyperkalemia can also result from excessive dietary potassium intake or severe acidosis.

Acid-base

The normal blood pH of turtles and most other reptiles ranges between 7.5 and 7.7 at 23–25°C.[24] The normal blood pH of some snakes and lizards may fall below 7.4. The blood pH of reptiles is labile, however, and it changes with fluctuations in temperature. An increase in temperature or excitement may cause the blood pH to decrease.[31] The blood pH may increase to 7.7–7.8 during anesthesia. As in mammals, the oxygen dissociation curve for reptilian hemoglobin shifts to the left as the pH increases, thereby producing an increased affinity of hemoglobin for oxygen but a decreased release to tissues. The buffering systems that regulate blood pH in mammals are most likely the same in reptiles, with the bicarbonate/carbonic acid buffer system being the most important because of the rapid rate of CO_2 elimination via

the lungs after conversion from H_2CO_3. Total plasma CO_2 or bicarbonate concentrations are rarely reported in reptiles; however, normal total CO_2 values for most reptiles are expected to range between 20 and 30 mmol/L. A marked fasting physiologic metabolic alkalosis occurs in postprandial alligators because of an anion shift, with bicarbonate replacing chloride in the blood as chloride is lost (as HCl) via gastric secretions.[10] Therefore, a postprandial decrease of chloride and increase of bicarbonate concentrations are seen in alligators and perhaps other reptiles.

Calcium and phosphorus

Both blood calcium metabolism and the amount of ionized calcium in reptilian plasma are mediated by parathormone (PTH), calcitonin, and activated vitamin D_3 (1,25-dihydrocholecalciferol). Other hormones, such as estrogen, thyroxin, and glucagon, may also influence calcium metabolism in reptiles. The primary function of PTH is to maintain normal blood calcium levels by its action on bone, kidneys, and intestinal mucosa. Low blood levels of ionized calcium stimulate the release of PTH, which results in calcium mobilization from bone, increased calcium absorption from the intestines, and increased calcium reabsorption from the kidneys.

The exact role of calcitonin in reptiles is unknown, but it most likely has a physiologic role opposite that of PTH (i.e., inhibiting calcium resorption from bone). Increases in the blood calcium level stimulate the release of calcitonin from the ultimobranchial gland.

The active form of vitamin D_3 stimulates the absorption of calcium and phosphorus by the intestinal mucosa. Photochemical production of the active form of vitamin D_3 by exposure to ultraviolet radiation (wavelength, 290–320 nm) is believed to be essential for normal calcium metabolism in reptiles, especially basking species.

Female reptiles exhibit features of calcium metabolism similar to those of birds during egg production. During egg development, female reptiles exhibit hypercalcemia in response to estrogen and reproductive activity. The increase in total plasma calcium is associated with an increase in protein-bound calcium during follicular development before ovulation, and the total plasma calcium level may increase by two- to fourfold or more.[15]

The normal plasma calcium concentration for most reptiles ranges between 8 and 11 mg/dL (2.0–2.7 mmol/L), and it varies both with the species and the physiologic status of the reptile. For example, some species of tortoises have low blood calcium concentrations (<8 mg/dL or 2.0 mmol/L).[8] Gender differences have been reported for plasma calcium concentration in free-ranging populations of reptiles where females exhibit significantly greater calcium concentrations than males. This difference is likely to be associated with reproductive activity (vitellogenesis) at the time of sample collection. Regardless of age and gender, plasma ionized calcium concentrations remain consistent in healthy reptiles. The normal plasma ionized calcium concentration for healthy green iguanas (*Iguana iguana*) has been determined to be 1.47 ± 0.105 mmol/L (5.9 mg/dL ± 0.42).[14]

In most reptiles hypocalcemia occurs when the plasma calcium concentration is less than 8 mg/dL (2.0 mmol/L). Hypocalcemia can occur with dietary calcium and vitamin D_3 deficiencies, excessive dietary phosphorus, alkalosis, hypoalbuminemia, or hypoparathyroidism. Secondary nutritional hyperparathyroidism is a common disorder of herbivorous reptiles such as green iguanas (*Iguana iguana*).[20,28,29] Diets fed to captive herbivores are often deficient in calcium and contain excessive amounts of phosphorus. In addition, dietary deficiency in vitamin D_3 or lack of proper exposure to ultraviolet light predisposes reptiles to hypocalcemia. Juvenile reptiles (especially green iguanas) with secondary nutritional hyperparathyroidism commonly develop metabolic bone disease with fibrous osteodystrophy and bone fractures.[7] Adult reptiles often develop muscle tremors, paresis, and seizures with hypocalcemia. Carnivorous reptiles that are fed all-meat, calcium-deficient diets also develop hypocalcemia associated with nutritional imbalances in calcium and phosphorus. Secondary renal hyperparathyroidism may result in hypocalcemia as well.

Hypercalcemia in reptiles is indicated by a plasma calcium concentration of greater than 20 mg/dL (5.0 mmol/L). Typically, this is an iatrogenic condition that is associated with oversupplementation with oral or parenteral calcium and vitamin D_3. Other differentials for hypercalcemia include primary hyperparathyroidism, pseudohyperparathyroidism, and osteolytic bone disease; however, these disorders are rarely reported in reptiles.

The normal plasma phosphorus concentration for most reptiles ranges between 1 and 5 mg/dL (0.3 and 1.6 mmol/L). Gender differences have been reported for plasma phosphorus concentration in free-ranging populations of reptiles where females exhibit significantly higher concentrations than males. This difference is likely to be associated with reproductive (vitellogenesis) activity at the time of sample collection.

Hypophosphatemia may result from starvation or a nutritional deficiency of phosphorus. Hyperphosphatemia is indicated by a plasma phosphorus concentration of greater than 5 mg/dL (1.6 mmol/L). Disorders resulting in hyperphosphatemia include excessive dietary phosphorus, hypervitaminosis D_3, and renal disease. Rare causes of hyperphosphatemia include severe tissue trauma and osteolytic bone disease. In mammalian blood samples, an artifactual hyperphosphatemia can occur when serum or plasma is not promptly separated from the clot, thereby allowing phosphorus to be released from erythrocytes. A few studies have suggested this may be less likely with reptilian blood samples; however,

hyperphosphatemia has been related to hemolysis in reptilian blood samples.[4] The ideal sample for biochemical testing is obtained by the immediate separation of the cells from plasma with no hemolysis.

Laboratory evaluation of the reptilian liver

Liver enzymes in reptiles appear to be similar to those in birds and mammals. The LD and AST activities are high in reptilian liver tissue, and although few critical studies have examined the biochemical testing of reptilian blood to evaluate hepatic disease, increases in the plasma activities of these enzymes may suggest hepatocellular disease.[6] The plasma AST activity is not considered to be organ specific, because activity for this enzyme can be found in many tissues. In general, normal plasma AST activity for reptiles is less than 250 IU/L. Increased plasma AST activity suggests hepatic or muscle injury. Generalized diseases such as septicemia or toxemia, however, may damage these tissues, thereby producing increased plasma AST activity. Increased AST activity in the plasma of healthy free-ranging tortoises may be related to muscle activity and injury due to increased male aggression during the breeding season.

The plasma LD activity is also considered to have a wide tissue distribution in reptiles. Therefore, increases in the plasma LD activity(>1000 IU/L) may be associated with damage to the liver, skeletal muscle, or cardiac muscle. Hemolysis also may result in increased plasma LD activity.

Like AST, plasma ALT is not considered to be organ specific in reptiles. The normal plasma ALT activity for reptiles is usually less than 20 IU/L. Although ALT activity occurs in the reptilian liver, increases in the plasma ALT activity may not be as reliable in the detection of hepatocellular disease compared with increases in the plasma AST or LD activity. However, it has been suggested that elevated plasma ALT activity can be associated with a prolonged diet of unnatural foods which causes liver disorders in captive tortoises.

Alkaline phosphatase (ALP) is also widely distributed in the reptilian body, and the plasma activity of this enzyme is not considered to be organ specific. Little information is available concerning the interpretation of increased plasma alkaline phosphatase activity in reptiles; however, increased activity may reflect increased osteoblastic activity rather than hepatobiliary disease. Increased plasma ALP has been associated with hyperparathyroidism and bone diseases, such as Paget's disease.

Biliverdin, a green bile pigment, is generally considered to be the primary end product of hemoglobin catabolism in reptiles. Green plasma results from the accumulation of biliverdin in reptilian blood, which is usually a pathologic finding that suggests hepatobiliary disease in these animals. A nonpathologic accumulation of biliverdin can occur in the blood of some reptilian species, such as arboreal scincid lizards (Scincidae) of the southwestern Pacific, which are rarely presented for clinical evaluation.[3] The physiologic advantage of this is not known. Biliverdin appears to be less toxic to tissues compared with bilirubin, and the normal biliverdin concentration in the plasma of some species of lizards (i.e., *Prasino haema*) can be greater than 1000 µmol/L.[3,5]

Laboratory evaluation of plasma and serum proteins

The plasma total protein concentration of normal reptiles generally ranges between 3 and 7 g/dL (30–70 g/L). Female reptiles demonstrate marked increases in their plasma total protein concentration during active folliculogenesis. This estrogen-induced hyperproteinemia is associated with increased levels of the proteins (primarily globulins) necessary for yolk production. The plasma total protein concentration returns to normal after ovulation. Captive reptiles may exhibit greater plasma total protein concentrations when compared to the same free-living species due to prolonged high-protein diets.[17]

The biuret method is the most accurate for determining the plasma or serum total protein concentration. The refractometer method, however, is commonly used to rapidly estimate the plasma protein concentration in reptilian blood. Although the refractometric method tends to overestimate the total protein value, it is useful for clinical decisions.

Protein electrophoresis provides an accurate assessment of the serum or plasma albumin and globulin concentrations in reptilian blood. Absolute concentrations of the various plasma proteins are obtained by determining the total protein concentration using the biuret method in conjunction with electrophoretic separation of the proteins.

In most reptiles hyperproteinemia is indicated by total protein values of greater than 7 g/dL (70 g/L); common causes include dehydration or hyperglobulinemia associated with chronic inflammatory diseases. The alpha, beta, and gamma globulins may increase with infectious diseases. A significant increase in total protein as measured by chemical analyzers can occur with hemolysis.[4]

Hypoproteinemia, as indicated by a total protein value of less than 3 g/dL (30 g/L), is commonly associated with chronic malnutrition in reptiles. Other causes, however, such as malabsorption, maldigestion, protein-losing enteropathy, severe blood loss, and chronic hepatic or renal disease, should also be considered.

Laboratory evaluation of serum and plasma lipids

The normal serum cholesterol concentration of reptiles varies depending upon the natural diet. In general, healthy

herbivorous reptiles are expected to have lower normal cholesterol concentrations (77–270 mg/dL or 2–7 mmol/L) compared to that of omnivores and carnivores. Low-density lipoprotein (LDL) is the major carrier of cholesterol in the serum of tortoises (*Agrionemys horsfieldi*, *Testudo graeca*, and *T. hermanni*) with high-density lipoprotein (HDL) representing a minor carrier.[2]

Seasonal and gender variation in the plasma lipid profile can occur in reptiles. For example, serum lipid concentrations tend to be higher in males than females. In one study, cholesterol represented 21% of the total lipid content in male Asian tortoises (*Agrionemys horsfieldi*) and only 14% in females.[2] Triglycerides and phospholipids are the major serum lipids in both sexes of these tortoises during reproduction (vitellogenesis). The lipid content of free-ranging tortoises changes immediately prior to hibernation as indicated by phospholipids and low-density lipoprotein cholesterol (LDL-C) being the major lipid fractions in serum, whereas triglycerides are very low.[2]

Laboratory evaluation of glucose metabolism

In general, the normal blood glucose concentration of most reptiles ranges between 60 and 100 mg/dL (3.33–5.55 mmol/L), but this is subject to marked physiologic variation. The blood glucose concentration of normal reptiles varies with species, nutritional status, and environmental conditions. For example, an increase in temperature produces hypoglycemia in turtles but hyperglycemia in alligators.[10] In aquatic reptiles, hypoxia associated with diving results in a physiologic hyperglycemia because of anaerobic glycolysis. Normal oral glucose tolerance curves in reptiles differ both among species and with temperature. A significant gender difference in plasma glucose concentration has been observed in free-ranging tortoises where males have higher concentrations than females.[16] The reason for this is not known.

Common causes of hypoglycemia in reptiles include starvation and malnutrition, severe hepatobiliary disease, and septicemia. Clinical signs associated with hypoglycemia in reptiles include tremors, loss of righting reflex, torpor, and dilated, nonresponsive pupils.

In mammals, prolonged exposure of the serum with erythrocytes results in a glucose concentration that decreases at a rate of approximately 10% per hour. Limited studies have shown that this does not occur in reptiles. A significant decrease in plasma glucose concentration may not occur until erythrocytes have been in contact with the plasma for 96 hours.[18] This is likely a result of slower reptilian erythrocyte metabolism compared to that of mammals.

Hyperglycemia in reptiles often results from the iatrogenic delivery of excessive glucose. A persistent, marked hyperglycemia and glucosuria are suggestive of diabetes mellitus, which is a rarely reported disorder of reptiles. Hyperglycemia also may occur with glucocorticosteroid excess.[12,33]

Laboratory detection of muscle injury

Creatinine kinase (CK) is considered to be a muscle-specific enzyme and is used to test for muscle cell damage. Increases in the plasma CK activity can result from muscle cell injury or exertion. Increased plasma CK activity is frequently observed in reptiles that are struggling to resist restraint during blood collection, or that are exhibiting seizure activity. Increased plasma CK activity resulting from muscle cell damage occurs with traumatic injury, intramuscular injections of irritating drugs or fluids, and systemic infections that affect skeletal or cardiac muscle. Brain tissue generally has high CK activity; however, whether brain lesions contribute significantly to plasma CK is not known.

Muscle injury also results in mild to moderate increases in plasma AST and LD activities. These enzymes are not organ specific for muscle, however, and their activities could increase with hepatobiliary disease. When plasma CK activity is not increased during increased AST and LD activity, hepatobiliary disease should be suspected. Damage to both liver and skeletal muscle can occur simultaneously, such as occurs with trauma and septicemia, which would result in elevated plasma AST, LD, and CK activities.

Laboratory evaluation of endocrine disorders

Laboratory evaluation of reptilian thyroid and adrenal function is uncommon. Because of the ectothermic nature of reptiles, their physiologic status, which includes endocrine physiology, is highly dependent on the external environment. Therefore, correction of environmental and nutritional deficits usually results in restoration of normal physiologic health.

The hypothalamo-pituitary-adrenal axis of reptiles appears to be typical of most vertebrates; therefore, a reptile's response to stress has an influence on glucose utilization and other metabolic activities modulated by the adrenal gland. Use of plasma corticosterone concentration as the sole measurement of stress is not recommended due to physiological variations. For example, there is a circadian and seasonal variation in plasma corticosterone concentrations in free-ranging reptiles, which tend to have higher concentrations than do captive reptiles of the same species. Because a twofold increase in corticosterone is expected to occur in 1 hour following capture with peak increases in 3 hours, it is recommended that baseline concentrations be obtained within 10 minutes of capture.

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37 Clinical Chemistry of Fish and Amphibians

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Fish

Blood biochemical evaluation is not routinely part of the clinical assessment of piscine patients most likely owing to the expense involved and lack of meaningful reference intervals. As a result, much of the blood biochemical studies have focused on economically important species, such as salmonids (salmon and trout), catfish, and cyprinids (carp, goldfish, and koi). Routine assay methods for the biochemical evaluation of mammalian blood appear to be useful for fish blood; however, interpretation of the results can be difficult. Many endogenous (i.e. species, age, nutritional status, gender, reproductive status) and exogenous factors (i.e. environmental conditions, population density, time of day (diel cycle), and method of capture) influence the plasma biochemistry results of fish. These factors should be taken into consideration when establishing reference intervals for fish.

Reference intervals are available for a few teleost fish. These include bass (*Morone saxatilis*);[13] goldfish (*Carassius auratus*);[6] koi (*Cyprinus carpio*);[23] rainbow trout (*Salmo gairdneri*);[11] red pacu (*Piaractus brachypomus*);[20] siraz (*Capoeta capoeta umbla*).[1] Blood biochemical reference values are also available for elasmobranch fish, such as the bonnethead shark (*Sphyrna tiburo*)[8] and southern stingray (*Dasyatis americana*).[3]

Sample collection and handling

Blood samples for biochemical studies of fish are collected in the same manner as that described for hematologic studies (see Chapter 21, Hematology of Fish). When collecting blood, emersion and handling of fish for as little as 30 seconds can elicit changes in plasma biochemical analytes such as electrolytes and ammonia.[7,10] Changes in plasma electrolyte concentrations resulting from transmembrane shifts of H^+, Na^+, Cl^-, and H_2O also may continue until the erythrocytes and plasma are separated. The magnitude of these changes varies directly with the handling time and with the time that elapses between blood collection and analysis. In vitro changes after blood collection can be minimized by separating plasma from the erythrocytes as soon as possible after the specimen is obtained.

Capture stress is unavoidable when dealing with wild-caught fish or removal of fish from large exhibits. The stress response will have a significant effect on some plasma biochemistry analytes.[7,10,17,19] For example, lactic acidosis may occur after 5 minutes of capture involving strenuous muscle activity as a result of lactate released from the white muscle of fish. The resulting intracellular fluid shift affects the majority of plasma constituents. Complete recovery of the blood parameters may require 2 weeks following two minute handling stress. Chemical restraint of captive fish may also affect plasma biochemical results. For example, tricaine methanesulfonate, a commonly used anesthetic for fish, can cause increases in plasma glucose and potassium and urinary electrolyte loss is teleost fish.

Biochemical evaluations on piscine blood can be performed using either serum or plasma.[16] Blood may be collected into an anticoagulant such as lithium heparin to harvest a plasma sample. Plasma is preferred over serum in some species of fish owing to the long time required for clot formation that may produce significant changes in some of the blood biochemical values. Furthermore, a larger sample volume can usually be obtained when performing biochemical tests on plasma versus serum; an important consideration when testing small fish. Collection of blood into lithium heparin also allows for evaluation of the hemogram and plasma chemistry parameters with use of only a single sample.

The sample size is often small, especially when blood is collected from small fish. Therefore, the clinician must

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decide which tests would be most beneficial in the evaluation of piscine patients. Blood biochemical tests that may be useful include those for total protein, glucose, aspartate aminotransferase (AST), ammonia, creatinine, calcium, sodium, chloride, potassium, and bicarbonate.

Laboratory evaluation of the piscine kidney

Both grossly and histologically, the anatomy of the piscine kidney varies among species.[12,22] Freshwater species have larger and more numerous glomeruli compared with those of marine species, some of which have aglomerular kidneys. When present, fish glomeruli resemble those of mammals. Fish kidneys lack a loop of Henle, and collecting ducts occur only in freshwater species. Fish also lack a true urinary bladder, although an enlargement of the distal ureter, which is of mesothelial rather than endothelial origin, resembles a bladder in some species. The primary urinary function occurs in the caudal kidney.

Normal renal physiology of freshwater fish

The kidney of freshwater teleosts (bony fish) have well-developed glomeruli, proximal and distal tubules, and collecting ducts. The proximal tubule has two subunits. The first (segment I) is homologous to the proximal tubule of tetrapod vertebrates, and the second (segment II) is found only in fish. Freshwater bony fish faced with a water volume load and salt loss maintain a high glomerular filtration rate (GFR) and urine production rate to counteract the marked osmotic uptake of water, whereas they conserve sodium chloride (NaCl) by reabsorption in the renal tubules and collecting ducts. The final processing of urine occurs in the water-impermeable "urinary bladder," where ion reabsorption is substantial.

Normal renal physiology of saltwater fish

The kidneys of marine teleosts have fewer and smaller glomeruli compared to those of freshwater species, and the distal tubule is usually missing. Glomeruli and proximal tubules also are missing in some marine species. Marine teleosts face water volume depletion and salt loading. In these fish, some reabsorption of urine occurs in the tubules and the "urinary bladder," which is permeable to water.

Normal renal physiology of sharks and rays

The kidneys of elasmobranchs (i.e., cartilaginous fish such as sharks, skates, and rays) are extremely complex and composed of glomeruli, proximal tubules and distal tubules that are divided into segments, and collecting tubules and ducts. The GFR of marine elasmobranchs approaches that of freshwater teleosts to balance the osmotic influx of water across the gill.[2] The proximal tubules of these fish can secrete NaCl, and both fluid and salt are reabsorbed in the distal tubule to establish an osmotic gradient, thereby facilitating a tubular countercurrent system to promote the passive reabsorption of urea. The high urea concentration of marine elasmobranchs causes the plasma to be slightly hyperosmotic to the surrounding seawater. Thus, marine elasmobranchs face a net osmotic influx of water, because their gill epithelium is permeable to water but not to NaCl. The high plasma urea concentration of these fish would be fatal without the presence of trimethylanine oxide (TMAO), which, when present at 50% of the urea concentration, counteracts the toxic effects of urea. Both plasma urea and TMAO are derived from hepatic biosynthesis, and the concentrations are maintained by low branchial (gill) permeability and renal tubular reabsorption. Freshwater rays have lost the ability to reabsorb urea and actually excrete urea to lower the plasma osmolarity; therefore they produce dilute urine similar in composition to that of freshwater teleosts with the exception that urea is the primary osmolyte.[14,15]

Plasma urea, uric acid, creatine, and creatinine

Piscine kidneys primarily are involved in ion excretion and osmoregulation.[5] Because these kidneys contribute little to the excretion of nitrogenous wastes, interpretation of the plasma concentrations of urea nitrogen, uric acid, and creatinine may not be useful in the evaluation of renal disease in fish. Urea is derived primarily from degradation of purines via uric acid. Most fish produce small amounts of urea with the exception of marine elasmobranchs, a few ureogenic teleosts, and coelacanths that produce urea as the major end product of nitrogen metabolism. Little is known concerning factors that regulate urea metabolism in teleosts. The gills, however, appear to predominate over the kidneys as the major organ of urea excretion in most fish (except perhaps marine elasmobranchs). Therefore, increases in the plasma urea concentration may be more indicative of branchial epithelial disease than of renal disease in teleost fish.[24] Freshwater teleosts living in alkaline lakes with high pH have high plasma urea concentrations because of a possible interaction of acid-base with urea production. Plasma urea concentrations increase in species such as the lungfish (*Protopterus* sp.), which can survive out of water (i.e., estivation) for extended periods. These fish primarily are ammoniotelic when living in water, but during estivation, the plasma ammonia concentration decreases to negligible levels and the urea concentration increases to avoid ammonia toxicity. The plasma urea concentration also increases in cyprinids (carp, goldfish, and koi) exposed to high environmental levels of ammonia.

The normal plasma urea concentration of freshwater and marine teleosts is less than 10 mg/dL (3.57 mmol/L) and 5 mg/dL (1.79 mmol/L), respectively. Marine elasmobranchs (sharks and rays) have a normal mean plasma urea concentration that ranges between 350 (125 mmol/L) and 1000 mg/dL (357 mmol/L). Decreases in the plasma urea concentra-

tion, especially in marine elasmobranchs, suggest hepatic disease or starvation.[2] Renal disease in marine elasmobranchs also may produce a decreased plasma urea concentration owing to reduced reabsorption. Decreases in food intake in marine elasmobranchs also results in decreases in plasma urea concentration.

Fish produce small amounts of uric acid, creatine, and creatinine, but little is known regarding their physiologic role. Uric acid, a degradation product of purine nucleotides and protein catabolism (via purines), produced primarily in the liver and white muscle of fish is generally converted to urea for excretion. Creatine, an endproduct of glycine, arginine, and methionine metabolism primarily in white muscle, represents more than 50% of the nitrogenous waste that is excreted through the kidney. Therefore, the plasma creatine concentration may be valuable in the assessment of renal disease among fish. Unfortunately, studies have not been performed to evaluate the use of creatine as an indicator of such renal disease, and most veterinary laboratories do not offer creatine assays.

Creatinine is formed from creatine and it also is secreted by piscine kidneys. The normal plasma creatinine concentration of teleosts ranges between 0.5 (44 µmol/L) and 2.0 mg/dL (177 µmol/L). In the English sole (*Parophrys vetulus*), increases in the plasma creatinine concentration have been associated with renal disease, although the urea concentrations remained normal.[4]

Divalent ions

Excess divalent and monovalent ions are excreted in marine teleosts by different routes after the oral ingestion of seawater. The kidneys excrete divalent ions such as magnesium and sulfate, and increases in the plasma concentrations of these ions may indicate renal disease in these fish.

Laboratory evaluation of electrolytes and acid/base balance

Osmoregulation

Teleost (bony fish) plasma is hyperosmotic to freshwater but hypoosmotic to seawater. Freshwater teleosts are hyperregulators, and they face hyperhydration and ion losses by diffusion. They maintain osmotic and ionic homeostasis by active uptake of ions across the intestinal and branchial epithelium. Because plasma sodium and chloride concentrations are more commonly measured than plasma osmolarity, the formula $Osm_{NaCl} = [Na^+ + Cl^-] \times 0.91$ is frequently used as a measurement of osmolarity in the literature.[5] Marine teleosts (Osm_{NaCl} greater than 300) are hyporegulators and maintain plasma osmolality at approximately one-third that of seawater and slightly greater than that of freshwater teleosts (Osm_{NaCl} = 195–252).[2] The resulting osmotic water loss compensated for by drinking seawater. A $Na^+/K^+/Cl^-$ cotransporter drives the water uptake in the intestinal epithelium, and the high uptake of monovalent ions is compensated for by excretion of these ions via the gills. Therefore, marine teleosts ingest saltwater to balance the osmotic loss of water across the gills, and freshwater teleosts excrete large volumes of dilute urine to balance the osmotic uptake of water.

Whereas plasma sodium chloride contributes to greater than 75% of the osmolarity of teleost fish, it contributes to only 50% or less in elasmobranchs (sharks, rays, and skates). Nonprotein nitrogen, primarily urea, makes up most of the balance in marine elasmobranchs to raise the osmotic pressure to slightly greater than that of the ambient seawater. Therefore, marine elasmobranchs, unlike marine teleosts, do not lose water across the gills; instead, they gain small amounts that allow for urine formation. Thus, marine elasmobranchs do not drink seawater. A decrease in plasma urea concentration and osmolarity occurs in marine elasmobranchs during fasting because of a decrease in urea biosynthesis. These decreases also occur when marine elasmobranchs move to environments with lower salinity because of increases in renal urea clearance.

Some freshwater elasmobranchs have higher plasma osmolarity (Osm_{NaCl} approximately 380) compared to that of other freshwater fish and yet maintain a high urea concentration, although it may be 50% that of marine elasmobranchs.[14,15] These fish are considered to be more recent emigrants to the freshwater environment. Freshwater rays, known to be long-term inhabitants of freshwater habitats, have a lower osmolarity (Osm_{NaCl} approximately 281) with urea having negligible participation.

Osmoregulation of marine elasmobranchs involves the kidneys, rectal glands, gills, and diet. A high level of urea is maintained by renal tubular reabsorption. In freshwater, urea is excreted to lower the osmolarity. Rays adapted to living in freshwater have lost the ability to reabsorb urea. The rectal gland of marine elasmobranchs is a salt secreting organ. When exposed to freshwater, these fish exhibit regression of the gland. Freshwater rays have no functional rectal gland. Two-thirds of the total sodium and chloride excreted by elasmobranchs occurs in the gills. Their gills have low permeability to urea. Finally, metabolic urea is directly related to food availability.

Sodium chloride

Na^+ and Cl^- are the major ions in the blood of all fish.[5] Marine teleosts display a higher branchial permeability to salt; therefore, the unidirectional Na^+ and Cl^- fluxes are 10- to 50-fold greater than those of freshwater teleosts. Ionic gradients across the gill epithelium are of the same magnitude as those of freshwater fish, but they are reversed in direction. Because the kidney of marine teleosts cannot produce urine, which is hyper-osmolar relative to plasma, extrarenal salt secretion must occur. The mitochondria-rich chloride cells of the gills are most likely the sites of ionic

and/or acid-base regulation involving Na/H [NH_4] and Cl/HCO_3 exchanges in fish.

The rectal gland is the site of extrarenal salt secretion in marine sharks and rays. This gland produces a solution that is iso-osmotic to the plasma but that contains more NaCl than seawater (in a manner similar to the NaCl transport system in the thick, ascending limb of the loop of Henle in mammals). An increase in plasma volume, rather than in NaCl concentration, appears to stimulate rectal gland secretions in marine elasmobranchs.[2]

Fish have adapted to marine or freshwater environments by using osmotic and ionic regulating mechanisms allowing them to maintain a relatively constant plasma and intracellular salt concentration as well as cellular volume. Whereas the kidney is the primary osmoregulatory organ of terrestrial vertebrates, fish use organs such as the gills, intestines, rectal glands, and to a lesser extent, the kidneys to regulate fluid volume and salt concentration.

The normal plasma sodium and chloride concentrations of freshwater teleosts and marine teleosts are approximately 150 mEq/L (mmol/l) and 130 mEq/L (mmol/L), respectively. Plasma sodium and chloride concentrations are affected by changes in ambient salinity, gill function, and stress. Within a few minutes of capture and handling trauma, catecholamines and cortisol are released along with lactic acid release from muscles. Stress-induced release of catecholamines causes an increase in blood pressure resulting in an increased electrolyte permeability of the gills that causes a rapid decrease in sodium and chloride in freshwater teleost fish and increase in those ions in marine teleost fish. Hyponatremia and hypochloremia in freshwater fish can be associated with gill and renal disease or with acidic or soft-water environments.

Potassium

The normal plasma potassium concentration of freshwater fish is approximately 3 mEq/L (mmol/L). Less than 2% of the total body potassium is found in extracellular fluids; therefore, plasma levels are unaffected by changes in gill electrolyte permeability.[5] Greater than 95% of the potassium ingested by marine fish is absorbed in the intestines, and the excess is excreted extrarenally as part of the slime coat. Hypokalemia may be associated with alkalosis, gastrointestinal or cutaneous potassium loss, or nitrite toxicity. Hyperkalemia may be associated with acidosis, such as occurs following strenuous muscle activity during capture and handling, and decreased renal secretion of potassium in freshwater teleosts. Hemolysis will also cause an artificial increase in plasma potassium.

Calcium

The normal plasma calcium concentration of teleosts is approximately 8–10 mg/dL (2–2.5 mmol/L). Because water is a readily available source of calcium, the plasma calcium concentration is influenced by the environmental calcium concentration. Fish have access to a continuous supply of calcium, so they must limit their calcium intake (unless the environmental calcium levels are low). In freshwater teleosts, calcium is transported by the chloride cells in the gills to the blood. Calcium ions enter these cells passively along the electrochemical gradient via calcium channels in the apical cell membrane. Stanniocalcin is a hormone that is unique to certain fish (e.g., teleosts) and that acts as a calcium-channel blocker to prevent the development of hypercalcemia.[5] Fish do not have parathyroid glands or a parathormone-like hormone. How fish that do not produce stanniocalcin regulate their blood calcium concentrations is not yet known.

In contrast to tetrapods, calcitonin whose role is to correct excessive calcium levels does not play a prominent role in calcium regulation in fish. Calcitonin is produced by the ultimobranchial bodies of fish and has a role in protecting the skeletal system during periods of increased demand for Ca^{2+} during active oogenesis.

In male and nongravid female freshwater teleost fish, 30–40% of the total plasma calcium is bound to protein. Approximately 22% of the total calcium is bound to protein in marine teleosts. Therefore, changes in plasma protein will affect the plasma total calcium concentration. For example, during vitellogenesis, a greater than threefold increase in total protein and calcium will be expected; however, free Ca^{++} concentration remains constant.

Magnesium

The plasma magnesium concentration of freshwater fish and marine elasmobranchs is generally lower than the calcium concentration; however, marine teleosts have magnesium concentrations that are greater than calcium. In general, inorganic ions, such as Na, Cl, and Ca, are kept below levels found in the ambient marine water. Approximately 25% of the plasma magnesium concentration is bound to protein; however, the mechanism of magnesium regulation is unknown. Because magnesium concentration within erythrocytes is nearly 10 times that of plasma, hemolysis would be expected to produce an artificial increase in plasma magnesium concentration.

Acid-base balance

Acid-base regulation in fish is more challenging compared with that of terrestrial animals, because the composition of water varies to a greater degree than that of air. Large and rapid changes in oxygen and carbon dioxide (CO_2) levels, electrolyte concentrations, and temperature are significant

challenges to acid-base regulation. The branchial epithelium is the site of gas exchange and principal ion regulation in fish; ions readily transfer across the gill surface. Therefore, changes in the water ionic composition affect the ionic transfer process across the branchial epithelium, which in turn affects osmotic and acid-base regulation.

Fish have a low blood CO_2 concentration compared with that of terrestrial animals.[18] This results from the high rate of gill ventilation and the much larger capacity of water for carbon monoxide (CO) dissolution. The small environmental CO_2 and arterial CO_2 differences limit the ability of fish to compensate for changes in arterial CO_2 by hyper- or hypoventilation. Therefore, changes in CO_2 are too small to contribute significantly to the acid-base balance in fish. However, even though respiratory regulation contributes little to acid-base balance, fish have a larger epithelial ionic transfer capacity than that of air-breathing mammals, and they also have the capacity for a net gain of bicarbonate from the environment to facilitate normalization of the acid-base status.[9] This epithelial ionic transfer is a function of the chloride cells located in juxtaposition to the secondary circulatory system of the central venous gill sinus. Ionic transfer for acid-base regulation also occurs, although to a lesser extent, across the skin and kidney of fish.

Laboratory evaluation of branchial epithelium

Because the gills of fish are important organs for osmotic, ionic, and acid-base regulation as well as for removal of nitrogenous waste, changes in the blood biochemistry may reflect damage to the branchial epithelium. Injury to gill tissue may result in thickening of the branchial epithelium and an increased distance for diffusion from blood to water. In turn, this may lead to an increased plasma concentration of analytes normally excreted by the branchial epithelium. Therefore, acid-base disturbances, electrolyte imbalances, and increases in the blood ammonia and urea concentrations may occur with damage to the branchial epithelium of fish.

Ammonia

Ammonia is the major end product of nitrogen metabolism in most fish except marine elasmobranchs. Ammonia is the most reduced and energy-efficient nitrogenous waste product of the biologic oxidation of dietary or structural proteins. The primary mechanism of ammonia excretion in freshwater teleosts is branchial excretion. The skin also contributes to ammonia excretion, especially in marine teleosts. The kidneys excrete less than 15% of ammonia.

The mechanism of branchial ammonia excretion primarily involves diffusion along a concentration gradient from blood to water and an electro-neutral Na^+/NH_4^+ exchange located on the apical membranes of the branchial epithelial cells.[12,24] Electro-neutral H^+/NH_4^+ exchange also may occur in the gill membranes of fish. Marine teleosts excrete ammonia by NH_4^+ diffusion along an electrochemical gradient from blood to water.

The inflammation, swelling, and mucinification that occur with gill damage result in an increased diffusion distance between blood and water, thereby creating an increased blood ammonia concentration. Environmental toxins, changes in the environmental pH and ammonia concentrations, or infections can damage the gills of fish, thus resulting in increased blood ammonia concentrations. Increases in the environmental pH and ammonia concentration also can increase the blood ammonia concentration by the inhibition of ammonia diffusion, thereby reversing the blood-to-water gradient.

Plasma total ammonia is highly variable and rarely used in diagnostic testing of piscine patients. Both the site of blood collection and the duration of restraint affect the blood ammonia concentration in fish. Total plasma ammonia concentration is higher in samples obtained by caudal venipuncture (prehepatic blood) compared to samples obtained by cardiocentesis (posthepatic blood). Venous blood contains 50–60% more ammonia than arterial blood. During restraint, the release of ammonia from hypoxic muscles and the interference with branchial excretion also can increase the blood ammonia concentration in fish. Plasma ammonia levels increase with feeding, exhaustive exercise, exposure to air, and under certain water quality parameters, such as an increase in water temperature, increase in ammonia concentration, and alkaline pH.

Laboratory evaluation of the piscine liver

Little information is available regarding laboratory evaluation of the liver in fish. The liver tissue of teleosts appears to be rich in aspartate aminotransferase (AST) and possible alanine aminotransferase (ALT). Therefore, plasma activity of these enzymes may elevate with severe hepatocellular disease in some piscine species.

There is a general lack of information regarding the influence of nonpathogenic factors on the activities of these enzymes in the plasma of fish; however, a few studies have suggested that plasma enzyme evaluation in fish may not be as straightforward as it is in mammals. For example, the high ammonia levels of fish may lead to high transaminase activites; therefore, the increase in activities may be associated with liver disease or changes in plasma ammonia concentration. High activities of AST and creatine kinase (CK) also occur in muscle of fish; therefore, elevated plasma activities of these enzymes will increase following muscle injury or strenuous muscle activity associated with capture and

restraint. Temperature changes have been reported to affect plasma enzyme activity of alkaline phosphatase (AP). The method of blood collection influences plasma lactate dehydrogenase (LDH) and CK activities in fish leading to the recommendation that blood collected by cardiocentesis be used for enzyme studies. Plasma LDH activity is also influenced by feeding and activity levels resulting in lower LDH values starvation and inactivity. It has also been shown that plasma LDH activity is positively correlated with water temperature and pH.

Bile pigments in most fish include both bilirubin and biliverdin; however, the percentages of these pigments vary between species. The serum usually is a light yellow color because of the presence of bilirubin. Hepatic disease in fish may not reliably cause an increased plasma bilirubin concentration. The serum from some fish (e.g., certain eels) is bluish green because of the presence of biliverdin.

There is little information about the normal bile acid metabolism in fish. Fish may continuously secrete bile acids into the intestines resulting in no change in plasma bile acid concentration associated with feeding.

Plasma glucose concentration in fish is variable and can be as low as 30 mg/dL (1.67 mmol/L) in some species. The source of plasma glucose in fish is hepatic glycogen metabolism; therefore depletion of hepatic glycogen reserves may result in hypoglycemia. Plasma glucose concentration in fish is highly dependent upon the activity level of the fish. For example, sluggish benthic species have lower plasma glucose concentrations compared to the more active pelagic species. Plasma glucose concentration also varies with age, nutritional and reproductive status, and stress. The duration and magnitude of postprandial hyperglycemia in fish depends upon dietary carbohydrate intake. The affect of starvation on plasma glucose concentration is species and time dependent because many species of fish exhibit normal blood glucose concentrations (up to 150 days) following prolonged starvation. The mechanism allowing for maintenance of normal blood glucose concentrations with prolonged starvation is not known. Variation in blood glucose concentration also occurs with the reproductive status of fish were lowest blood glucose values in males and females are associated with spawning.

Stress-induced hyperglycemia is a common occurrence in fish and the extent and duration is influenced by the severity of stress. The increased plasma concentrations of catecholamines and adrenocorticosteroids associated with stress changes the muscle and hepatic glycogen reserves in fishes. Catecholamines mobilize glycogen stores and corticosteroids induce glycogen synthesis. Therefore, the plasma hyperglycemia associated with marked glycogenolysis in liver and muscle is likely due to stress-induced increases in catecholamines.

It is not known whether changes in the plasma cholesterol concentration have significant meaning in regards to hepatic disease. Most fish, except for elasmobranchs, normally have higher blood cholesterol concentrations than do mammals. The majority of the blood cholesterol (60–90%) of fish is carried by high density lipoproteins (HDL). The blood cholesterol concentration of elasmobranchs is lower than that of teleosts and varies with gender and reproductive status. Males undergoing active spermatogenesis have higher cholesterol values compared to inactive males. Females have lower blood cholesterol concentrations compared to males were females undergoing active egg production have the lowest values.

Laboratory evaluation of endocrine disorders

The neuroendocrine system of fish is similar to those of other vertebrates. Because fish have a very close interaction with the ambient aquatic environment, their endocrine system may differ functionally from those of terrestrial animals. For example, hormones such as prolactin, growth hormone, cortisol, glucagon, and somatostatin have important ionic regulating functions in fish that are not observed in terrestrial vertebrates. Fish also have unique hormones, including somatolactin, melanophore-concentrating hormone, urotensin, and stanniocalcin. Parathormone and aldosterone are not found in fish, however, and this implies the absence of a requirement for these hormones because of their close association with their aquatic environment.

Commercial kits for the measurement of hormones in mammalian plasma have been used successfully in the determination of hormones that are common to both types of animals.[21] Homologous radioimmunoassays (RIA) have been developed to assay the blood hormones of a few species of fish (salmonids and cyprinids).

Thyroid

Fish thyroid tissue appears to behave similarly to that of terrestrial mammals. It is stimulated by a thyroid-stimulating hormone to release thyroxine (T_4), which is de-iodinated to triiodothyronine (T_3)in target organs such as the gills and liver. There are species differences associated with blood thyroid hormone concentrations. The plasma concentration of thyroid hormones may be influenced by plasma protein concentration as they are bound to transport proteins. Increases in plasma T_3 and T_4 concentrations are associated with significant physiologic functions in fish, such as the adaptation of salmonids to seawater. Decreases in T_4 concentration indicate either decreased thyroid secretion or increased conversion of T_4 to T_3. In general, both hormones are elevated during growth and decreased during conditions such as stress, starvation, and vitelogenesis.

Adrenal (interrenal tissue)

The interrenal tissue of fish is homologous to the adrenal tissues of higher vertebrates. The major corticosteroid produced by this tissue in most jawed fish is cortisol. The major corticosteroid in elasmobranchs is 1α-hydroxycorticosterone. Plasma cortisol concentration is cyclic and affected by the photoperiod (diel cycle) and time of feeding with peak concentrations occurring prior to the onset of light and increased locomotory activity. Cortisol is involved in energy metabolism, ion regulation, and response to stress. Cortisol secretion is stimulated by the stress response (i.e. capture, handling, crowding, transport, rapid changes in water quality, and other physical disturbances) mediated by ACTH resulting in a rapid hyperglycemia. The corticosteroid stress response of elasmobranchs is small compared to that of teleost fish.

Amphibians

Blood biochemical evaluation is not routinely part of the clinical assessment of amphibian patients. Routine assay methods for the biochemical evaluation of mammalian blood appear to be useful for amphibians. Interpretation of the results is difficult, however, because little information is available regarding plasma or serum chemistry values. Table 37.1 demonstrates the expected normal serum biochemical values in bullfrogs (*Rana catesbeiana*). Extrinsic factors such as environmental temperature and humidity, photoperiod, season, water-quality parameters, diet, and population density likely affect the normal plasma biochemistries. Intrinsic factors such as gender and age also likely influence the variation in plasma biochemistry values. As an example, female bullfrogs have higher plasma total protein, calcium, and sodium concentrations than male bullfrogs.

Table 37.1 Normal serum biochemistry reference values for American bullfrogs (*Rana catesbeiana*) kept at 20°C to 25°C.

Urea (mg/dL)	3.00 ± 1.00[a]
Creatinine (mg/dL)	0.99 ± 0.20
Uric acid (mg/dL)	0.06 ± 0.05
Total protein (g/dL)	4.40 ± 0.30 (females)
	3.70 ± 0.80 (males)
Albumin (g/dL)	1.60 ± 0.30
Aspartate aminotransferase (IU/L)	45 ± 21
Lactate dehydrogenase (IU/L)	33 ± 20
Calcium (mg/dL)	8.7 ± 0.6 (females)
	7.4 ± 0.6 (males)
Phosphorus (mg/dL)	3.3 ± 0.7
Sodium (mEq/L)	111 ± 3.0 (females)
	105 ± 4.0 (males)
Potassium (mEq/L)	2.7 ± 0.4
Chloride (mEq/L)	77 ± 6.0
Total carbon dioxide (mmol/L)	25 ± 4.5
Anion gap (calculated)	9.9 ± 6.5

Modified from Cathers T, Lewbart GA, Correa M, *et al*. (1997) Serum chemistry and hematology values for anesthetized American bullfrogs (*Rana catesbeiana*). *J Zool Wildl Med* 28: 171–4.
[a] All values represent meant +/– standard deviation.

Because adult newts and salamanders and gilled aquatic amphibian larvae are more fishlike than adult toads and frogs, the interpretation of changes in their plasma biochemistry profiles may be more like those in fish. The plasma biochemical changes in adult toads and frogs may be more like those of reptiles.

Blood samples for use in biochemical studies of amphibians are collected in the same manner as that described for hematologic studies (see Chapter 22). Blood to be evaluated for hematology and plasma biochemistry generally is collected into an anticoagulant (e.g., lithium heparin). Plasma is preferred to serum, because a larger sample volume usually can be obtained when collecting plasma.

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VI Clinical Case Presentations

Clinical Case Presentations

Introduction

This section presents a number of case studies taken from animal medical records. Each case is presented with its relevant clinicopathologic data. The cases are organized more or less by the primary disease or organ system involved in disease, with the understanding that many of them have multiple system abnormalities. For example, the first 14 cases focus on common primary hematologic disease. However, abnormal hematologic processes are dispersed throughout the remaining cases. The following is a directory of cases classified by the primary system abnormality or disease problem.

Clinical Case Presentations: Contents

Veterinary Hematology and Clinical Chemistry, Second Edition. Edited by Mary Anna Thrall, Glade Weiser, Robin W. Allison, and Terry W. Campbell.

acid-base disturbances are best considered with their primary disease process(es). Therefore, for more broad exposure to this category, also see:
Electrolyte abnormalities
Cases: #'s 11, 16, 24, 25, 30, 32, 34, 35, 41, 45, 47, 53, 54, 56, 57, 58, 63, 64, 65, 66
Acid base disturbances
Cases: #'s 19, 29, 31, 33, 40, 54, 56, 57, 58, 65

Hepatic Disease

Pancreatic & Gastrointestinal Disease and Glucose Metabolism

Endocrine Disorders

Perspective on use of this section

Interpretation of laboratory data is an art that is developed through accumulated experience. The interactions and patterns of data related to disease diagnosis are complex. One also must develop an appreciation for magnitudes of abnormality that influence interpretation of each measurement. This case discussion appendix is designed to provide the reader with both experience and guidance in beginning to learn the art of interpretation. This art is then continually cultivated through real-time experience in the clinical setting.

The laboratory data are presented for each case in a form that allows the reader to learn from making his or her own effort at describing and interpreting data. Please note the following formatting:

1. Data are presented in conventional units. In some areas, international (SI) units are given; these are shown in italics.
2. Laboratory values that are abnormal and central to the interpretation are given in bold type.

Following each data set, an interpretive discussion is presented. These narratives may be used by the reader for self-assessment of proficiency in interpretation of data.

List of abbreviations

The following list of abbreviations identifies test results that are typically used in laboratory reports. The user should refer to these as needed while learning the content of laboratory reports.

Alb	Albumin
ALP	Alkaline phosphatase
ALT	Alanine aminotransferase

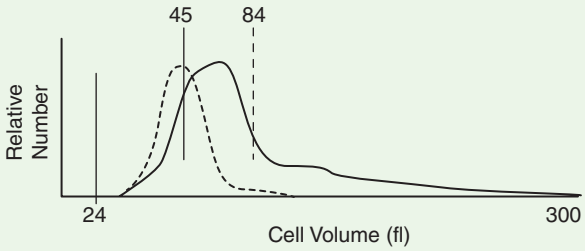
An. Gap	Anion gap
aPTT	Activated partial thromboplastin time
AST	Aspartate aminotransferase
Bands	Band neutrophils
BUN	Blood urea nitrogen
Ca	Calcium
Calc. Osmolality	Calculated osmolality
Chol	Cholesterol
CK	Creatine kinase
CL	Chloride
Creat	Creatinine
Eos	Eosinophils
Epith cells	Epithelial cells
GGT	Gamma glutamyl transferase
Glob	Globulin
Gluc	Glucose
Hgb	Hemoglobin
Hpf	High power fields
Lymphs	Lymphocytes
MCHC	Mean corpuscular hemoglobin concentration
MCV	Mean cell volume
Meas. Osmolality	Measured osmolality
Metas	Metamyelocytes
Monos	Monocytes
Na	Sodium
NCC	Total nucleated cell count (Also commonly called WBC for "white blood cell count".)
PCV	Packed cell volume
Phos	Phosphorus
PT	Prothrombin time
RBC	Red blood cells
Retics	Reticulocytes
SDH	Sorbitol dehydrogenase
Segs	Segmented neutrophils
Sp. Gr.	Specific gravity
T. Bili	Total bilirubin
TCO_2	Total CO_2
TP	Total protein
TP (P)	Total plasma protein
TP (S)	Total serum protein
Trig	Triglyceride
UN	Urea nitrogen (same as BUN)
WBCs	White blood cells
WRI	Within reference interval(s)

Case 1

Signalment and History: 11-year-old male cat. Lethargy and polydipsia. One month ago PCV was 38%.

Hematology		Reference Interval
PCV (%)	**13**	25–45
RBC ($\times 10^6/\mu L$)	**1.55**	5–11
Hgb (g/dL)	**4.0**	8–15
MCV (fL)	**84**	39–50
MCHC (g/dL)	31	33–37
Retics ($\times 10^3/\mu L$)	**155,000**	0–60,000
NCC ($\times 10^3/\mu L$)	**20.6**	5.5–19.5
Metas ($\times 10^3/\mu L$)	**0.4**	0
Bands ($\times 10^3/\mu L$)	**0.8**	0–0.3
Segs ($\times 10^3/\mu L$)	9.9	2.5–12.5
Lymphs ($\times 10^3/\mu L$)	**1.4**	1.5–7.0
Monos ($\times 10^3/\mu L$)	**3.1**	0–0.8
Eos ($\times 10^3/\mu L$)	0.2	0–1.5
Nucleated RBCs ($\times 10^3/\mu L$)	**4.8**	0
Platelets ($\times 10^3/\mu L$)	Adequate	150–700
TP (P) (g/dL)	**8.9**	6.0–8.5

Hemopathology: Many *Hemobartonella felis* (*Mycoplasma haemofelis*) organisms on erythrocytes. Occasional reactive lymphocyte.



Histogram Solid line = patient; Dashed line = normal.

Biochemical Profile		Reference Interval
Gluc (mg/dL)	**249**	67–124
BUN (mg/dL)	**96**	17–32
Creat (mg/dL)	**6.6**	0.9–2.1
Ca (mg/dL)	10.2	8.5–11
Phos (mg/dL)	**7.9**	3.3–7.8
TP (g/dL)	**8.4**	5.9–8.1
Alb (g/dL)	3.3	2.3–3.9
Glob (g/dL)	**5.1**	2.9–4.4
T. Bili (mg/dL)	0.3	0–0.3
Chol (mg/dL)	**386**	60–220
ALT (IU/L)	53	30–100
ALP (IU/L)	19	6–106
Na (mEq/L)	150	146–160
K (mEq/L)	4.9	3.7–5.4
CL (mEq/L)	127	112–129
TCO_2 (mEq/L)	**10**	14–23
An. gap (mEq/L)	18	10–27
Calc. osmolality (mOsm/kg)	**337**	290–310

Urinalysis (cystocentesis)

		Urine Sediment	
Color	Yellow	WBCs/hpf	6–8
Transparency	Cloudy	RBCs/hpf	1–2
Sp. gr.	**1.020**	Epith cells/hpf	1–3 transitional
Protein	Negative	Casts/lpf	0
Gluc	**2+**	Crystals	0
Bilirubin	Negative	Bacteria	0
Blood	Negative	Other	fat droplets
pH	5.0		
Ketones	Negative		

Interpretive discussion

Hematology

Packed cell volume, Hemoglobin, Red blood cell count: The cat is severely anemic; all measurements of red blood cell mass are decreased.

MCV: The mean cell volume is increased, which one would expect with a regenerative anemia. However, the increase is greater than can be accounted for by the reticulocytes, suggesting that agglutination is causing the increase, since doublets are being counted as one erythrocyte. This is confirmed by the histogram tailing to the right.

Reticulocytes are increased, indicating that the anemia is regenerative. Regenerative anemia is due to blood loss or blood destruction. The protein concentration suggests hemolysis and tends to rule out blood loss.

Nucleated RBCs are increased due to early marrow release, and are often present in a regenerative anemia.

Erythrocyte morphology: The presence of *Hemobartonella felis* (*Mycoplasma haemofelis*) organisms explains the anemia (blood destruction). Agglutination is likely due to the presence of antibodies against the organisms.

Monocytosis and increased immature (band) neutrophils are indicative of an inflammatory leukogram.

Lymphopenia is indicative of a stress component in the leukogram.

Total protein: Total protein is increased. In this patient, it is due to hyperglobulinemia (see biochemical profile interpretation below).

Biochemical profile

The serum glucose concentration is moderately increased. This could be due to stress (glucocorticoid release), as the lymphopenia suggests, but could also be due to diabetes mellitus.

The BUN and serum creatinine concentrations are increased, and in the face of a urine specific gravity of only 1.020, is indicative of renal azotemia.

The serum phosphorus concentration is mildly increased, and is compatible with decreased glomerular filtration rate.

The serum total protein concentration is increased due to an increase in the globulin concentration. The increase in globulin should trigger protein electrophoresis to determine if gammopathy is monoclonal or polyclonal.

The serum cholesterol concentration is moderately increased. This may be due to metabolic disorders associated with diabetes mellitus.

Serum total CO_2 is decreased, suggesting metabolic acidosis.

The increased calculated serum osmolality is primarily due to hyperglycemia and increased BUN.

Urinalysis

As evidenced by the relatively low urine specific gravity in the face of azotemia, the animal is not concentrating adequately, indicating renal dysfunction. The presence of glucose indicates that the renal threshold of glucose has been exceeded.

Summary

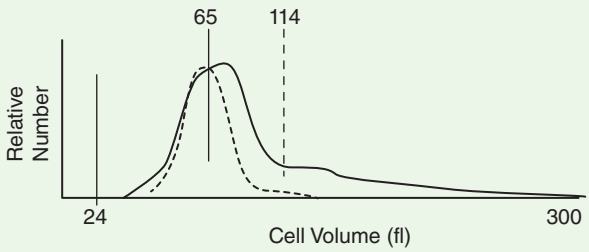
This animal had been previously diagnosed with diabetes mellitus and was not being controlled adequately. *Hemobartonella felis (Mycoplasma haemofelis*) is often an opportunist in cats that are immunosuppressed. The hyperglobulinemia was polyclonal, indicating antigenic stimulation, possibly due to *Mycoplasma felis.*

Case 2

Signalment: 5-year-old spayed female cocker spaniel
History: Acutely lethargic
Physical Examination: Pale, slightly icteric mucous membranes

Hematology		Reference Interval
PCV (%)	**12**	37–55
Hgb (g/dL)	**3.6**	12–18
RBC ($\times 10^6/\mu L$)	**0.95**	5.5–8.5
MCV (fL)	**114**	60–72
MCHC (g/dL)	**30**	34–38
Retics ($\times 10^3/\mu L$)	**123**	<60
NCC ($\times 10^3/\mu L$)	**96.1**	6–17
Segs ($\times 10^3/\mu L$)	**69.1**	3–11.5
Bands ($\times 10^3/\mu L$)	**6.7**	0–0.3
Metas ($\times 10^3/\mu L$)	**1.0**	0
Monos ($\times 10^3/\mu L$)	**5.8**	0.1–1.3
Lymphs ($\times 10^3/\mu L$)	0	1–4.8
Eos ($\times 10^3/\mu L$)	0	0.1–1.2
NRBCs ($\times 10^3/\mu L$)	**13.5**	0
Platelets ($\times 10^3/\mu L$)	284	200–500
TP (P) (g/dL)	6.8	6–8

Hemopathlogy: Polychromasia increased, agglutination present, many spherocytes present. Occasional Howell-Jolly body.



Histogram Solid line = patient; Dashed line = normal.

Biochemical Profile		Reference Interval
Gluc (mg/dL)	**143**	75–130
BUN (mg/dL)	**39**	7–28
Creat (mg/dL)	1.3	0.9–1.7
Ca (mg/dL)	9.0	9.0–11.2
Phos (mg/dL)	4.4	2.8–6.1
TP (g/dL)	6.5	5.4–7.4
Alb (g/dL)	3.3	2.7–4.5
Glob (g/dL)	3.2	1.9–3.4
T. Bili (mg/dL)	**4.7**	0–0.4
Chol (mg/dL)	269	130–370
ALT/µL (IU//L)	32	10–120
AST/µL (IU//L)	30	16–40
ALP (IU//L)	**438**	35–280
Na (mEq/L)	146	145–158
K (mEq/L)	5.0	4.1–5.5
CL (mEq/L)	118	106–127
TCO_2 (mEq/L)	14	14–27
An. gap (mEq/L)	19	8–25

Urinalysis (catheterized)

Color	Orange	**Urine Sediment**	
Transparency	Turbid	WBCs/hpf	0
Sp. Gr.	1.038	RBCs/hpf	**10**
Protein	1+	Epith Cells/hpf	0
Gluc	Negative	Casts/lpf	0
Bilirubin	**2+**	Crystals	Numerous **Bilirubin**
Blood	**1+**	Bacteria	0
pH	6.0		

Interpretive discussion

Hematology

Packed cell volume, Hemoglobin, Red blood cell count: The dog is very anemic, as all measurement of red blood cells mass are decreased. The RBC count is likely erroneously decreased, due to erythrocyte agglutination, and groups of red blood cells being counted as one red blood cell.

MCV: The mean cell volume is erroneously increased due to agglutination. Although the anemia is regenerative, the MCV is much higher than can be accounted for by an increase in reticulocytes. As one can see on the histogram, erythrocytes that are agglutinated are being counted as one large erythrocyte (note histogram tailing to right).

Reticulocytes are increased, indicating that the anemia is regenerative, suggesting either blood loss or blood destruction. The protein and RBC morphology findings indicate hemolysis. The decreased MCHC may be attributed to regeneration.

Nucleated RBCs are increased, likely due to early release from bone marrow in response to marked anemia. However, it is also possible that the dog has decreased splenic function secondary to glucocorticosteroid administration.

Erythrocyte morphology: Presence of spherocytes and agglutination, in the absence of a previous blood transfusion, are indicative of immune-mediated hemolytic anemia.

Neutrophilia, increased immature neutrophils, and monocytosis are indicative of a markedly inflammatory leukogram. The absence of lymphocytes is suggestive of stress or corticosteroids. Inflammatory leukograms are commonly seen in association with immune-mediated hemolytic anemia.

Biochemical profile

Glucose is mildly increased. Considering the lymphopenia, this may be due to stress or steroids.

BUN is mildly increased, suggesting decreased GFR or bleeding into the GI tract. Since the creatinine is within the reference interval, and the dog is concentrating urine, this is likely prerenal azotemia, due to GI hemorrhage (high protein diet) or decreased blood flow to the kidneys.

Bilirubin is increased, indicating either cholestasis or increased red blood cell destruction. Because the dog has immune-mediated hemolytic anemia, increased RBC destruction is most likely the cause.

Serum alkaline phosphatase activity is increased, which could be due to either cholestasis or previous treatment with corticosteroids.

Urinalysis

Bilirubinuria and the presence of bilirubin crystals reflect the increased serum bilirubin concentration. Conjugated bilirubin readily passes through glomeruli and is then excreted in the urine. Blood and protein may be present due to traumatic catheterization. The animal is concentrating, indicating that the increase in BUN is not due to renal dysfunction.

Summary

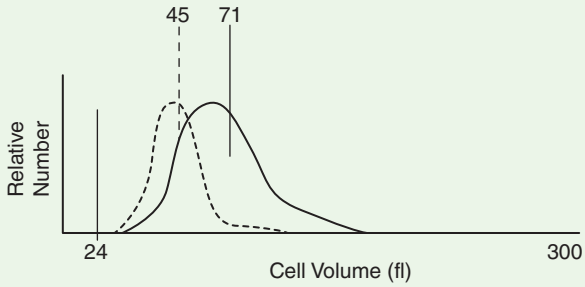
This is a typical case of immune-mediated hemolytic anemia. Dog was treated with prednisone and recovered. It had been previously treated with corticosteroids, accounting for the stress leukogram, hyperglycemia, and increased serum alkaline phosphatase activity.

Case 3

Signalment and History: 11-year-old DSH spayed female cat presented for anorexia and lethargy

Hematology		Reference Interval
PCV (%)	**13**	25–45
RBC ($\times 10^6/\mu L$)	**1.84**	5–11
Hgb (g/dL)	**4.2**	8–15
MCV (fL)	**71**	39–50
MCHC (g/dL)	32	33–37
Retics ($\times 10^3/\mu L$)	**7,360**	0–60,000
Nucleated cells ($\times 10^3/\mu L$)	**71.3**	5.5–19.5
Metas ($\times 10^3/\mu L$)	**0.7**	0
Bands ($\times 10^3/\mu L$)	**2.1**	0–0.3
Segs ($\times 10^3/\mu L$)	**33.2**	2.5–12.5
Lymphs ($\times 10^3/\mu L$)	2.8	1.5–7.0
Monos ($\times 10^3/\mu L$)	**6.9**	0–0.8
NRBCs ($\times 10^3/\mu L$)	**24.9**	0
Blasts ($\times 10^3/\mu L$)	**0.7**	0
Platelets ($\times 10^3/\mu L$)	Adequate	150–700
TP (P) (g/dL)	8.0	6.0–8.5

Hemopathology: Blasts appear to be rubriblasts. Many prorubricytes and rubricytes also present.



Histogram Solid line = patient; Dashed line = normal.

Interpretive discussion

Hematology

Packed cell volume, Hemoglobin, Red blood cell count: Cat is markedly anemic. Reticulocytes are not increased, indicating that the anemia is nonregenerative.

MCV is markedly increased, in the absence of reticulocytosis or agglutination. In a cat, this should trigger testing for feline leukemia virus, as the MCV may be increased as a result of viral-induced erythrodysplasia. Macrocytosis with widened histogram is often seen in FeLV positive cats with anemia.

Neutrophilia, increased band neutrophils and metamyelocytes, and monocytosis are indicative of inflammation.

Nucleated red blood cells are increased in the absence of reticulocytes. Moreover, many of these are quite immature, indicating that the cat has leukemia involving the erythrocytes.

Summary

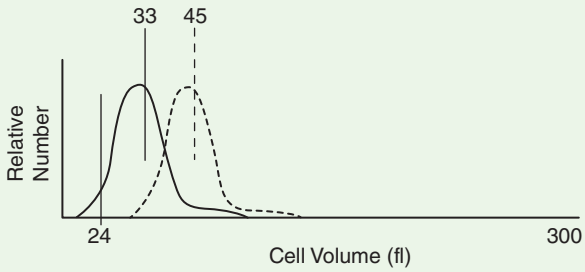
Myeloproliferative disorder, erythremic myelosis or M6(E).

Case 4

Signalment and History: 17-year-old male cat presented for lethargy and enlarged abdomen. Liver disease suspected, but biochemical profile normal.

Hematology		Reference Interval
PCV (%)	**24**	25–45
MCV (fL)	**33**	39–50
MCHC (g/dL)	32	33–37
Retics ($\times 10^3/\mu L$)	ND	0–60,000
Nucleated cells ($\times 10^3/\mu L$)	13.2	5.5–19.5
Bands ($\times 10^3/\mu L$)	**4.5**	0–0.3
Segs ($\times 10^3/\mu L$)	6.6	2.5–12.5
Lymphs ($\times 10^3/\mu L$)	**0.5**	1.5–7.0
Monos ($\times 10^3/\mu L$)	0.5	0–0.8
Eos ($\times 10^3/\mu L$)	0.3	0–1.5
Basophils ($\times 10^3/\mu L$)	**0.8**	rare
Platelets ($\times 10^3/\mu L$)	Adequate	150–700
TP (P) (g/dL)	6.6	6.0–8.5

Hemopathology: Many keratocytes, schistocytes.



Histogram Solid line = patient; Dashed line = normal.

Interpretive discussion

Hematology

Packed cell volume: The cat is mildly anemic.

MCV: The mean cell volume is prominently decreased. Decreased mean cell volume is almost always caused by iron deficiency anemia, which in adults is almost always secondary to chronic external blood loss. The borderline decrease in MCHC is not important diagnostically.

Erythrocyte morphology: Keratocytes are commonly associated with iron deficiency anemia. Iron deficiency anemia is not as common in cats as in dogs, and the few cases we have seen did not have increased central pallor.

The total leukocyte count and the mature neutrophil concentration are within the reference interval, but the increase in band neutrophils is indicative of inflammation. Lymphopenia is indicative of stress or previous corticosteroid administration.

Total protein is within the reference interval. Although one might expect total protein to be decreased with chronic blood loss, animals often compensate for this chronic loss of protein.

Summary

Owner declined further diagnostic evaluation. Chronic GI blood loss secondary to an intestinal tumor would be the most likely diagnosis in this aged patient with iron deficiency anemia.

Case 5

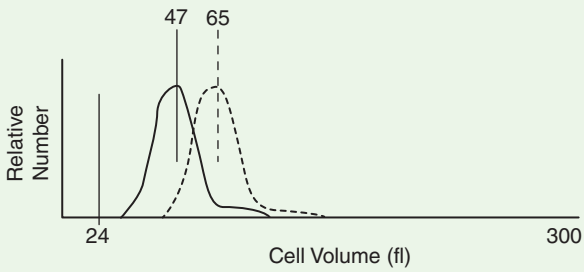
Signalment: 1-year-old pointer

History: Treated for neck or back pain with corticosteroids by referring veterinarian. Dog was thought to have GI parasites due to occult blood in feces, and was treated with anthelmintics. The dog returned 1 month later with a PCV of 15% and MCV of 40 fl. At that time the dog had an abdominal effusion.

Physical Examination: Painful abdomen, pale mucous membranes

Hematology		Reference Interval
PCV (%)	**18**	37–55
Hgb (g/dL)	**3.76**	12–18
RBC ($\times 10^6/\mu L$)	5.8	5.5–8.5
MCV (fL)	**47**	60–72
MCHC (g/dL)	33	33–38
Retics ($\times 10^3/\mu L$)	**18**	<60
NCC ($\times 10^3/\mu L$)	**40.1**	6–17
Segs ($\times 10^3/\mu L$)	**36.5**	3–11.5
Bands ($\times 10^3/\mu L$)	**0.4**	0–0.3
Metas ($\times 10^3/\mu L$)	**0.4**	0
Monos ($\times 10^3/\mu L$)	1.2	0.1–1.3
Lymphs ($\times 10^3/\mu L$)	1.2	1–4.8
Eos ($\times 10^3/\mu L$)	0.4	0.1–1.2
Platelets ($\times 10^3/\mu L$)	**623**	200–500
TP (P) (g/dL)	**5.9**	6–8

Hemopathology: Numerous keratocytes, few schistocytes, some RBCs appear hypochromic. Occasional lymphocyte with azurophilic granules.



Histogram Solid line = patient; Dashed line = normal:

Abdominal Fluid Analysis	
NCC (μL)	**90,000**
TP (g/dL)	**4.0**

Cytology: All cells are degenerate neutrophils. Bacteria of various types are present.

Interpretive discussion

Hematology

Packed cell volume, Hemoglobin: Both are decreased, indicating that the dog is anemic. The red blood cell count is within the reference interval, suggesting erythrocytes are small.

MCV: The mean cell volume is decreased. Decreased mean cell volume is almost always caused by iron deficiency anemia, which in adults is almost always secondary to chronic external blood loss.

Reticulocytes are not increased, indicating that the anemia is nonregenerative. While uncomplicated iron deficiency anemia is usually regenerative, this anemia may be nonregenerative due to the presence of inflammation (note inflammatory leukogram).

Erythrocyte morphology: Keratocytes, erythrocyte fragmentation, and increased central pallor are commonly associated with iron deficiency anemia.

Neutrophilia and increased immature neutrophils are indicative of a marked inflammatory leukogram. The inflammatory leukogram is compatible with the presence of inflammation in the peritoneal cavity, although one would usually expect to see more band neutrophils in dogs with peritonitis. The presence of this inflammation may be the explanation for the lack of a regenerative response to the anemia, as an anemia of inflammatory disease may be superimposed on the iron deficiency anemia. The noted occasional granulated lymphocyte is interpreted as an incidental finding.

Platelets are increased. Approximately half of all animals with iron deficiency anemia have increased platelets, probably in response to cytokines and growth factors.

Total protein: Total protein is slightly decreased, probably as a result of chronic blood loss.

Histogram confirms the presence of a population of microcytic cells (normal histogram represented by dashed line).

Abdominal fluid analysis

Suppurative septic inflammation. The presence of different types of bacteria suggests a GI source of bacteria.

Summary

The dog died, and on necropsy had an intestinal perforation secondary to an ulcer, chronic diffuse peritonitis, pyogranulomatous lymphadenitis and amyloidosis of the spleen, liver, and kidney. Presumably, the dog had been chronically bleeding from this ulcer, resulting in iron deficiency anemia.

Case 6

Signalment and History: 9 year-old female beagle presented for lethargy and pale mucous membranes. Owner reported that the dog occasionally had blood in feces.

Hematology		Reference Interval
PCV (%)	**12**	37–55
RBC ($\times 10^6/\mu L$)	**2.76**	5.5–8.5
Hgb (g/dL)	**3.2**	12–18
MCV (fL)	**40**	60–72
MCHC (g/dL)	**29**	34–38
Retics ($\times 10^3/\mu L$)	**242,880**	0–60,000
Nucleated cells ($\times 10^3/\mu L$)	**33.4**	6.0–17.0
Metas ($\times 10^3/\mu L$)	—	0
Bands ($\times 10^3/\mu L$)	—	0–0.3
Segs ($\times 10^3/\mu L$)	**30.7**	3.0–11.5
Lymphs ($\times 10^3/\mu L$)	**1.0**	1.0–4.8
Monos ($\times 10^3/\mu L$)	1.0	0.2–1.4
Eos ($\times 10^3/\mu L$)	—	0.1–1.2
NRBCs ($\times 10^3/\mu L$)	**0.7**	0
Platelets ($\times 10^3/\mu L$)	Adequate	200–500
TP (P) (g/dL)	6.3	6.0–8.0
Hemopathology: Increased central pallor, occasional keratocyte, giant platelets.		

Interpretive discussion

Hematology

Packed cell volume, Hemoglobin, Red blood cell count: The dog is markedly anemic; all measurements of red blood cell mass are decreased.

MCV: The mean cell volume is markedly decreased. Decreased mean cell volume is almost always caused by iron deficiency anemia, which in adults is almost always secondary to chronic external blood loss. The MCHC may be mildly decreased in iron deficiency as it is here. The reticulocytosis may also contribute to the decreased MCHC.

Reticulocytes are increased, indicating that the anemia is regenerative, suggesting blood loss or blood destruction. In this case, the decreased MCV strongly suggests iron deficiency anemia secondary to chronic blood loss. The presence of nucleated red blood cells is compatible with this degree of regenerative response.

Erythrocyte morphology: Keratocytes and increased central pallor are commonly associated with iron deficiency anemia.

Neutrophilia is indicative of inflammation, even though no band neutrophils are present, since the neutrophil concentration is greater than two fold upper reference interval. The lymphocyte count is in the low normal range, indicating that there may be a stress or steroid component to the leukogram.

Total protein is within the reference interval. Although one might expect total protein to be decreased with chronic blood loss, animals often compensate for this chronic loss of protein.

Summary

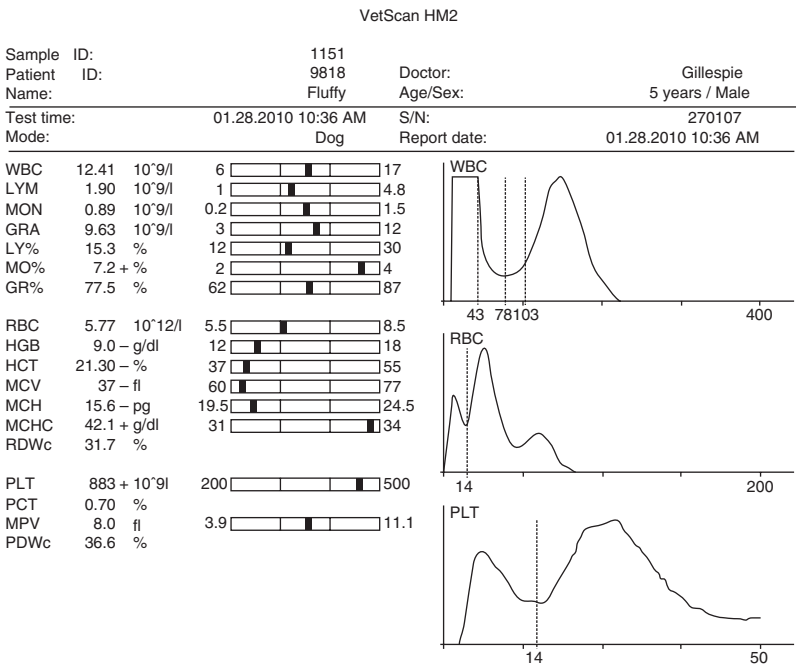
GI barium series performed and jejunal mass seen. At surgery, a mass in the mid-jejunum was resected and determined to be a leiomyosarcoma with clean surgical margins. The regenerative response in this case is in contrast to the previous case to make the point that iron deficiency anemia may be either regenerative or nonregenerative.

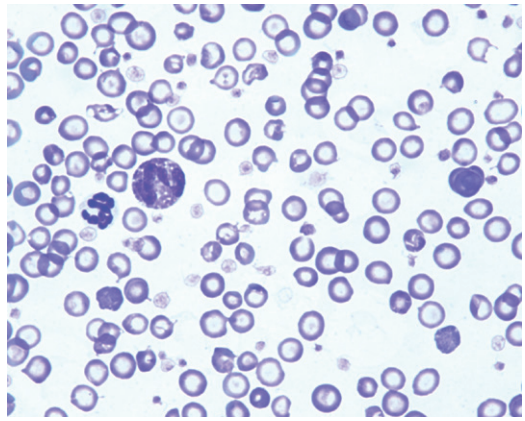
Case 7

Signalment Five-year-old mixed breed dog
History Lethargy
Physical examination Lethargic, pale mucous membranes

Hematology	1/20*	1/28	Reference Interval
Packed cell volume (%) (spun)	**14**	**30**	37–55
Hematocrit (%) (calculated)	**12.4**	**21.3**	37–55
Hgb (g/dL)	**4.4**	**9.0**	12–18
RBC (10^6/µL)	**4.26**	5.77	5.5–8.5
MCV (fL)	**29**	**37**	60–77
MCHC (g/dL)	35.2	**42.1**	31–34
RDW	**23.8**	**31.7**	12–15
Platelets(x10^3/µL)	**803**	**883**	200–500
Reticulocytes (x10^3/µL)	**102**	**403**	0–60
Total Protein (g/dL)	**5.3**	6.2	6–8
* sample moderately lipemic			

1/20 histogram and blood film



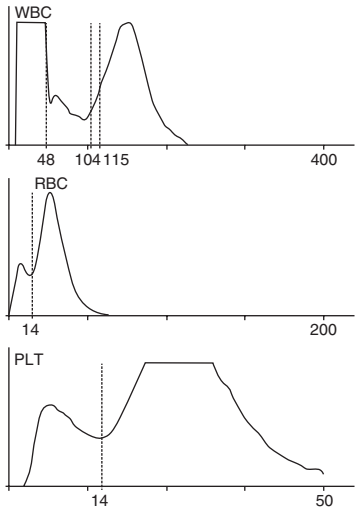


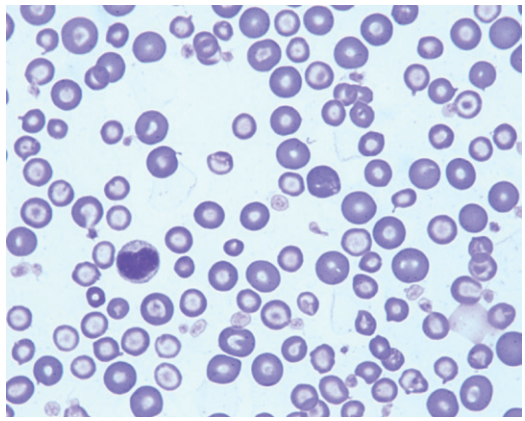
1/28 histogram and blood film

VetScan HM2

Sample ID:			1100		
Patient ID:			9818	Doctor:	Gillespie
Name:			Fluffy	Age/Sex:	5 years / Male
Test time:			01.28.2010 09:31 AM	S/N:	270107
Mode:			Dog	Report date:	01.28.2010 09:31 AM

WBC	8.86	10^9/l	6	17
LYM	2.03	10^9/l	1	4.8
MON	0.43	10^9/l	0.2	1.5
GRA	6.40	10^9/l	3	12
LY%	22.9	%	12	30
MO%	4.8 +	%	2	4
GR%	72.3	%	62	87
RBC	4.26 –	10^12/l	5.5	8.5
HGB	4.4 –	g/dl	12	18
HCT	12.39 –	%	37	55
MCV	29 –	fl	60	77
MCH	10.3 –	pg	19.5	24.5
MCHC	35.2 +	g/dl	31	34
RDWc	23.8	%		
PLT	803 +	10^9/l	200	500
PCT	0.67	%		
MPV	8.3	fl	3.9	11.1
PDWc	36.6	%		





Interpretive discussion

Hematology

PCV and Hematocrit: Decreased, indicating anemia. Note the marked discrepancy in the calculated hematocrit (HCT) and the spun packed cell volume (PCV). The HCT is calculated by the instrument using the following formula: (MCV x RBC)/10 = HCT(%). In the case of the sample from 1/28/10, the RBC count may be under-reporting the sample's RBC concentration since some small RBCs are likely appearing in the PLT channel. The lower reported RBC count is also likely due to the fact that two overlapping RBC peaks are now present in the RBC histogram from 1/28/10; the two overlapping peaks will be underestimated since the software expects only one RBC peak to be present and will not apply curve fitting algorithms here. Since the RBC count is underestimated, the HCT calculated here is lower than the reported PCV.

RBC

The RBCs are decreased as a result of anemia, but they are likely undercounted in this patient due to their small size, and are likely being counted in the platelet channel (see the platelet histogram below the erythrocyte histogram).

MCV

The decreased MCV indicates a severe microcytic anemia. The degree of microcytosis is illustrated by the RBC histogram on 1/20. The blood film contained many hypochromic erythrocytes, as well as keratocytes and schistocytes, erythrocyte shape changes and fragmentation that are very characteristic of iron deficient erythrocytes. The dog was treated with iron. Note that one week later on 1/28 the MCV increased, and the new normal-sized erythrocytes are evident in the erythrocyte histogram on 1/28. They are represented by the additional curve on the right side of the histogram as well as on the blood film made on 1/28.

MCHC

The MCHC is miscalculated to be high on 1/28 likely as a result of the undercounted RBCs. The MCHC is probably erroneously increased on 1/20 as well. The MCHC is calculated from the hemoglobin concentration and the HCT using the following formula: HGB (g/dL) divided by the HCT (%) x 100 = MCHC (g/dL). It provides an index for the quantity of hemoglobin relative to the volume of packed erythrocytes. It may be erroneously increased due to lipemia, hemolysis, or an erroneously low HCT. On 1/28, it is likely erroneously high due to the erroneously low HCT. On 1/20, it may also be erroneously high due to lipemia artifactually increasing the HGB.

RDW

The increased variation in erythrocyte size (anisocytosis) is represented by the increased red cell distribution width (RDW) on 1/28, a numerical expression (coefficient of variation) that correlates with the degree of anisocytosis and which the instrument determines by dividing the standard

deviation of the red cell size by the MCV. Both iron deficient microcytic erythrocytes and young macrocytic erythrocytes can also be seen on the blood film.

Platelets

The platelet concentration is markedly increased, as is often the case in patients with iron deficiency anemia. Some of the platelets are quite large. Also, some microcytic RBC are counted as PLT, falsely increasing the PLT concentration.

Reticulocytes

The reticulocyte concentration is increased, indicating a regenerative anemia.

Total protein

The total protein is slightly decreased on 1/20, likely due to chronic blood loss (protein is lost as well as erythrocytes).

Summary

Iron deficiency anemia as indicated by marked microcytosis.

Outcome

The diagnosis of iron deficiency anemia was confirmed by measuring serum iron, which was 27 µg/dL (Reference interval = 98–220 µg/dL). The source of chronic blood loss is usually the gastrointestinal tract. An occult blood test was performed on the feces on multiple occasions, and was consistently negative. An examination of feces for the eggs of parasites such as hookworms was also negative. Further history revealed that the owner had given the dog an anthelmintic two weeks prior to presentation, presumably eliminating a hookworm infestation. The dog was treated with 15 mg/kg iron dextran IM, and the owner was instructed to give him 162.5 mg Ferrous sulfate orally once daily for 4 weeks. The hematocrit and MCV were normal at his follow-up examination 6 months later.

Case 8

Signalment: 10-year-old castrated male Labrador retriever

History: Four episodes of acute weakness over past 3 months. At time of wellness exam 4 months ago, dog had PCV of 44% and T.P. of 8.2 g/dL.

Physical examination: Pale mucous membranes, abdomen slightly distended

Hematology		Reference Interval
PCV (%)	**16**	37–55
Hgb (g/dL)	**5.3**	12–18
RBC ($\times 10^6/\mu L$)	**2.48**	5.5–8.5
MCV (fL)	63	60–72
MCHC (g/dL)	34	34–38
Retics ($\times 10^3/\mu L$)	**342**	<60
NCC ($\times 10^3/\mu L$)	**39.1**	6–17
Segs ($\times 10^3/\mu L$)	**33.2**	3–11.5
Bands ($\times 10^3/\mu L$)	**1.2**	0–0.3
Monos ($\times 10^3/\mu L$)	**3.1**	0.1–1.3
Lymphs ($\times 10^3/\mu L$)	**0.4**	1–4.8
Eos ($\times 10^3/\mu L$)	0.4	0.1–1.2
NRBCs ($\times 10^3/\mu L$)	**0.8**	0
Platelets ($\times 10^3/\mu L$)	**130**	200–500
TP (P) (g/dL)	6.2	6–8
Hemopathology: Polychromasia increased, numerous acanthocytes and schistocytes. Numerous Howell-Jolly bodies		

Biochemical Profile
No abnormalities

Abdominal Fluid Analysis	
PCV (%)	**24**
NCC ($\times 10^3/\mu L$)	**34,000**
Cytology: 95% nondegenerate neutrophils; 5% macrophages, many of which have phagocytized erythrocytes	

Interpretive discussion

Hematology

Packed cell volume, Hemoglobin, Red blood cell count: The dog is anemic; all measurements of red blood cell mass are decreased.

MCV: The mean cell volume is normal. However, it is surprising that it is not higher considering that the reticulocyte count is increased.

Reticulocytes are increased, indicating that the anemia is regenerative, and is thus due to blood loss or blood destruction. The borderline low plasma protein suggests blood loss is likely; this will be confirmed by additional findings. Nucleated RBCs are increased due to early marrow release as part of regeneration.

Erythrocyte morphology: Acanthocytes are commonly seen in dogs with hemangiosarcoma. The schistocytes are suggestive of microangiopathy, which may also be associated with hemangiosarcoma.

Neutrophilia, increased immature (band) neutrophils, and monocytosis are indicative of an inflammatory leukogram, although a component of the mature neutrophilia is likely due to stress or corticosteroids. Lymphopenia is indicative of stress.

Platelets are slightly decreased. Considering the presence of schistocytes, the animal may have DIC.

Total protein: Total protein is within the reference interval. However, considering that it was 8.2 g/dL 4 months previously, it is likely decreased due to blood loss within the abdominal cavity.

Abdominal fluid analysis

Hemoabdomen.

Summary

The signalment (large breed, older dog), history (episodes of weakness), regenerative anemia, erythrocyte morphology, and the hemoabdomen are all suggestive of hemangiosarcoma. An exploratory was performed, and the dog had hemangiosarcoma of the spleen and liver, which had ruptured. Previous episodes of weakness were likely due to previous ruptures of the tumor, which had subsequently sealed, then ruptured again.

Case 9

Signalment: 15-year-old Staffordshire terrier
History: Lethargic
Physical examination: Pale mucous membranes

Hematology	January	October	Reference Interval
PCV (%)	**30**	**28**	37–55
RBC ($\times 10^6/\mu L$)	**4.70**	**4.44**	5.5–8.5
Hgb (g/dL)	**10.1**	**9.5**	12–18
MCV (fL)	61	64	60–72
MCHC (g/dL)	35	34	34–38
Retics ($\times 10^3/\mu L$)	**178,000**	**13,200**	0–60,000
NCC ($\times 10^3/\mu L$)	**23.4**	10.2	6.0–17.0
Bands ($\times 10^3/\mu L$)	**0.5**	0.2	0–0.3
Segs ($\times 10^3/\mu L$)	**15.7**	6.2	3.0–11.5
Lymphs ($\times 10^3/\mu L$)	**6.1**	1.5	1.0–4.8
Monos ($\times 10^3/\mu L$)	0.7	**1.7**	0.2–1.4
Eos ($\times 10^3/\mu L$)	—		0.1–1.2
NRBCs ($\times 10^3/\mu L$)	—		0
Platelets ($\times 10^3/\mu L$)	150	**12**	200–500
TP (P) (g/dL)	**8.2**	**5.6**	6.0–8.0
Alb	**1.5**		2.7–4.5
Glob	**6.0**		1.9–3.4

Hemopathology (January): Increased rouleaux, giant platelets, lymphs contain azurophilic granules.
Hemopathology (October): Increased rouleaux, lymphs contain azurophilic granules. numerous *Hemobartonella canis* (*Mycoplasma haemocanis*) organisms present.

Interpretive discussion

Hematology

Packed cell volume, Hemoglobin, Red blood cell count: The dog is anemic; all measurements of red blood cell mass are decreased.

Reticulocytes are increased in January, indicating that the anemia is regenerative, suggesting either blood loss or blood destruction. Although the PCV is further decreased in October, the anemia is no longer regenerative, suggesting bone marrow dysfunction.

Neutrophilic leukocytosis with left shift and monocytosis are indicative of an inflammatory leukogram (January).

Lymphocytosis in January is most suggestive of either lymphocytic leukemia or ehrlichiosis.

Platelets: The animal is markedly thrombocytopenic in October. The combination of thrombocytopenia and nonregenerative anemia should trigger a bone marrow aspirate examination and ehrlichia titer. Common causes of thrombocytopenia include ehrlichiosis, immune-mediated thrombocytopenia, and DIC.

Total protein: Total protein is increased. In this patient, it is due to hyperglobulinernia, which should trigger protein electrophoresis.

The presence of increased rouleaux is compatible with increased globulin. The presence of large granular lymphocytes is suggestive of certain types of antigenic stimulation, commonly ehrlichiosis, or a leukemia of LGL cells. The presence of *Hemobartonella canis* (*Mycoplasma haemocanis*) organisms in October suggests either a previous splenectomy or splenic dysfunction, since the erythrocyte parasite is rarely seen in dogs with functional spleens. The anemia is no longer regenerative in the face of anemia and this erythrocyte parasite, suggesting marrow impairment of some type, and a bone marrow aspirate is indicated.

Summary

In January, the anemia was possibly due to blood loss associated with a large hematoma of the spleen, and the dog was splenectomized. The lymphocytosis, hyperglobulinemia, and presence of large granular lymphocytes should have triggered an ehrlichia titer, but did not. The animal returned in October, severely anemic and thrombocytopenic. An ehrlichia titer was done at this time, and was strongly positive. The dog was treated for ehrlichiosis and hemobartonellosis, and recovered.

Case 10

Signalment and history: 9-year-old male castrated dog presented for lethargy

Hematology		Reference Interval
PCV (%)	**36**	37–55
RBC ($\times 10^6/\mu L$)	**5.42**	5.5–8.5
Hgb (g/dL)	13.2	12–18
MCV (fL)	66	60–72
MCHC (g/dL)	37	34–38
Retics ($\times 10^3/\mu L$)	0	0–60,000
NCC ($\times 10^3/\mu L$)	**96.4**	6.0–17.0
Metas ($\times 10^3/\mu L$)	—	0
Bands ($\times 10^3/\mu L$)	**7.7**	0–0.3
Segs ($\times 10^3/\mu L$)	**82.9**	3.0–11.5
Lymphs ($\times 10^3/\mu L$)	1.0	1.0–4.8
Monos ($\times 10^3/\mu L$)	**4.8**	0.2–1.4
Eos ($\times 10^3/\mu L$)	—	0.1–1.2
NRBCs ($\times 10^3/\mu L$)	—	0
Platelets ($\times 10^3/\mu L$)	39	200–500
TP (P) (g/dL)	6.2	6.0–8.0

Hemopathology: Decreased platelets, giant platelets, toxic neutrophils, numerous echinocytes, occasional schistocyte.

Interpretive discussion

Packed cell volume and hemoglobin are slightly decreased, indicating mild anemia.

Reticulocytes are not increased, indicating that the anemia is nonregenerative. Considering the inflammatory leukogram, this is most likely an anemia of inflammatory disease.

Marked neutrophilia with increased immature neutrophils and monocytosis is indicative of a chronic inflammatory leukogram.

Lymphopenia is indicative of a stress or steroid component to the leukogram.

Platelets are decreased. Thrombocytopenia is most commonly due to ehrlichiosis, immune-mediated thrombocytopenia or DIC. This should trigger other coagulation tests. The presence of giant platelets suggests that immature platelets are being released by the bone marrow, and the thrombocytopenia is not due to bone marrow dysfunction.

Summary

Anemia of inflammatory disease. Site of inflammation was a prostatic abscess. DIC was confirmed.

Case 11

Signalment: 4-year-old Doberman
History: Acutely ill, vomiting
Physical examination: Pendulous abdomen

Hematology	Day 1*	Day 2	Reference Interval
PCV (%)	50	**20**	37–55
Hgb (g/dL)	18.3	**7.5**	12–18
RBC ($\times 10^6/\mu L$)	7.70	**3.11**	5.5–8.5
MCV (fL)	66	66	60–72
MCHC (g/dL)	36	37	34–38
Retics ($\times 10^3/\mu L$)	ND	**124**	<60
NCC ($\times 10^3/\mu L$)	6.6	14.7	6–17
Segs ($\times 10^3/\mu L$)	**0.4**	4.1	3–11.5
Bands ($\times 10^3/\mu L$)	**3.1**	**7.9**	0–0.3
Metas ($\times 10^3/\mu L$)	**0.1**	**1.5**	0
Monos ($\times 10^3/\mu L$)	0.5	0.3	0.1–1.3
Lymphs ($\times 10^3/\mu L$)	2.1	**0.4**	1–4.8
Eos ($\times 10^3/\mu L$)	0.1	0.1	0.1–1.2
Platelets ($\times 10^3/\mu L$)	**193**	**90**	200–500
TP (P) (g/dL)	**5.9**	**4.0**	6–8

Hemopathology: marked toxic neutropils on Days 1 and 2.
*Had abdominal exploratory surgery the evening of Day 1; treated with fluids between Day 1 and Day 2

Biochemical Profile	Day 1*	Day 2	Reference Interval
Gluc	**26**	**36**	75–130
BUN (mg/dL)	**45**	**62**	7–28
Creat (mg/dL)	**0.6**	**1.8**	0.9–1.7
Ca (mg/dL)	**8.2**	**7.6**	9.0–11.2
Phos (mg/dL)	5.9	**11.0**	2.8–6.1
TP (g/dL)	**4.5**	**2.6**	5.4–7.4
Alb (g/dL)	**1.8**	**1.0**	2.7–4.5
Glob (g/dL)	2.7	**1.0**	1.9–3.4
T. Bili (mg/dL)	0.1	**3.0**	0–0.4
Chol (mg/dL)	145	140	130–370
ALT (IU/L)	20	**328**	10–120
AST (IU/L)	**77**	**775**	16–40
ALP (IU/L)	208	**440**	35–280
GGT	1	1	0–6
Na (mEq/L)	**136**	143	145–158
K (mEq/L)	4.1	5.8	4.1–5.5
CL (mEq/L)	**100**	106	106–127
TCO_2 (mEq/L)	**9.4**	19.4	14–27
An. gap (mEq/L)	**31**	23	8–25

*Had abdominal exploratory surgery the evening of Day 1; treated with fluids between Day 1 and Day 2

Abdominal Fluid Analysis	
NCC (/μL)	**93,000**
TP (g/dL)	1.5

Cytology: 100% degenerate neutrophils; various types of bacteria phagocytized and extracellular.

Interpretive discussion

Hematology

Packed cell volume, Hemoglobin, Red blood cell count: Within or near reference intervals on Day 1, markedly decreased on Day 2 following blood loss that occurred at the time of surgery. Dog seemed to bleed excessively during the surgery.

Reticulocytes are increased on Day 2, indicating that the anemia is regenerative. This regenerative response is earlier than is typically seen, in that reticulocytes don't usually increase until 24 to 72 hours following the onset of anemia.

Neutropenia is present on Day 1, with an increase in immature neutrophils, indicating in this case that the mature neutrophils are being consumed in an inflammatory process, and the marrow is not meeting the consumption demand. On Day 2, the mature neutrophils have increased, as have the immature neutrophils (bands and metamyelocytes). This indicates that the consumptive process has decreased (source of inflammation) or that the marrow has increased production, or both.

Lymphopenia is indicative of a stress or steroid component to the leukogram.

Platelets are mildly decreased on Day 1, and more markedly decreased on Day 2. While some platelets may have been consumed in clotting process secondary to surgery related blood loss, it is also possible that the animal has DIC, particularly with the history of excessive bleeding during surgery. This should trigger additional tests such as FDPs, PT, APTT, and activated clotting time.

Total protein: Total protein is decreased on Day 1 and Day 2. In this patient, this is likely due to loss into the abdominal cavity on Day 1, compounded by blood loss on Day 2. Fluid administration may also be diluting the PCV and plasma protein on Day 2.

Biochemical profile

The serum glucose concentration is markedly decreased, both on Day 1 and Day 2. In this patient, considering the leukogram, this is most likely due to sepsis. Other differentials should include insulinoma, although this is a relatively young dog for an insulinoma.

The BUN is increased on both Day 1 and Day 2, and the serum creatinine is increased on Day 2. This may be either prerenal azotemia or renal azotemia. A urinalysis was not performed.

The serum calcium is decreased on both Days 1 and 2, and is due to the hypoalbuminemia.

The serum phosphorus concentration is increased on Day 2 and is compatible with decreased glomerular filtration rate.

The serum total protein concentration is decreased due to hypoalbuminemia on Day 1, and both hypoalbuminemia and hypoglobulinemia on Day 2 (see explanation above).

The serum bilirubin concentration is increased on Day 2, likely due to cholestasis related to septicemia.

The ALT and AST activity on Day 2 indicates hepatocellular injury, possibly related to anemia, shock, surgery, or septicemia.

The ALP activity is increased on Day 2, possibly related to endogenous corticosteroids or cholestasis.

Both sodium and chloride are decreased on Day 1, possibly due to loss of electrolytes into abdominal effusion, or loss due to vomiting.

Serum total CO_2 is decreased on Day 1, suggesting metabolic acidosis. This has been corrected by Day 2, likely due to fluid therapy.

The anion gap is increased on Day 1, likely due to lactic acid.

Abdominal fluid

The nucleated cell count is very high and all of the cells present are neutrophils, indicating suppurative inflammation or peritonitis. The total protein may be low because the serum protein is decreased, or it may he diluted in the large volume of fluid. The presence of different types of bacteria suggests that the source of bacteria is the gastrointestinal tract.

Summary

This dog had a consumptive inflammatory leukogram and hypoglycemia due to sepsis. On exploratory, the abdominal cavity contained 1400 mL of fluid, and a toothpick was found to have perforated the intestine. Dog died on the evening of Day 2 as a result of septic peritonitis.

Case 12

Signalment 11-year-old female spayed border terrier dog

History Owner noticed intermittent nose bleeds for a few days

Physical examination Few petechial hemorrhages noted on mucous membranes, otherwise normal

Hematology		Reference Interval
Plasma protein g/dL	**6.2**	6.0–8.0
PCV (%)	**24**	40–55
Hgb (g/dL)	**8.4**	12.0–18.0
RBC ($\times 10^6/\mu L$)	**3.34**	5.5–8.5
MCV (fL)	72	62–73
MCHC (g/dL)	35	33–36
Reticulocytes ($\times 10^3/\mu L$)	**149**	<60
NCC ($\times 10^3/\mu L$)	11.5	4.5–15.0
Bands ($\times 10^3/\mu L$)	0.1	0–0.2
Neutrophils ($\times 10^3/\mu L$)	9.0	2.6–11.0
Lymphocytes ($\times 10^3/\mu L$)	1.4	1.0–4.8
Monocytes ($\times 10^3/\mu L$)	0.7	0.2–1.0
Eosinophils ($\times 10^3/\mu L$)	0.1	0.1–1.2
NRBC ($\times 10^3/\mu L$)	0.2	0
Platelets ($\times 10^3/\mu L$)	**7**	200–500
MPV (fL)	**22**	7.5–14.6
Hemopathology noted on blood film: • Moderate polychromasia • No platelet clumps found • Few macroplatelets on scanning		

Interpretive discussion

The erythrocyte values indicate moderate anemia. The anemia is regenerative as indicated by reticulocytosis, along with a few nucleated red cells. While the plasma protein concentration is seemingly normal, there is a reasonable probability it is decreased for this patient. The reason is that older dogs tend to have higher protein concentrations and 6.2 is regarded as low normal; the dog may have had a protein between 7 and 8 g/dL before bleeding occurred. The triad of anemia, regeneration, and decreasing protein is classical for blood loss.

The cause of the blood loss is thrombocytopenia. This magnitude of thrombocytopenia is expected to result in both petechial hemorrhages and blood loss that may not be physically visible.

Examination of the data from the bone marrow perspective indicates that the marrow is producing erythrocytes (regeneration) and neutrophils appropriately. The thrombocytopenia present is therefore a selective, specific cytopenia. When present at this magnitude, typically less than $20 \times 10^3/\mu L$, immune-mediated thrombocytopenia is by far the most likely cause or diagnosis. Furthermore, the increased mean platelet volume (MPV) corroborated by macroplatelets on the blood film suggests accelerated marrow thrombopoiesis. This is the expected response to a consumptive thrombocytopenia.

Summary

The pattern present is characteristic of immune-mediated thrombocytopenia, with bone marrow response to hemorrhage.

Case 13

Signalment Nine-year-old male castrated beagle dog
History Owner complaint of lethargy
Physical examination Mildly enlarged peripheral lymph nodes, somewhat thin

Hematology		Reference Interval
Plasma protein g/dL	7.3	6.0–8.0
PCV (%)	**32**	40–55
Hgb (g/dL)	**11.5**	13.0–20.0
RBC ($\times 10^6/\mu L$)	**4.80**	5.5–8.5
MCV (fL)	69	62–73
MCHC (g/dL)	35	33–36
Reticulocytes ($\times 10^3/\mu L$)	**49**	0–100
NCC ($\times 10^3/\mu L$)	**83.7**	4.5–15.0
Bands ($\times 10^3/\mu L$)	0	0–0.2
Neutrophils ($\times 10^3/\mu L$)	**16.7**	2.6–11.0
Lymphocytes ($\times 10^3/\mu L$)	**64.4**	1.0–4.8
Monocytes ($\times 10^3/\mu L$)	1.6	0.2–1.0
Eosinophils ($\times 10^3/\mu L$)	0.1	0.1–1.2
NRBC ($\times 10^3/\mu L$)	0.8	0
Platelets ($\times 10^3/\mu L$)	**139**	200–500
MPV (fL)	**11.4**	7.5–14.6

Hemopathology noted on blood film:
- Many of the lymphoid cells are large with fine chromatin and classified as prolymphocytes and lymphoblasts
- No platelet clumps found

Interpretive discussion

There is marked leukocytosis with the predominant abnormality being marked lymphocytosis. Further, the morphology indicates the presence of large lymphocyte forms. The magnitude of lymphocytosis is clearly interpreted as lymphocytic leukemia, with morphologic features of a blastic form that some would term "acute." There is a mild mature neutrophilia and monocytosis that is difficult to interpret. These could be related to steroid release, with neoplastic lymphocytosis masking the expected steroid-induced lymphopenia. Alternatively, there may be a compensated inflammatory stimulus. There is a disproportionate number of NRBC that may be related to either marrow and/or splenic injury associated with lymphoma/leukemia.

The erythrocyte values indicate mild anemia. The anemia is poorly regenerative as indicated by the reticulocyte concentration. There is mild thrombocytopenia. The presence of two cell lines that potentially have decreased production along with evidence of leukemia suggests that marrow may be involved.

Summary

The pattern present is characteristic of lymphocytic leukemia. Cytometric analysis is recommended if treatment is considered.

Case 14

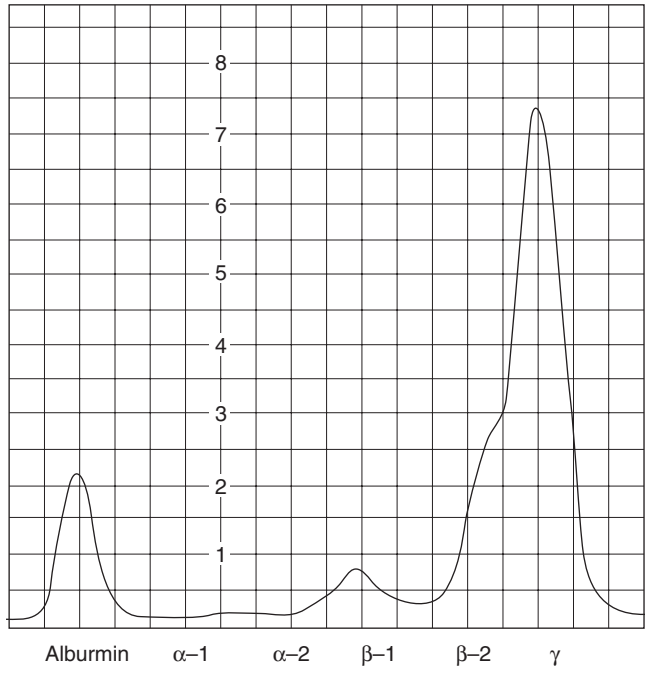
Signalment 14-year-old CM mixed-breed dog
History Polydipsia and polyuria, bloody diarrhea, weight loss
Physical exam Lethargic, bilateral masses in perianal region

Hematology		Reference Interval
Packed cell volume (%)	**33**	37–55
Hemoglobin (g/dL)	**11.1**	12–18
RBC (10^6/μL)	5.77	5.5–8.5
MCV (fL)	**57**	60–72
MCHC (g/dL)	34	34–38
Total nucleated cell count ($\times10^3$/μL)	5.6	6–17
Segmented neutrophils ($\times10^3$/μL)	4.6	3–11.5
Monocytes ($\times10^3$/μL)	0.7	0.2–1.4
Lymphocytes ($\times10^3$/μL)	**0.6**	1–4.8
Platelets ($\times10^3$/μL)	190	200–500
Plasma protein (g/dL)	**11.4**	6–8
Hemopathology: Marked rouleaux		

Biochemical Profile		
Glucose (mg/dL)	93	65–122
Blood Urea Nitrogen (mg/dL)	19	7–28
Creatinine (mg/dL)	1.2	0.6–1.5
Calcium (mg/dL)	**14**	9.0–11.2
Phosphorus (mg/dL)	6.0	2.8–6.1
Total Protein (g/dL)	**12.0**	5.4–7.4
Albumin (g/dL)	**1.7**	2.7–4.5
Globulin (g/dL)	**10.3**	1.9–3.4
Total Bilirubin (mg/dL)	0.4	0–0.4
Cholesterol	154	130–300
Alanine aminotransferase (IU/L)	49	10–120
Aspartate aminotransferase (IU/L)	41	16–40
Alkaline phosphatase (IU/L)	**249**	18–141
Gamma glutamyl transferase (IU/L)	5	0–6
Sodium (mEq/L)	144	145–158
Potassium (mEq/L)	4.2	4.1–5.5
Chloride (mEq/L)	117	106–127
HCO_3 (mEq/L)	**13.1**	14–27
Anion gap	18	8–25

Urinalysis (cystocentesis)			
Color	pale yellow	**Urine Sediment**	
Transparency	clear	WBCs/hpf	0–1
Specific Gravity	**1.012**	RBCs/hpf	0–1
pH	5.0	Epithelial cells/hpf	0
Glucose	neg	Casts/lpf	0
Bilirubin	neg	Crystals	few amorphous
Blood	1+	Bacteria	0
Protein	**3+**		
Ketones	neg		

Serum protein electrophoresis tracing



Interpretive discussion

Hematology

There is a mild normochromic, microcytic anemia that appears nonregenerative (no increased polychromasia is noted on the blood film). A reticulocyte count should be done to confirm this. In a sick dog, anemia of chronic disease should be considered; however, anemia of chronic disease is not usually microcytic. Given that the dog has bloody diarrhea, iron deficiency due to GI blood loss should also be considered as a cause of the microcytosis.

The lymphopenia suggests a stress/steroid response, although the expected neutrophilia is not observed. The slight thrombocytopenia is not clinically significant. Marked rouleaux seen on the blood film is related to the markedly increased plasma protein concentration (discussed further below).

Biochemical profile

The increased total protein concentration is due to marked hyperglobulinemia. This degree of hyperglobulinemia is usually caused by lymphoid neoplasia (such as multiple myeloma), but can also be seen with ehrlichiosis in dogs. A serum protein electrophoresis is indicated to evaluate for monoclonal versus polyclonal gammopathy (discussed later), and evaluation for tick-borne diseases should be pursued. The albumin concentration is moderately decreased, which may be from decreased production in response to the hyperglobulinemia. However, there is also evidence for urinary protein loss that may be contributing to the hypoalbuminemia.

The other significant abnormality present is hypercalcemia. Since this dog has palpable perianal masses and adenocarcinoma of the anal sac is a common cause of paraneoplastic hypercalcemia in dogs, aspiration cytology or biopsy of those masses should be performed. Hypercalcemia may also accompany lymphoid neoplasia, which is another possible differential in this dog based on the hyperglobulinemia. Ionized calcium could be measured to confirm the hypercalcemia, but was not done in this case.

The slight increase in ALP suggests induction due to cholestasis or steroids. Decreased bicarbonate concentration with a normal anion gap is consistent with a secretional metabolic acidosis, and can be explained by the diarrhea (GI loss of bicarbonate).

Urinalysis

The urine is in the isosthenuric range, which can be explained by the hypercalcemia. Hypercalcemia interferes with ADH action in the renal tubules, preventing adequate urine concentration and causing polyuria with polydipsia. The pH is acid, consistent with the metabolic acidosis. The 3+ protein is significant given the urine specific gravity and inactive sediment. A urine protein : creatinine ratio could have been performed, but was not in this case. Possibilities for the proteinuria include a prerenal proteinuria due to glomerular overload (paraproteinuria associated with multiple myeloma) or renal proteinuria due to glomerular disease.

Serum protein electrophoresis

There is a distinct monoclonal peak in the gamma region, suggestive of a neoplastic monoclonal gammopathy. However, some cases of canine ehrlichiosis have apparent monoclonal gammopathies, so further diagnostics are warranted to confirm lymphoid neoplasia.

Summary

The hypercalcemia was consistent with the clinical suspicion of anal sac adenocarcinoma, but the hyperglobulinemia suggested a second pathologic process. Fine needle aspirates of the perianal masses were consistent with anal sac adenocarcinoma, which was later confirmed by surgical removal and histopathology. Radiographs of the thoracic cavity showed no evidence of pulmonary metastases, however pathologic fracture of the right 6th rib was identified. Pending tickborne disease titers, a bone marrow aspirate was performed and was diagnostic for plasma cell myeloma (64% of marrow cells were plasma cells). An immunoelectrophoresis identified the paraprotein as IgA. Ehrlichial titers were negative. This dog was treated with melphalan and prednisone, and did well at home for about one year.

Case 15

Signalment: 8-year-old spayed female golden retriever
History: Presented for lethargy, anorexia, a mass over the humeroradial joint, and prolonged bleeding at a biopsy site
Physical examination: Fever, icterus, and an enlarged liver and spleen

Hematology		Reference Interval
PCV (%)	**23**	37–55
Hgb (g/dL)	**8.5**	12–18
RBC ($\times 10^6/\mu L$)	**3.27**	5.5–8.5
MCV (fL)	71	60–72
MCHC(g/dL)	37	34–38
Retics ($\times 10^3/\mu L$)	**130.8**	<60
NCC ($\times 10^3/\mu L$)	**45.4**	6–17
Segs ($\times 10^3/\mu L$)	**41.8**	3–11.5
Bands ($\times 10^3/\mu L$)	**0.5**	0–0.3
Monos ($\times 10^3/\mu L$)	**3.2**	0.1–1.3
Lymphs ($\times 10^3/\mu L$)	**0.0**	1–4.8
Eos ($\times 10^3/\mu L$)	0.0	0.1–1.2
Platelets ($\times 10^3/\mu L$)	**25**	200–500
TP (P) (g/dL)	**4.6**	6–8
Hemopathology: Increased polychromasia and giant platelets		

Biochemical Profile		Reference Interval
Gluc (mg/dL)	70	65–122
BUN (mg/dL)	11	7–28
Ca (mg/dL)	**8.2**	9.0–11.2
Phos (mg/dL)	4.0	2.8–6.1
TP (g/dL)	**5.0**	5.4–7.4
Alb (g/dL)	**2.2**	2.7–4.5
Glob (g/dL)	2.8	1.9–3.4
T. Bili (mg/dL)	**7.6**	0–0.4
Chol (mg/dL)	329	130–370
ALT (IU/L)	58	10–120
ALP (IU/L)	**775**	35–280
Na (mEq/L)	**144**	145–158
K (mEq/L)	**4.0**	4.1–5.5
CL (mEq/L)	109	106–127
TCO_2 (mEq/L)	16.6	14–27
An. gap (mEq/L)	22	8–25

Urinalysis			
Color	Yellow	**Urine Sediment**	
Transparency	Clear	WBCs/hpf	0–2
Sp. Gr.	1.012	RBCs/hpf	0–1
Protein	–	Epith cells/hpf	0
Gluc	–	Casts/lpf	0
Bilirubin	**4+**	Crystals	0
Blood	Trace	Bacteria	0
pH	5.5	Other	1 + fat

Coagulation Data		Reference Interval
Activated clotting time (seconds)	**>300**	72–86
PT (seconds)	**14.5**	6.4–7.4
aPTT (seconds)	**32.3**	9–11
FDPs (µg/mL)	**>80**	<10

Interpretative discussion

Hematology

There is anemia that is regenerative as evidenced by the significant reticulocytosis and polychromasia. Anemia in combination with low total protein suggests that the cause is blood loss. There is a mixed inflammatory and stress (steroid) leukogram evidenced by the neutrophilia with band neutrophils, monocytosis, and absence of lymphocytes and eosinophils. The thrombocytopenia in conjunction with the large platelets suggests increased consumption and production of platelets.

Biochemical profile

Hypocalcemia corrects into the normal range, and thus is due to hypoalbuminemia. Hypoproteinemia is due to blood loss. The hyperbilirubinemia may result from increased erythrocyte destruction or cholestasis. Increased alkaline phosphatase activity supports cholestasis. Very mild hyponatremia and hyperkalemia are probably insignificant in this case.

Urinalysis

The urine specific gravity is isosthenuric, but the urea nitrogen is normal, so the specific gravity may not be significant. Water deprivation and ensuing specific gravity would determine renal function reserve. Hyperbilirubinuria is a consequence of hyperbilirubinemia.

Coagulation data

Decreased platelets, prolonged ACT, PT, aPTT, and increased FDPs support disseminated intravascular coagulation. Erythrocytes may be destroyed during disseminated intravascular coagulation, thus contributing to elevated total bilirubin.

Summary

The mass was diagnosed as malignant histiocytosis, with nodules in the liver, spleen, mediastinum, and peripheral lymph nodes at necropsy. A likely scenario is that extensive tumor mass developed necrosis and/or inflammation that triggered hypercoagulability leading to disseminated intravascular coagulation. Involvement of liver likely explains the hypoalbuminemia and other liver changes.

Case 16

Signalment: 5-month-old female dog
History: The puppy bleeds excessively when it loses teeth
Physical examination: The mucous membranes were pale. There is moderate bleeding evident at the site of a recent tooth loss.

Hematology		Reference Interval
PCV (%)	**19**	37–55
Hgb (g/dL)	**6.1**	12–18
Retics ($\times 10^3/\mu L$)	**188**	<60
NCC ($\times 10^3/\mu L$)	**35.4**	6–17
Segs ($\times 10^3/\mu L$)	**29.7**	3–11.5
Bands ($\times 10^3/\mu L$)	**2.5**	0–0.3
Monos ($\times 10^3/\mu L$)	**3.2**	0.1–1.3
Lymphs ($\times 10^3/\mu L$)	**0.0**	1–4.8
Eos ($\times 10^3/\mu L$)	0.0	0.1–1.2
Platelets ($\times 10^3/\mu L$)	**915**	200–500
TP (P) (g/dL)	6.5	6–8
Hemopathology: Moderate polychromasia and anisocytosis is present.		

Coagulation Data		Reference Interval
Activated clotting time	**>180**	72–86
PT (seconds)	6.8	6.4–7.4
aPTT (seconds)	**>120**	9–11
Fibrinogen (mg/dL)	200	100–400
Bleeding Time (minutes)	3	1–5

Interpretative discussion

Hematology

The anemia is regenerative as the reticulocyte count is increased and there is polychromasia and anisocytosis on the blood film. The cause of anemia is not determined, but is likely due to hemolysis or blood loss since it is regenerative. The clinical findings of hemorrhage suggest that blood loss is the cause. Thrombocytosis is common in iron deficiency anemia. Microcytosis is evident in chronic iron deficiency, and may contribute to anisocytosis. Size of erythrocytes is not known, since the MCV is not provided. Serum iron and iron binding capacity would be useful in determination of the cause of anemia. The neutrophilia, left shift, and monocytosis indicate an inflammatory leukogram. Lymphopenia is indicative of a concurrent stress/steroid mediated response.

Coagulation data

The coagulation profile suggests a deficiency of one or multiple coagulation factors in the intrinsic pathway. Platelet concentration is increased in number, and no large forms are seen in peripheral blood. Bleeding time is normal, and in the face of adequate platelet concentration, indicates that platelet function is normal. The most common cause of a severe coagulopathy with normal platelet concentration, normal hepatic enzyme activity, and a prolongation of the aPTT with normal PT is factor 8 deficiency. The occurrence is less common in females, and to have an affected female requires that the sire also be affected.

Summary

This dog was tested for factor 8 plasma activity and was found to have 21% of normal activity, which is diagnostic for factor 8 deficiency or hemophilia A. This is compatible with the major abnormalities in the ACT and APTT and the clinical description of bleeding in a young dog.

Case 17

Signalment: 7-year-old female Walker hound
History: The owner noticed a swelling on the right front leg on the day of admission
Physical examination: The mucous membranes were pale. There was a subcutaneous swelling in the right ventral thoracic area, with some dried blood on all four legs.

Hematology		Reference Interval
PCV (%)	**25**	37–55
Hgb (g/dL)	**8.4**	12–18
RBC ($\times 10^6/\mu L$)	**4.03**	5.5–8.5
MCV (fL)	62	60–72
MCHC (g/dL)	34	34–38
Retics ($\times 10^3/\mu L$)	44	<60
NCC ($\times 10^3/\mu L$)	14.4	6–17
Segs ($\times 10^3/\mu L$)	**12.2**	3–11.5
Monos ($\times 10^3/\mu L$)	**1.6**	0.1–1.3
Lymphs ($\times 10^3/\mu L$)	**0.6**	1–4.8
Platelets ($\times 10^3/\mu L$)	315	200–500
TP (P) (g/dL)	**4.6**	6–8
Hemopathology: 1+ leptocytosis and anisocytosis.		

Biochemical Profile		Reference Interval
Gluc (mg/dL)	88	65–122
BUN (mg/dL)	17	7–28
Creat (mg/dL)	1.1	0.9–1.7
Ca (mg/dL)	10.2	9.0–11.2
Phos (mg/dL)	3.5	2.8–6.1
TP (g/dL)	**4.1**	5.4–7.4
Alb (g/dL)	**2.3**	2.7–4.5
Glob (g/dL)	**1.8**	1.9–3.4
T. Bili (mg/dL)	0.3	0–0.4
Chol (mg/dL)	188	130–370
ALT (IU/L)	35	10–120
ALP (IU/L)	40	35–280
Na (mEq/L)	**144**	145–158
K (mEq/L)	**4.0**	4.1–5.5
CL (mEq/L)	107	106–127
TCO_2 (mEq/L)	18	14–27

Coagulation Data		Reference Interval
Activated clotting time (sec)	**>180**	72–86
PT (seconds)	**>180**	6.4–7.4
aPTT (seconds)	**>180**	9–11
Fibrinogen (mg/dL)	300	100–400
Bleeding Time (minutes)	**4**	1–5

Interpretive discussion

Hematology

The anemia is nonregenerative as the erythrocyte indices are normal and the reticulocyte count is normal. The plasma and serum protein are low, with equal deficiency of globulin and albumin, suggesting blood loss as the cause of anemia. The anemia is likely too acute for there to be a regenerative response. Aspiration of the subcutaneous mass confirmed the presence of blood. Mild neutrophilia, monocytosis, and lymphopenia are indicative of a stress leukogram.

Biochemical profile

The protein changes discussed above indicate subacute blood loss, with fluid shifting and dilution of plasma protein resulting in anemia and hypoproteinemia. The mild decrease in sodium and potassium are insignificant.

Coagulation data

The coagulation data indicates either a deficiency of multiple coagulation factors, or a single factor deficiency of the common pathway. Platelets are normal in number, and, no large forms are seen in peripheral blood. Bleeding time is normal and in the face of normal platelet concentration, indicates that platelet function is also normal. The most common cause of a severe coagulopathy with normal platelets and normal hepatic enzyme activities is vitamin K antagonism.

Summary

This dog was exposed to diphacinone, a rodentocide that is a vitamin K antagonist; coagulation times returned to normal following administration of vitamin K.

Case 18

Signalment: 2-month-old female horse
History: Off feed
Physical examination: Depressed, evidence of diarrhea

Hematology		Reference Interval
PCV (%)	**14**	32–52
Hgb (g/dL)	**6.5**	11–19
NCC ($\times 10^3/\mu L$)	6.5	5.5–12.5
Segs ($\times 10^3/\mu L$)	4.7	2.7–6.7
Monos ($\times 10^3/\mu L$)	0.1	0–0.8
Lymphs ($\times 10^3/\mu L$)	1.6	1.5–5.5
NRBCs ($\times 10^3/\mu L$)	**0.1**	0
Platelets ($\times 10^3/\mu L$)	**14**	100–600
TP (P) (g/dL)	6.3	6–8
Hemopathology: mod toxic neutrophils, few reactive lymphs, mod Howell-Jolly bodies, few echinocytes, marked anisocytosis.		

Biochemical Profile		Reference Interval
Gluc (mg/dL)	91	70–110
BUN (mg/dL)	**40**	14–27
Creat (mg/dL)	**2.1**	1.1–2.0
Ca (mg/dL)	**9.7**	11.0–13.7
Phos (mg/dL)	**6.3**	1.9–4.1
TP (g/dL)	**4.6**	5.8–7.6
Alb (g/dL)	**2.2**	2.7–3.7
Glob (g/dL)	**2.4**	2.6–4.6
T. Bili (mg/dL)	**3.2**	0.6–2.1
AST (IU/L)	280	185–300
GGT (IU/L)	**28**	7–17
SDH (IU/L)	**27**	0–9
CK (IU/L)	169	130–470
Na (mEq/L)	**120**	133–145
K (mEq/L)	3.8	2.2–4.6
CL (mEq/L)	**84**	100–111
TCO_2 (mEq/L)	**11.0**	24–34
An. gap	**28.8**	5–15
Calc. Osmolality (mOsm/kg)	**250**	280–310
Amylase (IU/L)	34	0–87
Lipase (IU/L)	534	ND*
Grossly lipemic serum		
*ND—Not determined for foals		

Blood Gas Data (arterial)		Reference Interval
pH	**7.282**	7.38–7.46
pCO_2 (mmHg)	**20.6**	35–47
pO_2 (mmHg)	**60.9**	67–96
HCO_3 (mEq/L)	**9.3**	22–30

Coagulation Profile		Reference Interval
PT (seconds)	**14.6**	9.5–11.5
aPTT (seconds)	39.8	24–45
Fibrinogen (mg/dL)	500	100–400
FDPs (µg/mL)	>10 & <40	ND*
*ND—Not determined for foals		

Abdominal Fluid Analysis	
Color	**Red**
Clarity	**Opaque**
NCC (/µL)	**16,000**
TP (g/dL)	**5.7**
PCV	**13%**
Comments: Erythrophagia and platelets noted in film.	

Interpretive discussion

Hematology

There is a marked anemia. While it is not unusual for neonatal animals to have a "congenital anemia" due to iron deficiency, the PCV is much lower than is typically encountered by this physiological change. The presence of anisocytosis leads one to suspect that there may be a regenerative response, for which evaluation of the MCV and RBC histogram would be useful. The presence of nucleated erythrocytes in the peripheral blood is uncommon in horses, but occasionally seen in foals with profound regenerative responses, or with damage to the bone marrow endothelium, as might occur with sepsis. Combined decreases in PCV and serum proteins may indicate hemorrhage. There is a marked thrombocytopenia, which may be due to decreased production or increased consumption; thrombocytopenia is severe enough to be resulting in blood loss. Refer to the discussion of the coagulation profile for more on this matter.

Biochemical profile

The BUN, serum creatinine, and phosphorus concentrations are increased, but the nature of this azotemia cannot be definitively differentiated without a urinalysis.

There is hypocalcemia and hyperphosphatemia. This combination of mineral abnormalities may be seen in nutritional secondary hyperparathyroidism due to excessive dietary phosphorus. However, higher serum phosphorus concentrations are commonly observed in growing animals, and hypocalcemia may also be due to uptake by widespread damaged tissues, decreased intake with anorexia, or to an apparent decrease due to hypoalbuminemia.

Serum total protein concentration is decreased, including both hypoalbuminemia and hypoglobulinemia. Low serum albumin may be hepatocellular dysfunction or cachexia and decreased albumin synthesis. Alternatively, there may be pathologic albumin loss due to gastrointestinal or renal disease. Low serum globulin in a 2-month-old foal is not due to failure of passive transfer, but may be due to decreased production, malnutrition, or pathologic loss. Loss of all proteins would be expected with hemorrhage, which could also account for the profound anemia. This is the most likely cause.

The serum total bilirubin concentration is increased, with only a mild increase in serum GGT activity. This may reflect hyperbilirubinemia of fasting in an equine anorexic patient. However, SDH activity is increased, indicating hepatocellular damage.

Serum sodium and chloride concentrations are decreased. This is commonly observed in young animals with an enterotoxigenic or secretory diarrhea. This may also be due to gastrointestinal stasis, a third space accumulation in the abdominal cavity, as well as to decreased intake. One would typically expect a hyperkalemia to occur in secretory diarrhea, owing to acidosis-induced intercompartmental exchange. Hypokalemia may be expected in third space syndromes, owing to potassium loss and renal decompensation. It is possible to observe normokalemia with concomitant potassium loss and metabolic acidosis, wherein redistribution of potassium from the intracellular to the extracellular fluid compartment obscures the whole body potassium deficit. There is evidence in support of abdominal hemorrhage and third spacing due either to acute pancreatitis or a gastric ulcer (see below).

Marked lipemia is often seen in ponies with starvation and metabolic disease, but is unusual in horses. In other species, hyperlipidemia may occur due to impaired triglyceride clearance associated with endotoxemia. One should consider other potential causes of hyperlipidemia such as pancreatitis. In this case, serum amylase activity is normal, but lipase activity may be increased. There is evidence of recent hemorrhage into the abdominal cavity, which could be related to acute pancreatitis, but is more often due, in diseased foals, to a bleeding gastric ulcer. The low calculated osmolality would be expected, given the hyponatremia and hypochloremia.

Blood gas data

There is an increased anion gap metabolic acidosis with respiratory compensation. This is consistent with a secretory diarrhea, complicated by hypovolemia and/or sepsis. If there were gastrointestinal stasis, one might expect an alkalosis. If there were a ruptured urinary bladder, one might expect a metabolic acidosis with hyperkalemia. The increased anion gap may result from sepsis, with hypovolemia and lactic acidosis due to reduced tissue perfusion and/or the metabolic effects of endotoxemia. The decreased oxygen tension may indicate respiratory compromise as well.

Coagulation data

The prothrombin time is prolonged slightly, the activated partial thromboplastin time is normal, and the FDP concentration is in an intermediate range. These findings may indicate disseminated intravascular coagulation, particularly in light of the severe thrombocytopenia, wherein Factor VII levels are becoming depleted, thereby prolonging the PT, but other coagulation factor concentrations are adequate to maintain a normal APTT. The concurrent observation of thrombocytopenia and findings consistent with blood loss anemia support a diagnosis of DIC with pathologic hemorrhage.

Summary

Enterotoxigenic *E. coli* diarrhea, pancreatitis, hepatitis, and DIC were findings confirmed at necropsy. On necropsy, the pancreas was 5 to 6 times normal size and the liver was swollen. Histopathology showed necrosis and inflammation of the pancreas, diffuse mesenteric steatitis, fat necrosis and fat saponification, inflammation of the liver with thrombi in central veins and associated focal ischemic necrosis. Inflammatory disease is often not reflected in equine leukograms.

Case 19

Signalment: 3-year-old spayed female cocker spaniel
History: Left in owner's car in shopping mall parking lot for approximately 3 hours on a hot summer afternoon
Physical examination: Depressed and mildly dehydrated

Hematology		Reference Interval
PCV (%)	**58**	37–55
NCC ($\times 10^3/\mu L$)	16.0	6–17
Segs ($\times 10^3/\mu L$)	**13.4**	3–11.5
Monos ($\times 10^3/\mu L$)	**1.6**	0.1–1.3
Lymphs ($\times 10^3/\mu L$)	1.0	1–4.8
Platelets ($\times 10^3/\mu L$)	Adequate	200–500

Biochemical Profile		Reference Interval
Gluc (mg/dL)	**142**	65–122
BUN (mg/dL)	**62**	7–28
Creat (mg/dL)	**3.0**	0.9–1.7
Ca (mg/dL)	**8.4**	9.0–11.2
Phos (mg/dL)	4.9	2.8–6.1
TP (g/dL)	**9.4**	5.4–7.4
Alb (g/dL)	**5.4**	2.7–4.5
Glob (g/dL)	**4.0**	1.9–3.4
T. Bili (mg/dL)	0.4	0–0.4
Chol (mg/dL)	160	130–370
ALT (IU/L)	**178**	10–120
ALP (IU/L)	60	35–280
Na (mEq/L)	**164**	145–158
K (mEq/L)	5.4	4.1–5.5
CL (mEq/L)	124	106–127
TCO_2 (mEq/L)	14	14–27
An. gap (mEq/L)	**31.4**	8–25
Meas. Osmolality (mOsm/kg)	**358**	290–310
Calc. Osmolality (mOsm/kg)	**344**	290–310
Osmol gap (mOsm/kg)	**14**	0–10

Blood Gas Data (arterial)		Reference Interval
pH	**7.09**	7.33–7.45
PCO_2 (mmHg)	**46**	24–39
HCO_3 (mEq/L)	**13**	14–24

Urinalysis			
Color	Dk yellow	**Urine Sediment**	
		WBCs/hpf	2–3
Transpareney	Cloudy	RBCs/hpf	4–5
Sp. Gr.	**1.011**	Epith cells/hpf	2–3
Protein	**1+**	Casts/lpf	**2–3 fine granular**
Gluc	Neg		
Bilirubin	Neg	Crystals	2+ Ca oxalate
Blood	Neg	Bacteria	0
pH	5.5		

Interpretive discussion

Hematology

Hemoconcentration is indicated by the increased PCV and physical signs of dehydration. Mild neutrophilia, monocytosis, and borderline lymphopenia is interpreted as a stress leukogram.

Biochemical profile

There is a mild hyperglycemia, which may be due to a catecholaminergic or steroid stress response.

The BUN and serum creatinine concentrations are increased. See discussion of urinalysis below to explain whether the azotemia is likely prerenal, renal, or postrenal.

Hyperalbuminemia with hyperproteinemia indicates dehydration. In this case, hyperglobulememia is also likely caused by dehydration.

There is a mild hypocalcemia in the face of hyperalbuminemia due to dehydration. Thus, serum calcium concentration is truly decreased. This is often seen in heat-stressed animals, subsequent to widespread tissue damage and precipitation of calcium salts in ischemic areas.

The small increase in serum ALT activity may not be significant, or may reflect some hepatocellular damage.

The hypernatremia, in concert with other signs of dehydration, indicates a hypertonic dehydration. This is commonly seen in heat-stressed dogs owing to increased insensible losses of water, in excess of solute, due to hyperventilatory evaporation.

The measured and calculated osmolality values are increased, consistent with hypertonic dehydration. However, the osmol gap is also increased, indicating the accumulation of unmeasured osmotically active solutes in the blood. The anion gap is likewise increased, and given the dehydration and probable tissue hypoperfusion, some degree of lactic acidosis is likely.

Blood gas data

There is a combined metabolic (decreased bicarbonate) and respiratory (increased pCO_2) acidosis. The metabolic acidosis results from lactic acidosis due to tissue hypoperfusion. The respiratory acidosis suggests compromised pulmonary function.

Urinalysis

The presence of 1+ proteinuria with a specific gravity of 1.011 indicates significant urinary protein loss. The fine granular casts indicate tubular epithelial damage. The isosthenuric specific gravity in the face of dehydration and azotemia, yet in the absence of electrolyte depletion, suggests renal disease as well. This is likely a case of acute renal failure secondary to heat stress. The presence of calcium oxalate crystals may have no importance, or may represent one potential route of calcium loss due to renal tubular damage associated with the hypocalcemia.

Summary

Heat stress, hypertonic dehydration, and acute renal failure. If one did not have the history given, or doubted its veracity, laboratory findings like these would strongly suggest antifreeze intoxication. One could analyze serum for ethylene glycol concentration to definitively rule this possibility in or out.

Case 20

Signalment: 4-year-old intact male dog
History: Experiencing intermittent periods of weakness and lameness
Physical examination: Mild dehydration, foul smelling breath, teeth covered with tartar

Hematology		Reference Interval
PCV (%)	**11**	37–55
Hgb (g/dL)	**4.0**	12–18
RBC ($\times 10^6$/µL)	**1.64**	5.5–8.5
MCV (fL)	67	60–72
MCHC (g/dL)	36	34–38
Retics ($\times 10^3$/µL)	**13.1**	<60
NCC ($\times 10^3$/µL)	8.7	6–17
Segs ($\times 10^3$/µL)	7.7	3–11.5
Bands ($\times 10^3$/µL)	0.1	0–0.3
Monos ($\times 10^3$/µL)	0.3	0.1–1.3
Lymphs ($\times 10^3$/µL)	**0.4**	1–4.8
Eos ($\times 10^3$/µL)	0.2	0.1–1.2
Platelets ($\times 10^3$/µL)	370	200–500
TP (P) (g/dL)	6.8	6–8

Hemopathology: Slight anisocytosis and slight polychromasia.

Biochemical Profile		Reference Interval
Gluc (mg/dL)	91	65–122
BUN (mg/dL)	**183 *(65.3)***	7–28 *(2.5–10.0 mmol/L)*
Creat (mg/dL)	**8.1 *(716)***	0.9–1.7 *(79–150 µmoJ/L)*
Ca (mg/dL)	**8.2 *(2.05)***	9.0–11.2 *(2.25–2.8 mmol/L)*
Phos (mg/dL)	**17.2 *(5.5)***	2.8–6.1 *(0.9–2.9 mmol/L)*
TP (g/dL)	5.8	5.4–7.4
Alb (g/dL)	3.2	2.7–4.5
Glob (g/dL)	2.6	1.9–3.4
T. Bili (mg/dL)	0.4	0–0.4
Chol (mg/dL)	180	130–370
ALT (IU/L)	19	10–120
AST (IU/L)	17	16–40
ALP (IU/L)	40	35–280
Na (mEq/L)	146	145–158
K (mEq/L)	5.0	4.1–5.5
Cl (mEq/L)	115	106–127
TCO_2 (mEq/L)	16	14–27
An. gap (mEq/L)	20	8–25

Urinalysis (catheterized)			
Color	Yellow	**Urine Sediment**	
Transparency	Clear	WBCs/hpf	3–5
Sp. Gr.	**1.008**	RBCs/hpf	2–3
Protein	Trace	Epith cells/hpf	0
Gluc	Negative	Casts/lpf	0
Bilirubin	Negative	Crystals	0
Blood	Negative	Bacteria	0
pH	5.0		

Interpretive discussion

Hematology

The nonregenerative anemia is secondary to chronic renal disease. Decreased erythropoietin production by the kidneys is a major factor leading to anemia in animals with chronic renal disease. The severity of anemia is unusual for chronic renal disease. Such anemias are typically of mild to moderate severity. Other causes of nonregenerative anemia should also be considered in this case.

The cause of the lymphopenia is increased blood steroid concentration associated with stress. The leukocyte response in not a typical steroid-mediated response in that a mature neutrophilia typically accompanies lymphopenia. It is likely that the animal's resting neutrophil concentration was low normal and it has approximately doubled due to the steroid influence.

Biochemical profile

The triad of BUN, creatinine, and phosphorus concentrations is markedly increased indicating decreased glomerular filtration. These products are passively filtered by the glomerulus, and any cause of decreased glomerular filtration will result in retention of these analytes in the blood. In light of the urine specific gravity in the isosthenuric range, primary renal azotemia is interpreted.

At least two mechanisms have played a role in causing the hypocalcemia. The phosphorus concentration is markedly increased, and the Ca × P product is 141. When this product exceeds 70, calcium and phosphorus precipitate in soft tissues, decreasing the serum calcium concentration. In addition, chronic renal disease may result in decreased activation of vitamin D by the kidneys (i.e., conversion of 25-hydroxycholecalciferol to 1,25-dihydroxycholecalciferol). Decreased activated vitamin D results in decreased absorption of calcium from the intestinal tract.

Urinalysis

A urine specific gravity in the isosthenuric range in an azotemic animal suggests an inability to concentrate urine. Animals with prerenal azotemia due to decreased renal perfusion (e.g., dehydration, cardiac insufficiency, circulatory shock) should be conserving water and concentrating urine. The urine specific gravity is a key to properly interpreting the cause of the azotemia in this case.

Urine sediment—Small numbers of leukocytes and erythrocytes are normal in urine. These numbers must be interpreted in light of the urine concentration and the technique used to concentrate the sediment. Leukocyte numbers may be slightly increased in this case, suggesting minimal inflammation in the urinary tract.

Summary

These data indicate chronic renal failure. Chronicity is suggested by the nonregenerative anemia, which would not be present with acute renal failure. Postmortem diagnosis in this case was chronic interstitial nephritis or end-stage renal disease. No lesions suggesting suppurative inflammation in the urinary tract were found.

Case 21

Signalment: 9-year-old intact female dog
History: Abscess on rear leg 2 months ago. Intermittent vomiting began 2 days ago.
Physical examination: Popliteal and cervical lymph nodes are enlarged

Hematology		Reference Interval
PCV(%)	**35**	37–55
Hgb (g/dL)	12.1	12–18
RBC ($\times 10^6/\mu L$)	5.6	5.5–8.5
MCV (fL)	62	60–72
MCHC (g/dL)	36	34–38
Retics ($\times 10^3/\mu L$)	22.4	<60
NCC ($\times 10^3/\mu L$)	13	6–17
Segs ($\times 10^3/\mu L$)	9.4	3–11.5
Bands ($\times 10^3/\mu L$)	0.1	0–0.3
Monos ($\times 10^3/\mu L$)	0.8	0.1–1.3
Lymphs ($\times 10^3/\mu L$)	2.4	1–4.8
Eos ($\times 10^3/\mu L$)	0.3	0.1–1.2
Platelets ($\times 10^3/\mu L$)	250	200–500
TP (P) (g/dL)	6.2	6–8
Hemopathology: Normal.		

Biochemical Profile		Reference Interval
Gluc (mg/dL)	89	65–122
BUN (mg/dL)	**114 *(40.7)***	7–28 *(2.5–10.0 mmol/L)*
Creat (mg/dL)	**3.2 *(283)***	0.9–1.7 *(79–150 µmol/L)*
Ca (mg/dL)	**8.5 *(2.12)***	9.0–11.2 *(2.25–2.8 mmol/L)*
Phos (mg/dL)	**8.8 *(2.84)***	2.8–6.1 *(0.9–2.9 mmol/L)*
TP (g/dL)	**5.2**	5.4–7.4
Alb (g/dL)	**1.2**	2.7–4.5
Glob (g/dL)	**4.0**	1.9–3.4
T. Bili (mg/dL)	0.3	0–0.4
Chol (mg/dL)	**582 *(15.1)***	130–370 *(3.4–9.6 mmol/L)*
ALT (IU/L)	18	10–120
AST (IU/L)	20	16–40
ALP (IU/L)	22	35–280
Na (mEq/L)	**142**	145–158
K (mEq/L)	4.7	4.1–5.5
CL (mEq/L)	120	106–127
TCO_2 (mEq/L)	18	14–27
An. gap (mEq/L)	9	8–25
Amylase (IU/L)	**1530**	50–1250
Lipase (IU/L)	**720**	30–560

Urinalysis (catheterized)			
Color	Yellow	**Urine Sediment**	
Transparency	Cloudy	WBCs/hpf	0
Sp. Gr.	**1.021**	RBCs/hpf	0
Protein	**4+**	Epith cells/hpf	0
Gluc	Negative	Casts/lpf	2–3 granular
Bilirubin	Negative	Crystals	0
Blood	Negative	Bacteria	0
pH	6.0		
protein/ creatinine ratio	5.4		

Interpretive discussion

Hematology

A mild nonregenerative anemia (reticulocyte count is in the normal range) is present. This should prompt an evaluation for endocrine disease, renal disease, and chronic inflammatory disease as potential causes. In this case, chronic renal disease is probably the underlying cause. There is no evidence of inflammatory disease in the leukogram.

Biochemical profile

The azotemia indicated by increased concentrations of BUN, creatinine, and phosphorus should be classified as renal since urine concentration is not adequate (i.e., <1.030), suggesting a loss of renal concentrating ability.

The hypocalcemia is probably due to two factors. The calcium × phosphorus product is 75. When this product exceeds 70, precipitation of calcium and phosphorus in soft tissues can occur, and decreased serum calcium concentrations may result. In addition, activation of vitamin D by the kidney is decreased in chronic renal disease, resulting in decreased absorption of calcium from the small intestine.

The hyperphosphatemia is due to decreased glomerular filtration rate (GFR). In this case, glomerular disease has caused decreased GFR and subsequent hyperphosphatemia.

In light of the marked proteinuria, the most likely cause of the hypoproteinemia and hypoalbuminemia is renal protein loss, due to glomerular disease. The hyperglobulinemia most likely resulted from chronic antigenic stimulation. History of a previous abscess and subsequent lymph node enlargement are compatible with such antigenic stimulation (i.e., the original infection may not have been completely eliminated, resulting in chronic antigenic stimulation and hyperplasia in lymphoid tissue). Such chronic antigenic stimulation can predispose to some forms of glomerular disease.

Hypercholesterolemia is interpreted as a component of nephrotic syndrome. Nephrotic syndrome, a group of abnormalities that may be associated with serious glomerular disease, includes hypoalbuminemia, proteinuria, hypercholesterolemia and edema. In this case, edema was not observed; however, presence of the other three components is still suggestive of this syndrome. Edema is not likely to occur until the albumin is below 1.0 g/dL. The mechanism causing hypercholesterolemia in this syndrome has not been identified.

The cause of mild hyponatremia is not certain in this case. Renal Na loss is a possible cause. If edema were present, it is possible that dilution of extracellular Na in this fluid (third-spacing) could result in decreased serum Na concentration. Edema was, however, not evident in this case, and, even in animals with edema, hyponatremia is not common.

Serum amylase and lipase activities are commonly increased in animals with decreased GFR. Although other causes of increased activities such as pancreatitis could be considered in this case, the clinical presentation and other laboratory data are more compatible with decreased GFR resulting in mildly increased amylase and lipase activities.

Urinalysis

Urine concentrating ability is inadequate. If the azotemia in this dog were due to prerenal causes such as dehydration, cardiac insufficiency, or circulatory shock, urine specific gravity should be >1.030. The specific gravity suggests inadequate concentrating ability and primary renal azotemia. Post-renal azotemia is ruled out by the demonstration of a patent urethra via catheterization and by the absence of evidence of urine leakage into tissues or the abdomen. Lack of concentrating ability results from loss of nephrons and/or tubular damage. Both of these alterations are probably occurring in this dog. Although the disease is primarily glomerular, severe damage to glomeruli results in secondary tubular damage and in loss of nephrons.

A 4+ protein in a moderately dilute urine and a urine protein/creatinine ratio (UPC) of 5.4 are evidence of severe proteinuria. In the absence of evidence of hemorrhage or inflammation (i.e., increased erythrocyte or leukocyte numbers in the urine sediment), a UPC >1.0 is abnormal in the dog, and a UPC >5.0 is indicative of glomerular disease. A UPC of >15 is diagnostic for glomerular disease.

Summary

Renal biopsy revealed amyloidosis. Chronic infection resulting in chronic antigenic stimulation probably predisposed the dog to this disease. The lymph node enlargement was most likely due to hyperplasia secondary to chronic antigenic stimulation. The combination of hypoalbuminemia, proteinuria, and hypercholesterolemia suggest imminent onset of nephrotic syndrome.

Case 22

Signalment: 13-year-old castrated male cat
History: Rear leg paralysis, dyspnea, vomiting
Physical examination: Lethargy and dyspnea. Systolic murmur detected.

Hematology	Day 1	Reference Interval
PCV (%)	35	24–45
Hgb (g/dL)	11.3	8–15
RBC ($\times 10^6/\mu L$)	8.05	5–11
MCV (fL)	44	39–50
MCHC (g/dL)	32	33–37
NCC ($\times 10^3/\mu L$)	18.1	5.5–19.5
Segs ($\times 10^3/\mu L$)	**16.3**	2.5–12.5
Monos ($\times 10^3/\mu L$)	0.5	0–0.8
Lymphs ($\times 10^3/\mu L$)	**0.9**	1.5–7.0
Basophils ($\times 10^3/\mu L$)	0.2	Rare
NRBC ($\times 10^3/\mu L$)	**0.2**	0
Platelets ($\times 10^3/\mu L$)	Adequate	
TP (P) (g/dL)	7.2	6–8
Hemopathology: Normal		

Biochemical Profile	Day 1	Day 3	Reference Interval
Gluc (mg/dL)	**153** ***(8.4)***	**360** ***(19.8)***	67–124 *(3.7–6.8 mmol/L)*
BUN (mg/dL)	**46** ***(16.4)***	**137** ***(48.9)***	17–32 *(6.1–11.4 mmol/L)*
Creat (mg/dL)	**2.9** ***(256)***	**9.8** ***(866)***	0.9–2.1 *(80–186 mmol/L)*
Ca (mg/dL)	**8.4** ***(2.12)***	**4.9** ***(1.22)***	8.5–11 *(2. 12–2.75 mmol/L)*
Phos (mg/dL)	**8.0** ***(2.6)***	**16.1** ***(5.2)***	3.3–7.8 *(1.1–2.5 mmol/L)*
TP (g/dL)	6.9	**5.4**	5.9–8.1
Alb (g/dL)	2.8	2.4	2.3–3.9
Glob (g/dL)	4.1	3.0	2.9–4.4
T. Bili (mg/dL)	0.2	0.3	0–0.3
Chol (mg/dL)	192	151	60–220
ALT (IU/L)	**158**	**294**	30–100
AST (IU/L)	**461**	**643**	14–38
ALP (IU/L)	54	25	6–106
GGT (IU/L)	0	1	0–1
CK (IU/L)	**45,313**	**350,930**	60–300
Na (mEq/L)	150	**139**	146–160
K (mEq/L)	4.9	**6.6**	3.7–5.4
CL (mEq/L)	119	**99**	112–129
TCO_2(mEq/L)	19.2	15.9	14–23
An. gap (mEq/L)	17	**31**	10–27

Urinalysis (cystocentesis)	Day 1	Day 3	Urine Sediment	Day 1	Day 3
Color	Dark yellow	Light yellow	WBCs/hpf	**15–20**	0–1
Transparency	Hazy	Clear	RBCs/hpf	**35–50**	5–10
Sp. Gr.	1.050	**1.010**	Epith cells/hpf	0	0
Protein	**2+**	**1+**	Casts/lpf	**Few granular**	0
Gluc	**4+**	**4+**	Crystals	0	0
Ketones	**2+**	Negative			
Bilirubin	Negative	Negative	Bacteria	0	0
Blood	**4+**	**4+**			
pH	5.5	5.0			

Fractional excretion	Day 1	Reference Interval
Na (%)	**7.2**	<1.0
K (%)	**165.1**	5–20
P (%)	**68.6**	<7–21
Ca (%)	**10.5**	<1.0

Coagulation Data	Day 1	Day 3	Reference Interval
PT (seconds)	10.0	8.9	7–11.5
aPTT (seconds)	**8.2**	16.5	10–18

Endocrine Data	Day 1	Day 3	Reference Interval
Total T4 (µg/dL)		1.34	1.2–4.8

Interpretive discussion

Hematology

In light of normal values for other erythrocyte measurements, the slightly decreased MCHC is not significant. Mature neutrophilia and lymphopenia are typical of a stress leukogram. The basophils are not significant. Occasionally, nucleated RBCs may be found in the blood of normal animals. In the absence of anemia or other erythrocyte abnormalities, the few nucleated RBCs noted in this cat are not important.

Biochemical profile

The cat is hyperglycemic on Days 1 and 3. This abnormality could be due to severe excitement or stress with resulting increased epinephrine or corticosteroid levels, respectively. The leukogram is suggestive of stress. The presence of ketonuria on Day 1 suggests that diabetes mellitus should also be considered. Although this cat's blood glucose concentration on Day 1 is not above the renal threshold, detection of glucosuria on this day suggests that the cat may have had periods with higher blood glucose concentrations or that this cat has an abnormally low renal threshold for glucose.

The cat has an azotemia which progresses from mild to severe. Since urine specific gravity is high on Day 1, the azotemia on that day appears to be prerenal. Urine specific gravity is in the isosthenuric range on Day 3 and may be of renal origin; however, the cat had received fluid therapy, and this, rather than renal failure, likely caused the low urine specific gravity on this day.

Hypocalcemia progresses from mild on Day 1 to marked on Day 3. While ethylene glycol toxicosis may result in hypocalcemia and causes severe azotemia, rear leg paralysis and increased CK activity are not associared with ethylene glycol toxicosis. The Ca × P product on Day 1 is 67 on Day 1 and 79 by Day 3. Precipitation of Ca and P in the tissues may, therefore, be occurring on Day 3 and may, in part, explain the decreasing Ca concentration. Massive muscle tissue destruction, as evidenced by increased CK activity, may have resulted in calcium preciptitation in damaged tissues and subsequent hypocalcemia.

Hyperphosphatemia resulted from decreased glomerular filtration rate. Maintenance of normal serum P concentration depends on normal glomerular clearance of P.

Total serum protein concentration was normal on Day 1, but decreased by Day 3. Although both albumin and globulin concentrations remained within reference intervals, concentrations of both of these proteins decreased due to fluid therapy and subsequent expansion of blood volume. In light of normal serum albumin and globulin concentrations, the significance of the hypoproteinemia is borderline.

Increased serum ALT activity suggests mild hepatocyte injury that progressed to moderate.

The combination of increased serum AST and CK activities indicates muscle injury. Since CK has a short half-life (less than four hours), the extremely high CK activity implies active muscle damage. AST is also present in hepatocytes, and hepatic injury is an alternate explanation for the increased serum AST activity, but, in light of the increased serum CK activity, muscle origin is most likely.

Hyponatremia on Day 3 may be due to renal loss (see fractional excretion results). Since hypochloremia is also evident, vomiting could also be a cause of Na loss. Hypochloremia on Day 3 may be due to both renal loss and vomiting.

Hyperkalemia on Day 3 may be due to several different causes. Since the cat is in renal failure, kidneys may not be excreting K normally. This cat also had a significant degree of tissue necrosis that could have resulted in release of K from dead or dying cells.

The increased anion gap suggests increased concentrations of anions such as ketones, uremic acids, phosphate, sulfate, or lactate. Ketones are not present in the urine of this cat on Day 3, and a significant ketosis is, therefore, not likely. Since the cat is severely azotemic, concentrations of uremic acids are probably increased. Serum phosphorus concentration confirms that increased phosphate is contributing to the anion gap. The final diagnosis suggested that this cat had significant tissue damage, and this probably increased serum sulfate concentrations. Hypoxia was also a component of this cat's disease; therefore, lactic acidosis was also occurring.

Urinalysis

The implications of the urine specific gravities were discussed in the interpretation of this cat's azotemia. The cat has proteinuria and hematuria on both days and pyuria on Day 1. These abnormalities suggest urinary tract inflammation. Cystitis or pyelonephritis are possible causes of this inflammation. The protein concentration decreased between Days 1 and 3, but this probably reflects the change in the concentration of the urine with more dilution of protein on Day 3. Both pyuria and hematuria probably contributed to the proteinuria. Other causes of proteinuria such as glomerular or tubular disease cannot be eliminated. The dipstick test for blood was equally increased on Days 1 and 3, but the RBC concentration decreased markedly between these days. This suggests that the positive test is due to either hemoglobinuria or myoglobinuria. In light of the apparent muscle injury (increased CK), myoglobinuria is most likely.

Glucosuria is marked on both days. On Day 3, this reflects a blood glucose concentration which exceeds the renal threshold. The glucosuria is more difficult to explain on Day 1, when the blood glucose is below the renal threshold. While it is possible that this cat has a lowered renal threshold, it is also possible blood glucose concentrations were fluctuating on Day 1 with periods above the renal threshold occurring.

Presence of a few granular cast suggests tubular damage.

The significance of the positive urine ketone reaction is considered in the discussion of hyperglycemia.

Fractional excretions of Na, K, P, and Ca are increased. This indicates abnormal reabsorption of these electrolytes and, in this case, is probably due to acute renal damage.

Coagulation data

The activated partial thromboplastin time (APTT) is slightly decreased on Day 1 and probably reflects this cat's hypercoagulable condition. The mechanism of this change is not known but may be related to this cat's cardiac problem (see Summary). This cat was treated with streptokinase between Days 1 and 3, and this treatment increases APTT and PT, and return of APTT to within the reference interval on Day 3 may have resulted from this treatment; however, the absence of a longer PT on Day 1 as compared to Day 3 makes a significant effect of streptokinase treatment less certain.

Summary

Clinical diagnosis was restrictive cardiomyopathy with aortic thromboemboli (saddle and renal thrombosis and pulmonary thromboembolism). Restrictive cardiomyopathy predisposes to thrombosis. In this case, the thrombotic disease involved the kidneys and resulted in acute renal failure. In addition, hypoxia occurred in other tissues including the muscles of the rear legs. This resulted in increased serum activities of AST and CK. Necropsy examination was not performed.

Case 23

Signalment: 11-year-old FS canine
History: Weight loss and polyuria
Physical examination: Thin, slightly dehydrated

Hematology		Reference Interval
PCV (%)	**36.0**	37–55
Hgb (g/dL)	12.5	12–18
RBC ($\times 10^6$/μL)	**5.38**	5.5–8.5
MCV (fL)	67.0	60–72
MCHC (g/dL)	35.0	34–38
NCC ($\times 10^3$/μL)	7.0	6–17
Segs ($\times 10^3$/μL)	6.1	3–11.5
Bands ($\times 10^3$/μL)	0.1	0–0.3
Monos ($\times 10^3$/μL)	0.2	0.1–1.3
Lymphs ($\times 10^3$/μL)	**0.5**	1–4.8
Eos ($\times 10^3$/μL)	0.1	0.1–1.2
Platelets ($\times 10^3$/μL)	400	200–500
TP (P) (g/dL)	8.1	6–8

Biochemical Profile		Reference Interval
Gluc (mg/dL)	112	65–122
BUN (mg/dL)	**216 (77.1)**	7–28 *(2.5–10.0 mmol/L)*
Creat (mg/dL)	**15.6 (1379)**	0.9–1.7 *(79–150 μmol/L)*
Ca (mg/dL)	**12.1 *(3.0)***	9.0–11.2 *(2.25–2.8 mmol/L)*
Phos (mg/dL)	**20.9 (6.75)**	2.8–6.1 *(0.9–2.9 mmol/L)*
TP (g/dL)	6.9	5.4–7.4
Alb (g/dL)	4.0	2.7–4.5
Glob (g/dL)	2.9	1.9–3.4
T. Bili (mg/dL)	0.4	0–0.4
Chol (mg/dL)	335	130–370
ALT (IU/L)	73	10–120
AST (IU/L)	25	16–40
ALP (IU/L)	**662**	35–280
GGT (IU/L)	**8**	0–6
Na (mEq/L)	**144**	145–158
K (mEq/L)	**6.2**	4.1–5.5
CL (mEq/L)	**98**	106–127
TCO_2 (mEq/L)	**13.1**	14–27
An. gap (mEq/L)	**39**	8–25
Amylase (IU/L)	866	50–1250
Lipase (IU/L)	386	30–560

Urinalysis			
Color	Yellow	**Urine Sediment**	
Transparency	Cloudy	WBCs/hpf	1–2
Sp. Gr.	**1.011**	RBCs/hpf	1–2
Protein	**3+**	Epith cells/hpf	5–8
Gluc	Negative	Casts/lpf	**0–1 coarse granular and waxy**
Bilirubin	1+	Crystals	Negative
Blood	Trace	Bacteria	Negative
pH	6.0		
UPC	**11.1**		

Interpretive discussion

Hematology

The PCV is marginally decreased, but without reticulocyte count it is difficult to classify the regenerative response. A marginal normocytic, normochromic anemia is observed in renal failure, for which there are other indications in the laboratory data.

The lymphopenia indicates a steroid response.

Biochemical profile

The serum glucose concentration is normal.

The BUN, serum creatinine, and serum phosphorus values are markedly increased. These findings are consistent with decreased glomerular filtration rate. However, one cannot differentiate the nature of the azotemia (prerenal, renal, or postrenal) based on these findings alone. Refer to the discussion of urinalysis results for further interpretation.

Serum total calcium is mildly increased, for which one should consider hypercalcemia of malignancy, hypoadrenocorticism, renal failure, vitamin D toxicosis, or primary hyperparathyroidism.

A significant increase in serum ALP activity and mild increase in GGT activity is consistent with cholestasis. Because AST and ALT activities are normal, there is not likely any hepatocellular damage. ALP and GGT activities may also be increased by corticosteroids.

Serum Na and Cl are decreased In concentration, while serum K is increased. The Na:K ratio is 23.2, which may indicate hypoadrenocorticism. Alternatively, renal disease may result in a functional hypoadrenocorticism due to inability of the damaged renal tubules to respond appropriately to mineralocorticoids or may be due to a simple loss of sodium and retention of potassium because of renal disease and oliguria. The serum total CO_2 is decreased, indicating a metabolic acidosis, while the anion gap is increased, indicating the accumulation of organic anions. Acidosis may result in hyperkalemia as well.

The serum calculated osmolality is increased, predominantly because of the profound azotemia. Likewise, the increased anion gap is due to retention of urinary metabolic products.

Serum amylase and lipase activities are normal, and while not definitive, lessen the probability for pancreatitis.

Urinalysis

The urinary specific gravity is in the isosthenuric range, and there is 3+ proteinuria in the absence of significant hematuria or pyuria. The urinary protein:creatinine ratio is 11.1, which is significantly increased. The mild bilirubinuria is likely significant considering the low specific gravity. The coarse granular and waxy casts also definitively indicate renal tubular damage. Together with the marked azotemia, these findings support a diagnosis of renal disease.

Summary

Malignant fibrous histiocytoma of both kidneys identified at postmortem examination. This accounted for chronic renal failure.

Case 24

Signalment: 12-year-old Quarter horse gelding
History: Losing weight and recently has loose stools
Physical examination: Thin to poor body condition, mild dependent edema all four limbs. Horse developed watery diarrhea during hospitalization.

Hematology		Reference Interval
Packed cell volume (%)	**23**	32–52
Hemoglobin (g/dL)	**7.9**	11–18
RBC (10^6/μL)	**4.41**	6.5–10.5
MCV (fL)	52	36–52
MCHC (g/dL)	34	34–39
Total nucleated cell count ($\times10^3$/μL)	2.5	5.5–12.5
Segmented neutrophils ($\times10^3$/μL)	**0.10**	2.7–6.7
Band neutrophils ($\times10^3$/μL)	**0.025**	0–0.1
Monocytes ($\times10^3$/μL)	0.150	0–0.8
Lymphocytes ($\times10^3$/μL)	2.20	1.5–5.5
Eosinophils ($\times10^3$/μL)	0.025	0–0.9
Platelets ($\times10^3$/ μL)	217	150–500
Plasma protein (g/dL)	**4.6**	6–8
Note: Toxic changes in neutrophils		

Biochemical Profile		Reference Interval
Glucose (mg/dL)	**153**	70–110
Blood Urea Nitrogen (mg/dL)	**254**	14–27
Creatinine (mg/dL)	**23**	1.1–2.0
Calcium (mg/dL)	**16.7**	11–13.7
Phosphorus (mg/dL)	**1.2**	1.9–4.1
Total Protein (g/dL)	**4.1**	5.8–7.6
Albumin (g/dL)	**1.7**	2.7–3.7
Globulin (g/dL)	**2.4**	2.6–4.6
Total Bilirubin (mg/dL)	**5.2**	0.6–2.1
Aspartate aminotransferase (IU/L)	229	185–300
Alkaline phosphatase (IU/L)	255	90–290
GGT (IU/L)	**23**	7–17
CK (IU/L)	**2341**	130–470
Sodium (mEq/L)	**125**	133–145
Potassium (mEq/L)	**8.5**	2.2–4.6
Chloride (mEq/L)	**95**	100–111
Total CO_2 (mEq/L)	**17**	24–34
Anion Gap (mEq/L)	**22**	5–15

Urinalysis voided

		Urine Sediment	
Color	yellow	WBCs/hpf	0–3
Transparency	clear	RBCs/hpf	0–5
Specific Gravity	**1.009**	Epithelial cells/hpf	none
Protein	**2+**	Casts/lpf	neg
Glucose	neg	Crystals	calcium carbonate
Ketones	neg		
Blood	neg		

Interpretive discussion

Hematology

The PCV, hemoglobin, and total RBC count are decreased, indicating a moderate anemia is present. It cannot be determined if the anemia is regenerative because reticulocytes are not released from marrow in the horse. Given the biochemistry profile the anemia is likely due to chronic renal failure (CRF). The mild to moderate degree of anemia fits with CRF as severe anemia is not seen with renal failure unless there is a second problem, e.g., blood loss. If this horse is dehydrated the anemia is more severe as are the protein losses. The plasma protein is markedly decreased, likely due to diarrhea and GI loss. There is severe, life-threatening leukopenia, neutropenia with a left shift and toxic changes in neutrophils. This is seen with acute diarrheal disease in horses due to endotoxemia and or overwhelming sepsis. The bone marrow cannot meet demands and a likely source of the problem is enteric salmonellosis with or without septicemia.

Biochemistry profile

This horse has GI and renal disease, both appear to be severe. Marked azotemia, isosthenuria and hypercalcemia with hypophosphatemia are diagnostic for renal failure in the horse. Suspect chronic renal failure due to poor body condition, history, anemia, hypoalbuminemia, electrolyte abnormalities and an inactive urine sediment. Additionally, chronic renal failure is more common than acute renal failure in horses. UN and Ct are about as high as possible in a living patient; clearly this is not due to a prerenal cause. Isosthenuria confirms renal and postrenal is rare in an adult horse, especially one that is urinating, perhaps even with an increased volume of urine. Lesion in the kidneys is likely to be end-stage, small shrunken kidneys with fibrosis and little to no chance of regeneration or long term survival. Lesion may have started as glomerulonephritis or amyloidosis as hypoalbuminemia is moderate but it can also be seen in severe chronic renal failure from any cause that compromises glomerular function. Peripheral edema is attributed to hypoalbuminemia and decreased colloidal osmotic pressure. Horses tend to develop peripheral edema rather then ascites with hypoalbuminemia.

Hypercalcemia and hypophosphatemia are only seen with renal failure in equidae, all other species develop hyperhosphatemia even if hypercalcemia is present. The only other differentials for hypercalcemia and hypophosphatemia are a malignancy and primary hyperparathyroidism. Hypercalcemia of malignancy (HHM) is uncommon in horses but has been reported in horses with gastric carcinoma and lymphoma. Primary hyperparathyroidism is very rare in horses

and would only be pursued if renal failure and HHM are ruled out first. All three differentials are associated with dilute urine due to inhibition of ADH by hypercalcemia. If mild or moderate azotemia is present with dilute urine in a horse with hypercalcemia and hypophosphatemia it can be difficult to distinguish HHM and chronic renal failure. The easiest diagnostic tests are to first search for cancer (enlarged lymph nodes and endoscopy to look for gastric SCC), rectal and or ultrasound examination of kidneys to determine if they are small and shrunken. If this does not clarify then consider protein creatinine ratio and or fractional excretion of sodium, if <1% rule out renal if >1% rule in renal. Always favor renal failure over HHM in horses.

Hyponatremia and hypochloremia can be attributed to chronic renal failure and or GI loss, the latter is more likely. The hyperkalemia is severe and life threatening. This is unusual as adult horses tend to develop hypokalemia with GI disease but young horses with diarrhea will have hyperkalemia. The most likely explanation is metabolic acidosis even though it does not appear that severe in this horse. The pattern of hyponatremia and hyperkalemia can be seen with renal failure, urinary bladder rupture and hypoadrenocorticism. Decreased bicarbonate (TCO_2) is due to GI and or renal loss. Increased anion gap is due to retained uremic acids, shock and anaerobic glycolysis with accumulation of lactic acid. Horses with diarrheal diseases usually have a metabolic acidosis as does this horse. Hypoalbuminemia and concurrent decreased serum globulin is due to GI disease, suspect salmonellosis. Hypoalbuminemia is moderate and more severe than the decrease in globulins which may be due to renal loss of albumin in addition to the loss in GI tract. Proteinuria without blood is present in the urine and supports renal loss of albumin. Increased bilirubin is due to anorexia, which is the most common cause of icterus in horses. Although hepatic disease and cholestasis are possible explanations they are too unlikely given all the clinical and biochemistry data (liver enzymes WRI). The mild increase in GGT may be spurious rather than a true indicator of liver issues. GGT seems to increase in horses easily and is not reliable indicator of hepatic problems if increased without any increases in other hepatic parameters.

Increase in CPK is mild for a horse and is due to recumbency, it is too low to consider a primary muscle disease. The AST is WRI so it is not a muscle problem in which the CPK is decreasing while the AST is still increased. The urine is yellow, no evidence of brown color or blood, rule out myoglobin induced renal damage with these values. Urine is not concentrated; a second check to confirm isosthenuria is usually recommended but in this case is not needed. Proteinuria with no evidence of hemorrhage or active sediment in an animal with hypoalbuminemia is due to renal loss. The proteinuria is more severe than 2+ since the urine is dilute. A protein to creatinine ratio could help assess the degree of severity but is not needed in this case, given all the data. The few white and red blood cells are inconsequential, may be due to voided urine and the calcium carbonate crystals are normal for a horse.

Summary

Marked chronic renal failure and salmonellosis

Outcome

Horse was euthanized and chronic renal failure and salmonellosis were confirmed at autopsy. Initially the horse presented primarily for weight loss, while it was being evaluated it developed profuse diarrhea in the hospital. The horse was sick and stressed and likely had a recrudescence of salmonella. The horse may have been a carrier of salmonella.

Both kidneys were small, knobby and looked like irregular bovine kidneys due to marked fibrosis. Microscopically there was severe glomerulonephritis, chronic interstitial nephritis and even oxalate crystals in many tubules. The oxalate crystals were attributed to either being a horse or endogenous formation of oxalates which happens in chronic renal failure in dogs and other species as well. It was not ethylene glycol toxicity. Glomerulonephritis was considered the primary lesion but it was difficult to determine if it preceded the interstitial nephritis. Pulmonary thrombi were present and one large thrombus was present in the pulmonary artery. These thrombi were likely due to hypoalbuminemia and decreased AT III (not measured) which lead to a state of increased coagulability. Pulmonary thrombosis associated with decreased AT III and glomerular lesions is seen most frequently, or documented most frequently in dogs.

Hypercalcemia and hypophosphatemia are present in some horses with renal failure, empirically, perhaps about one third. The pathogenesis is not known and multiple theories are postulated. These electrolyte changes will even occur with nephrectomy in the horse. Some horses may excrete excess dietary calcium (from alfalfa-rich diet) in the urine and retain phosphorus. Hypercalcemia is then attributed to decreased excretion by the failing kidneys and hypophosphatemia to increased excretion. However, if the kidneys are removed it seems impossible to explain how they would develop hypophosphatemia. Certainly there cannot be increased renal loss as the kidneys have been removed so it must be increased GI loss, but this is unproven. Many horses with chronic renal failure will have hyperphosphatemia and normo or hypocalcemia, similar to other species.

Case 25

Signalment: 6-year-old MC feline DSH
History: Approximately 1 month's duration of intermittent weakness, exercise intolerance, poor hair coat
Physical examination: Cat is near collapse and approximately 10% dehydrated. There is definite cervical ventroflexion.

Hematology		Reference Interval
PCV (%)	41.0	24–45
Segs ($\times 10^3/\mu L$)	**18.0**	2.5–12.5
Monos ($\times 10^3/\mu L$)	0.7	0–0.8
Lymphs ($\times 10^3/\mu L$)	**0.5**	1.5–7.0
Platelets ($\times 10^3/\mu L$)	Adequate	150–700

Biochemical Profile		Reference Interval
Gluc (mg/dL)	98	67–124
BUN (mg/dL)	**68 *(24.3)***	17–31 *(6.1–11.4 mmol/L)*
Creat (mg/dL)	**2.8 *(247)***	0.9–2.1 *(80–186 µmol/L)*
Ca (mg/dL)	10.9	8.5–11
Phos (mg/dL)	6.8	3.3–7.8
TP (g/dL)	**9.3**	5.9–8.1
Alb (g/dL)	**5.3**	2.3–3.9
Glob (g/dL)	4.0	2.9–4.4
T. Bili (mg/dL)	0.3	0–0.3
Chol (mg/dL)	180	60–220
ALT (IU/L)	52	30–100
ALP (IU/L)	48	6–106
CK (IU/L)	**2419**	60–300
Na (mEq/L)	157	146–160
K (mEq/L)	**2.0**	3.7–5.4
CL (mEq/L)	114	112–129
T CO_2 (mEq/L)	15	14–23
An. gap (mEq/L)	**30**	10–27

Blood Gas Data (arterial)		Reference Interval
pH	**7.130**	7.33–7.44
pCO_2 (mmHg)	**44.0**	35–42
HCO_3 (mEq/L)	**14.0**	16–22

Urinalysis			
Color	Yellow	**Urine Sediment**	
Transparency	Cloudy	WBCs/hpf	0–2
Sp. Gr.	**1.014**	RBCs/hpf	0–2
Protein	Trace	Epith cells/hpf	0–2
Gluc	Negative	Casts/lpf	Negative
Bilirubin	Negative	Crystals	Negative
Blood	Negative	Bacteria	Negative
pH	5.5	Other	

Fractional excretion		Reference Interval
Na (%)	0.55	<1.0
K (%)	**37.7**	<20.0

Interpretive discussion

Hematology

There is a mature neutrophilia and lymphopenia, indicating a stress leukogram. Other components of the hemogram are normal.

Biochemical profile

The BUN and serum creatinine concentrations are mildly increased. These findings are consistent with decreased glomerular filtration rate. However, one cannot differentiate the nature of the azotemia (prerenal, renal, or postrenal) based on these findings alone. Refer to the discussion of urinalysis results for further interpretation. The normal serum phosphorus and total calcium concentrations do not contribute to the characterization of renal disease.

Serum total protein and albumin concentrations are increased; this documents marked dehydration or hemoconcentration.

Serum CK activity is increased significantly and is indicative of muscle damage.

Serum Na and Cl concentrations are normal, but serum K concentration is markedly decreased. This is especially significant in light of the acidosis, which results in a shift of potassium from within cells to extracellular fluid and suggests a marked potassium deficit.

Blood gas data

There is prominent acidosis. This is due to a combined metabolic (decreased HCO_3) and respiratory (increased pCO_2) acidosis, with an increased anion gap. It would not be unusual for this degree of dehydration to lead to hypovolemia-induced lactic acidosis. It is also possible that this degree of hypokalemia may have caused sufficient respiratory muscle dysfunction to impair normal ventilation.

Urinalysis

The urinary specific gravity is in the isosthenuric range. Given the azotemia and normal serum Na and Cl concentrations, this indicates probable renal disease. However, hypokalemia can also impair ADH responsiveness by the kidneys, so that urine concentration should be evaluated following rehydration and K repletion.

The urinary FE_{Na} is 0.55%, which speaks against a generalized renal tubular disease. However, the FE_K is 37.7%, which is markedly increased, especially for a cat with this degree of hypokalemia.

Summary

The combined observations of azotemia, hypokalemia, acidosis, and hyperkaluria in a cat with cervical ventroflexion and evidence of widespread muscle damage support a diagnosis of feline kaliopenic polymyopathy/nephropathy syndrome. In this case, it was completely corrected by dietary change (non-acidifying, higher K diet). This syndrome is no longer seen, as dietary imbalances in commercial cat food were corrected.

Case 26

Signalment: 2-year-old male canine, West Highland white terrier
History: Polyuria, polydipsia

Hematology		Reference Interval
PCV (%)	**33.0**	37–55
Hgb (g/dLO)	**11.3**	12–18
RBC ($\times 10^6/\mu L$)	**4.45**	5.5–8.5
MCV (fL)	**74.0**	60–72
MCHC (g/dL)	35.0	33–38
NCC ($\times 10^3/\mu L$)	5.9	6–17
Segs ($\times 10^3/\mu L$)	3.9	3–11.5
Monos ($\times 10^3/\mu L$)	0.4	0.1–1.3
Lymphs ($\times 10^3/\mu L$)	1.2	1–4.8
NRBC ($\times 10^3/\mu L$)	**0.4**	0
Platelets ($\times 10^3/\mu L$)	425	200–500
TP (P) (g/dL)	6.7	6–8
Hemopathology: Few acanthocytes and schistocytes		

Biochemical Profile		Reference Interval
Gluc (mg/dL)	108	65–122
BUN (mg/dL)	**65 *(23.2)***	7–28 *(2.5–10.0 mmol/L)*
Creat (mg/dL)	**2.0 *(176.8)***	0.9–1.7 *(79–150 µmol/L)*
Ca (mg/dL)	**7.2 *(1.8)***	9.0–11.2 *(2.25–2.8 mmol/L)*
Phos (mg/dL)	6.1	2.8–6.1
TP (g/dL)	5.8	5.4–7.4
Alb (g/dL)	3.7	2.7–4.5
Glob (g/dL)	2.1	1.9–3.4
T. Bili (mg/dL)	0.3	0–0.4
Chol (mg/dL)	**382 *(9.9)***	130–370 *(3.4–9.6 mmol/L)*
ALT (IU/L)	56	10–120
ALP (IU/L)	137	35–280
Na (mEq/L)	147	145–158
K (mEq/L)	**3.0**	4.1–5.5
CL (mEq/L)	115	106–127
TCO_2 (mEq/L)	22.3	14–27
An. gap (mEq/L)	12.7	8–25
Calc. osmolality (mOsm/kg)	**317**	290–310

Blood Gas Data (arterial)		Reference Interval
pH	7.349	7.33–7.45
PO_2 (mmHg)	80.1	67–92
PCO_2 (mmHg)	39.1	24–39
HCO_3 (mEq/L)	21.0	14–24
ionized Ca++ (mEq/L)	**3.44**	4.5–5.6

Urinalysis			
Color	Yellow	**Urine Sediment**	
Transparency	Clear	WBCs/hpf	3–6
Sp. Gr.	1.028	RBCs/hpf	3–6
Protein	**2+**	Epith cells/hpf	0–2
Gluc	**3+**	Casts/lpf	**Rare fine gran**
Bilirubin	1+	Crystals	Negative
Blood	Negative	Bacteria	Negative
pH	5.0		
Ketones	Trace		
osmolality	358 (mOsm/L)		
UPC	**1.75**		

Fractional excretion		Reference Interval
Na (%)	**1.62**	<1.0
Ca (%)	**7.47**	<1.0

Interpretive discussion

Hematology

The packed cell volume, erythrocyte count, and hemoglobin concentration are decreased, indicating an anemia. Observed red blood cell morphologic abnormalities include acanthocytes and schistocytes. These may be observed when there is erythrocytic membrane damage due to free radical or lipid metabolic abnormalities, or when there is microangiopathic pathology due to vascular disease or neoplasia. Although a reticulocyte count has not been provided, the increased erythrocyte MCV and nucleated erythrocytes are consistent with a regenerative responce. There are no other hematologic abnormalities.

Biochemical profile

Serum glucose concentration is normal, and its importance in the interpretation of the glucosuria is discussed below.

The BUN and serum creatinine concentrations are increased, while the serum phosphorus value is at the upper limit of the reference interval. These findings are consistent with decreased glomerular filtration rate. However, one cannot differentiate the nature of the azotemia (prerenal, renal, or postrenal) based on these findings alone. Refer to the discussion of urinalysis results for further interpretation.

The serum total calcium concentration is decreased. The ionized calcium concentration reported with the blood gas panel is likewise less than normal, indicating a true hypocalcemia. In this case, excessive loss of calcium in the urine is the likely cause (see urinalysis discussion).

Serum total protein, albumin, and globulin concentrations are within the reference interval. This observation suggests that there is not hemoconcentration due to dehydration, although a concurrent protein-losing disorder might exist. Thus, the azotemia noted above is less likely due to dehydration, and more likely renal in origin.

Serum cholesterol is increased, whereas other indices of hepatic function are normal. There are no other indicators of a primary metabolic disease like diabetes mellitus, but it is possible, nevertheless, that this dog has hypothyroidism or hyperadrenocorticism.

The serum sodium and chloride concentrations are normal, yet there is hypokalemia. Possible causes in this case might include hyperadrenocorticism, chronic renal disease, or urinary potassium wasting associated with diuresis. Calculated serum osmolality is mildly increased due to the azotemia.

Blood gas data

Indices of acid-base metabolism (pH, pCO_2, HCO_3, and anion gap) are normal.

Urinalysis

Although the urinary specific gravity indicates some concentrating ability, one would expect this to be greater if the azotemia were pre-renal in origin. It is also possible for the specific gravity to be increased by the presence of solutes which do not contribute to renal concentration capacity (glucose, protein, amino acids). Concomitant determination of urinary osmolality (358 mOsm/L) confirms that the urine is not being adequately concentrated relative to the calculated osmolality of the serum. Inability to concentrate the urine may be due to central diabetes insipidus (a defect in hypothalamic/pituitary antidiuretic hormone release), or nephrogenic diabetes insipidus (ADH is released, but the kidney is unable to respond). The latter may be caused by anatomic pathology or functional impairment of renal tubular actions necessary to maintain a medullary concentration gradient and water reabsorption. This finding may indicate that the observed azotemia is renal in origin.

The presence of proteinuria on the dipstick was followed by a chemical determination of urinary protein concentration. When indexed to the urinary creatinine value, the urinary protein : creatinine ratio is 1.75. While this value is probably abnormal, it is not sufficiently high to indicate glomerular protein loss. Values in the range of 1.0 to 2.0 have been associated epidemiologically with tubular or inflammatory causes of proteinuria. The absence of significant numbers of leukocytes suggests there is no inflammatory disease. The presence of fine granular casts is indicative of renal tubular damage, and may explain the proteinuria.

Glucosuria concomitant to euglycemia may be explained by three mechanisms. (1) There is a Fanconi's-type syndrome wherein tubular malfunction leads to loss of glucose, protein, and other solutes which would otherwise be reabsorbed from the glomerular filtrate. This is supported by the findings of modest proteinuria and increased urinary fractional excretion of electrolytes. Fanconi's syndromes may be inherited (as reported in Basenjis and Whippets) or acquired (as reported following exposure to nephrotoxicants, including aminoglycoside antibiotics and heavy metals). (2) There was an earlier episode of hyperglycemia which exceeded the renal threshold for glucose reabsorption, during which time the urine analyzed was produced. Depending on the rate of urine formation, a single void may represent blood chemistry-related changes for many hours prior to specimen collection. (3) A laboratory error was made in the determination of either the serum glucose (improper preservation of the blood sample or analytical error) or the urinary glucose (cross-contamination of dipstick reaction squares by excess urine or operator error in interpreting the color change).

The FE_{Na} is 1.62%. This may be indicative of renal tubular disease or dysfunction due to mineralocorticoid deficiency or transport malfunction. The FE_{Ca} is 7.47%. This is particularly inappropriate given the hypocalcemia, and may well

be the cause of calcium loss from the body. This may be indicative of renal tubular disease or dysfunction due to parathyroid hormone deficiency or transport malfunction. Increased urinary excretion of both of these electrolytes may be observed in renal failure (consider the azotemia and impaired urinary concentrating ability) or in Fanconi's syndrome, wherein proximal renal tubule reabsorptive function is impaired (consider the euglycemic glucosuria).

Summary

This is a case of congenital Fanconi's syndrome which did not resolve following supportive treatment for renal failure. Other tests one should perform include those that evaluate the parathyroid gland.

Case 27

Signalment: 8-year-old male canine
History: Polydipsia
Physical Examination: Slightly dehydrated

Hematology		Reference Interval
PCV (%)	38.0	37–55
Hgb (g/dL)	12.0	12–18
RBC ($\times 10^6/\mu L$)	5.51	5.5–8.5
MCV (fL)	69.0	60–72
NCC ($\times 10^3/\mu L$)	**18.2**	6–17
Segs ($\times 10^3/\mu L$)	**2.0**	3–11.5
Monos ($\times 10^3/\mu L$)	0.6	0.1–0.3
Lymphs ($\times 10^3/\mu L$)	**13.8**	1–4.8
Platelets ($\times 10^3/\mu L$)	298	200–500
TP (P) (g/dL)	**8.8**	6–8
Hemopathlogy: clumped platelets		

Biochemical Profile		Reference Interval
Gluc (mg/dL)	91	65–122
BUN (mg/dL)	**33 *(11.8)***	7–28 *(2.5–10.0 mmol/L)*
Creat (mg/dL)	**2.9 *(256)***	0.9–1.7 *(80–150 µmol/L)*
Ca (mg/dL)	**15.4 *(3.85)***	9.0–11.2 *(2.25–2.80 mmol/L)*
Phos (mg/dL)	**7.1 *(2.3)***	2.8–6.1 *(0.9–2.0 mmol/L)*
TP (mg/dL)	**7.9**	5.4–7.4
Alb (g/dL)	4.0	2.7–4.5
Glob (g/dL)	**3.9**	1.9–3.4
T. Bili (mg/dL)	**1.0 *(17)***	0–0.4 *(0–6.8 µmol/L)*
Chol (mg/dL)	291	130–370
ALT (IU/L)	**152**	10–120
AST (IU/L)	**64**	16–40
ALP (IU/L)	**361**	35–280
GGT (IU/L)	**14**	0–6
Na (mEq/L)	154	145–158
K (mEq/L)	**5.8**	4.1–5.5
CL (mEq/L)	109	106–127
TCO_2 (mEq/L)	**12.1**	14–27
An. gap (mEq/L)	**38.7**	8–25

Urinalysis		Urine Sediment	
Color	Straw	WBCs/hpf	2–3
Transparency	Clear	RBCs/hpf	1–2
Sp. Gr.	**1.011**	Epith cells/hpf	Negative
Protein	**2+**	Casts/lpf	Negative
Gluc	Negative	Crystals	Negative
Bilirubin	**2+**	Bacteria	Negative
Blood	Negative		
pH	6.5		
UPC	**2.6**		

Fractional excretion		Reference Interval
Na (%)	**1.73**	<1.0
Ca (%)	**3.37**	<1.0

Interpretive discussion

Hematology

The nucleated cell count is mildly increased, but there is a neutropenia and marked lymphocytosis. Other hematologic parameters, including cell morphology, are normal. However, the concurrent observation of marked lymphocytosis and neutropenia should alert one to the possibility of lymphocytic leukemia, lymphoma with bone marrow involvement, or ehrlichiosis. The concurrent observation of marked lymphocytosis and hypercalcemia should likewise lead to consideration of lymphoma and humoral hypercalcemia of malignancy.

Biochemical profile

The BUN, creatinine, and phosphorus concentrations are mildly increased. These findings are consistent with decreased glomerular filtration rate. However, one cannot differentiate the nature of the azotemia (pre-renal, renal, or post-renal) based on these findings alone. Refer to the discussion of urinalysis results for further interpretation.

The serum total calcium concentration is markedly increased. In light of the lymphocytosis, humoral hypercalcemia of malignancy is most likely. PTHrP could be measured to support this interpretation. The Ca × P product is increased at 109, indicating likely soft tissue mineralization.

The serum total protein and globulin concentrations are slightly increased. Increased globulin concentration may occur in dogs with lymphoproliferative disorders.

The total bilirubin concentration is increased, as are the serum ALP and GGT activities. These findings are evidence of cholestasis. There are mild increases in the serum activities of ALT and AST, so there may be some hepatocellular damage as well.

The increase in serum potassium is probably due to redistribution of intracellular potassium to the extracellular space secondary to acidosis. The serum total CO_2 concentration is mildly decreased, indicating a metabolic acidosis. A complete blood gas panel is required to completely evaluate acid-base status.

Urinalysis

The urine specific gravity is in the isosthenuric range. The dog does not appear to be dehydrated, and it is possible for a normal dog to produce urine with a specific gravity in this range. However, this dog is azotemic. Dilute urine in the face of azotemia usually indicates renal disease, but hypercalcemia interferes with concentrating ability by antagonizing the actions of ADH. Hypercalcemia may also cause damage to the kidney, especially when phosphorus is concurrently increased. There is significant proteinuria of 2+ on the dipstick, and a UPC of 2.6. In the absence of significant sediment changes, this is indicative of renal protein loss, probably glomerular in origin. The FE_{Na} is 1.73%, indicating tubular dysfunction. Increased fractional excretion of Ca is expected given the hypercalcemia.

Summary

This is a case of lymphoma with hypercalcemia of malignancy and hypercalcemic nephropathy.

Case 28

Signalment: 9-year-old female dog
History: Polydipsia, polyuria
Physical Examination: Mass in pelvic inlet

Biochemical Profile		Reference Interval
Gluc (mg/dL)	106	65–122
BUN (mg/dL)	8	7–28
Creat (mg/dL)	1.4	0.9–1.7
TP (g/dL)	**7.7**	5.4–7.4
Alb (g/dL)	**5.2**	2.7–4.5
Ca (mg/dL)	**16.4** ***(4.5)***	9.0–11.2 *(2.25–2.80 mmol/L)*
Phos (mg/dL)	3.5	2.8–6.1
T. Bili (mg/dL)	0.2	0–0.4
ALT (IU/L)	43	10–120
ALP (IU/L)	**428**	35–280
Na (mEq/L)	155	145–158
K (mEq/L)	3.9	4.1–5.5
CL (mEq/L)	119	106–127
TCO_2 (mEq/L)	21.6	14–27

Urinalysis	
Specific gravity	**1.014**

Interpretive discussion

Biochemical profile

Hyperproteinemia is due to hyperalbuminemia, indicating dehydration. There is marked hypercalcemia and this magnitude of increase is suggestive of hypercalcemia of malignancy or primary hyperparathyroidism. Hypercalcemia of this magnitude may result in renal injury leading to azotemia and loss of concentrating ability. A dehydrated animal should be maximally concentrating its urine, and this dog's urine is nearly isosthenuric. This could be consistent with renal disease (early, prior to development of azotemia), but hypercalcemia alone is sufficient to explain this abnormality (due to antagonism of ADH at the renal tubules).

A slight increase in alkaline phosphatase activity (ALP) suggests cholestasis or drug induction (corticosteroids, anticonvulsants). If cholestasis is present it is not of sufficient magnitude to affect the serum bilirubin. A more likely explanation for the increased alkaline phosphatase is increased bone turnover secondary to increased serum concentration of PTH or PTHrp, which could be measured.

Summary

The mass in the pelvis was aspirated, and appeared neuroendocrine, rather than lymphoid. The mass was surgically removed and confirmed by histopathology to be an apocrine gland adenocarcinoma of the anal sac. Following surgery, the calcium normalized, but later metastasis to the lungs resulted in return of hypercalcemia. In contrast to the previous case, the hypercalcemia has not resulted in sufficient renal injury to cause azotemia.

Case 29

Signalment: 6-month-old DSH female cat
History: Vomiting, weakness, acute onset
Physical examination: Tachypnea for 24 hours, weakness

Hematology		Reference Interval
PCV (%)	40	24–45
WBC ($\times 10^3/\mu L$)	**21.0**	5.5–19.5
Segs ($\times 10^3/\mu L$)	**20.2**	2.5–12.5
Bands ($\times 10^3/\mu L$)	0	0–0.3
Lymphs ($\times 10^3/\mu L$)	**0.2**	1.5–7.0
Monos ($\times 10^3/\mu L$)	0.6	0–0.85

Biochemical Profile		Reference Interval
Gluc (mg/dL)	**150 *(8.2)***	67–124 *(3.7–6.8 mmol/L)*
BUN (mg/dL)	**45 *(16.1)***	17–32 *(6.1–11.4 mmol/L)*
Creat (mg/dL)	**2.2 *(194)***	0.9–2.1 *(80–186 µmol/L)*
Ca (mg/dL)	**18 *(4.5)***	8.5–11 *(2.12–2.75 mmol/L)*
Phos (mg/dL)	**9.5 *(3.1)***	3.3–7.8 *(1.1–2.5 mmol/L)*
TP (g/dL)	8.0	5.9–8.1
Alb (g/dL)	**4.2**	2.3–3.9
Glob (g/dL)	3.8	2.9–4.4
T. Bili (mg/dL)	0.2	0–0.3
Chol (mg/dL)	120	60–270
ALT (IU/L)	100	30–100
ALP (IU/L)	25	11–210
Na (mEq/L)	159	146–160
K (mEq/L)	**6.4**	3.7–5.4
CL (mEq/L)	112	112–129
TCO_2 (mEq/L)	16.8	14–24
An. gap (mEq/L)	**37**	10–27

Blood Gas Data (arterial)		Reference Interval
pH	**6.926**	7.33–7.44
PCO_2 (mmHg)	**72.1**	35–42
PO_2 (mmHg)	**65**	80–95
HCO_3 (mEq/L)	**14.9**	16–22

Urinalysis	
Sp. Gr.	**1.020**
Gran casts/hpf	**2**

Interpretive discussion

Hematology

There is a stress leukogram indicated by mature neutrophilia and lymphopenia.

Biochemical profile

Increased glucose is compatible with stress identified in the leukogram. The BUN and creatinine are mildly increased, indicating azotemia. The urine specific gravity is less than what one would expect in a cat with prerenal azotemia, therefore renal azotemia should be considered. However, hypercalcemia alone can interfere with normal concentrating ability. With a calcium of 18 mg/dL, renal dysfunction is likely occurring due to soft tissue mineralization. The total protein and albumin are increased, particularly for a young cat, indicating dehydration.

The calcium is markedly increased. Primary causes of this degree of hypercalcemia are hypercalcemia of malignancy, primary hyperparathyroidism, and hypervitaminosis D. Vitamin D toxicosis should be very high on the differential list, due to the age of the cat and the acute onset. Because of acidosis, ionized calciun is likely very high. Phosphorus is mildly increased. This could be in part due to the young age of the cat or may be due to decreased GFR. It is also seen with hypervitaminosis D. The Ca × P product is markedly increased at 171, which will result in calcification of renal tubules, lungs and other soft tissues.

Potassium is increased. This may be due to acidosis and an associated shift of K out of cells or the animal may be becoming oliguric. Increased anion gap indicates increase in unmeasured anions. Possible unmeasured anions are lactic acid or uremic acids. Increased phosphates are also adding to the anion gap.

Blood gas data

The pH is extremely decreased indicating severe acidemia. The pCO_2 is the major abnormality in the balance between bicarbonate and CO_2. Therefore, respiratory acidosis is the major component of the acidosis. The bicarbonate is also decreased indicating a component of metabolic acidosis is superimposed. Hypoxemia is also present. The combined hypoxemia and retention of CO_2 indicate a severe ventilation abnormality, probably due to calcification of lungs. The metabolic acidosis is probably a result of renal failure.

Summary

The cat was diagnosed with renal disease with severe acidemia, both metabolic and respiratory. The respiratory component may be due to calcification of lungs. Cholecalciferol toxicosis was diagnosed, as the cat had an opportunity to ingest a rodenticide containing cholecalciferol.

Case 30

Signalment: 3-year-old male cat
History: Acute lethargy, vomiting, and anorexia
Physical examination: Obese, almost comatose

Hematology		Reference Interval
PCV (%)	**50**	24–45
NCC ($\times 10^3/\mu L$)	**24.0**	5.5–19.5
Segs ($\times 10^3/\mu L$)	**23.0**	2.5–12.5
Monos ($\times 10^3/\mu L$)	0.7	0–0.88
Lymphs ($\times 10^3/\mu L$)	**0.3**	1.5–7.0
Platelets ($\times 10^3/\mu L$)	Adequate	200–500

Biochemical Profile		Reference Interval
Gluc (mg/dL)	**285**	67–124
BUN (mg/dL)	**110**	17–32
Creat (mg/dL)	**7.5**	0.9–2.1
Ca (mg/dL)	**6.5**	8.5–11
Phos (mg/dL)	**14**	3.3–7.8
TP (g/dL)	**9.0**	5.9–8.1
Alb (g/dL)	**4.9**	2.3–3.9
Glob (g/dL)	4.1	2.9–4.4
T. Bili (mg/dL)	0.3	0–0.3
ALT (IU/L)	35	30–100
ALP (IU/L)	45	11–210
Na (mEq/L)	**165**	146–160
K (mEq/L)	**6.8**	3.7–5.4
CL (mEq/L)	**107**	112–129
TCO_2 (mEq/L)	**10**	14–23
An. gap (mEq/L)	**55**	10–27
Calc. Osmolality (mOsm/kg)	**394**	290–310
Meas. Osmolality (mOsm/kg)	**440**	290–310
Osmolal Gap (mOsm/kg)	**46**	>10

Urinalysis (cystocentesis)

Color	Yellow	**Urine Sediment**	
Transparency	Cloudy	WBCs/hpf	2–3
Sp. Gr.	**1.016**	RBCs/hpf	2–3
Protein	**1+**	Epith cells/hpf	1–3 transitional
Gluc	**1+**	Casts/lpf	0
Bilirubin	Negative	Crystals	**Calcium oxalate monohydrate**
Blood	**1+**	Bacteria	0
pH	5.0		

Interpretive discussion

Hematology

PCV is slightly increased. This is likely due to dehydration, considering that the albumin is also increased. The mature neutrophilia and lymphopenia are suggestive of a stress or corticosteroid leukogram.

Biochemical profile

The serum glucose concentration is increased. Differentials should include stress or corticosteroids, excitement and diabetes mellitus. Excitement is less likely than the others, since the cat does not have an excitement leukogram and there is glucosuria. (See summary for further discussion of hyperglycemia.)

The BUN and creatinine are increased, and considering that the cat is not concentrating its urine, this is most likely a renal azotemia. Since the cat is dehydrated, a prerenal component to the azotemia may be present as well. Because the cat is not anemic, is obese, and the history is acute, this is most likely acute renal failure. Phosphorus is increased due to decreased glomerular filtration rate.

The serum calcium is decreased. Considering that the cat likely has acute renal failure, the most likely cause of the hypocalcemia is formation of calcium oxalate crystals associated with ethylene glycol toxicosis. Oxalate is one of the metabolites of ethylene glycol and combines with calcium to form calcium oxalate crystals.

Hyperproteinemia is due to hyperalbuminemia, indicating dehydration.

The sodium is increased, likely due to dehydration. Chloride would be expected to increase with sodium, but is selectively decreased in this case, probably due to vomiting of gastric HCl. This causes a hypochloremic alkalosis. However, TCO_2 is decreased and the anion gap is increased, suggesting concurrent metabolic acidosis and a mixed acid-base disorder. A blood gas would more fully characterize the acid-base status.

The increased anion gap indicates increased concentrations of anions other than those used in the formula to calculate the anion gap (chloride and HCO_3^-). In this case, uremic acids, phosphate, albumin, and most importantly, metabolites of ethylene glycol are probably contributing to the anion gap and a high-gap metabolic acidosis.

The calculated osmolality is increased, since the substances that are included in the formula to calculate osmolality are increased (glucose, urea, sodium, potassium). However, the measured plasma osmolality is much higher than the calculated osmolality, since a substance is present in the blood that is not used in the formula to calculate osmolality. The most common cause of an increased osmole gap is the pres-

ence of ethylene glycol, which contributes to plasma osmolality due to its low molecular weight.

Urinalysis

The urine specific gravity of 1.016 in an azotemic dehydrated cat indicates that the cat is not capable of concentrating urine, and that renal dysfunction is present. The presence of calcium oxalate monohydrate crystals in a cat with acute renal failure is very suggestive of ethylene glycol toxicosis. The renal threshold for glucose has been exceeded, resulting in glucosuria. The 1+ proteinuria is probably significant in light of the low urine specific gravity and probably resulted from tubular damage.

Summary

The cat died, and necropsy revealed renal tubular necrosis and the presence of calcium oxalate crystals in the tubules due to ethylene glycol toxicosis. The cat had access to antifreeze shortly before it became ill. Approximately 50% of dogs and cats with ethylene glycol induced renal failure have hyperglycemia, probably due to a combination of stress and the formation of aldehyde, a metabolite of ethylene glycol that interferes with glucose metabolism. While diabetes mellitus could cause hyperglycemia and metabolic acidosis, the presence of acute renal failure and calcium oxalate crystalluria should prompt consideration of ethylene glycol toxicosis.

Case 31

Signalment: 3-month-old Saint Bernard
History: Stumbling for 4 hours
Physical examination: Cannot stand, in a stupor
Hematology: No abnormalities

Biochemical Profile		Reference Interval
Gluc (mg/dL)	**129**	65–122
BUN (mg/dL)	20	7–28
Creat (mg/dL)	1.6	0.9–1.7
Ca (mg/dL)	11.2	9.0–11.2
Ionized calcium (mg/dL)	5.6	4.5–5.6
Phos (mg/dL)	**10.2**	2.8–6.1
TP (g/dL)	5.8	5.4–7.4
Alb (g/dL)	2.9	2.7–4.5
Glob (g/dL)	2.4	1.9–3.4
T. Bili (mg/dL)	0.2	0–0.4
Chol (mg/dL)	220	130–37
ALT (IU/L)	60	10–120
AST (IU/L)	30	16–40
ALP (IU/L)	**300**	35–280
GGT (IU/L)	2	0–6
Na (mEq/L)	148	145–158
K (mEq/L)	5.2	4.1–5.5
CL (mEq/L)	105	106–127
HCO_3 (mEq/L)	15.1	14–27
An. gap (mEq/L)	**33**	8–25
Meas. Osmolality (mOsm/kg)	**442**	290–310
Calc. Osmolality (mOsm/kg)	**330**	290–310
Osmole gap (mOsm/kg)	**112**	0–10
Serum ethylene glycol concentration (mg/dL)	**>250**	0

Blood Gas Data (arterial)		Reference Interval
Blood pH	7.305	7.33–7.44
HCO_3 (mEq/L)	**13.7**	16–22
PCO_2 (mm/Hg)	**29**	35–42

Urinalysis	
Urine specific gravity	**1.012**
Urine pH	5

Interpretive discussion

Biochemical profile

The serum glucose concentration is slightly increased. This may be due to stress, although the leukogram is normal. Aldehydes, a metabolite of ethylene glycol (see later discussion), are reported to interfere with glucose metabolism.

The BUN and creatinine are normal in this dog that has a high serum ethylene glycol concentration. In dogs, azotemia begins between 24 and 36 hours following ingestion. The history suggests that this dog ingested antifreeze approximately 5 hours prior to the time of these laboratory data.

Phosphorus is markedly increased. Hyperphosphatemia may be due to the young age of the dog, but is somewhat high for this. In this case the serum phosphorus increase was likely due to phosphate rust inhibitors present in most commercial antifreeze.

Serum alkaline phosphatase activity is mildly increased, likely due to the bone isoform that is increased in growing dogs.

The anion gap is increased, likely due to either phosphates or metabolites of ethylene glycol, which are anions. The calculated osmolality is slightly increased. However, the actual (measured) plasma osmolality is much higher than the calculated osmolality, resulting in a large osmole gap, since a substance is present in the blood that is not used in the formula to calculate osmolality. The most common cause of an increased osmole gap is the presence of ethylene glycol, which contributes to plasma osmolality due to its low molecular weight. This was confirmed by measuring serum ethylene glycol concentration.

Blood gas data

The blood pH is slightly low and HCO_3 is decreased, indicating metabolic acidosis. Metabolites of ethylene glycol are acids. Decreased pCO_2 is consistent with a compensatory respiratory alkalosis. The blood gases were determined about one hour following the biochemical profile, which probably accounts for the discrepancy between the HCO_3 determined on the biochemical panel, and that from the blood gas machine.

Urinalysis

The urine specific gravity of 1.012 in this patient is likely due to ethylene glycol causing osmotic diuresis. It is also possible that concentrating ability has been impaired, but the animal is not yet azotemic.

Summary

The dog was treated with fomepizole, an alcohol dehydrogenase inhibitor, approximately 7 to 8 hours following antifreeze ingestion, and did not become azotemic. In contrast to the previous case, the biochemical profile is often not diagnostic in acute ethylene glycol poisoning, and other tests, such as serum ethylene glycol concentration or measured osmolality must be used to confirm the diagnosis. The acute onset of stumbling and stupor triggered suspicion of ethylene glycol toxicosis.

Case 32

Signalment: Five-day-old male foal
History: Foal was fine at birth, now will not eat
Physical examination: Acts colicky, seems constipated

Hematology		Reference Interval
PCV (%)	32	28–46
Hgb (g/dL)	11	11–16
TP (P) (g/dL)	**5.6**	6–8

Biochemical Profile		Reference Interval
Gluc (mg/dL)	80	70–110
UN (mg/dL)	**32**	7–27
Creat (mg/dL)	**4.8**	1.1–2.0
Ca (mg/dL)	**9.6**	11–13.7
Phos (mg/dL)	**10**	1.9–3.6
TP (g/dL)	5.9	5.8–7.6
Alb (g/dL)	3.0	2.7–3.7
Glob (g/dL)	2.9	2.6–4.6
T. Bili (mg/dL)	**3.8**	0.6–2.5
AST (IU/L)	229	185–300
ALP (IU/L)	**340**	66–180
CK (IU/L)	237	130–470
Na (mEq/L)	**118**	133–145
K (mEq/L)	**7.1**	2.2–4.6
CL (mEq/L)	**92**	98–103
TCO_2 (mEq/L)	**18**	24–29
An. gap (mEq/L)	15	10–15

Interpretive discussion

Hematology

There is a mild decrease in plasma proteins; concerned about failure of passive transfer of colostrum but globulins on chemistry panel are adequate.

Biochemical profile

Azotemia is present but there is no urine specific gravity to help determine if this is prerenal or renal azotemia. Recommend catheterization to collect urine and determine if the foal can urinate on its own. UN is mildly increased but creatinine (Ct) is moderately increased, the ratio of UN/Ct is 6. This can happen with recent fluid therapy and a more rapid decrease in UN than Ct, but no fluids have been administered. It could happen with a hepatic shunt and decreased synthesis of UN and with noncreatinine chromogens in horses and cattle. The latter is more likely but confuses the interpretation of the severity of the azotemia. The increase in phosphorus is consistent with a decreased GFR; prerenal, renal, or postrenal causes can do this.

The key abnormalities are the hyponatremia, hyperkalemia and hypochloremia. The Na:K ratio is low at 16 (see comments section). In a foal the most likely differentials are diarrhea, ruptured urinary bladder, renal failure and hypoadrenocorticism due to a septicemia. There is no evidence of diarrhea at this time; renal failure is a possible explanation. To evaluate uroabdomen recommend abdominocentesis and determination of Ct (and or UN) on the abdominal fluid and in a concurrent serum sample to compare Ct in abdominal fluid to serum; they should be equal if the bladder wall is intact. Ct (and or UN) will be higher in abdominal fluid than serum if bladder has ruptured. Hypoadrenocorticism is unlikely; only pursue if other differentials are ruled out. Bicarbonate (TCO_2) is decreased, AG is high end of reference interval, and there likely is a metabolic acidosis, possibly due to dehydration and decreased tissue perfusion. ALP is mildly increased probably due to bone (growth) or liver source; does not seem important to pursue at this time. The bilirubin is increased which could be due to a liver problem but anorexia is the more likely cause (anorexia is number one reason for hyperbilirubinemia in horses).

Abdominocentesis	
Clear, slight yellow color	
TNCC 8500/μL 50:50 neutrophils and mononuclear cells	
Total protein (refractive index) 2.8 g/dL	
Creatinine (mg/dL)	
Abdominal fluid	9.2
Serum	4.8

Summary and comment: Uroabdomen

There was a hole in the dorsal aspect of the urinary bladder. It was surgically repaired and the bladder wall appeared healthy at surgery. The foal recovered.

Rupture of the urinary bladder is usually caused by an obstruction (calculi) in males or excessive trauma (hit by car) in all species except horses. Typically the problem is seen in male foals that appeared fine at birth but gradually develop anorexia and other problems. The male urethral lumen is small and apparently does not allow expulsion of urine easily enough during birth, and the back pressure caused by the mare's strong contractions during parturition causes the bladder to rupture. There are no calculi obstructing outflow. Usually there is a history of dribbling urine or that the foal was seen to urinate. The hole is almost always located dorsally due to the musculature of the bladder wall and therefore patients may still urinate. If contrast dyes are used to determine bladder integrity the dye may still be retained for the same reason. Comparison of abdominal Ct concentration to serum Ct is the diagnostic test of choice. Abdominal Ct does not have to be twice as great as serum Ct to rule in a ruptured bladder, it just needs to be several mg/dL higher than the serum Ct. If the bladder wall is intact the serum and abdominal Ct will be similar or identical. If the patient is not azotemic then both samples will have Ct concentrations WRI, if the bladder wall is intact. If the patient has renal failure and is azotemic than the abdominal Ct concentration will be increased to a comparable value, if the bladder wall is intact.

The serum electrolytes and Na : K ratio of less than 20 are key to consider this differential diagnosis. In dogs the following are the four most likely differentials and the tests to rule in or rule out each differential for this electrolyte pattern:

Diagnosis	Test of choice
Hypoadrenocorticism	Basal cortisol; ACTH stim
Renal failure	Fluid therapy; complete UA; fractional excretion of Na
Uroabdomen	Compare abdominal and serum Ct concentrations
GI – whipworms, *Salmonella*	Fecal floatation; fecal culture

Case 33

Signalment 10-year-old castrated male domestic short hair cat
History Anorexia, lethargy
Physical examination Dehydrated

Miscellaneous tests		Reference Interval
Plasma glucose (mg/dL)	**328**	67–124
Urinalysis dipstick abnormalities	glucosuria and ketonuria	
Na (mEq/L)	**130**	146–160
K (mEq/L)	**2.2**	3.7–5.4
CL (mEq/L)	**74**	112–129
pH	**7.28**	7.33–7.44
HCO_3 (mEq/L)	**9.2**	16–20
pCO_2 (mmHg)	**20**	28–34

Interpretive discussion

There is moderate hyperglycemia that exceeds the renal glucose threshold. There is resultant glucosuria. Ketonuria indicates impaired glucose utilization by tissues suggesting insulin deficiency. This establishes a working diagnosis of diabetes mellitus.

There is marked hypokalemia in the face of acidosis (see blood gas discussion), which would be expected to increase potassium due to an intracellular to extracellular shift. It would be suspected that total body potassium is depleted and there should be caution in insulin administration that would drive potassium into cells, resulting in weakness due to even more severe hypokalemia. There is also hyponatremia and disproportionate hypochloremia. The hyponatremia may be due to a combination of urinary and gastrointestinal loss. The disproportionate hypochloremia is of a magnitude that suggests upper gastrointestinal chloride loss due to vomiting.

The acid-base data show a low pH and decreased bicarbonate, consistent with a metabolic acidosis. The decreased pCO_2 is a compensatory response (respiratory alkalosis). There is also a likely hidden component of metabolic alkalosis (hypochloremic alkalosis), resulting in a mixed acid-base disorder. The loss of HCl in gastric fluid will metabolically generate bicarbonate. The generation of ketoacids that require buffering by bicarbonate is judged to be more severe in the balance between bicarbonate utilization and production.

Summary

Diabetic ketoacidosis pattern, largely compensated.

Case 34

Signalment: 5-year-old spayed female Manx cat
History: Decreased appetite of approximately 2 weeks' duration. Fluid draining from a fistula over the terminal coccygeal vertebrae of 8 months' duration.
Physical examination: Approximately 6% dehydrated. Coccygeal vertebrae were noted to terminate cranial to the anal sphincter. The fistula was noninflamed and draining a clear, colorless fluid.

Hematology		Reference Interval
PCV (%)	**49**	24–45
NCC ($\times 10^3/\mu L$)	11.6	5.5–19.5
Segs ($\times 10^3/\mu L$)	9.6	2.5–12.5
Monos ($\times 10^3/\mu L$)	0.6	0–0.8
Lymphs ($\times 10^3/\mu L$)	**1.4**	1.5–7.0
Platelets ($\times 10^3/\mu L$)	Adequate	200–500

Biochemical Profile		Reference Interval
Gluc (mg/dL)	91	67–124
BUN (mg/dL)	**82**	17–32
Creat (mg/dL)	**2.2**	0.9–2.1
Ca (mg/dL)	**7.3**	8.5–11
Phos (mg/dL)	5.2	3.3–7.8
TP (g/dL)	**8.4**	5.9–8.1
Alb (g/dL)	**4.1**	2.3–3.9
Glob (g/dL)	4.3	2.9–4.4
T. Bili (mg/dL)	0.1	0–0.3
Chol (mg/dL)	153	60–220
ALT (IU/L)	40	30–100
Na (mEq/L)	**131**	146–160
K (mEq/L)	4.6	3.7–5.4
CL (mEq/L)	**101**	112–129
TCO_2 (mEq/L)	16	14–23
An. gap (mEq/L)	18.6	10–27

Urinalysis	
Color	Straw
Transparency	Clear
Sp. Gr.	**1.015**
Protein	Negative
Gluc	Negative
Bilirubin	Negative
Blood	Negative
pH	6.5

Fractional excretion		Reference Interval
Na (%)	0.03	<1.0
CL (%)	0.08	<1.0

Interpretive discussion

Hematology

The increased PCV is consistent with hemoconcentration due to dehydration. Other data are unremarkable.

Biochemical profile

The azotemia (increased BUN and serum creatinine concentrations) may be prerenal and/or renal. Refer to the discussion of urinalysis below.

There is hypocalcemia, despite hyperproteinemia due to hemoconcentration, suggesting that serum calcium concentration may be truly decreased. An ionized calcium could be measured for confirmation. Hypocalcemia could have occurred secondary to chloride depletion and loss of the electrochemical gradient needed to support calcium absorption from the glomerular filtrate in the Loop of Henle of the renal tubules.

Serum sodium and chloride are decreased in concentration. This usually reflects increased loss, compounded by reduced intake in sick, anorexic patients. In this case, there is physical evidence of cerebrospinal fluid loss from a draining meningomyelocele. Cerebrospinal fluid contains higher sodium and chloride concentrations than the blood, owing to the active chloride transport mechanism employed by the choroid plexus for secretion. Draining CSF from the body will create electrolyte depletion in excess of water, an otherwise classic scenario for hypotonic dehydration. Although this cat had been losing CSF for some time, the development of anorexia probably precipitated an imbalance between these pathologic losses and replacement of the electrolytes, resulting in the clinical presentation.

Urinalysis

The urinary fractional excretion values for sodium and chloride were well within the normal reference interval, thereby ruling out renal loss as a cause for the electrolyte depletion. The only significant abnormality is a urine specific gravity of 1.015. Dehydration should stimulate antidiuretic hormone release from the hypothalamus, and increased water reclamation by the renal tubules. However, electrolyte loss in this type of hypotonic dehydration often leads to medullary solute depletion and a loss of the renal concentration gradient. Another alternative is that there is renal disease, due to renal hypoperfusion, sepsis, etc., resulting in both azotemia and loss of concentrating ability.

Summary

Sodium chloride depletion in a manx cat with a fistulated meningomyelocele (Hall JA, MJ Fettman, JT Ingram. Sodium chloride depletion in a cat with fistulated meningomyelocele. J Am Vet Med Assoc 1988;192:1445–1448).

Case 35

Signalment: 10-year-old female horse
History: Abdominal pain
Physical examination: Tense abdomen, slight fever

Hematology		Reference Interval
PCV (%)	52.0	32–52
Hgb (g/dL)	18.1	11–19
RBC ($\times 10^6/\mu L$)	11.15	6.5–12.5
MCV (fL)	46.0	36–52
MCHC (g/dL)	34.0	34–39
NCC ($\times 10^3/\mu L$)	**14.2**	5.5–12.5
Segs ($\times 10^3/\mu L$)	**11.8**	2.7–6.7
Monos ($\times 10^3/\mu L$)	0.3	0–0.8
Lymphs ($\times 10^3/\mu L$)	2.1	1.5–5.5
Platelets ($\times 10^3/\mu L$)	162	100–600
TP (P) (g/dL)	7.0	6–8
Fibrinogen (mg/dL)	200	100–400

Biochemical Profile		Reference Interval
Gluc (mg/dL)	**166**	70–110
BUN (mg/dL)	23	14–27
Creat (mg/dL)	**4.2**	1.1–2.0
Ca (mg/dL)	**10.5**	11.0–13.7
Phos (mg/dL)	**4.5**	1.9–4.1
TP (g/dL)	7.1	5.8–7.6
Alb (g/dL)	3.2	2.7–3.7
Glob (g/dL)	3.9	2.6–4.6
T. Bili (mg/dL)	1.4	0.6–2.1
AST (IU/L)	**430**	185–300
GGT (IU/L)	8	7–17
SDH (IU/L)	**99**	0–9
CK (IU/L)	**8422**	130–470
Na (mEq/L)	140	133–145
K (mEq/L)	3.5	2.2–4.6
CL (mEq/L)	**86**	100–111
TCO_2 (mEq/L)	**22.6**	24–34
An. gap	**35**	5–15

Abdominal Fluid Analysis	
Fluid color	Straw
Fluid clarity	Hazy
Supernatant color	Straw
Supernatant clarity	Clear
TP (g/dL)	1.3
NCC (/μL)	300

Cytology: There are approximately equal numbers of neutrophils and large mononuclear cells. Although the overall cellularity and protein are low, some of the neutrophils are degenerate and bacteria are seen extracellularly, predominantly rods. Some of the macrophages and neutrophils contain cytoplasmic material suggestive of bacterial remnants. There are moderate numbers of lymphocytes and rare mast cells seen. There is debris present in the background.

Interpretive discussion

Hematology

There is a neutrophilic leukocytosis with low normal lymphocyte numbers, which most likely reflects stress, rather than inflammation. The fibrinogen is within normal limits. The PCV is at the top of the reference interval and serum proteins are normal, suggesting possible splenic contraction.

Biochemical profile

There is a mild hyperglycemia, which is consistent with stress. The increases in serum creatinine and serum phosphorus are likely the result of decreased glomerular filtration rate. Creatinine is a more sensitive marker for decreased GFR in horses because of their ability to excrete urea into the GI tract. A urinalysis might help differentiate prerenal from renal azotemia, but the low chloride (discussed later) might affect urinary concentrating ability. There may be a prerenal component (dehydration) to the azotemia since the PCV is at the top of the reference interval. However, the normal albumin does not support dehydration, and there does not appear to be much protein loss into the abdominal effusion (which would lower the serum albumin).

There is a slight hypocalcemia, which may be due to deposition in injured tissue.

Increased CK activity indicates muscle cell damage. The increased SDH activity is specific for hepatocellular injury. The slight increase in AST activity could be from muscle or hepatocellular injury.

There is a selective hypochloremia, indicating a hypochloremic alkalosis. Selective chloride loss can result from upper GI loss or sequestration of CL and excessive sweating in horses. In addition, the decreased TCO_2 and increased anion gap indicates a high-gap metabolic acidosis; thus there is a mixed acid-base disorder. Uremic acids and lactic acid, secondary to hypoperfusion, are likely contributors to the anion gap. A blood gas profile is needed to fully assess acid-base status in this horse.

Abdominal fluid analysis

Although the quantitative indices are all within normal limits, the presence of degenerate inflammatory cells, bacteria, and debris are all consistent with an acute rupture of the intestinal tract.

Summary

This mare experienced intestinal colic, followed by acute rupture of the involved strangulated intestine. There had not been time for an inflammatory leukogram to develop.

Case 36

Signalment: 11-year-old intact male miniature schnauzer

History: Intermittent vomiting and diarrhea for last two weeks

Physical examination: Tense, painful abdomen. Very fat.

Hematology		Reference Interval
PCV (%)	38	37–55
Hgb (g/dL)	13.2	12–18
RBC ($\times 10^6/\mu L$)	5.7	5.5–8.5
MCV (fL)	67	60–72
MCHC (g/dL)	35	33–38
NCC ($\times 10^3/\mu L$)	**17.9**	6–17
Segs ($\times 10^3/\mu L$)	**14.2**	3–11.5
Bands ($\times 10^3/\mu L$)	**0.5**	0–0.3
Monos ($\times 10^3/\mu L$)	0.7	0.1–1.3
Lymphs ($\times 10^3/\mu L$)	2.5	1–4.8
Platelets ($\times 10^3/\mu L$)	250	200–500
TP (P) (g/dL)	**9.0**	6–8
Hemopathology: Moderate polychromasia		

Biochemical Profile (serum was lipemic)		Reference Interval
Gluc (mg/dL)	**124 *(6.8)***	65–122 *(3.5–6.7 mmol/L)*
BUN (mg/dL)	**42 *(15)***	7–28 *(2.5–10.0 mmol/L)*
Creat (mg/dL)	1.2	0.9–1.7
Ca (mg/dL)	9.8	9.0–11.2
Phos (mg/dL)	5.8	2.8–6.1
TP (g/dL)	**7.7**	5.4–7.4
Alb (g/dL)	3.7	2.7–4.5
Glob (g/dL)	**4.0**	1.9–3.4
T. Bili (mg/dL)	**10.8 *(184.7)***	0–0.4 *(0–6.8 µmol/L)*
Chol (mg/dL)	**1230 *(32)***	130–370 *(3.4–9.6 mmol/L)*
ALT (IU/L)	**600**	10–120
AST (IU/L)	**540**	16–40
ALP (IU/L)	**660**	35–280
Na (mEq/L)	148	145–158
K (mEq/L)	4.3	4.1–5.5
CL (mEq/L)	110	106–127
TCO_2 (mEq/L)	24	14–27
An. gap (mEq/L)	18	8–25
Amylase (IU/L)	510	50–1250
Lipase (IU/L)	120	30–560

Urinalysis (voided)			
Color	Yellow	**Urine Sediment**	
Transparency	**Cloudy**	WBCs/hpf	**>50**
Sp. Gr.	**1.022**	RBCs/hpf	0–1
Protein	**3+**	Epith cells/hpf	0
Gluc	Negative	Casts/lpf	0
Bilirubin	**2+**	Crystals	0
Blood	Negative	Bacteria	**Many bacilli**
pH	7.0		

Interpretive discussion

Hematology

The PCV, hemoglobin concentration, and RBC count are at the lower end of the reference interval, and it is possible that an anemia has been masked by dehydration. With a history of vomiting and diarrhea and an increased plasma protein concentration, it is possible that this animal is dehydrated. However, we have no additional support for dehydration in these data. Because the serum is lipemic, a likely explanation for the high total plasma protein as measured by refractometry is the presence of lipids, which interfere with the reading. The presence of moderate polychromasia suggests a regenerative response. Blood loss may have caused a regenerative anemia in this dog (see summary).

Neutrophilia with a left shift indicates an inflammatory leukogram.

Biochemical profile

The BUN concentration is only mildly increased and the serum creatinine concentration is normal. Urine specific gravity indicates inadequate urine concentrating ability in the face of possible dehydration (if present) and azotemia, suggesting possible renal disease. Increased BUN with normal creatinine also prompts consideration of prerenal azotemia secondary to GI tract hemorrhage (which acts as a high protein meal).

Both plasma and serum protein concentrations are increased, but the plasma protein concentration is much higher than the serum protein concentration. Because fibrinogen is present in plasma but not in serum, one would expect the plasma protein concentration to be 0.2 to 0.4 g/dL higher than that of serum. However, the difference is often greater because plasma protein concentration is estimated using a refractometer, while serum protein is measured spectrophotometrically. Increased plasma concentration of lipids may falsely increase the protein estimate determined by a refractometer. The increased difference between these protein concentrations in this case is likely due to lipemia.

The hyperglobulinemia may be the result of chronic antigenic stimulation with subsequent increase in antibody production.

The combination of hyperbilirubinemia and increased serum ALP activity is typical of cholestasis. The increased bilirubin concentration in the urine reflects the hyperbilirubinemia; conjugated bilirubin is cleared by the glomerulus and excreted in the urine. Although hypercholesterolemia is a nonspecific problem, cholestasis is a common cause of this abnormality and may be an explanation in this case. The magnitude of the hypercholesterolemia is unusual for cholestasis alone. Since this dog is a miniature schnauzer, and the serum is lipemic, suggesting hypertriglyceridemia and/or chylomicronemia, idiopathic hyperlipidemia is likely.

Increased serum AST and ALT activities indicate hepatocyte injury. Both of these enzymes are leakage enzymes and are present in significant concentrations in hepatocytes. AST is also present in high concentrations and ALT in low concentrations in muscle, but muscle is an unlikely source of these enzymes in this case. In light of the evidence for cholestasis, hepatic origin is most likely for these enzymes in this dog.

Urinalysis

Proteinuria, pyuria and bacteruria suggest inflammation in the urinary tract. Since these are found in a voided urine sample, reproductive tract origin must also be considered. Bacteria in a voided urine sample may be contaminants but are more significant when accompanied by pyuria. Culture of this urine sample is indicated.

Summary

This dog had a suppurative cholangiohepatitis, a duodenal ulcer, and pyelonephritis. The cholangiohepatitis resulted in the cholestasis and damage to hepatocytes. The chronic antigenic stimulation caused by both cholangiohepatitis and pyelonephritis resulted in hyperglobulinemia. The mild azotemia could have resulted from pyelonephritis or GI hemorrhage secondary to the duodenal ulcer.

Case 37

Signalment: Six-year-old CM German shepherd
History: Receiving prednisone for inflammatory bowel disease. Losing weight for 6 months.
Physical examination: Thin, with multiple hairless scaly skin lesions. Hepatomegaly.

Hematology		Reference Interval
PCV (%)	38	36–60
Hgb (g/dL)	13.1	12–18
RBC ($\times 10^6/\mu L$)	4.9	4.8–9.3
MCV (fL)	79	58–79
MCHC (g/dL)	34	33–38
NCC ($\times 10^3/\mu L$)	**27.8**	4–15.5
Segs ($\times 10^3/\mu L$)	**25.5**	2–10.5
Bands ($\times 10^3/\mu L$)	0	0–0.3
Monos ($\times 10^3/\mu L$)	**2.0**	0–0.9
Lymphs ($\times 10^3/\mu L$)	**0.3**	1–4.5
Eos ($\times 10^3/\mu L$)	0	0.1–1.2
Platelets ($\times 10^3/\mu L$)	374	200–500
Hemopathology: Moderate acanthocytes, few echinocytes, keratocytes, schistocytes.		

Biochemical Profile		Reference Interval
Gluc (mg/dL)	103	70–138
BUN (mg/dL)	11	6–25
Creat (mg/dL)	0.5	0.5–1.6
Ca (mg/dL)	**8.4**	8.9–11.4
Phos (mg/dL)	4.4	2.5–6.0
TP (g/dL)	5.3	5.0–7.4
Alb (g/dL)	**2.3**	2.7–4.4
Glob (g/dL)	3.0	1.6–3.6
T. Bili (mg/dL)	**1.6**	0.1–0.3
Chol (mg/dL)	121	92–324
Trig (mg/dL)	102	29–291
ALT (IU/L)	**1041**	12–128
AST (IU/L)	**101**	15–66
ALP (IU/L)	**640**	5–131
GGT (IU/L)	**237**	1–12
CK (IU/L)	174	59–895
Na (mEq/L)	149	139–154
K (mEq/L)	4.9	3.6–5.5
CL (mEq/L)	108	102–120
TCO_2 (mEq/L)	23	15–25

Coagulation Profile		Reference Interval
PT (seconds)	7.0	6–12
aPTT (seconds)	15.9	10–25

Interpretive discussion

Hematology

There is a moderate leukocytosis characterized by a mature neutrophilia, lymphopenia, and monocytosis. This is most consistent with a steroid/stress leukogram, which is expected given the history of prednisone administration. The magnitude of the mature neutrophilia is slightly greater than that usually seen with a stress response alone, so chronic inflammation should also be considered.

Although there is no anemia present there are significant erythrocyte membrane changes observed. Acanthocytes may be seen with liver disease and hemangiosarcoma. Keratocytes and schistocytes suggest there is some red cell fragmentation occurring.

Biochemical profile

The most significant abnormalities relate to the liver. Increased activities of the leakage enzymes ALT and AST indicate there has been hepatocellular injury. Increased activities of the inducible enzymes ALP and GGT may be due to cholestasis and/or drug induction, given the history of steroid administration. The increased total bilirubin concentration supports cholestasis, since there is no indication of hemolysis, and indicates that hepatic function has been compromised.

Albumin is slightly decreased, most likely due to the ongoing inflammatory bowel disease. Decreased production due to liver failure is less likely since glucose, BUN, and cholesterol are normal. The mild decrease in calcium is likely due to a decrease in protein-bound calcium secondary to hypoalbuminemia. An ionizd calcium could be measured to confirm this.

Summary

There is laboratory evidence for hepatocellular injury and cholestasis severe enough to affect hepatic function. Given the history of prednisone administration, a steroid hepatopathy is most likely. Red cell membrane changes may be due to hepatic disease or potentially hemangiosarcoma. A liver aspirate was performed and revealed marked indistinct hepatic vacuolization consistent with glycogen, and numerous bile casts indicating cholestasis. These findings were confirmed on a liver biopsy; there was no evidence of neoplasia or inflammation. Biopsies of the skin lesions revealed pyogranulomatous dermatitis with intralesional pigmented fungal hyphae. Presumably immune suppression due to the prolonged steroid administration predisposed to the fungal infection.

Case 38

Signalment: 5-year-old FS canine
History: On phenobarbital to control seizures for 2.5 years. Vomiting daily and lethargic for about 1 month.
Physical examination: Lethargic, icteric, pendulous abdomen; arthritic and appears older than stated age

Hematology		Reference Interval
PCV (%)	40.0	37–55
Hgb (g/dL)	13.6	12–18
RBC ($\times 10^6/\mu L$)	5.53	5.5–8.5
MCV (fL)	72.0	60–72
MCHC (g/dL)	34.0	33–38
NCC ($\times 10^3/\mu L$)	**47.2**	6–17
Segs ($\times 10^3/\mu L$)	**40.1**	3–11.5
Bands ($\times 10^3/\mu L$)	**0.9**	0–0.3
Monos ($\times 10^3/\mu L$)	**4.7**	0.1–1.3
Lymphs ($\times 10^3/\mu L$)	**0.9**	1–4.8
Eos ($\times 10^3/\mu L$)	0.5	0.1–1.2
Platelets ($\times 10^3/\mu L$)	299	200–500
TP (P) (g/dL)	**5.5**	6–8
Hemopathology: slt toxic neutrophils, many echinocytes		

Biochemical Profile		Reference Interval
Gluc (mg/dL)	69	65–122
BUN (mg/dL)	**5 *(1.78)***	7–28 *(2.5–10.0 mmo1/L)*
Creat (mg/d1)	**0.6**	0.9–1.7
Ca (mg/dL)	**8.1 *(2.02)***	9.0–11.2 *(2.25–2.80 mmol/L)*
Phos (mg/dL)	5.1	2.8–6.1
TP (g/dL)	**4.8**	5.4–7.4
Alb (g/dL)	**2.0**	2.7–4.5
Glob (g/dL)	2.8	1.9–3.4
T. Bili (mg/dL)	**4.5 *(77)***	0–0.4 *(0–6.8 µmol/L)*
Chol (mg/dL)	**126 *(3.28)***	130–370 *(3.4–9.6 mmol/L)*
ALT (IU/L)	**348**	10–120
AST (IU/L)	**176**	16–40
ALP (IU/L)	**4503**	35–280
GGT (IU/L)	**426**	0–6
Na (mEq/L)	**142**	145–158
K (mEq/L)	**3.3**	4.1–5.5
CL (mEq/L)	114	106–127
TCO_2 (mEq/L)	14.8	14–27
An. gap (mEq/L)	16.5	8–25
Lipase (IU/L)	**575**	30–560

Urinalysis			
Color	Orange	**Urine Sediment**	
Transparency	**Cloudy**	WBCs/hpf	**8–10**
Sp. Gr.	1.015	RBCs/hpf	0–2
Protein	**2+**	Epith cells/hpf	**80–100**
Gluc	Negative	Casts/lpf	Negative
Bilirubin	**4+**	Crystals	Negative
Blood	**3+**	Bacteria	**4+ rods**
pH	6.0		
Ketones	**3+**		

Coagulation Data		Reference Interval
PT (seconds)	9.8	7.5–10.5
aPTT (seconds)	14.0	10.5–16.5

Interpretive discussion

Hematology

There is a moderate neutrophilia with a mild left shift, monocytosis, and slightly toxic neutrophils were observed in the blood film. This is an inflammatory leukogram, but the lymphopenia indicates a concurrent steroid-induced component. Monocytosis is consistent with the combined leukocyte response.

Biochemical profile

The serum glucose concentration is at the low end of the reference interval and the BUN is decreased. These findings may indicate hepatic functional impairment, particularly in light of the observation of a potential stress leukogram (stress would be expected to increase the glucose concentration). See discussion of serum protein below. Decreased creatinine likely reflects decreased muscle mass.

The serum total protein and albumin concentrations are decreased. Considerations in this case include renal loss (see urinalysis discussion) and, more likely, decreased production secondary to hepatic disease (discussed more later).

There is a mild hypocalcemia that is likely secondary to hypoalbuminemia, and therefore not clinically significant. An ionized calcium could be measured to confirm this.

Serum cholesterol is decreased. While one should not overinterpret decreases in some analytes, this is commonly observed in end-stage liver disease, owing to impaired hepatic lipid synthesis. This is particularly notable given the degree of hyperbilirubinemia and increases in enzyme activ-

ities indicative of cholestasis (ALP and GGT). The magnitude of increase in serum ALP activity is large enough to warrant consideration of corticosteroid induction. Likewise, the degree of increase in GGT activity may be related to steroid induction rather than cholestasis alone. The serum ALT and AST activities are moderately increased, indicating hepatocellular damage. Phenobarbital may induce increased production of several liver enzymes.

The serum sodium and potassium are decreased, and one should consider typical causes for electrolyte depletion, including pathologic losses from the gastrointestinal and urinary systems, as well as third space syndromes. Hypokalemia is a frequent observation in hepatic disease, often due to anorexia and vomiting.

Coagulation data

The APTT and PT are normal. If hepatic disease or end-stage liver failure has progressed sufficiently, as suggested by even lower values for glucose, BUN, albumin, and cholesterol, one might expect these indices of coagulation factor synthesis to become abnormal as well.

Urinalysis

The urinary specific gravity indicates the urine is poorly concentrated and may reflect impaired concentrating ability. This may be due to the decreased BUN, since urea also plays a role in urine concentration. The concentrations of protein, ketones, bilirubin, and blood are particularly notable given this weak urine concentration. The proteinuria is explained by the urinary tract inflammation/infection as indicated by the significant pyuria, bacteriuria, and presence of marked occult blood. The presence of bilirubin is not surprising given the degree of hyperbilirubinemia. The presence of ketonuria in the absence of glucosuria is unusual. Ketosis is a possible sequela to impaired oxidative lipid metabolism by the diseased liver, especially when triglyceride absorption from the GI tract or mobilization from peripheral stores is greater than hepatic functional capacity for processing.

Summary

There is biochemical evidence of chronic liver failure, cholestasis, and hepatocellular injury. Phenobarbital-induced hepatopathy was considered. Ultrasound of the liver showed an enlarged liver with numerous well-defined hypoechoic foci throughout. Masses throughout the cranial mid-abdomen had similar echogenicity as masses within liver. Cytology of a liver aspirate showed vacuolated hepatocytes, bile stasis, and a population of nonhepatic cells with a high nucleus:cytoplasm ratio, most of which were broken. Numerous cells in mitosis were observed, and neoplasia was diagnosed. Biopsy of liver revealed adenocarcinoma which effaced and replaced hepatic parenchyma, and glucocorticoid hepatopathy with severe bile stasis. The neoplasm had a neuroendocrine (potentially adrenal) pattern, and was possibly causing the steroid hepatopathy. Endocrine panel was not performed. Dog was euthanized; necropsy was not allowed.

Case 39

Signalment: 6-year-old spayed female dog
History: Struck by car 1 month ago. Not taken to veterinarian. Dyspnea since accident. Anorexia.
Physical examination: Emaciated and lethargic. Intestinal sounds auscultated in thorax.

Hematology		Reference Interval
PCV (%)	37	37–55
Hgb (g/dL)	12.3	12–18
RBC ($\times 10^6/\mu L$)	6.1	5.5–8.5
MCV (fL)	61	60–72
MCHC (g/dL)	33	33–38
NCC ($\times 10^3/\mu L$)	16.1	6–17
Segs ($\times 10^3/\mu L$)	**13.5**	3–11.5
Bands ($\times 10^3/\mu L$)	0.2	0–0.3
Monos ($\times 10^3/\mu L$)	1.0	0.1–1.3
Lymphs ($\times 10^3/\mu L$)	**0.6**	1–4.8
Eos ($\times 10^3/\mu L$)	0.8	0.1–1.2
Platelets ($\times 10^3/\mu L$)	330	200–500
TP (P) (g/dL)	**3.3**	6–8
Hemopathology: Normal		

Biochemical Profile		Reference Interval
Gluc (mg/dL)	77	65–122
BUN (mg/dL)	**3 *(1.07)***	7–28 *(2.5–10.0 mmol/L)*
Creat (mg/dL)	1.5	0.9–1.7
Ca (mg/dL)	**6.3**	9.0–11.2
Phos (mg/dL)	**4.4**	2.8–6.1
TP (g/dL)	**2.9**	5.4–7.4
Alb (g/dL)	**0.6**	2.7–4.5
Glob (g/dL)	2.3	1.9–3.4
T. Bili (mg/dL)	**3.0 *(51.3)***	0–0.4 *(0–6.8 µmol/L)*
Chol (mg/dL)	**102 *(2.65)***	130–370 *(3.4–9.6 mmol/L)*
ALT (IU/L)	**170**	10–120
AST (IU/L)	**72**	16–40
ALP (IU/L)	**540**	35–280
Na (mEq/L)	146	145–158
K (mEq/L)	**6.0**	4.1–5.5
CL (mEq/L)	118	106–127
TCO_2 (mEq/L)	**11**	14–27
An. gap (mEq/L)	23	8–25
Plasma ammonia (mg/dL)	**150**	0–90

Urinalysis (catheterized)			
Color	Yellow	**Urine Sediment**	
Transparency	Clear	WBCs/hpf	0
Sp. Gr.	1.035	RBCs/hpf	0
Protein	Negative	Epith cells/hpf	0
Gluc	Negative	Casts/lpf	0
Bilirubin	**1+**	Crystals	**Bilirubin**
Blood	Negative	Bacteria	0
pH	5.5		

Interpretive discussion

Hematology

This dog's erythrocyte measurements are near the lower end of their reference intervals, and there is no evidence of a regenerative response. This may be normal for this dog, or it is possible that this dog is developing a nonregenerative anemia secondary to chronic disease.

Leukocyte abnormalities are a mature neutrophilia and lymphopenia, typical of a corticosteroid-mediated leukogram.

Biochemical profile

Decreased BUN concentration can be caused by hepatic failure, diuresis, decreased protein intake, or treatment with anabolic steroids. BUN concentration below the reference interval can also occur in normal animals. In light of other laboratory findings in this case, the decreased BUN concentration is probably due to hepatic failure and resulting failure of hepatocytes to synthesize urea. Anorexia resulting in decreased protein intake may have also contributed to this abnormality.

This dog has hypocalcemia; however, it also has severe hypoalbuminemia. Hypoalbuminemia, and resultant decreased protein-bound calcium, may be the cause for the hypocalcemia, in which case it is not clinically significant. Ionized calcium could be measured to confirm this. While not always necessary, determining the ionized calcium would be recommended in this case because of the magnitude of the hypocalcemia and the critical condition of the patient.

Both plasma and serum protein concentrations are decreased. These decreases are a result of hypoalbuminemia. When interpreted in combination with other laboratory data, this abnormality is probably due to decreased albumin synthesis by the liver. Decreased protein intake can result in hypoalbuminemia and may also have played a role in this case. The albumin concentration is low enough to lead to ascites; however, ascites was not noted in this animal.

In dogs, hyperbilirubinemia can result from hemolysis, failure of hepatocyte uptake and metabolism of bilirubin, or failure to excrete bilirubin due to cholestasis or other disruption of bile flow. In this case, failure of hepatic uptake and metabolism of bilirubin is probably the major abnormality leading to hyperbilirubinemia. It is also probable the bile duct is partially blocked and cholestasis is playing a role in producing this abnormality. The increased serum ALP activity suggests cholestasis is present in this dog.

Hypocholesterolemia is probably another result of hepatic failure. The liver is a major site of cholesterol synthesis and excretion. Abnormalities of these two processes have opposite effects on serum cholesterol concentrations. In this case, synthetic failure is apparently more severe than failure to excrete cholesterol.

Both serum ALT and AST activities are mildly increased. These enzymes leak from injured hepatocytes, and liver injury is the appropriate interpretation in this case. AST is also present in muscle cells, and muscle injury cannot be ruled out, but the mild increase of AST activity in conjunction with the increased ALT activity suggests the AST has leaked from the liver in this case.

Increased ALP activity most often results from either cholestasis or increased blood corticosteroid concentrations. In combination with other laboratory data suggesting hepatic disease, cholestasis is the most important cause of the increased ALP in this case. This dog probably had an increased blood corticosteroid concentration as suggested by the leukogram, and this may have also played a role in increasing the serum ALP activity.

Hyperkalemia may be a result of metabolic acidosis-induced shifting of potassium from within cells to extracellular fluid. In animals with metabolic acidosis, hydrogen ions enter cells in an attempt to equalize their concentrations in the intracellular and extracellular compartments. In order to maintain electrical neutrality, potassium ions must leave the cells. The net result is increased extracellular and, therefore, serum potassium concentrations.

The cause of the decreased total CO_2 is not certain. Since this animal has a compromised respiratory system, it is reasonable to assume that it has a respiratory acidosis. However, the total CO_2 concentration would be expected to increase in compensation for the respiratory acidosis. Since this concentration decreased rather than increased, it is reasonable to assume the dog has another abnormality causing metabolic acidosis. Although the anion gap is normal, the markedly low albumin may be masking an increase in unmeasured anions, thus there could be a hidden high-gap acidosis. Abnormal renal regulation of acid-base balance is another possible cause of decreased total CO_2, but there is no evidence of renal dysfunction in this case. A blood gas analysis would be helpful to further evaluate this dog's acid-base status.

The hyperammonemia is a result of hepatic failure. Ammonia is normally absorbed from the digestive tract and transported to the liver by the portal circulation. The liver is responsible for removing and metabolizing this ammonia. Alterations in blood flow to the liver and/or markedly decreased numbers of functional hepatocytes can result in increased blood ammonia concentrations.

Urinalysis

Bilirubinuria and the presence of bilirubin crystals are the only abnormalities in the urine. These changes reflect the increased serum bilirubin concentration. Conjugated bilirubin readily passes through glomeruli and is then excreted in the urine. The very mild increase in urine bilirubin suggests that most of the serum bilirubin is unconjugated. Interestingly, this dog is concentrating urine in the face of a very low bun.

Summary

Exploratory surgery revealed a diaphragmatic hernia through which the liver and a portion of the GI tract had passed. The liver was decreased in size and firm. Many fibrous adhesions were present. On the surgeon's recommendation, the dog was euthanized.

This dog had hepatic failure due to chronically decreased blood supply to the liver. Decreased BUN, albumin, and cholesterol concentrations suggested decreased synthetic function by the liver. Increased bilirubin and ammonia concentrations resulted from decreased delivery of these substances to the liver and, therefore, decreased removal from the blood as well as due to decreased functional hepatic mass. Cholestasis resulting from partial occlusion of the bile duct also contributed to hyperbilirubinemia. Since this was an end-stage liver disease, leakage of ALT and AST from hepatocytes was minimal due to the small number of hepatocytes remaining, and serum activities of ALT and AST were, therefore, only slightly increased.

Case 40

Signalment: Ten-year-old spayed female Doberman dog
History: Lethargy, weight loss, diarrhea, and poor appetite
Physical examination: Depressed, dehydrated, hypotensive, and icteric

Hematology		Reference Interval
TP (P) (g/dL)	**4.7**	6.0–8.0
PCV (%)	41	40–55
Hgb (g/dL)	14.6	13.0–20.0
RBC ($\times 10^6/\mu L$)	6.07	5.5–8.5
MCV (fL)	67	62–73
MCHC (g/dL)	36	33–36
NCC ($\times 10^3/\mu L$)	**26.6**	4.5–15.0
Bands ($\times 10^3/\mu L$)	**1.3**	0–0.2
Segs ($\times 10^3/\mu L$)	**12.2**	2.6–11.0
Lymphs ($\times 10^3/\mu L$)	**11.9**	1.0–4.8
Monos ($\times 10^3/\mu L$)	**1.1**	0.2–1.0
Eos ($\times 10^3/\mu L$)	0	0.1–1.2
Platelets ($\times 10^3/\mu L$)	**90**	200–500
MPV (fL)	13.9	7.5–14.6

Hemopathology noted on blood film:
- Most lymphoid cells are large with fine granular chromatin; interpreted as mostly prolymphocytes with some blasts.
- No platelet clumps found, occasional giant platelets.

Biochemical Profile		Reference Interval
Gluc (mg/dL)	65	75–130
BUN (mg/dL)	**69**	7–32
Creat (mg/dL)	1.5	0.4–1.5
Phos (mg/dL)	**13.2**	2.1–6.0
Ca (mg/dL)	9.4	9.2–11.7
Mg (mg/dL)	**3.4**	1.8–2.5
TP (g/dL)	**3.9**	5.3–7.2
Alb (g/dL)	**2.4**	2.5–4.0
Glob (g/dL)	**1.5**	2.0–3.8
Chol (mg/dL)	**102**	130–300
T. Bili (mg/dL)	**12.6**	0–0.3
ALP (IU/L)	**1717**	20–142
ALT (IU/L)	**590**	10–110
AST (IU/L)	**401**	16–50
GGT (IU/L)	5	0–8
Na (mEq/L)	**138**	142–152
K (mEq/L)	4.3	3.5–5.2
CL (mEq/L)	**100**	108–120
Bicarbonate (mEq/L)	**10.5**	16–25
An. gap (mEq/L)	**32**	13–22

Blood Gas Analysis–venous		Reference Interval
pH	**6.92**	7.33–7.45
pCO2 (mmHg)	**57.3**	24–39
pO2 (mmHg)	75.9	67–92
HCO_3 (mEq/L)	**11.3**	15–24
Lactate (mmol/L)	**8.4**	0.2–1.4

Interpretive discussion

Hematology

The hematocrit is low normal, but some degree of anemia is probable given physical dehydration; the baseline hematocrit is not known.

There is moderate hypoproteinemia, to be considered in chemistry interpretation.

The leukogram indicates moderate lymphocytosis. The presence of mostly abnormal lymphocyte forms indicates lymphproliferative disease that is leukemic. The left shift with mild neutrophilia indicates concurrent inflammatory stimulus. Minimal monocytosis accompanies the inflammatory pattern.

There is thrombocytopenia with some giant platelets, indicating active thrombopoiesis. A consumptive process should be considered.

Chemistry

Renal analytes

The following processes are identified:

- Moderate azotemia indicated by BUN and prominent hyperphosphatemia. The creatinine has likely increased in this individual, within the reference interval. Increased magnesium is due to decreased GFR.
- Likely a prerenal component given dehydration and other evidence of poor perfusion such as lactic acidosis (later). A urinalysis, especially specific gravity, would be helpful.
- Possible renal component requires urinalysis for further characterization.
- The Ca × P is 124 indicating calcification will be occurring.

Liver

The following processes are identified:

- There is hepatocellular injury indicated by increased activities of ALT/AST.
- There is evidence of liver function failure in the form of probable decreased cholesterol synthesis and marked hyperbilirubinemia in the face of near normal HCT.

• Cholestasis and/or steroid induced ALP are considerations given the ALP magnitude; cholestasis is a likely contributing factor to the hyperbilirubinemia.
• Mild hypoglycemia and hypoalbuminemia may also be related in part to function failure.

Protein

There is marked hypoproteinemia due to panhypoproteinemia. All potential causes of loss should be evaluated, particularly GI given the observation of diarrhea.

Acid-base/electrolyte

The following processes are identified:
• There is severe, life-threatening acidemia. This is a mixed acidosis of two processes.
• There is metabolic acidosis indicated by decreased bicarbonate. This is most likely related to poor tissue perfusion and development of lactate acidemia, as well as uremic acids.
• There is also a component of respiratory acidosis indicated by the prominent increase in pCO_2. The respiratory component is likely related to terminal respiratory failure; this would prompt evaluation of cardiopulmonary function.
• Both the pCO_2 and bicarbonate are counter to any detectable compensation.
• The increased anion gap is attributable to retention of renal-excreted anions such as phosphate and sulfate, and lactate is a contributing factor.

Summary

• Biochemical evidence of liver function failure, with hepatocellular injury and cholestasis. Hepatic infiltrate with lymphoma should be considered given that there is blood evidence of lymphoproliferative disease.
• Azotemia likely prerenal; kidneys should be investigated further.
• Severe mixed acidemia compatible with multiple causes of metabolic acid and respiratory acid formation.
• Lymphoproliferative disorder.
• Marked panhypoproteinemia.

Recommendations for further characterization would include:
• Urinalysis.
• Evaluation of liver and kidney size with sampling for possible infiltrative disease (lymphoma).
• Bone marrow for possible infiltrate.
• Cytometric analysis of blood leukocytes if treatment is contemplated.

As follow-up, the dog was euthanized. Necropsy findings included splenomegaly, hepatomegaly, lymphadenopathy with marked involvement with lymphoma. The severity of this change in liver would explain the biochemical evidence of function failure.

Case 41

Signalment: 9-month-old intact female dog
History: Struck by car 3 weeks ago. Treated for shock and released. Listless since then.
Physical examination: Abdomen distended and fluid-filled

Hematology		Reference Interval
PCV (%)	**30**	37–55
Hgb (g/dL)	**10.3**	12–18
RBC ($\times 10^6/\mu L$)	**5.45**	5.5–8.5
MCV (fL)	**55**	60–72
MCHC (g/dL)	34	33–38
Retics (/µL)	42	<60
NCC ($\times 10^3/\mu L$)	16	6–17
Segs ($\times 10^3/\mu L$)	**12.8**	3–11.5
Bands ($\times 10^3/\mu L$)	**0.5**	0–0.3
Lymphs ($\times 10^3/\mu L$)	2.7	1–4.8
Platelets ($\times 10^3/\mu L$)	270	200–500
TP (P) (g/dL)	6.5	6–8
Hemopathology: Slight hypochromasia, moderate number of keratocytes		

Biochernical Profile		Reference Interval
Gluc (mg/dL)	65	65–122
BUN (mg/dL)	25	7–28
Creat (mg/dL)	1.2	0.9–1.7
Ca (mg/dL)	**8.4 *(2.1)***	9.0–11.2 *(2.25–2.80 mmol/L)*
Phos (mg/dL)	6.0	2.8–6.1
TP (g/dL)	5.8	5.4–7.4
Alb (g/dL)	**2.5**	2.7–4.5
Glob (g/dL)	3.3	1.9–3.4
T. Bili	**0.5 *(8.5)***	0–0.4 *(0–6.8 µmol/L)*
Chol (mg/dL)	170	130–370
ALT (IU/L)	23	10–120
AST (IU/L)	28	16–40
ALP (IU/L)	51	35–280
Na (mEq/L)	**139**	145–158
K (mEq/L)	5.2	4.1–5.5
CL (mEq/L)	**105**	106–127
TCO_2 (mEq/L)	15	14–27
An. gap (mEq/L)	24	8–25

Urinalysis (voided)

Color	Dark yellow	**Urine Sediment**	
Transparency	Clear	WBCs/hpf	0
Sp. Gr.	1.030	RBCs/hpf	0
Protein	Negative	Epith cells/hpf	0
Gluc	Negative	Casts/lpf	0
Bilirubin	**3+**	Crystals	**Bilirubin**
Blood	Negative	Bacteria	0
pH	6.0		

Body Fluid Analysis

Color	**Red-brown**	**Differential**	
Transparency	Hazy	Neutrophils	74%
TP (g/dL)	**3.8**	Lymphs	5%
NCC (/µL)	**8800**	Macrophages	21%

Other observations: Neutrophils are nondegenerate. Lymphocytes are uniformly small. Large mononuclear cells are a mixture of reactive mesothelial cells and macrophages. Macrophages contain large amounts of blue-green pigment, suggestive of bile. No microorganisms are evident. Moderate numbers of erythrocytes are present.

Interpretive discussion

Hematology

This dog has a nonregenerative anemia. The indices reveal that this anemia is microcytic and borderline hypochromic. These abnormalities, in combination with the presence of hypochromasia and keratocytes observed on the blood film, indicate iron deficiency. Serum iron concentration should be measured in this dog. Although the most common cause of iron deficiency is chronic blood loss, there is no history of such blood loss in this case. In most such cases, examination of feces will reveal the presence of blood. GI parasites such as hookworms, should also be considered.

Borderline neutrophilia with a slight left shift suggests a mild tissue demand for neutrophils, and, therefore, a mild inflammatory process. It is likely that the anemia is nonregenerative due to the presence of inflammation (anemia of inflammatory disease).

Biochemical profile

The blood glucose concentration is at the bottom of the reference interval. Decreased carbohydrate intake or decreased hepatic production are possible causes. Since there is little evidence of hepatic failure in this case, decreased intake appears to be the most likely explanation. Alternatively, it may be normal for this dog.

The hypocalcemia may be a result of hypoalbuminemia, in which case it is not clinically significant. An ionized calcium could be measured to confirm this.

The mild hypoalbuminemia is probably due to decreased protein intake or decreased amino acid absorption from the GI tract. Evidence of hepatic failure is not present, and urine protein concentration is normal; therefore, decreased albumin production by the liver and increased albumin loss through the kidneys are unlikely. In light of the anemia and the evidence of iron deficiency, chronic blood loss should be considered as a cause of hypoalbuminemia in this case; however, globulin concentration usually decreases proportionally with albumin concentration during blood loss. The globulin concentration may, however, have been increased in this dog due to chronic antigenic stimulation, and this would explain a normal globulin concentration despite blood loss severe enough to result in hypoalbuminemia.

The combination of increased serum and urine bilirubin concentrations suggests disruption in the hepatic excretion of conjugated bilirubin. The serum bilirubin concentration, while increased, appears inappropriately low in light of the marked increase in urine bilirubin concentration. Dogs have a low renal threshold for bilirubin, and, in dogs with abnormalities of hepatic conjugated bilirubin excretion, urine bilirubin concentration may increase prior to increases in serum biliruhin concentration, or urine bilirubin concentration may be markedly increased while serum bilirubin concentration is only mildly increased.

Hyponatremia and hypochloremia were probably caused by dilution of these electrolytes in an increased volume of extracellular fluid. This increased fluid volume is a result of accumulation of fluid in the peritoneal cavity. This "third-spacing" phenomenon commonly results in hyponatremia and hypochloremia.

Urinalysis

Marked bilirubinuria and presence of bilirubin crystals are the only abnormalities in the urinalysis. These changes probably resulted from increased passage of conjugated bilirubin into the blood with subsequent renal excretion. Either cholestasis or rupture of the bile duct or gall bladder could be an underlying cause.

Abdominal fluid analysis

Based on the total nucleated cell count and on the predominance of neutrophils, the peritoneal fluid should be classified as an exudate. Since neutrophils are nondegenerate and bacteria are absent, this is probably a nonseptic exudate. The pigment noted in macrophages is suggestive of bile and, therefore, gall bladder or bile duct rupture are likely.

Summary

Exploratory surgery revealed a ruptured gall bladder. Due to severe adhesions throughout the peritoneal cavity, the owner was offered a guarded prognosis. The owner opted for euthanasia.

Bilirubin leaking with bile into this dog's peritoneal cavity was reabsorbed through the peritoneal wall. The bilirubin entered the blood and was efficiently excreted by the kidneys. As a result, serum bilirubin concentration increased only slightly while urine bilirubin concentration increased markedly. Serum activities of the hepatic leakage enzymes, ALT and AST, did not increase since there was no direct liver injury. Serum activity of ALP did not increase since there was no cholestasis.

Case 42

Signalment: 2-year-old male mixed breed dog
History: Weight loss, lethargy
Physical examination: Thin dog, pendulous abdomen
Hematology: Unremarkable

Biochemical Profile		Reference Interval
Gluc (mg/dL)	**64 *(3.5)***	65–122 *(3.5–6.7 mmol/L)*
BUN (mg/dL)	**6 *(2.l)***	7–28 *(2.5–10.0 mmol/L)*
Creat (mg/dL)	1.0	0.9–1.7
Ca (mg/dL)	**7.4 *(1.85)***	9.0–11.2 *(2.25–2.80 mmol/L)*
Phos (mg/dL)	2.8	2.8–6.1
TP (g/dL)	**4.2**	5.4–7.4
Alb (g/dL)	**1.2**	2.7–4.5
Glob (g/dL)	3.0	1.9–3.4
T. Bili (mg/dL)	0.4	0–0.4
Chol (mg/dL)	**65 *(1.7)***	130–370 *(3.4–9.6 mmol/L)*
ALT (IU/L)	**30**	10–120
ALP (IU/L)	260	35–280
Bile Acids (µmol/L)	**30**	3.0–9.0
Na (mEq/L)	146	145–158
K (mEq/L)	4.1	4.1–5.5
CL (mEq/L)	115	106–127

Abdominal Fluid Analysis	
TP (g/dL)	1.0
NCC (/µL)	1500
Segs (%)	60
Lymphs (%)	22
Macrophages (%)	18
Morphology: neutrophils nondegenerate	

Interpretive discussion

Biochemical profile

A number of factors in the profile suggest liver failure. These include a borderline low glucose, low BUN, hypoproteinemia characterized by severe hypoalbuminemia, and a markedly low cholesterol concentration. Hepatic enzymes are often normal with end-stage liver disease. An alternative, but less likely, possibility for this pattern is severe starvation. The increased bile acids indicate decreased liver function and help confirm end-stage liver disease.

Hypocalcemia may be due to hypoalbuminemia, in which case it is clinically insignificant. An ionized calcium could be measured to confirm this.

Body fluid analysis

The abdominal fluid has the typical features of a transudate. With end-stage liver disease this is due to a combination of hypoalbuminemia and increased portal blood pressure resulting in transudation of fluid into the cavity.

Summary

Hepatic cirrhosis; end-stage liver disease.

Case 43

Signalment: 3-month-old intact female dog
History: Anorexia, depression, and diarrhea of one week duration. Poor growth rate.
Physical examination: Severe, diffuse dermatitis with multifocal ulcerative lesions

Hematology		Reference Interval
PCV (%)	**13**	37–55
Hgb (g/dL)	**4.5**	12–18
RBC ($\times 10^6/\mu L$)	**2.5**	5.5–8.5
MCV (fL)	**52**	60–72
MCHC (g/dL)	35	33–38
Retic ($\times 10^3/\mu L$)	2.5	<60
NCC ($\times 10^3/\mu L$)	**1.6**	6–17
Segs ($\times 10^3/\mu L$)	**0.5**	3–11.5
Bands ($\times 10^3/\mu L$)	0.1	0–0.3
Monos ($\times 10^3/\mu L$)	0.1	0.1–1.3
Lymphs ($\times 10^3/\mu L$)	**0.9**	1–4.8
Platelets ($\times 10^3/\mu L$)	340	200–500
TP (P) (g/dL)	**3.4**	6–8
Hemopathology: Markedly toxic neutrophils, few RBC fragments.		

Biochemical Profile		Reference Interval
Gluc (mg/dL)	**40 (*2.2*)**	65–122 (*3.5–6.7 mmol/L*)
BUN (mg/dL)	**4 (*1.43*)**	7–28 (*2.5–10.0 mmol/L*)
Creat (mg/dL)	**0.3**	0.9–1.7
Ca (mg/dL)	**7.8 (*1.95*)**	9.0–11.2 (*2.25–2.80 mmol/L*)
Phos (mg/dL)	**2.0 (*0.65*)**	2.8–6.1 (*0.9–2.0 mmol/L*)
TP (g/dL)	**2.9**	5.4–7.4
Alb (g/dL)	**1.7**	2.7–4.5
Glob (g/dL)	**1.2**	1.9–3.4
T. Bili (mg/dL)	0.2	0–0.4
Chol (mg/dL)	142	130–370
ALT (IU/L)	15	10–120
AST (IU/L)	22	16–40
ALP (IU/L)	63	35–280
GGT (IU/L)	6	0–6
Na (mEq/L)	**141**	145–158
K (mEq/L)	**3.7**	4.1–5.5
CL (mEq/L)	114	106–127
TCO_2 (mEq/L)	17	14–27
An. gap (mEq/L)	**14**	8–25
Fasting Bile acids (µmol/L)	**88**	<10
Iron (µg/dL)	**50 (*8.95*)**	60–110 (*10.7–19.7 µmol/L*)

Urinalysis (cystocentesis)			
Color	Yellow	**Urine Sediment**	
Transparency	Clear	WBCs/hpf	0–2
Sp. Gr.	1.029	RBCs/hpf	0
Protein	Negative	Epith cells/hpf	0
Gluc	**2+**	Casts/lpf	0
Bilirubin	Negative	Crystals	0
Blood	Negative	Bacteria	0
pH	5.0		

Interpretive discussion

Hematology

This dog has a severe nonregenerative anemia. The anemia in this dog is microcytic, and the serum iron concentration is decreased, suggesting iron deficiency secondary to chronic blood loss. Alternately, microcytic anemia is also seen in dogs with portosystemic shunt, in which case serum iron may or may not be decreased, and anemia may be secondary to other abnormalities in iron metabolism. Red blood cell fragmentation is a typical finding in iron deficiency anemia. While iron deficiency anemia may be regenerative, this dog's bone marrow is not adequately responding, perhaps due to viral damage or concurrent anemia of chronic disease.

Severe leukopenia has resulted from a combination of neutropenia and lymphopenia. In a young dog with diarrhea as well as neutropenia and lymphopenia, parvovirus infection with virus-induced bone marrow damage should be a strong consideration. Acute bacterial enteritis resulting in endotoxemia may result in a similar leukogram. The presence of toxic neutrophils suggests that the bone marrow is rapidly producing neutrophils, and this may signal early recovery of previously suppressed neutrophil production, or may be a response to loss of neutrophils due to rapid, destruction or emigration into tissues as would occur with endotoxemia or overwhelming tissue demand for neutrophils, respectively.

Platelets are adequate, indicating chronic marrow failure is not present.

Biochemical profile

Hypoglycemia probably resulted from decreased hepatic glucose production. Numerous diseases can result in hypoglycemia, but, in light of other laboratory data, hepatic failure is the most likely cause of hypoglycemia in this dog. The decreased blood supply to the liver which occurs with portosystemic shunts can result in liver atrophy. Such a liver cannot play its normal role in maintenance of blood glucose concentrations. A second possibility, in light of the decreased neutrophil concentration, is that the dog has bacteremia or endotoxemia which may result in hypoglycemia. A third possibility is that glucose is being lost through the urinary tract (see discussion of glucosuria).

Both the BUN and serum creatinine concentrations are decreased. Since there is evidence of hepatic failure, it is likely that the decreased BUN concentration resulted from decreased liver production of urea. Decreased creatinine reflects decreased muscle mass.

This dog has both hypocalcemia and hypoalbuminemia. The hypocalcemia may be caused by decreased protein-bound calcium secondary to the hypoalbuminemia, in which case it is clinically insignificant. An ionized calcium could be measured to confirm this.

Hypophosphatemia occurs most commonly in hypercalcemic disorders such as primary hyperparathyroidism and pseudohyperparathyroidism, but these are unlikely in a 3-month-old dog. Other causes include dietary phosphate or vitamin D deficiency, malabsorption syndrome, diabetes mellitus, and canine Fanconi-like syndrome. This dog appears to have a renal tubular defect (see discussion of glucosuria), and it is possible that this defect is part of a canine Fanconi-like syndrome. In such a syndrome, inadequate tubular reabsorption of phosphate results in excessive loss of phosphate in the urine.

The hypoproteinemia is the result of both hypoalbuminemia and hypoglobulinemia. These abnormalities, in combination with anemia, indicate that blood loss should be considered. In this case, it is likely that chronic liver disease is also contributing to hypoalbuminemia.

This dog's serum bilirubin concentration, serum alkaline phosphatase (ALP) activity, and gamma glutamyltransferase (GGT) activity are normal, suggesting that cholestasis is not occurring. While most forms of liver failure result in some degree of cholestasis, liver failure resulting from a portosystemic shunt usually does not. These normal values, in combination with the history and other laboratory abnormalities, suggest that a portosystemic shunt is likely. Since this is a young dog, a slight increase in ALP of bone origin would not have been unusual. Despite evidence of hepatic disease, serum ALT and AST activities are normal. Serum activities of hepatic leakage enzymes such as ALT and AST may be normal to increased in dogs with portosystemic shunts.

The hyponatremia and hypokalemia may have resulted from diuresis induced by glucosuria, or losses associated with diarrhea. It is also possible this dog's tubular function defect includes abnormal reabsorption of Na and K. Decreased intake likely contributed to the hypokalemia.

Markedly increased fasting bile acid concentration can result from decreased hepatic blood flow, hepatic failure, or cholestasis. In this case, decreased hepatic blood flow and subsequent hepatic failure are the most likely explanations.

Blood loss is the most common cause of decreased serum iron concentration in animals, although nursing animals have low serum iron due to inadequate dietary intake. In this case, however, the decreased serum iron concentration is probably due to the presence of a portosystemic shunt. Decreased serum iron concentration does not always occur in dogs with portosystemic shunts and the cause is not known, but it appears to he related to iron sequestration in tissues such as liver and/or defects in the transport of iron. Some cases also have intermittent gastrointestinal bleeding associated with pica.

Urinalysis

Moderate glucosuria in an animal with a low or normal blood glucose suggests a lowered renal threshold for glucose

and, therefore, a renal tubular absorption defect. Such a defect may be confined to glucose absorption only, or may include defective absorption of several substances. As previously noted, this dog may also have defective absorption of phosphate, sodium, and potassium. If this is the case, this is probably a form of canine Fanconi-like syndrome. Measurement of the fractional excretion of phosphate, sodium, and potassium would have helped in assessing this possibility.

Summary

This dog had a portosystemic shunt. Hypoglycemia, decreased BUN concentration, hypoalbuminemia, and increased serum bile acid concentrations resulted from decreased hepatic blood flow and subsequent hepatic failure. Microcytosis has been reported as common in dogs with portosystemic shunts. This dog also has a renal tubular defect. Glucosuria in the presence of hypoglycemia, hypophosphatemia, hyponatremia, and hypokalemia are probably a result of defective tubular reabsorption of these substances. This defect is probably a canine Fanconi-like syndrome. Neutropenia does not typically occur in either portosystemic shunts or canine Fanconi-like syndrome, and this dog may have a concurrent enteric infection, most likely caused by parvovirus or endotoxin-producing bacteria, resulting in this abnormality.

Case 44

Signalment: 6-month-old intact male dog
History: Struck by a car on Day 1

Physical examination: Pale mucous membranes. Day 1 blood sample obtained 12 hours after accident.

Hematology	Day 1	Day 6	Reference Interval
PCV (%)	**29**	**35**	37–55
Hgb (g/dL)	**9.6**	**11.5**	12–18
RBC ($\times 10^6/\mu L$)	**4.7**	**5.1**	55–8.5
MCV (fL)	62	69	60–72
MCHC (g/dL)	33	33	33–38
Retics ($\times 10^3/\mu L$)	**47**	**304**	<60
NCC ($\times 10^3/\mu L$)	**22.7**	**20.0**	6–17
Segs ($\times 10^3/\mu L$)	**22.0**	**12.0**	3–11.5
Bands ($\times 10^3/\mu L$)	0	**2.0**	0–0.3
Monos ($\times 10^3/\mu L$)	0	1.0	0.1–1.3
Lymphs ($\times 10^3/\mu L$)	**0.7**	**5.0**	1–4.8
Platelets ($\times 10^3/\mu L$)	340	460	200–500
TP (P) (g/dL)	**5.4**	6.5	6–8
Hemopathology: No abnormalities on Day 1. Moderate anisocytosis and polychromasia on Day 6.			

Biochemical Profile	Day 1	Day 6	Reference Interval
Gluc (mg/dL)	**125 (*6.9*)**	**105**	65–122 (*3.5–6.7 mmol/L*)
BUN (mg/dL)	9	13	7–28
Creat (mg/dL)	1.1	1.3	0.9–1.7
Ca (mg/dL)	**8.9 (*2.22*)**	9.3	9.0–11.2 (*2.25–2.80 mmol/L*)
Phos (mg/dL)	5.5	5.6	2.8–6.1
TP (g/dL)	**5.0**	6.0	5.4–7.4
Alb (g/dL)	3.4	4.0	2.7–4.5
Glob (g/dL)	**1.6**	2.0	1.9–3.4
T. Bili (mg/dL)	0.3	0.4	0–0.4
Chol (mg/dL)	210	180	130–370
ALT (IU/L)	**1098**	**150**	10–120
AST (IU/L)	**948**	**80**	16–40
ALP (IU/L)	**302**	**295**	35–280
Na (mEq/L)	150	147	145–158
K (mEq/L)	4.8	4.7	4.1–5.5
CL (mEq/L)	120	121	106–127
TCO_2 (mEq/L)	**12**	21	14–27
An. gap (mEq/L)	23	10	8–25

Urinalysis (catheterized)—obtained on Day 1

Color	Yellow	**Urine Sediment**	
Transparency	Clear	WBCs/hpf	1–2
Sp. Gr.	1.019	RBCs/hpf	**3–5**
Protein	Trace	Epith cells/hpf	0
Glucose	Negative	Casts/lpf	0
Bilirubin	Negative	Crystals	0
Blood	Negative	Bacteria	0
pH	6.5		

Interpretive discussion

Hematology

This dog is anemic on both days. The anemia is more severe on Day 1 and is nonregenerative. Since the Day 1 blood sample was obtained 12 hours after the accident, it is likely that the anemia is due to acute blood loss. The concurrent hypoproteinemia (see discussion below) also supports blood loss as the cause of this anemia. Increased polychromasia and reticulocyte count are not evident in blood until 2 to 4 days following acute blood loss. While the anemia appears nonregenerative on the initial sample, by Day 6, the erythrocyte values have increased, and there is evidence of increased erythrocyte production (increased polychromasia and reticulocyte count). This dog is, therefore, responding appropriately to the blood loss.

Although normal on both days, the MCV increased between Day 1 and Day 6, probably due to increased erythrocyte production resulting in increased number of large, immature erythrocytes.

The dog has a mature neutrophilia and lymphopenia on Day 1. This is compatible with a corticosteroid-mediated leukogram, resulting from stress associated with pain or trauma.

Neutrophilia and a left shift on Day 6 are typical of an inflammatory leukogram. Tissue injury associated with the accident probably incited an inflammatory response. An infectious etiology cannot be excluded, however.

Biochemical profile

Mild hyperglycemia on Day 1 resulted from stress. The presence of a stress leukogram supports this explanation.

Slight hypocalcemia may be normal in this dog since young animals commonly have slightly lower serum Ca concentrations than adults. However, the serum Ca concentration returned to within the reference interval on Day 6, suggesting that this is the more normal value for this dog. It is possible that the hypocalcemia on day 1 resulted from loss of albumin and albumin-bound Ca during hemorrhage.

Hypoproteinemia and hypoglobulinemia on Day 1 probably resulted from loss of protein during hemorrhage. Although the serum albumin concentration is in the reference interval, this might actually be low for this animal. The serum albumin concentration increased by Day 6, implying that this is the more normal concentration for this dog. All protein concentrations returned to within the reference intervals by Day 6, indicating that compensatory mechanisms had replaced the protein lost through hemorrhage.

Both serum ALT and AST activities are markedly increased on Day 1, but decrease to nearly normal by Day 6. These increases suggest liver and/or muscle injury. High concentrations of ALT are present in the liver and lower concentrations are present in muscle. The marked increase in ALT in this case, therefore, probably resulted from liver injury, but muscle injury may have also contributed. Aspartate aminotransferase (AST) is present in high concentrations in both liver and muscle, and both tissues may be sources of AST in this case. Liver and muscle trauma may explain these increased enzyme activities on Day 1. Shock with subsequent hypoxia and tissue injury can also result in leakage and increased serum activities of both enzymes. Regardless of the underlying cause of their leakage, the decreasing activities of both enzymes by Day 6 imply the damage was acute, and it is no longer active.

Increased alkaline phosphatase (ALP) activities are likely normal for this dog. Young, growing animals commonly have slightly to moderately increased serum ALP activity since, due to active bone growth, increased amounts of ALP are released from osteoblasts.

There is a slight decrease in the total CO_2 on Day 1, suggesting metabolic acidosis. Hypovolemic shock leading to tissue hypoxia may have resulted in production of acid metabolites, and decreased renal blood flow may have interfered with renal acid-base regulation. The anion gap, while still within the reference interval, is higher on Day 1 as compared to Day 6, and this may have resulted from increased blood concentrations of anions such as lactate.

Urinalysis

In light of the relatively dilute urine (specific gravity = 1.019), the urine concentration of erythrocytes may be slightly increased. Mild hematuria may have resulted from trauma.

Summary

This dog had a dislocated hip and broken femur. Surgery was performed between Days 1 and 6. The dog's recovery was uneventful. This case demonstrates a normal response to acute blood loss. It also demonstrates the importance of serial measurement of serum enzyme activities in animals with increases of these activities. Steady or increasing activities of these enzymes indicates active and continuing damage to the tissue(s) of origin. Decreasing activities usually indicate that the injury has ceased and/or is resolving.

Case 45

Signalment: 5-year-old cocker spaniel

History: Presented for anorexia and dark orange urine and feces. Dog had ITP 2 years previously, and has been given phenobarbital (100 mg bid) for epilepsy for several years.

Hematology		Reference Interval
PCV (%)	**13**	37–55
RBC ($\times 10^6/\mu f$)	**1.95**	5.5–8.5
Hgb (g/dL)	**4.6**	12–18
MCV (fL)	67	60–72
MCHC (g/dL)	35	33–38
Retics (/μL)	**0**	0–60,000
NCC ($\times 10^3/\mu L$)	**54.9**	6.0–17.0
Metas ($\times 10^3/\mu L$)	**1.1**	0
Bands ($\times 10^3/\mu L$)	**6.0**	0–0.3
Segs ($\times 10^3/\mu L$)	**43.4**	3.0–11.5
Lymphs ($\times 10^3/\mu L$)	1.1	1.0–4.8
Monos ($\times 10^3/\mu L$)	**2.2**	0.2–1.4
Eos ($\times 10^3/\mu L$)	0.5	0.1–1.2
NRBCs ($\times 10^3/\mu L$)	**0.5**	0
Platelets ($\times 10^3/\mu L$)	260	200–500
TP (P) (g/dL)	6.3	6.0–8.0
Hemopathology: occasional imperfect spheres, slight agglutination.		
Coombs test: **positive**		

Bone marrow aspirate
Megakaryocytes present. Myeloid and erythroid hyperplasia, with normal maturation up to metarubricyte stage. M : E ratio decreased slightly. Rare erythrophagocytosis.

Biochemical Profile		Reference Interval
Gluc (mg/dL)	**56 (*3.1*)**	65–122 (*3.5–6.7 mmol/L*)
BUN (mg/dL)	**56 (*19.9*)**	7–28 (*2.5–10 mmol/L*)
Creat (mg/dL)	0.6	0.6–1.5
Ca (mg/dL)	**8.5 (*2.1*)**	9.0–1.12 (*2.25–2.80 mmol/L*)
Phos (mg/dL)	**6.4 (*2.1*)**	2.8–6.1 (*0.9–2.0 mmol/L*)
TP (g/dL)	**3.8**	5.4–7.4
Alb (g/dL)	**1.5**	2.7–4.5
Glob (g/dL)	2.3	1.9–3.4
T. Bili (mg/dL)	**35.8 (*612.2*)**	0–0.4 (*0–6.84 μmol/L*)
Chol (mg/dL)	**64 (*1.6*)**	130–370 (*3.4–9.6 mmol/L*)
ALT (IU/L)	**70**	16–40
ALP (IU/L)	**566**	18–141
GGT (IU/L)	**15**	0–6
Na (mEq/L)	**160**	145–158
K (mEq/L)	**3.2**	4.1–5.5
CL (mEq/L)	**135**	106–127
TCO_2 (mEq/L)	**9.5**	14–27
An. gap (mEq/L)	16	8–26

Urinalysis	
Color	**brown**
Transparency	**cloudy**
Sp. Gr.	1.022
Bilirubin	++++
No other abnormal findings.	

Interpretive discussion

Hematology

The dog is markedly anemic. Reticulocytes are not increased, indicating that the anemia is not regenerative. The presence of the imperfect spherocytes and agglutination is suggestive of immune-mediated hemolytic anemia, possibly very acute, or with destruction of precursors. An unexplained nonregenerative anemia, when platelets and neutrophils are normal and increased, respectively, triggered a bone marrow aspirate. The bone marrow aspirate findings further substantiated immune-mediated hemolytic anemia with destruction of polychromatophilic cells.

Neutrophilia, increased immature neutrophils, and monocytosis are indicative of inflammation.

If the animal has not received a previous transfusion, a positive Coombs' test is suggestive of immune-mediated hemolytic anemia.

Bone marrow

In light of marked erythroid response in marrow, anemia is either very acute, and will respond, or precursors are being destroyed. Because imperfect spherocytes are present on blood film, the latter is more likely.

Biochemical profile

The serum glucose concentration is decreased. Differentials should include insulinoma and, in this patient, end-stage liver disease, since the animal is also hypoalbuminemic and hypocholesterolemic.

The BUN is increased, and although the creatinine is within the reference interval, one would expect the animal to be concentrating greater than 1.022 if the azotemia is prerenal. One should consider if the animal is bleeding into the GI tract, increasing the BUN, or since IMHA is suspected based on the hematology, if the animal has hemolysis with subsequent hemoglobinuric nephrosis. If the animal does have end-stage liver disease, one would expect the BUN to be decreased as well, so the increase in BUN is slightly confusing. The mild increase in serum phosphorus suggests decreased glomerular filtration rate.

The serum calcium is decreased, likely due to hypoalbuminemia. An ionized calcium could be measured to confirm this.

The serum total protein concentration is decreased due to hypoalbuminemia. Since the globulin is within the reference interval, liver failure would be the best differential, since the animal is not proteinuric. Another consideration would be that blood loss is causing the anemia and hypoproteinemia, but there is no clinical evidence of blood loss.

The serum bilirubin concentration is markedly increased and may be due to hemolysis, liver failure, or cholestasis or some combination of the three. The ALT is only slightly increased, indicating mild hepatocellular damage. The ALP activity is increased, as is GGT activity, indicating cholestasis. Alternatively, enzymes may be induced by phenobarbital.

Serum total CO_2 is decreased, suggesting metabolic acidosis. This may be secondary to lactic acidosis associated with marked anemia. Increased sodium and chloride suggest hypertonic dehydration or administration of hypertonic fluid. The hypokalemia in conjunction with metabolic acidosis (which should have caused an increased potassium) suggests whole body potassium depletion.

Urinalysis

The marked bilirubinuria reflects conjugated hyperbilirubinemia. Specific gravity is discussed above.

Summary

Immune-mediated hemolytic anemia, liver failure, and renal dysfunction. The dog died, and necropsy showed severe chronic micronodular cirrhosis and cholestasis, possibly related to phenobarbital. Bone marrow showed myeloid and erythroid hyperplasia. Examination of the kidneys revealed severe hemoglobinemic nephrosis with mild chronic interstitial nephritis.

Case 46

Signalment: 8-month-old German shepherd
History: Lethargic, "poor doer," weight loss

Hematology		Reference Interval
PCV (%)	**34**	37–55
MCV (fL)	**52**	60–72
NCC ($\times 10^3/\mu L$)	**44.6**	6–17
Segs ($\times 10^3/\mu L$)	**38.0**	3–11.5
Bands ($\times 10^3/\mu L$)	**2.2**	0–0.3
Lymphs ($\times 10^3/\mu L$)	3.1	1.0–4.8
Monos ($\times 10^3/\mu L$)	0.9	0.2–1.4
Eos ($\times 10^3/\mu L$)	0.4	0.1–1.2
Platelets ($\times 10^3/\mu L$)	Adequate	200–500

Biochemical Profile		Reference Interval
Gluc (mg/dL)	87	65–122
BUN (mg/dL)	**6 (*2.1*)**	7–28 (*2.5–10.0 mmol/L*)
Creat (mg/dL)	**0.5**	0.9–1.7
Ca (mg/dL)	**8.6 (*2.15*)**	9.0–11.2 (*2.25–2.80 mmol/L*)
Phos (mg/dL)	5.6	2.8–6.1
TP (g/dL)	**4.3**	5.4–7.4
Alb (g/dL)	**2.4**	2.7–4.5
Glob (g/dL)	1.9	1.9–3.4
T. Bili (mg/dL)	0.4	0–0.4
Chol (mg/dL)	**75 (1.95)**	130–370 (*3.4–9.6 mmol/L*)
ALT (IU)	**250**	10–120
ALP (IU)	129	35–280
GGT (IU)	**7**	0–6
Na (mEq/L)	154	145–158
K (mEq/L)	4.1	4.1–5.5
CL (mEq/L)	126	106–127
TCO_2 (mEq/L)	22.3	14–27
An. gap (mEq/L)	10	8–26
Bile acids, fasting (µmol/L)	**88.5**	<10
Serum iron (µg/dL)	**22**	60–100

Interpretive discussion

Hematology

The dog has mild anemia that is not characterized by a reticulocyte count. The anemia is microcytic suggesting iron deficiency. Microcytosis should also prompt consideration of a portosystemic shunt in a young dog. Although some dogs with portosystemic shunt will have low serum iron concentrations, marked hypoferremia suggests iron deficiency is the cause of the microcytosis. There is a marked leukocytosis characterized by neutrophilia with a left shift; this is interpreted as an inflammatory leukogram.

Biochemical profile

The decreased BUN suggests reduced biosynthesis of urea by the liver. The same may be interpreted for cholesterol and albumin. There is a slight increase in ALT suggesting a mild degree of hepatocellular injury. The markedly increased bile acid concentration confirms a defect in hepatic function. The bilirubin and ALP do not indicate cholestasis. The slight increase in GGT is of questionable significance.

Hypocalcemia may be due to hypoalbuminemia, in which case it is clinically insignificant. An ionized calcium could be measured to confirm this.

Decreased creatinine reflects decreased muscle mass.

Summary

The findings of reduced hepatic biosynthesis with retention of bile acids in a young dog are highly suggestive of portosystemic shunt.

Case 47

Signalment: 8-year-old male Samoyed
History: Diarrhea
Physical examination: Recumbent, arrested prior to treatment

Hematology		Reference Interval
PCV (%)	**18**	37–55
Retics (×10³/μL)	**197,830 (*7.3%*)**	<60,000
MCV (fL)	66	60–72
NCC (×10³/μL)	**78.0**	6–17
Segs (×10³/μL)	**44.5**	3–11.5
Bands (×10³/μL)	**14.8**	0–0.3
Metas (×10³/μL)	**3.9**	0
Myelocytes (×10³/μL)	**0.8**	0
Monos (×10³/μL)	0.8	0.1–1.3
Lymphs (×10³/μL)	3.1	1–4.8
NRBC (×10³/μL)	**9.4**	0
Platelets (×10³/μL)	158	200–500
Hemopathology: Increased polychromasia, target cells, giant platelets, toxic neutrophils.		

Biochemical Profile		Reference Interval
Gluc (mg/dL)	**580 (*31.9*)**	65–122 (*3.5–6.7 mmol/L*)
BUN (mg/dL)	**98 (*35*)**	7–28 (*2.5–10.0 mmol/L*)
Creat (mg/dL)	**3.1 (*274*)**	0.9–1.7 (*80–150 μmol/L*)
Ca (mg/dL)	9.6	9.0–11.2
Phos (mg/dL)	**13.1 (*4.2*)**	2.8–6.1 (*0.9–2.0 mmol/L*)
TP (g/dL)	**4.7**	5.4–7.4
Alb (g/dL)	**2.4**	2.7–4.5
T. Bili (mg/dL)	**0.6 (*10.3*)**	0–0.4 (*0–6.8 μmol/L*)
Chol (mg/dL)	246	130–370
ALT (IU/L)	**1031**	10–120
ALP (IU/L)	**2500**	35–280
Na (mEq/L)	**130**	145–158
K (mEq/L)	**6.5**	4.1–5.5
CL (mEg/L)	**87**	106–127
TCO_2 (mEq/L)	**10.6**	14–27
An. gap (mEq/L)	**39**	8–26

Urinalysis	
Sp. Gr.	**1.017**
Gluc	**2+**
Protein	0
Ketones	0
No other abnormalities present.	

Interpretive discussion

Hematology

There is moderate regenerative anemia. Considering the hypoproteinemia, hemorrhage is the most likely cause. The nucleated RBC are interpreted as part of the regenerative response. There is a marked leukocytosis characterized by prominent neutrophilia with toxic change and a left shift to myelocytes indicating inflammation. The mild thrombocytopenia may be caused by hemorrhage, and giant platelets indicate active thrombopoiesis.

Biochemical profile and urinalysis

There is marked hyperglycemia. This is associated with an expected glucosuria. The magnitude of hyperglycemia should prompt consideration of diabetes mellitus. The lack of urine ketones makes the diagnosis more difficult.

Moderate azotemia is indicated by increased concentrations of BUN and creatinine. The specific gravity indicates minimal concentrating ability in the face of azotemia. This suggests an element of primary renal disease. However, electrolyte depletion (see later) may be contributing to the decreased concentrating ability. The increased phosphorus is compatible with decreased glomerular filtration.

The hypoproteinemia along with regenerative anemia is compatible with blood loss.

There is a marked increase in ALT activity indicating hepatocellular injury. Diabetes is associated with fat mobilization to the liver; this may result in modest ALT activity increases. The magnitude of this ALT suggests more severe injury. There is also an element of cholestasis indicated by the marked increase in ALP and a minimal increase in bilirubin.

The hyponatremia is likely due to urinary sodium loss secondary to glucosuria (osmotic diuresis). Losses associated with diarrhea may have contributed. Additionally, cellular water may move from the intracellular compartment into the extracellular fluid compartment, diluting serum sodium (expect 1.6 mEq/L decrease in sodium for every 100 mg/dL increase in glucose). The hyperkalemia is probably due to a shift of potassium ions out of cells in exchange for hydrogen ions, which enter cells during metabolic acidosis. Another possibility is that the animal is becoming oliguric and retaining potassium.

Increased anion gap is due to the presence of "unmeasured" anions. In this dog, these likely include phosphates, as well as lactate, since the dog is markedly anemic. In addition, because this dog is diabetic, ketones may contribute to unmeasured anions. Since beta hydroxybutyrate is not detected by routine urine dipstick methods, ketonuria may actually be present.

Summary

Further evaluation led to the findings of diabetes mellitus and hepatic lipidosis. The enlarged, fragile liver had led to a fractured liver. This latter injury likely contributed to the magnitude of the ALT increase.

Case 48

Signalment: 4-year-old DSH cat
History: Anorexia, weight loss, depression
Physical examination: Thin, icteric mucous membranes

Hematology		Reference Interval
PCV (%)	29	24–45
NCC ($\times 10^3/\mu L$)	13.7	5.5–19.5
NRBC ($\times 10^3/\mu L$)	**0.1**	0
Segs ($\times 10^3/\mu L$)	11.6	2.5–12.5
Bands ($\times 10^3/\mu L$)	0.1	0–0.3
Monos ($\times 10^3/\mu L$)	0.4	0–0.8
Lymphs ($\times 10^3/\mu L$)	**0.7**	1.5–7
Eos ($\times 10^3/\mu L$)	0.8	0–1.5
Platelets ($\times 10^3/\mu L$)	304	200–500
Morphology: Many acanthocyte-like RBCs, occasional fragmented RBC.		

Biochemical Profile		Reference Interval
Gluc (mg/dL)	67	67–124
BUN (mg/dL)	**14**	17–32
Creat (mg/dL)	1.2	0.9–2.1
Ca (mg/dL)	9.0	8.5–11
Phos (mg/dL)	5. I	3.3–7.8
TP (g/dL)	6.2	5.9–8.1
Alb (g/dL)	3.0	2.3–3.9
T. Bili (mg/dL)	**6.3 (*108*)**	0–0.3 (*0–5.1 µmol/L*)
ALT (IU/L)	**332**	30–100
ALP (IU/L)	**2185**	11–210
Na (mEq/L)	149	146–160
K (mEq/L)	5.2	3.7–5.4
CL (mEq/L)	**109**	112–129
TCO_2 (mEq/L)	19	14–23

Interpretive discussion

Hematology

The leukogram shows a lymphopenia with a high normal concentration of mature neutrophils; this is interpreted as a stress or steroid leukogram. There are acanthocyte-like or spiculated cells present. These are commonly observed in cats with liver disease or hepatic lipidosis.

Biochemical profile

The mildly decreased BUN may be insignificant or may be due to decreased hepatic urea production or decreased protein intake. The combination of hyperbilirubinemia and increased ALT and ALP activities is characteristic of hepatic lipidosis in cats. The combination of the hepatocellular injury (indicated by increased ALT) and cholestasis (indicated by increased ALP) lead to failure of bilirubin clearance and hyperbilirubinemia. This degree of increase in ALP activity is unusual in cats, other than in association with hepatic lipidosis. Lipidosis is thought to occur as a result of massive fat mobilization from adipocytes in association with anorexia of several days duration or acute diabetes mellitus.

Summary

The biochemical findings are characteristic of hepatic lipidosis, which was confirmed by liver aspiration cytology.

Case 49

Signalment Five-year-old female alpaca in the late stage of gestation
History Lethargy, anorexia
Physical Thin, depressed

Hematology		Reference Interval
PCV (%)	28	24–35
Hgb (g/dL)	12.7	11–19
RBC ($\times 10^6/\mu L$)	12.1	8.8–15.4
MCV (fL)	23	21–30
MCHC (g/dL)	45.9	39.2–46.1
NCC ($\times 10^3/\mu L$)	8.4	5.2–15.7
Segs ($\times 10^3/\mu L$)	6.0	2.1–9.5
Monos ($\times 10^3/\mu L$)	0.6	0–0.6
Lymphs ($\times 10^3/\mu L$)	1.3	0.9–4.4
Eos ($\times 10^3/\mu L$)	0.4	0–3.3
Platelets ($\times 10^3/\mu L$)	2,141	206–3,600
TP (P) (g/dL)	**9.0**	5.4–7.2
Hemopathology: few reactive lymphocytes, mild anisocytosis. Grossly lipemic plasma.		

Biochemical Profile		Reference Interval
Gluc (mg/dL)	129	100–132
BUN (mg/dL)	14	12–33
Creat (mg/dL)	1.7	1.3–2.7
Ca (mg/dL)	8.9	8.0–10.4
Phos (mg/dL)	3.9	2.5–8.6
TP (g/dL)	6.3	5.3–7.6
Alb (g/dL)	3.6	2.6–4.7
Glob (g/dL)	2.7	2.7–2.9
T. Bili (mg/dL)	0.1	0–0.2
Chol (mg/dL)	**364**	12–58
Trig (mg/dL)	**4,330**	5–30
β-hydroxybutyrate (mmol/L)	**26.0**	0.2–1.1
AST (IU/L)	**474**	110–250
SDH (IU/L)	**17.6**	3–10
GGT (IU/L)	**76**	10–42
ALP (IU/L)	105	20–150
CK (IU/L)	45	40–500
Na (mEq/L)	146	142–156
K (mEq/L)	3.8	3.6–6.5
CL (mEq/L)	112	108–122
TCO_2 (mEq/L)	**13**	19–29
An. Gap (mEq/L)	25	12–25
Grossly lipemic serum		

Urinalysis (catheter)

		Urine Sediment	
Color	Light yellow	WBCs/hpf	Rare
Transparency	Clear	RBCs/hpf	None seen
Specific Gravity	1.006	Epithelial cells/hpf	0–1
Protein	Negative	Casts/lpf	0
Glucose	Negative	Crystals	0
Bilirubin	Negative	Bacteria	0
Blood	Negative		
pH	9.0		
Ketones	**1+**		

Interpretive discussion

Hematology

The plasma protein determined by refractometer is significantly higher than the total protein determined on the biochemistry panel. This is due to the marked lipemia of the sample. Lipemia occurs when triglyceride concentrations are increased. The refractometer estimates plasma proteins by the bending of light in relation to the concentration of solutes in the sample. Hyperlipidemia will cause an artifactual increase in the plasma protein measurement. Although hypercholesterolemia does not cause visible lipemia, if markedly increased it can artifactually increase the refractometric plasma protein measurement.

Biochemical profile

There is a marked increase in triglycerides and cholesterol, compatible with hyperlipidemia that may develop in sick camelids. In this case, negative energy balance accompanying late term pregnancy likely precipitated this condition. The glucose in this case is still normal.

Increased activity of AST in combination with a normal creatine kinase is compatible with hepatocellular injury rather than muscle origin of the AST. Increased activity of SDH also suggests hepatocellular damage while GGT is an indicator of cholestasis. Increased AST, SDH, GGT, triglycerides and cholesterol are common findings in camelids with hepatic lipidosis. Neither ALP nor bilirubin are increased and have been shown to be less reliable indicators of hepatic lipidosis in camelids.

The alpaca is ketotic as evidenced by the increase in serum β-hydroxybutyrate and the presence of ketones in the urine. The low TCO2 indicates a decrease in bicarbonate and a metabolic acidosis. A blood gas profile is needed to completely assess acid/base status.

Urinalysis

The hyposthenuria is of unknown significance at this time. Camelids with hepatic lipidosis are at risk for developing renal failure secondary to accumulation of lipid in the renal parenchyma. Although her BUN and creatinine are normal, her renal function should be carefully monitored. The alkaline urine pH is normal for herbivores. However, this is somewhat surprising in the face of metabolic acidosis and ketonuria.

Summary

Increased activities of AST, SDH and GGT along with hypertriglyceridemia and hypercholesterolemia are common findings in camelids with hepatic lipidosis. Ketosis and metabolic acidosis also can develop in these patients. Although not measured in this case, nonesterified fatty acids (NEFA) are expected to be increased as a result of mobilization of fat. Hepatic lipidosis can be precipitated by severe negative energy balance associated with late term pregnancy, stress, or anorexia. Fat is mobilized to supply fatty acids for energy production. However, the supply of fatty acids exceeds the ability to utilize them in the tricarboxylic acid (TCA) cycle. Fatty acids in the liver are incorporated into triglycerides and released as very low density lipoproteins (VLDL), resulting in the hypertriglyceridemia. However, hepatic production of triglycerides exceeds the ability to export them as VLDL so triglycerides accumulate in the cells. Fatty acids also will be shunted into ketogenesis, resulting in the observed increase in β-hydroxybutyrate and ketonuria.

Case 50

Signalment: 7-year-old female border collie
History: Depression, anorexia
Physical examination: Ascites, dermatitis of face and genital area

Hematology		Reference Interval
PCV (%)	**15**	37–55
MCV (fL)	**57**	60–72
Retics ($\times 10^3/\mu L$)	**118**	<60
NCC ($\times 10^3/\mu L$)	9.5	6–17
Segs ($\times 10^3/\mu L$)	4.3	3–11.5
Bands ($\times 10^3/\mu L$)	**2.2**	0–0.3
Metas ($\times 10^3/\mu L$)	**0.6**	0
Monos ($\times 10^3/\mu L$)	0.8	0.1–1.3
Lymphs ($\times 10^3/\mu L$)	**0.7**	1–4.8
NRBC ($\times 10^3/\mu L$)	**0.9**	0
Platelets ($\times 10^3/\mu L$)	**20**	200–500
Hemopathology: target cells, acanthocytes, schistocytes, toxic neutrophils, giant platelets.		

Biochemical Profile		Reference Interval
Gluc (mg/dL)	**45**	65–122
BUN (mg/dL)	16	7–28
Creat (mg/dL)	1.0	0.9–1.7
Ca (mg/dL)	9.2	9.0–11.2
Phos (mg/dL)	3.8	2.8–6.1
TP (g/dL)	**4.5**	5.4–7.4
Alb (g/dL)	**1.7**	2.7–4.5
Glob (g/dL)	2.8	1.9–3.4
T. Bili (mg/dL)	**3.3**	0–0.4
Chol (mg/dL)	**86**	130–370
ALP (IU/L)	**1391**	35–280
ALT (IU/L)	**239**	10–120
Na (mEq/L)	147	145–158
K (mEq/L)	**2.6**	4.1–5.5
CL (mEq/L)	122	106–127
TCO_2 (mEq/L)	**8.5**	14–27

Fluid Analysis (abdominal)	
Color	Straw
Transparency	Clear
NCC (/μL)	1300
TP (g/dL)	1.5

Coagulation Data		
PT (sec)	**20**	6.5–9.0
aPTT (sec)	**36**	12–16

Interpretive discussion

Hematology

PCV is decreased, indicating anemia. Reticulocytes are increased, indicating that the anemia is somewhat regenerative. MCV is decreased, particularly in light of increased reticulocytes, suggesting iron deficiency anemia secondary to chronic blood loss.

Inflammatory leukogram is present, as evidenced by the marked left shift and toxic change in neutrophils. In light of low normal number of segmented neutrophils, sepsis or endotoxemia may be present. Lymphopenia suggests a concurrent stress response.

The combination of thrombocytopenia, schistocytes, and prolonged PT and APTT suggests disseminated intravascular coagulopathy (DIC). Alternatively, this degree of thrombocytopenia may be seen with immune-mediated destruction or ehrlichiosis.

Biochemical profile

Hypoglycemia may be due to sepsis (leukogram is suggestive of sepsis or endotoxemia), end-stage liver disease, insulinonia, or other type of neoplasia, such as a large hepatoma.

Hypoalbuminemia, in conjunction with low cholesterol, is indicative of GI disease (malabsorption, maldigestion, protein losing enteropathy) or end-stage liver disease. Another possible cause of low total protein is blood loss, since MCV indicates iron deficiency anemia. However, albumin is relatively lower than globulin.

Total bilirubin is increased. While the animal is anemic, and blood destruction is a possible cause, the MCV suggests blood loss. Therefore the bilirubin is probably increased due to cholestasis or hepatocellular dysfunction. Increased alkaline phosphatase activity suggests cholestasis.

Cholesterol is decreased, likely due to end-stage liver disease (see hypoalbuminemia discussion).

Hypokalemia may be due to decreased intake. In face of acidosis, it indicates total body depletion of potassium.

Decreased total CO_2 indicates metabolic acidosis. The decrease is likely due to lactic acidosis in this patient, since the dog is not uremic and there is no evidence of diabetic ketoacidosis.

Abdominal fluid analysis

Transudate, likely due to liver disease and hypoalbuminemia.

Coagulation data

While prolonged PT and APPT may be due to lack of synthesis of coagulation factors by the liver, another explanation is DIC, in light of the decreased platelets.

Summary

End-stage liver disease; cholestasis
DIC
Inflammation, possibly sepsis
Iron deficiency anemia

Dermatitis was determined to be necrolytic migratory erythema (superficial necrolytic dermatitis), which is associated with hyperglucagonemia, often seen with severe hepatic disease (hepatocutaneous syndrome).

Case 51

Signalment: 10-year-old spayed female miniature schnauzer

History: Polydipsia, polyuria, weight loss, abdominal "cramping" for 1 month

Physical Examination: Tense abdomen, thin with mild truncal alopecia and comedones on dorsal midline

Hematology		Reference Interval
PCV (%)	48	37–55
NCC ($\times 10^3/\mu L$)	**34.4**	6–17
Segs ($\times 10^3/\mu L$)	**29.0**	3–11.5
Bands ($\times 10^3/\mu L$)	**2.0**	0–0.3
Monos ($\times 10^3/\mu L$)	**3.4**	0.1–1.3
Lymphs ($\times 10^3/\mu L$)	0	1–4.8
Platelets ($\times 10^3/\mu L$)	Adequate	200–500
TP* (g/dL))	**9.0***	6–8
*although dog fasted, plasma is markedly lipemic, so refractometric measurement of total protein may be falsely increased		

Biochemical Profile		Reference Interval
Gluc (mg/dL)	**353 *(19.4)***	65–122 *(3.7–6.8 mmol/L)*
BUN (mg/dL)	**35 *(12.5)***	7–28 *(6.1–11.4 mmol/L)*
Creat (mg/dL)	1.2	0.9–1.7
Ca (mg/dL)	11.0	9.0–11.2
Phos (mg/dL)	6.0	2.8–6.1
TP (g/dL)	6.0	5.4–7.4
Alb (g/dL)	2.7	2.7–4.5
Glob (g/dL)	3.3	1.9–3.4
T. Bili (mg/dL)	**1.2 *(26.5)***	0–0.4 *(0–6.8 µmol/L)*
Chol (mg/dL)	**900 *(23.4)***	130–370 *(3.4–9.6 mmol/L)*
ALT (IU/L)	**987**	10–120
ALP (IU/L)	**1200**	35–280
Na (mEq/L)	**139**	145–158
K (mEq/L)	3.1	4.1–5.5
CL (mEq/L)	**100**	106–127
TCO_2 (mEq/L)	12.2	14–27
An. gap (mEq/L)	**30**	8–25
Lipase (IU/L)	**3500**	30–560

Urinalysis	
Color	Yellow
Transparency	Clear
Sp. Gr.	1.035
Protein	Neg
Gluc	2+
Ketones	Neg
Bilirubin	+
Blood	Neg
pH	6.0

Endocrine Data		Reference Interval
ACTH stimulation:		
serum cortisol (µg/dL)-(pre)	**4.5 *(124)***	1–4 *(25–110 nmol/L)*
serum cortisol (µg/dL)-(post)	14.6	<20
Low dose dexamethasone suppression test:		
serum cortisol (µg/dL)-(pre)	3.5	1–4
serum cortisol (µg/dL)-(8-hour post)	1.5	<1.5

Interpretive discussion

Hematology

Lymphopenia is indicative of increased endogenous (stress or hyperadrenocorticism) or exogenous corticosteroids. Increased immature neutrophil concentration is indicative of inflammation. Neutrophilia may be due to inflammation or stress. In summary, an inflammatory and stress (steroid) leukogram is present.

Biochemical profile

Hyperglycemia is of the magnitude that diabetes mellitus should be suspected. Hyperglycemia may also be secondary to hyperadrenocorticism; therefore, adrenocorticotropic hormone (ACTH) stimulation and low dose dexamethasone suppression tests (LDDS) are indicated.

BUN is increased, but creatinine is within the reference interval. Urine specific gravity indicates kidneys are capable of concentrating, thus the azotemia is prerenal, perhaps clue to dehydration. However, albumin is within reference interval. The PCV is normal, suggesting that GI bleeding is not the cause of the increased BUN.

Total bilirubin is increased suggesting cholestasis, because anemia is not present. Alkaline phosphatase activity is increased, which is also suggestive of cholestasis. Another consideration is hyperadrenocorticism, with an increase in the corticosteroid-induced alkaline phosphatase isoenzyme. Increased cholesterol of this magnitude is probably due to lipidemia, although some component of the increase could also be due to cholestasis. ALT activity is increased, which is indicative of hepatocellular damage.

Sodium and chloride concentrations are decreased. Sodium may be lost through the kidney, although this animal is capable of concentrating. Although it is not mentioned in the history, abdominal pain may have been associated with vomiting, which would result in electrolyte loss. Hyperglycemia results in increased serum osmolality with a shift of intracellular fluid to extracellular fluid in an attempt to decrease extracellular fluid solute concentration. Sodium can be expected to decrease by 1.6 mEq/L for every 100 mg/dL increase in glucose.

Total CO_2 is decreased, indicating metabolic acidosis. The anion gap is increased, indicating increased unmeasured anions are present. In this case, unmeasured anions might be ketones, although they are not present in the urine. Urine ketone tests that use the nitroprusside reaction do not detect β-hydroxybutyric acid; therefore, the presence of this ketone cannot be ruled out. Other possibilities include lactic acidosis.

Serum lipase activity is increased. In this patient, this increase could partially be due to decreased GFR, as indicated by azotemia. However, the inflammatory leukogram, increased bilirubin, increased alkaline phosphatase activity, hyperglycemia, and lipernia are also suggestive of pancreatitis. This magnitude of lipase increase is highly supportive of pancreatitis. Prerenal azoternia due to hemoconcentration and poor renal perfusion is a common complication of pancreatitis. Likewise, so is hepatocellular injury and cholestasis.

Urinalysis

Urine specific gravity of 1.035 indicates the dog is capable of concentrating, thus the increase in BUN is prerenal (perhaps dehydration). Glucosuria and bilirubinuria are to he expected in light of the serum concentrations.

Endocrine data

ACTH stimulation test: Baseline cortisol is slightly above normal. Normal animals stimulate to around 10 to 16 µg/dL. Low dose dexamethasone suppression test: Baseline cortisol is normal. Dog suppressed marginally at 8 hours. The endocrine data are not supportive of hyper-adrenocorticism.

Summary

This dog has primary hyperlipidemia, which has been shown to be familial in miniature schnauzers (Rogers WA, EF Donovan, GJ Kociba. Idiopathic hyperlipoproteinenia in dogs. J Am Vet Med Assoc 1975;166:1087–1091), and pancreatitis with secondary diabetes mellitus. Dogs with hyperlipidemia are predisposed to development of pancreatitis. While diabetes mellitus may be transitory, treatment is indicated. Some abnormalities (hyperglycemia, stress leukogram, increased alkaline phosphatase activity, lipemia, history, and physical appearance) were suggestive of hyperadrenocorticism. This possibility was ruled out by the ACTH stimulation and LDDS test. Imaging revealed evidence of swelling in the area of the pancreas.

Case 52

Signalment: 9-year-old SF canine, miniature schnauzer
History: Not eating, vomited a few times
Physical examination: Tense abdomen

Hematology		Reference Interval
PCV (%)	**32.0**	37–55
MCV (fL)	68.0	60–72
NCC (×10³/μL)	**5.2**	6–17
Segs (×10³/μL)	**2.7**	3–11.5
Bands (×10³/μL)	**1.4**	0–0.3
Monos (×10³/μL)	0.2	0.1–1.3
Lymphs (×10³/μL)	**0.6**	1–4.8
Basophils (×10³/μL)	0.1	rare
Platelets (×10³/μL)	**111**	200–500
Hemopathology: marked toxic neutrophils, glant platelets, hemolyzed and lipemic		

Biochemical Profile		Reference Interval
Gluc (mg/dL)	**226 *(12.4)***	65–122 *(3.5–6.7 mmol/L)*
BUN (mg/dL)	20	7–28
Creat (mg/dL)	1.2	0.9–1.7
Ca (mg/dL)	**8.2 *(2.0)***	9.0–11.2 *(2.2–2.8 mmol/L)*
Phos (mg/dL)	5.1	2.8–6.1
TP (g/dL)	**5.0**	5.4–7.4
Alb (g/dL)	**1.8**	2.7–4.5
Glob (g/dL)	3.2	1.9–3.4
T. Bili (mg/dL)	**1.4 *(23.9)***	0–0.4 *(0.6–8.4 μmol/L)*
Chol (mg/dL)	**666 *(17.3)***	130–370 *(3.4–9.6 mmol/L)*
ALT (IU/L)	33	10–120
AST (IU/L)	51	16–40
ALP (IU/L)	**1282**	35–280
GGT (IU/L)	5	0–6
Na (mEq/L)	152	145–158
K (mEq/L)	**3.7**	4.1–5.5
CL (mEq/L)	116	106–127
TCO_2 (mEq/L)	14	14–27
An. gap (mEq/L)	25	8–25
Amylase (IU/L)	**2421**	50–1250
Lipase (IU/L)	**2256**	30–560
Triglycerides (mg/dL)	**2884**	ND*
*Not Determined		

Urinalysis			
Color	Golden	**Urine Sediment**	
Transparency	Cloudy	WBCs/hpf	2–3
Sp. Gr.	1.034	RBCs/hpf	3–5
Protein	**2+**	Epith cells/hpf	Negative
Gluc	**4+**	Casts/lpf	2
Bilirubin	**3+**	Crystals	Negative
Blood	**2+**	Bacteria	Negative
pH	8.0		
ketones	Negative		

Coagulation Data		Reference Interval
PT (seconds)	9.3	7.5–10.5
aPTT (seconds)	**19.5**	10.5–16.5

Abdominal Fluid Analysis	
Color	Red
Supernatant	Light yellow
Refractometric protein (g/dL)	**7.2**
NCC (×10³/μL)	2.0
Triglyceride (mg/dL)	**257**
Chol (mg/dL)	**728**

Interpretive discussion

Hematology

The PCV is mildly decreased, no polychromasia was noted in the blood film, and the MCV is normal, indicating a mild nonregenerative anemia. Marked lipemia and hemolysis may have resulted in in vitro hemolysis, but this typically does not result in an important decrease in the PCV. There is a neutropenia with increased bands and marked numbers of toxic neutrophils. This suggests consumption as a result of severe inflammatory disease. Lymphopenia indicates a stress component. The thrombocytopenia is discussed with the coagulation data.

Biochemical profile

The serum glucose concentration is moderately increased. In this range, it is possible that this is a stress hyperglycemia, but is more likely due to some metabolic or endocrine abnormality.

The BUN and serum creatinine concentrations are normal. The serum phosphorus is normal, but there is a mild decrease in serum total calcium concentration. Given the degree of hypoalbuminemia, it is wise to attempt to correct the total calcium for the hypoproteinemia. In this case, the corrected value is 9.9 mg/dL (8.2–1.8 + 3.5), which is normal.

The serum cholesterol concentration is markedly increased. While this may be associated with cholestasis, given the degree of increase in cholesterol one should also consider other metabolic abnormalities including hepatic disease, disorders of lipoprotein metabolism, or endocrinopathies. The serum triglyceride concentration is markedly increased, and further supports a diagnosis of a metabolic and/or endocrinologic disorder. Cholestasis is indicated by the increased total bilirubin and ALP activity. The serum ALT, AST, and GGT activities are normal or near normal, reducing the likelyhood of hepatocellular injury,

The serum amylase and lipase activities are significantly increased, and in the absence of azotemia suggest acute pancreatitis. This is a frequent complication of severe prolonged hypertipidemia. The concurrent findings of hyperlipidemia and pancreatitis in a miniature schnauzer should alert one to the potential diagnosis of a primary dyslipidemia.

Coagulation data

The coagulation profile includes a normal PT, but prolonged APTT. While it is more common for the PT to become prolonged first when there is impaired coagulation factor synthesis by the liver, incipient DIC (note the thrombocytopenia) or heparinization of the patient may result in changes in the APTT alone.

Abdominal fluid analysis

Abdominal fluid chemical analysis similarly indicates accumulation of excess lipids in the peritoneal cavity. It is likely that the increased total protein by refractometry is spuriously elevated by this lipid. The cell concentration suggests a modified transudate.

Urinalysis

The urine specific gravity indicates that the kidneys are capable of concentrating, and the number of leukocytes and erythrocytes are not significant. However, there is 2+ proteinuria, some occult blood, and some hyaline and fine granular casts. Thus, there may be mild tubular and/or glomerular disease. In addition, there is significant glucosuria, which is explained by the hyperglycemia, It would be useful to evaluate the UPC in order to determine the magnitude of the proteinuria. Given the hypoalbuminemia and hypercholesterolemia, one should consider, the possibility of nephrotic syndrome; there may be a protein-losing glomerulopathy without azotemia.

Summary

Minature schnauzer hyperlipidemia and acute pancreatitis.

Case 53

Signalment: 11-year-old castrated male cat
History: Polyuria and polydipsia for 2 months, anorexia and lethargy more recently
Physical examination: Presented in lateral recumbency, 10% dehydrated

Hematology		Reference Interval
PCV (%)	40	24–45
Hgb (g/dL)	12.8	8–15
RBC ($\times 10^6/\mu l$)	8.64	5–11
MCV (fL)	46	39–50
MCHC (g/dL)	34	33–37
NCC ($\times 10^3/\mu l$)	18.7	5.5–19.5
Segs ($\times 10^3/\mu l$)	**15.0**	2.5–12.5
Bands ($\times 10^3/\mu l$)	**2.4**	0–0.3
Monos ($\times 10^3/\mu l$)	0.2	0–0.8
Lymphs ($\times 10^3/\mu l$)	**0.9**	1.5–7.0
Eos ($\times 10^3/\mu l$)	0.2	0–1.5
Platelets ($\times 10^3/\mu l$)	375	200–500
TP (P) (g/dL)	**11.7**	6–8
Hemopathology: Slightly toxic neutrophils, many echinocytes.		

Biochemical Profile		Reference Interval
Gluc (mg/dL)	**766 *(42.7)***	67–124 *(3.7–6.8 mmol/L)*
BUN (mg/dL)	**127 *(45.3)***	17–32 *(6.1–11.4 mmol/L)*
Creat (mg/dL)	**6.4 *(566)***	0.9–2.1 *(78–186 µmol/L)*
Ca (mg/dL)	10.1	8.5–11
Phos (mg/dL)	**7.9 *(10.0)***	3.3–7.8 *(1.1–2.5 mmol/L)*
TP (g/dL)	**9.7**	5.9–8.1
Alb (g/dL)	**4.4**	2.3–3.9
Glob (g/dL)	**5.3**	2.9–4.4
T. Bili (mg/dL)	0.3	0–0.3
Chol (mg/dL)	**388 *(10.1)***	60–220 *(1.6–5.7 mmol/L)*
ALT (IU/L)	**124**	30–100
AST (IU/L)	**354**	14–38
ALP (IU/L)	65	6–106
GGT (IU/L)	1	0–1
Na (mEq/L)	**172**	146–160
K (mEq/L)	5.1	3.7–5.4
CL (mEq/L)	**132**	112–129
TCO_2 (mEq/L)	**10.9**	14–23
An. gap (mEq/L)	**34**	10–27
Calc. Osmolarity (mOsm/L)	**417**	290–310

Urinalysis (cystocentesis)			
Color	Yellow	**Urine Sediment**	
Transparency	Cloudy	WBCs/hpf	**6–8**
Sp. Gr.	1.034	RBCs/hpf	2–3
Protein	**2+**	Epith cells/hpf	1–3 transitional
Gluc	**2+**	Casts/lpf	0
Bilirubin	Negative	Crystals	0
Blood	**4+**	Bacteria	0
pH	5.0	Ketones	Negative
		Other	Small amt of fat

Interpretive discussion

Hematology

Leukogram abnormalities include neutrophilia, a left shift, lymphopenia, and slightly toxic neutrophils. This is an inflammatory leukogram indicating a tissue demand for neutrophils. The lymphopenia suggests concurrent increase in corticosteroid concentrations due to stress. Toxic neutrophils indicate a rapid rate of neutrophil production.

Echinocyte formation can be an artifact, but in this case, it may have resulted from the marked hyperosmolality and electrolyte abnormalities. These may have caused movement of water from the cytoplasm of erythrocytes to the plasma with resulting shrinkage and crenation of erythrocytes.

Biochemical profile

The serum glucose concentration is markedly increased. The most likely cause of hyperglycemia of this magnitude is diabetes mellitus. Severe, acute excitement with release of catecholamines can cause marked hyperglycemia in cats, but serum glucose concentration is seldom greater than 400 mg/dL in such cats. This cat is azotemic, and decreased renal excretion of glucose, secondary to decreased glomerular filtration rate, may have augmented the magnitude of the hyperglycemia. Moreover, the cat does not have an excitement leukogram (lymphocytosis).

Both BUN and serum creatinine concentrations are increased. Since the urine specific gravity suggests adequate renal concentrating ability (i.e., the specific gravity is greater than 1.030), this appears to be a prerenal ozotemia. However, the marked hyperproteinemia and hypernatremia suggest severe dehydration, and an even higher urine specific gravity would be expected in this situation. It is, therefore, possible that this cat has some loss of urine concentration ability. Alternatively, osmotic diuresis due to glucosuria may have contributed to the lower than expected urine specific gravity. The hyperphosphatemia is a result of a decreased glomerular filtration rate. Maintenance of normal serum phosphorus

concentrations depends on phosphorus excretion through the kidney.

Hyperproteinemia (both plasma and serum protein) with concurrent hyperalbuminemia and hyperglobulinemai is typical of dehydration. Contraction of plasma water volume results in proportional increases in concentrations of both albumin and globulin. Although other abnormalities can cause hyperglobulinemia, dehydration is the only cause of hyperalbuminemia. Diuresis secondary to glucosuria is common in diabetes mellitus and can result in dehydration.

The serum cholesterol concentration is increased. In this case, this abnormality is probably secondary to diabetes mellitus and related abnormalities in lipid metabolism.

Serum activities of both ALT and AST are increased. The increased serum ALT activity is due to hepatocyte injury and subsequent leakage of this enzyme. This injury was probably caused by fatty change which developed secondary to the metabolic abnormalities of diabetes mellitus. The increased serum AST activity may also be due to leakage of AST from injured hepatocytes, but the higher activity of AST as compared to ALT suggests that there is also an extrahepatic source. This source may be muscle, and may have resulted from muscle injury secondary to hypoperfusion, since the cat is very dehydrated.

Hypernatremia and hyperchloremia are probably due to severe dehydration. Glucosuria causes diuresis resulting in Na and Cl loss through the kidneys in nondehydrated or mildly dehydrated, diabetic animals. This can lead to hyponatremia and hypochloremia. When such animals become severely dehydrated, however, diuresis no longer occurs, and hypernatremia and hyperchloremia develop. These changes, in combination with hyperglycemia and azotemia, result in severe hyperosmolality.

Decreased serum total CO_2 concentration probably represents a primary metabolic acidosis. Serum total CO_2 concentration may also decrease as a compensatory reaction in animals with primary respiratory alkalosis, but in animals with diabetes mellitus, metabolic acidosis is more likely to be the primary alteration. Increased serum concentrations of ketones are a common cause of acidosis in diabetic animals, but the absence of urine ketones suggests that this cat is probably not ketotic. Urine ketone tests that use the nitroprusside reaction do not detect β-hydroxybutyric acid, therefore, the presence of this ketone cannot be ruled out. Increased serum lactate concentration may be contributing to the acidosis in this cat. The cat is markedly dehydrated and is, therefore, probably experiencing tissue hypoxia which may lead to increased lactate production.

The anion gap is increased. In most diabetic animals, increased ketoacid concentration in the blood is the major cause of this abnormality. In this cat, which is apparently not ketotic, increased blood lactate concentration is probably contributing to this gap.

The calculated osmolarity is increased and, in combination with other laboratory changes, suggests this cat has diabetic nonketotic hyperosmolar syndrome (see summary).

Urinalysis

This cat has a proteinuria with a mild pyuria. It is possible that the protein exuded into the urine as part of the inflammatory process; however, the degree of proteinuria appears to be excessive compared to the degree of pyuria. Other causes of proteinuria such as glomerular and tubular disease should be considered in this case. Although glomerular disease has been associated with diabetes mellitus in humans, this has not been documented in animals.

The strongly positive reaction on the chemical test for blood in combination with normal numbers of erythrocytes suggests that the positive reaction is due to either free hemoglobin or myoglobin. It is unlikely that this represents a hematuria with subsequent lysis of erythrocytes since such lysis is unlikely in urine with a high specific gravity. Absence of anemia suggests a significant hemolytic problem is not occurring in this cat. Myoglobinuria is a possible explanation, and severe muscle hynoxia secondary to hypovolemia may have occurred in this cat. However, the serum AST activity, while increased, does not suggest such massive muscle injury.

Glucosuria is a result of the serum glucose concentration exceeding the renal threshold.

Summary

The clinical diagnosis was diabetic nonketotic hyperosmolar syndrome. This syndrome is characterized by marked hyperglycemia (blood glucose concentration >600 mg/dL), hyperosmolarity (>350 mOsm/l), and absence of ketosis in a diabetic animal. Such animals commonly have prerenal or renal azotemia. The hyperosmolarity results in dehydration of neurons and subsequent neurologic signs. This syndrome is associated with a high fatality rate.

After a brief, unsuccessful attempt to decrease serum glucose concentrations with insulin therapy and to improve the cat's electrolyte and fluid balance by administration of fluids, the owner elected euthanasia. Necropsy revealed severs islet cell degeneration and amyloidosis and severe hepatocytic vacuolar degeneration. A few mineralized casts were present in renal tubules, but the kidneys were otherwise normal, and the azotemia was probably prerenal in this case. The cause of the inflammatory leukogram was not determined.

Case 54

Signalment: 10-year-old MC feline DSH
History: Not eating well, lethargic
Physical examination: Slightly dehydrated

Hematology	Day1	Reference Interval
PCV (%)	38.0*	24–45
Hgb (g/dL)	12.8	8–15
RBC ($\times 10^6/\mu L$)	9.25	5–11
MCV (fL)	44.0	39–50
MCHC (g/dL)	35.0	33–37
Retics ($\times 10^3/\mu L$)	80,000	0–60,000
NCC ($\times 10^3/\mu L$)	12.9	5.5–19.5
Segs ($\times 10^3/\mu L$)	12.5	2.5–12.5
Lymphs ($\times 10^3/\mu L$)	**0.3**	1.5–7.0
Platelets ($\times 10^3/\mu L$)	Adequate	200–500
TP (P) (g/dL)	**9.0**	6–8
Hemopathology: giant platelets, slight increase in polychromasia, slightly toxic neutrophils, **2+ Heinz bodies**		
*PCV was 27% on Day 5, and 17% on Day 7		

Biochemical Profile	Day 1	Reference Interval
Gluc (mg/dL)	**328 *(18.0)***	67–124 *(3.7–6.8 mmol/L)*
BUN (mg/dL)	29	17–32
Creat (mg/dL)	1.5	0.9–2.1
Ca (mg/dL)	9.4	8.5–11
Phos (mg/dL)	**1.9 *(0.6)***	3.3–7.8 *(1.1–2.5 mmol/L)*
TP (g/dL)	8.0	5.9–8.1
Alb (g/dL)	**4.3**	2.3–3.9
Glob (g/dL)	3.7	2.9–4.4
T. Bili (mg/dL)	**2.1 *(35.9)***	0–0.3 *(0–5.1 mmol/L)*
Chol (mg/dL)	**512 *(13.3)***	60–220 *(1.6–5.7 mmol/L)*
ALT (IU/L)	**282**	30–100
ALP (IU/L)	99	6–106
Na (mEq/L)	**130**	146–160
K (mEq/L)	**2.2**	3.7–5.4
CL (mEq/L)	**74**	112–129
TCO_2 (mEq/L)	**10.5**	14–23
An. gap (mEq/L)	**47.7**	10–27
Lipase (IU/L)	**161**	3–125

Blood Gas Data (arterial)		Reference Interval
pH	**7.280**	7.33–7.44
PCO_2 (mmHg)	**20.0**	35–42
PO_2 (mmHg)	85.5	73–92
HCO_3 (mEq/L)	**9.2**	16–22
ionized Ca++ (mg/dL)	**4.64**	4.8–5.3

Urinalysis			
Color	Yellow	**Urine Sediment**	
Transparency	Clear	WBCs/hpf	0–1
Sp. Gr.	1.033	RBCs/hpf	0–1
Protein	**1+**	Epith cells/hpf	0–1
Gluc	**4+**	Casts/lpf	**3–4 granular**
Bilirubin	**1+**	Crystals	Negative
Blood	**1+**	Bacteria	Negative
pH	6.0	Other	
Ketones	**3+**		

Interpretive discussion

Hematology

The packed cell volume, hemoglobin, and total RBC count are normal, but given the degree of hemoconcentration represented by the hyperproteinemia, it is possible that the PCV is actually lower. There is a slight increase in polychromasia and mild reticulocytosis. The anemia is rapidly progressive over a 1 week period of time. The presence of 2+ Heinz bodies indicates significant oxidative damage to the red blood cells, and is commonly observed in cats with diabetic ketoacidosis; however, the owner should be questioned as to whether the cat has received acetaminophen or other oxidant drugs or chemicals. Another potential cause of hemolytic anemia in this patient is hypophosphatemia. There is a stress leukogram, as indicated by the high normal neutrophil count and lymphopenia.

Biochemical profile

The serum glucose concentration is moderately increased. While a glucose concentration of this magnitude may be encountered due to extreme excitement (sympathetic activation) or stress (glucocorticoid release), diabetes mellitus is more likely. Evidence against excitement-induced hyperglycemia is the lack of an excitement leukogram (lymphocytosis). The BUN and serum creatinine concentrations are normal.

The serum phosphorus concentration is decreased, and given the degree of hyperglycemia, one should consider diabetic ketoacidosis-induced urinary phosphate loss. The serum total calcium concentration is normal, reducing the possibility of an endocrine abnormality causing the change in serum phosphorus. The serum total protein concentration is at the upper end of the reference interval, and serum albumin is increased, indicating hemoconcentration due to dehydration.

The serum cholesterol concentration is moderately increased. While this may be associated with cholestasis, as indicated by the increased total bilirubin, the ALP activity is normal. Given the degree of increase in cholesterol, one should consider metabolic abnormalities including hepatic disease, disorders of lipoprotein metabolism, or endocrinopathies. If not due to cholestasis, then the increase in bilirubin may be due to hemolysis. The serum ALT activity is increased modestly which indicates hepatocellular damage. ALP is not induced by steroids in cats, thus, hyperadrenocorticism is a possibility. Serum lipase activity is only slightly increased, possibly reducing the probability for concurrent pancreatitis; however, increased lipase activity is not a reliable marker for feline pancreatitis.

Serum Na, K, and Cl concentration are decreased significantly. One should consider typical causes for electrolyte depletion, including pathologic losses from the gastrointestinal and urinary systems, as well as a shift to third space. The marked hyperglycemia should initiate consideration of diabetic ketoacidosis with subsequent urinary electrolyte loss. There is a marked decrease in serum total CO, suggesting metabolic acidosis. The increase in the anion gap is likely due to the presence of ketones, which are unmeasured anions.

Blood gas data

The blood gas panel indicates a metabolic acidosis (decreased pH and HCO_3) with respiratory compensation (decreased pCO_2). Ionized calcium is marginally decreased.

Urinalysis

The urinary specific gravity is normal. However, with marked increases in the concentration of solutes, such as glucose, not pertinent to urinary concentration capacity, one might question the accuracy of this measure, and consider determining urinary osmolality to address urinary concentration capacity specifically. The presence of 1+ protein and coarse granular casts is consistent with renal tubular disease. The absence of more significant proteinuria speaks against the possibility of glomerular protein loss, but a urinary protein : creatinine ratio should be determined to confirm this. In either case, urinary tract inflammation is not a likely cause of the observed changes, as there is only a small amount of occult blood and no pyuria. The presence of significant amounts of glucose and ketones supports a diagnosis of diabetic ketoacidosis. The mild bilirubinuria is a result of the increased serum bilirubin and subsequent renal excretion.

Summary

Diabetic ketoacidosis; Heinz body anemia

Case 55

Signalment Eight-year-old CM Labrador retriever

History Two months of decreased activity, progressing to muscle fasciculations and mild intermittent seizures. Referring DVM prescribed phenobarbital and prednisone 5 days previously.

Physical examination Obese, reluctant to move. Normal body temperature, heart rate, respiratory rate. Normal chest radiographs and abdominal ultrasound.

Hematology		Reference Interval
Packed cell volume (%)	40	37–55
Hemoglobin (g/dL)	14	12–18
RBC ($\times 10^6/\mu L$)	5.75	5.5–8.5
MCV (fL)	69	60–72
MCHC (g/dL)	35	34–38
Total nucleated cell count ($\times 10^3/\mu L$)	14.5	6–17
Segmented neutrophils ($\times 10^3/\mu L$)	**12.5**	3–11.5
Band neutrophils ($\times 10^3/\mu L$)	0	0–0.3
Monocytes ($\times 10^3/\mu L$)	1.3	0.1–1.3
Lymphocytes ($\times 10^3/\mu L$)	**0.7**	1–4.8
Eosinophils ($\times 10^3/\mu L$)	0	0.1–1.2
Platelets ($\times 10^3/\mu L$)	463	200–500
Plasma protein (g/dL)	7.0	6–8

Biochemical Profile		Reference Interval
Glucose (mg/dL)	**24**	65–122
Blood Urea Nitrogen (mg/dL)	16	7–28
Creatinine (mg/dL)	1.2	0.9–1.7
Calcium (mg/dL)	10.5	9.0–11.2
Phosphorus (mg/dL)	4.5	2.8–6.1
Total Protein (g/dL)	6.8	5.4–7.4
Albumin (g/dL)	3.5	2.7–4.5
Globulin (g/dL)	3.3	1.9–3.4
Total Bilirubin (mg/dL)	0.3	0–0.4
Cholesterol (mg/dL)	256	130–370
Alanine aminotransferase (IU/L)	110	10–120
Aspartate aminotransferase (IU/L)	32	16–40
Alkaline phosphatase (IU/L)	**602**	13–141
Gamma glutamyl transferase (IU/L)	**9**	0–6
Sodium (mEq/L)	151	145–158
Potassium (mEq/L)	4.1	4.1–5.5
Chloride (mEq/L)	116	106–127
Total CO_2 (mEq/L)	17	14–27
Anion Gap	22	8–25
Other		
Serum insulin (µU/mL)	**46.2**	5–25

Interpretive discussion

Hematology

The only abnormalities are a mild mature neutrophilia and lymphopenia, consistent with a stress/steroid leukogram. This is not surprising given the history of prednisone administration.

Biochemical profile

Increased ALP and GGT activities are most likely due to steroid induction given the history of prednisone administration. There is no other evidence to support cholestasis or liver disease.

Hypoglycemia is pronounced. Appropriate sample handling procedures were followed, ruling-out artifactual hypoglycemia due to delayed removal of serum from the red cells, and episodic hypoglycemia fits the clinical signs. There is no evidence for sepsis (no inflammatory leukogram) or liver failure (BUN, cholesterol, albumin are normal).

Serum insulin concentration is increased at the same time this dog is hypoglycemic, which is an inappropriate response. Normally, feedback mechanisms result in low serum insulin concentrations when hypoglycemia exists. Uncontrolled insulin production from a neoplasm is most likely. The most common tumor associated with hypoglycemia in dogs is insulinoma, a neoplasm of pancreatic β-cells.

Summary

An exploratory laparotomy was performed and a small pancreatic mass was identified and removed. Small nodules were present in the liver, and regional lymph nodes were enlarged. Aspirates from an enlarged node were taken intraoperatively, and a metastatic endocrine tumor was diagnosed by cytology. Histopathology confirmed a β-cell carcinoma in the pancreas with metastases to liver and lymph node. It is important to measure serum insulin concentrations at the same time the dog is hypoglycemic, preferably when the blood glucose is <50 mg/dL. Under these conditions, a serum insulin concentration that is increased or in the upper half of the reference interval indicates a relative insulin excess, suggesting uncontrolled insulin production.

Case 56

Signalment: 6-day-old female Holstein
History: Scours
Physical examination: Severe dehydration

Hematology		Reference Interval
PCV (%)	**58.0**	24–46
Hgb (g/dL)	**19.0**	8–15
RBC ($\times 10^6/\mu L$)	**17.1**	5.0–10.0
MCV (fL)	**34.0**	37–53
MCHC (g/dL)	33.0	33–38
NCC ($\times 10^3/\mu L$)	5.0	4.0–12.0
Segs ($\times 10^3/\mu L$)	3.2	0.6–4.0
Monos ($\times 10^3/\mu L$)	**1.7**	0–0.8
Lymphs ($\times 10^3/\mu L$)	**0.1**	2.5–7.5
Platelets ($\times 10^3/\mu L$)	288	200-800
Fibrinogen (mg/dL)	600	200–600
TP (P) (g/dL)	**10.9**	6–8
Hemopathology: many acanthocytes and keratocytes, RBC fragments, hypochromic RBCs.		

Biochemical Profile		Reference Interval
Gluc (mg/dL)	**31**	55–95
BUN (mg/dL)	**87**	7–20
Creat (mg/dL)	**4.6**	1.0–1.8
Ca (mg/dL)	**7.8**	8.2–9.9
Phos (mg/dL)	6.9	4.3–7.0
TP (g/dL)	**10.3**	6.3–7.6
Alb (g/dL)	**5.3**	2.5–4.3
Glob (g/dL)	**5.0**	2.6–5.0
T. Bili (mg/dL)	**0.8**	0.1–0.4
CK (IU/L)	**352**	57–280
AST (IU/L)	**286**	40–130
GGT (IU/L)	14	10–26
SDH (IU/L)	17	8–23
Na (mEq/L)	**129**	136–147
K (mEq/L)	**6.7**	3.6–5.2
CL (mEq/L)	**91**	95–105
TCO_2 (mEq/L)	**17.0**	24–32
An. gap (mEq/L)	**27.7**	14–26

Blood Gas Data (venous)		Reference Interval
pH	**7.140**	7.32–7.45
pCO_2 (mmHg)	**45.7**	34–44
HCO_3 (mEq/L)	**15.3**	23–31

Urinalysis			
Color	Yellow	**Urine Sediment**	
Transparency	Clear	WBCs/hpf	0–1
Sp. Gr.	**1.014**	RBCs/hpf	0–1
Protein	Negative	Epith cells/hpf	1–2
Gluc	Negative	Casts/lpf	Negative
Bilirubin	Negative	Crystals	Negative
Blood	Negative	Bacteria	Negative
pH	5.0		

Interpretive discussion

Hematology

There is a monocytosis and a lymphopenia that represent the effects of stress. The plasma protein concentration is increased, most probably due to dehydration. Erythrocyte indices reflect hemoconcentration as well, as evidenced by the increased RBC count, hemoglobin concentration, and PCV. The MCV is decreased, which may be due to an underlying iron-deficiency anemia of the newborn that is obscured by hemoconcentration. The presence of several erythrocyte morphologic abnormalities supports this. Iron deficiency is frequently associated not only with a microcytic anemia, but also with oxidative damage to the erythrocytes, resulting in membrane abnormalities and fragmentation changes.

Biochemical profile

There is a profound hypoglycemia, which in a neonatal calf with diarrhea is most probably related to decreased food intake, as well as the possibility of sepsis. Sepsis is unlikely, considering the normal neutrophil concentration.

The BUN and serum creatinine concentrations are increased, but the origin of this azotemia cannot be discerned from this data alone. Refer to the discussion in the urinalysis section below.

Serum calcum is mildly decreased, possibly due to decreased milk intake. The serum total protein and albumin concentrations are increased, further reflecting hemoconcentration due to dehydration. The serum CK and AST activities are modestly increased, which may be related to muscle damage subsequent to prolonged recumbency or hypoperfusion. The total bilirubin is increased. Together with the increased AST activity, this may indicate hepatocellular damage. Alternatively, there may be cholestasis due to dehydration or prehepatic icterus due to increased destruction of oxidatively-damaged iron-deficient erythrocytes.

The serum sodium and chloride concentrations are decreased, reflecting decreased intake and/or increased loss from the body. *E. coli*-associated diarrhea in neonatal calves commonly results from increased sodium chloride loss induced by the enterotoxin that promotes active secretion into the gut lumen. Increased water loss follows this osmotic gradient. Bicarbonate is also lost in the feces, and hypovolemia may lead to tissue hypoperfusion, lactic acidosis, and decreased bicarbonate concentration as well. Fecal potassium loss is typically increased, but concomitant metabolic acidosis results in exchange of intracellular potassium for extraceliular protons, and a redistributional hyperkalemia.

Blood gas data

There is a combined metabolic (decreased bicarbonate) and respiratory (increased pCO_2) acidosis. The metabolic acidosis results from bicarbonate loss in the diarrhea and from lactic acidosis due to tissue hypoperfusion. The increased anion gap reflects the accumulation of unmeasured anions such as lactate. The mild respiratory acidosis indicates pulmonary dysfunction. Early pneumonia or decreased pulmonary perfusion secondary to dehydration are possible explanations.

Urinalysis

The only significant abnormality is a urine specific gravity of 1.014. Six-day-old calves, unlike neonates of many other species, should have mature capacity to concentrate urine. Dehydration should stimulate antidiuretic hormone release from the hypothalamus, and increased water reclamation by the renal tubules. However, electrolyte loss in this type of hypotonic dehydration often leads to medullary solute depletion and a loss of the renal concentration gradient. Another alternative is that there is renal disease, due to renal hypoperfusion, sepsis, etc., resulting in both azotemia and loss of concentrating ability.

Summary

Secretory diarrhea and hypotonic dehydration in a neonatal calf.

Case 57

Signalment: 9-month-old bull
History: Anorexia, depression
Physical examination: Enlarged abdomen

Hematology		Reference Interval
PCV (%)	**19**	24–46
MCV (fL)	**31**	37–53
NCC ($\times 10^3/\mu L$)	**18.0**	4.0–12.0
Segs ($\times 10^3/\mu L$)	**10.5**	0.6–4.0
Bands ($\times 10^3/\mu L$)	**2.5**	0–0.1
Monos ($\times 10^3/\mu L$)	1.0	0–0.8
Lymphs ($\times 10^3/\mu L$)	3.5	2.5–7.5
Eos ($\times 10^3/\mu L$)	0.5	0–2.4
Platelets ($\times 10^3/\mu L$)	Adequate	200–800
Fibrinogen (mg/dL)	**1000**	200–600
Hemopathology: Numerous schistocytes, keratocytes		

Biochemical Profile		Reference Interval
Gluc (mg/dL)	**618**	55–95
BUN (mg/dL)	**90**	7–20
Creat (mg/dL)	**6.1**	1.0–1.8
Ca (mg/dL)	**7.8**	8.2–9.9
Phos (mg/dL)	**14.1**	4.3–7.0
TP (g/dL)	**10.1**	6.3–7.6
Alb (g/dL)	**4.5**	2.5–4.3
Glob (g/dL)	**5.6**	2.6–5.0
T. Bili (mg/dL)	**0.8**	0.1–0.4
CK (IU/L)	**1100**	57–280
AST (IU/L)	**350**	40–130
Na (mEq/L)	**130**	136–147
K (mEq/L)	**3.1**	3.6–5.2
CL (mEq/L)	**47**	95–105
TCO_2 (mEq/L)	**50**	24–32

Blood Gas Data (Venous)		Reference Interval
HCO_3 (mEq/L)	**49.3**	23–31
pH	7.412	7.32–7.45
pCO_2 (mmHg)	**80**	34–44

Interpretive discussion

Hematology

PCV is decreased, indicating anemia.

MCV is decreased, suggesting iron deficiency anemia secondary to chronic blood loss.

Neutrophilia, increased band neutrophils, and monocytosis are indicative of chronic inflammation.

Increased fibrinogen also suggests inflammation.

Keratocytes and schistocytes are commonly seen with iron deficiency anemia.

Biochemical profile

Glucose is markedly increased, perhaps a sympathoadrenal response that can be seen in severely ill cattle. Other possibilities include prior treatment with glucose-containing fluids, diabetes mellitus, or acute pancreatitis. Other lab data supports proximal duodenal obstruction, in which marked hyperglycemia is a consistent finding. This may be due to a combination of stress-induced hyperglycemia and poor peripheral perfusion, so that the glucose isn't used. Also low K may result in decreased cell uptake of glucose.

BUN, creatinine, and phosphorus are increased. Urine specific gravity would help determine if renal or pre-renal. Because of severe dehydration as indicated by increased albumin, at least a pre-renal component is likely. Phosphorus may also be increased due to high GI obstruction, which is likely the diagnosis.

Calcium is slightly decreased. Phosphorus is excreted in the saliva of ruminants; with GI obstruction, elimination of phosphorus via the GI tract is decreased. Mild hypocalcemia has been reported with abomasal and forestomach disease.

Total protein and albumin are increased, indicating dehydration. Globulin is increased, which may be due to dehydration or antigenic stimulation.

Bilirubin is increased, which in this patient may be due to cholestasis or anorexia.

Serum creatine kinase activity is increased, probably indicative of myopathy. AST is mildly increased, either from myopathy or hepatocellular damage.

Marked hypochloremia is probably due to abomasal acid secretion into the lumen. Obstruction of abomasal out flow and distention exacerbates. Chloride is decreased more than would be expected with abomasal displacement or volvulus; this degree of hypochloremia is indicative of high GI obstruction. Potassium is likely decreased for the same reason.

Sodium is low and is perhaps being lost in urine. This may be due to hyperglycemia resulting in osmotic diuresis and thus increasing urinary losses of electrolytes.

Hyperosmolality may also be contributing to hyponatremia, as a result of cellular water moving into extracellular

fluid compartment, diluting serum sodium (1.6 mEq/L decrease in Na for every 100 mg/dL increase in glucose)

Total CO_2 and HCO_3 are increased, indicating marked hypochloremic metabolic alkalosis. pH is in the high normal range as a result of compensatory respiratory acidosis (increased pCO_2). Remarkable hypochloremia and alkalosis indicated that there is obstruction of abomasal out flow, preventing re-exchange of chloride and bicarbonate.

Increased anion gap (36 mEq/L) also indicates increase in unmeasured anions. Most of the anions contributing to this are not truly "unmeasured," but are the increased phosphates and protein. Additionally, there may be increased lactate due to decreased tissue perfusion, or increased sulfates due to tissue breakdown.

Summary

This animal had a high GI obstruction (foreign body), thus explaining many of the abnormalities.

Azotemia was probably prerenal due to dehydration, although there are abnormalities in distal tubular transport which may be due to hypochloremia; osmotic diuresis may be also contributing to these abnormalities.

Inflammation is present, perhaps associated with the GI obstruction.

Iron deficiency anemia from chronic blood loss is present (perhaps abomasal ulcer, GI parasites).

Other tests that should be performed include urinalysis, especially specific gravity, and fecal occult blood.

Case 58

Signalment: 9-day-old female Holstein
History: Several days duration of diarrhea, anorexia, extreme weakness
Physical examination: Hypothermic, 12% dehydrated

Hematology		Reference Interval
PCV (%)	**51**	24–46
NCC ($\times 10^3/\mu L$)	**19.7**	4.0–12.0
Segs ($\times 10^3/\mu L$)	**11.4**	0.6–4.0
Monos ($\times 10^3/\mu L$)	**2.0**	0–0.8
Lymphs ($\times 10^3/\mu L$)	6.3	2.5–7.5
Platelets ($\times 10^3/\mu L$)	Adequate	200–800

Biochemical Profile		Reference Interval
Gluc (mg/dL)	**46**	55–95
BUN (mg/dL)	**63**	7–20
Great (mg/dL)	**3.7**	1.0–1.8
Ca (mg/dL)	**5.9**	8.2–9.9
Phos (mg/dL)	**14.5**	4.3–7.0
TP (g/dL)	**3.0**	6.3–7.6
Alb (g/dL)	**1.9**	2.5–4.3
Glob (g/dL)	**1.1**	2.6–5.0
T. Bili (mg/dL)	0.2	0.1–0.4
CK (IU/L)	**7819**	57–280
AST (IU/L)	**177**	40–130
GGT (IU/L)	**28**	10–26
Na (mEq/L)	**158**	136–147
K (mEq/L)	**7.9**	3.6–5.2
CL (mEq/L)	**117**	95–105
TCO_2 (mEq/L)	**15**	24–32
An. gap (mEq/L)	**33.9**	14–26

Blood Gas Data (Venous)		Reference Interval
pH	**7.140**	7.32–7.45
pCO_2 (mmHg)	**45.7**	34–44
HCO_3 (mEq/L)	**15.3**	23–31

Interpretive discussion

Hematology

There is a neutrophilia and monocytosis, indicating an inflammatory leukogram. The PCV is increased, reflecting hemoconcentration due to dehydration.

Biochemical profile

There is a profound hypoglycemia, which in a neonatal calf with diarrhea is most probably related to decreased food intake, as well as the possibility of sepsis. Considering the increased neutrophil concentration, sepsis is unlikely.

The BUN and serum creatinine concentrations are increased, but the origin of this azotemia cannot be discerned without a urinalysis. However, given the other evidence of hemoconcentration, prerenal azotemia is the most likely cause. Although higher serum phosphorus concentrations are common in young animals, this degree of hyperphosphatemia is more likely related to decreased glomerular filtration rate. There is a marked hypocalcemia, but this may be due solely to the hypoalbuminemia; i.e., the ionized calcium concentration may be normal, but the protein-bound fraction is decreased.

There is marked hypoproteinemia, despite the severe degree of dehydration. This is due both to hypoalbuminemia and hypoglobulinemia. The former may be due to liver disease, inanition, or intestinal loss associated with the diarrhea. The latter is very likely due to lack of passive transfer, which would have subsequently predisposed this neonate to infections, resulting in diarrhea and sepsis.

The increased serum CK and AST activities may be due to muscle damage, to prolonged recumbency, or hypoperfusion. The very slight increase in GGT activity may be due to absorption of a small amount of colostrum, which is high in GGT activity in ruminants.

The increased serum sodium and chloride indicate that this calf is hypertonically dehydrated. One typically expects hypotonic dehydration to develop in a neonatal calf with scours, owing to electrolyte loss in the secretory diarrhea. Thus, it is more likely that this is not a secretory diarrhea, but rather another infectious cause of diarrhea, with or without septicemia. Water, loss in excess of solute may be compounded by reduced water consumption, increased insensible losses due to fever, and/or exudation (along with albumin) across a damaged intestinal mucosa. Although there may have been significant potassium loss in

the diarrhea, redistributional hyperkalemia is commonly observed in cases like this owing to exchange of intracellular potassium for extracellular protons (H^+) in response to the metabolic acidosis. The respiratory acidosis suggests inadequate pulmonary perfusion.

Blood gas data

There is a combined metabolic (decreased bicarbonate) and respiratory (increased pCO_2) acidosis. The metabolic acidosis results from bicarbonate loss in the diarrhea and from lactic acidosis due to tissue hypoperfusion. The increased anion gap reflects the accumulation of unmeasured anions like lactate.

Summary

Nonsecretory diarrhea and hypertonic dehydration in a neonatal calf following failure of passive transfer.

Case 59

Signalment Six-year-old male castrated male fox terrier.

History Intermittent vomiting and diarrhea for past seven weeks, seizured for a few minutes a few hours before presentation on 8/8.

Physical examination Lethargic, weak, "bloated"

Hematology	8/8	8/17	Reference Interval
Packed cell volume (%)	**28**	**22**	40–55
Hgb (g/dL)	**8.9**	**7.0**	13–20
RBC (106/μL)	**3.81**	**2.95**	5.5–8.5
MCV (fL)	73	73	62–73
MCHC (g/dL)	**32**	**32**	33–36
Total nucleated cell count ($\times10^3$/μL)	**17.3**	**17.9**	4.5–15
Segmented neutrophils ($\times10^3$/μL)	**14.4 (83%)**	**16.3**	2.6–11
Band neutrophils ($\times10^3$/μL)	**0.3** (2%)		0–0.2
Monocytes ($\times10^3$/μL)	0.3 (2%)	1.3	0.2–1.0
Lymphocytes ($\times10^3$/μL)	1.9 (11%)	**0.2**	1–4.8
Eosinophils($\times10^3$/μL)		0.2	0.1–1.2
Platelets ($\times10^3$/ μL)	302	323	200–500
Plasma protein (g/dL)	**2.5**	**2.9**	6–8
Reticulocytes ($\times10^3$)	**80,000** (2.1%)	**209,450** (7.1%)	0–60,000
Hemopathology: slight polychromasia, slt toxic neuts			

Biochemical Profile	slightly hemolyzed		Reference Interval
Glucose (mg/dL)	123	99	75–130
Blood Urea Nitrogen (mg/dL)	8	9	7–28
Creatinine (mg/dL)	**0.5**	**0.4**	0.7–1.9
Calcium (mg/dL)	**4.4**	**5.1**	9.0–11.7
Phosphorus (mg/dL)	**1.7**	**1.5**	2.1–6.0
Magnesium (mg/dL)	**0.9**	**0.7**	1.8–2.5
Total Protein (g/dL)	**2.1**	**2.3**	5.4–7.4
Albumin (g/dL)	**1.2**	**1.2**	2.7–4.5
Globulin (g/dL)	**0.9**	**1.1**	2.0–3.8
Total Bilirubin (mg/dL)	0.1	0.1	0–0.3
Cholesterol (mg/dL)	**69**	**71**	130–300
Alanine aminotransferase (IU/L)	**600**	**274**	10–110
Aspartate aminotransferase (IU/L)	**540**	**163**	16–50
Alkaline phosphatase (IU/L)	**660**	**405**	20–142
Creatine kinase (IU/L)	**1343**	**356**	50–275
GGT (IU/L)	**77**	**108**	0–9
Sodium (mEq/L)	**136**	**140**	142–152
Potassium (mEq/L)	**2.9**	3.6	3.5–5.2
Chloride (mEq/L)	**106**	109	108–120
Bicarbonate (mEq/L)	17.6	20.6	16–25
Anion Gap (mEq/L)	15	14	13–22
Calc. osmolality (mosm/L)	**267**	275	284–304
Iron	100	140	75–280
TIBC	110	153	
Sat percent	91	92	
UIBC	<10	13	
Ionized calcium (mmol/L)	0.96	0.80	1.30–1.46

Coagulation		Reference Interval
Protime (sec)	17.5	7.5–10.5
APTT (sec)	52.9	10.5–16.5
Antithrombin 45% of normal pooled sera		

Interpretive discussion

Hematology

Regenerative anemia is present as evidenced by reticulocytosis. This may be due to blood loss or blood destruction. Hypomagnesemia has been reported to cause hemolytic anemia in humans. The total protein is not useful to differentiate hemolysis from loss in this case, as it is likely decreased due to other causes. Blood loss could be though the GI tract.

An inflammatory leukogram is present as evidenced by neutrophilia and increased bands on 8/8.

A stress leukogram is present on 8/17 as evidenced by mature neutrophilia and lymphopenia. A monocytosis may be a component of a stress leukogram.

Plasma protein by refractometry is markedly decreased (see biochemical profile interpretation).

Biochemical profile

Numerous biochemical profiles were performed over several weeks. The common abnormalities are marked hypoproteinemia, hypoalbuminemia, hypoglobulinemia, and hypocalcemia, Hypocholesterolemia is present in three of the profiles, as well. All but the first biochemical profile also showed increased ALT, AST, ALP, and CK. Sodium and potassium are decreased in the last three profiles, and serum magnesium is markedly decreased. Serum creatinine is also decreased on the last two profiles. These abnormalities are discussed below.

Panhypoproteinemia

The most likely differential for a decrease of this magnitude in both serum albumin and globulin, particularly in a dog with a history of diarrhea, is protein-losing enteropathy. Other possible causes would be blood loss or loss of protein into the abdominal cavity due to inflammation. The PCV is not low enough to explain this degree of hypoproteinemia.

Hypocalcemia

While some of the hypocalcemia can be explained by hypoalbuminemia, this cannot account for this degree of hypocalcemia. When corrected for hypoalbuminemia, the calcium is still low. (For example, in the profile on 8/17, 5.1 $-1.2 + 3.5 = 7.4$.) The decreased ionized calcium further substantiates that both ionized and bound calcium are decreased.

There is probably no differential for this degree of hypocalcemia other than the hypocalcemia that may occur in patients with protein losing enteropathy, since primary hypoparathyroidism should result in increased phosphorus concentration. While hypocalcemia with protein losing enteropathy is usually because of hypoalbuminemia, decreased ionized calcium may also occur in these patients as a result of decreased GI calcium absorbtion and also because of decreased vitamin D absorption. Hypomagnesemia may also result in hypocalcemia by leading to decreased synthesis or release of PTH or by leading to decreased responsiveness to PTH by skeletal and renal tissue. Moreover, hypomagnesemia can cause decreased activation of vitamin D by kidneys.

The hypomagnesemia is severe and in this case is likely due to loss through the GI tract. Decreased vitamin D may result in decreased magnesium absorption.

(Other possible causes of hypomagnesemia include loss through the kidney or shifts of magnesium from extracellular to intracellular, but there is no evidence for renal disease. Other disorders associated with hypomagnesemia include diuresis, diabetic ketoacidosis, pancreatitis, sepsis, and primary hyperparathyroidism, but again, there is no history or evidence of these.)

Hypocholesterolemia is usually due to either decreased intake through the GI tract, or decreased production as a result of liver failure. In this patient, it is likely due to increased intake, secondary to protein losing enteropathy.

Increased ALT and AST in later profiles are indicative of hepatocellular damage, although the AST may be increased due to muscle damage, as CK is increased. ALT may be induced by steroids, as well.

Increased GGT and ALP may be due to cholestasis or induced by corticosteroids. The serum alkaline phosphatase and GGT were not increased in the initial biochemical profile performed by the referring veterinarian. Additional history revealed that the dog had been receiving injectable glucocorticosteroids, which are likely responsible for the increase in these enzymes. The dog likely has a steroid hepatopathy at this time.

Hyponatremia and hypokalemia are also likely due to losses through the GI tract.

The increase in APPT and PT are likely due to decreased vitamin K absorption, as it is also a fat soluble vitamin.

The decreased antithrobin III is likely due to concurrent albumin losses.

Additional tests needed

Parathyroid hormone assay, vitamin D assay.

Summary

The most likely diagnosis in this dog is protein losing enteropathy with resulting severe hypocalcemia and hypomagnesemia, and likely secondary hypoparathyroidism.

Outcome

PTH was low normal and vitamin D was decreased. Small bowel biopsy showed mixed inflammation, dilated crypts, necrosis, bacterial overgrowth, and mild lymphangiectasia. Dog had positive occult blood in feces. It was treated by changing diet (Eukanuba low-residue dry), Prednisone (1.3 mg/kg bid), Tums, and enrofloxacin (Baytril), and coconut oil (10–20 mL per day added to food).

Further reading

Kimmel SE, Waddel LS, Michel KE (2000) Hypomagnesemia and hypocalcemia associated with protein-losing enteropathy in Yorkshire trriers: five cases (1992–1998). *J Am Vet Med Assoc* 217: 703–6.

Bush WW, Kimmel Se, Wosar MA, *et al*. (2001) Secondary hypoparathyroidism attributed to hypomagnesemia in a dog with protein-losing enteropathy. *J Am Vet Med Assoc* 219: 1732–4. Explains in detail how hypomagnesemia results in hypoparathyroidism (decreases cAMP generation and blunts release of PTH) and also results in blunted end organ response to PTH by decreasing phosphoinositide-specific phospholipase C in cell membranes.

Case 60

Signalment Ten-year-old, spayed female, mixed breed dog

History Chronic weight loss, chronic voluminous diarrhea, ravenous appetite

Physical Bright and alert, thin with a 1/5 body condition score, 5% dehydrated.

Hematology		Reference Interval
Packed cell volume (%)	37	36–54
Plasma protein (g/dL)	**5.2**	5.4–7.2

Biochemical Profile		Reference Interval
Glucose (mg/dL)	94	77–126
Blood Urea Nitrogen (mg/dL)	17	5–20
Creatinine (mg/dL)	1.1	0.6–1.6
Calcium (mg/dL)	9.7	9.3–11.6
Phosphorus (mg/dL)	4.1	3.2–8.1
Total Protein (g/dL)	5.3	5.1–7.1
Albumin (g/dL)	3.0	2.9–4.2
Globulin (g/dL)	2.3	2.2–2.9
Total Bilirubin (mg/dL)	0.1	0.1–0.4
Cholesterol (mg/dL)	**49**	80–315
ALT (IU/L)	44	10–55
AST(IU/L)	23	12–40
ALP (IU/L)	66	15–120
Creatine Kinase (IU/L)	81	50–400
Sodium (mEq/L)	145	143–153
Potassium (mEq/L)	4.1	4.1–5.4
Chloride (mEq/L)	112	109–120
TCO_2 (meq/L)	22	16–25
Anion Gap	15	15–25

Other Data		
Bile acids–fasting (µmol/L)	1.0	<15.5
Bile acids–post prandial (µmol/L)	7	5–20
Folate (µg/L)	20.4	7.7–24.4
Cobalamine-B12 (ng/L)	**154**	251–908
Trypsin-Like Immunoreactivity (TLI)–fasting (µg/L)	**0.2**	5.7–45.2
Fecal float	negative	

Interpretive discussion

The decrease in the plasma protein may be compatible with loss of protein or decreased protein production. On the biochemical profile, total protein, albumin and globulins are at the low end of the reference interval. Because the dog is dehydrated, it is possible that these values will decrease below the reference interval when the animal is rehydrated. Given the history of chronic diarrhea and the low body condition score, protein losing enteropathy or exocrine pancreatic insufficiency (EPI) are primary differential diagnoses.

The hypocholesterolemia can be the result of malabsorption, maldigestion, protein losing enteropathy, or liver failure. Given the normal liver enzymes and total bilirubin, there is no evidence of hepatocellular damage or cholestasis. However, liver enzymes do not measure liver function: fasting and postprandial bile acids were done to evaluate for hepatic insufficiency. Given the normal fasting and postprandial bile acids, decreased production of cholesterol secondary to hepatic insufficiency is unlikely.

The low normal total protein, albumin, and globulin in conjunction with hypocholesterolemia is compatible with protein losing enteropathy or EPI resulting in malabsorption and maldigestion, respectively. Both of these conditions are associated with diarrhea and weight loss. Differentiating these two conditions requires additional testing. The normal serum folate in conjunction with decreased cobalamnie-B12 is compatible with distal small intestinal disease or EPI. The low TLI is diagnostic for EPI.

Summary

This dog was diagnosed with exocrine pancreatic insufficiency (EPI), and she responded to dietary supplementation with pancreatic enzymes and cobalamine injections. The syndrome of EPI results from inadequate production and release of pancreatic enzymes into the intestinal tract. Maldigestion and malabsorption of nutrients results in diarrhea that is often voluminous. Steatorrhea and hypocholesterolemia are consequences of maldigestion and malabsorption of fats. Unlike patients with protein losing enteropathy, many patients with EPI maintain their serum protein within the reference interval. Intrinsic factor is required for absorption of dietary cobalamine/vitamin B12. Because the pancreas is the source of intrinsic factor in the dog, cobalamine deficiency may develop as a result of malabsorption secondary to EPI and is reflected in the low serum cobalamine levels seen in this patient. Pancreatic ascinar atrophy is the most common cause of EPI in younger dogs and is seen most commonly in the German shepherd breed. When pancreatic ascinar atrophy occurs in an older dog such as this patient, other causes of EPI such as pancreatitis or neoplasia should be explored.

Case 61

Signalment: 3-year-old castrated male golden retriever
History: Lethargic, heat seeking
Physical examination: Obese, poor hair coat, tailhead alopecia

Hematology		Reference Interval
PCV (%)	**34**	37–55
MCV (fL)	65	60–72
MCHC (g/dL)	35	34–38
Retics ($\times 10^3/\mu L$)	**2**	<60
NCC ($\times 10^3/\mu 1$)	12.5	6–17
Segs ($\times 10^3/\mu 1$)	9.3	3–11.5
Monos ($\times 10^3/\mu 1$)	1.0	0.1–1.3
Lymphs ($\times 10^3/\mu 1$)	2.2	1–4.8
Platelets ($\times 10^3/\mu 1$)	Adequate	200–500
TP (P) (g/dL)	7.5	6–8
Hemopathology: Numerous leptocytes ("target cells") present		

Biochemical Profile		Reference Interval
Gluc (mg/dL)	105	65–122
BUN (mg/dL)	20	7–28
Creat (mg/dL)	1.2	0.9–1.7
Ca (mg/dL)	10.5	9.0–11.2
Phos (mg/dL)	4.0	2.8–6.1
TP (g/dL)	7.0	5.4–7.4
Alb (g/dL)	3.7	2.7–4.5
Glob (g/dL)	3.3	1.9–3.4
T. Bili (mg/dL)	0.2	0–0.4
Chol (mg/dL)	**720 *(18.7)***	130–370 *(3.4–9.6 mmol/L)*
ALT (IU/L)	110	10–120
AST (IU/L)	35	16–40
ALP (IU/L)	220	35–280
Na (mEq/L)	**143**	145–158
K (mEq/L)	4.5	4.1–5.5
CL (mEq/L)	107	106–127
TCO_2 (mEq/L)	20	14–27

Endocrine Data		Reference Interval
TT4 (µg/dL)	1.6	1.4–4.0
Free T4 (ng/dL)	**0.24 *(3.0)***	1.2–3.4 *(15.4–4.8 pmol/L)*
Endogenous TSH (ng/mL) (Immulite)	**0.5**	0.1–0.45

Interpretive discussion

Hematology

A mild nonregenerative, normocytic, normochromic anernia is the only abnormality in the CRC. "Target cells" are common and are not very diagnostically useful. They commonly present in animals with hypercholesterolemia.

Biochemical profile

The only abnormalities present are hypercholesterolemia and mild hyponatremia. Hypercholesterolemia is marked, and in conjunction with the history, physical examination, and mild anemia, is very suggestive of hypothyroidism. Mild hyponatremia has been reported in approximately 30% of dogs with hypothyroidism.

Endocrine data

Total T4 is within the reference interval. However, since many variables affect TT4, and this dog has clinical and laboratory findings that are suggestive of hypothyroidism a free T4 and endogenous TSH are indicated. The decreased FT4 and increased endogenous TSH are diagnostic for hypothyroidism.

Summary

Early primary hypothyroidism.

Case 62

Signalment: 13-year-old MC dog
History: Polyuria, frequent urination with small volumes
Physical examination: Overweight

Hematology		Reference Interval
PCV (%)	**36.0**	37–55
Hgb (g/dL)	13.4	12–18
RBC ($\times 10^6/\mu L$)	**5.26**	5.5–8.5
MCV (fL)	69.0	60–72
MCHC (g/dL)	37.0	34–38
NCC ($\times 10^3/\mu L$)	**18.1**	6–17
Segs ($\times 10^3/\mu L$)	**16.7**	3–11.5
Monos ($\times 10^3/\mu L$)	1.3	0.1–1.3
Lymphs ($\times 10^3/\mu L$)	**0.2**	1.0–4.8
Platelets ($\times 10^3/\mu L$)	452	200–500
TP (P) (g/dL)	**8.2**	6–8
Hemopathology: few Howell-Jolly bodies		

Biochemical Profile		Reference Interval
Gluc (mg/dL)	**806 *(44.3)***	65–122 *(3.5–6.7 mmol/L)*
BUN (mg/dL)	**81 *(28.9)***	7–28 *(2.5–10.0 mmol/L)*
Creat (mg/dL)	1.6	0.9–1.7
Ca (mg/dL)	**8.4 *(2.1)***	9.0–11.2 *(2.25–2.80 mmol/L)*
ionized Ca++ (mg/dL)	**3.56**	4.5–5.6
Phos (mg/dL)	**7.2 *(2.3)***	2.8–6.1 *(0.9–2.0 mmol/L)*
TP (g/dL)	6.0	5.4–7.4
Alb (g/dL)	3.3	2.7–4.5
Glob (g/dL)	2.7	1.9–3.4
T. Bili (mg/dL)	**1.3 *(22.2)***	0–0.4 *(0–6.8 µmol/L)*
Chol (mg/dL)	**467 *(12.1)***	130–370 *(3.4–9.6 mmol/L)*
ALT (IU/L)	**1355**	0–120
AST (IU/L)	**341**	16–40
ALP (IU/L)	**4660**	35–280
GGT (IU/L)	**373**	0–6
CK (IU/L)	**266**	50–250
Na (mEq/L)	**144**	145–158
K (mEq/L)	**3.8**	4.1–5.5
CL (mEq/L)	**98**	106–127
TCO_2 (mEq/L)	18.5	14–27
An. gap (mEq/L)	**31.3**	8–25
Amylase (IU/L)	**1687**	50–1250
Lipase (IU/L)	**3746**	30–560

Urinalysis			
Color	Yellow	**Urine Sediment**	
Transparency	Cloudy	WBCs/hpf	**50–100**
Sp. Gr.	**1.014**	RBCs/hpf	**>100**
Protein	**2+**	Epith cells/hpf	Negative
Gluc	**4+**	Casts/lpf	Negative
Bilirubin	Negative	Crystals	Negative
Blood	**4+**	Bacteria	**3+ rods**
pH	5.0		
ketones	Negative		

Coagulation Data		Reference Interval
PT (seconds)	7.5	7.5–10.5
aPPT (seconds)	**18.2**	10.5–16.5
FDPs (µg/mL)	**1:12**	**<1:10**

Endocrine Data		Reference Interval
Free T4 (ng/dL)	**<0.15**	1.2–3.4
total T4 (µg/dL)	**0.85**	1.5–3.5
endog TSH (ng/mL)	**0.05**	0.1–0.45

Interpretive discussion

Hematology

The PCV and total RBC count are marginally decreased, with no abnormalities in red blood cell size, hemogiobin content, or morphology. One should consider recent blood loss (particularly GI hemorrhage) even though plasma protein concentration is normal. Alternatively, there may be a mild normochromic, normocytic anemia associated with renal failure. The leukocyte count is increased, with a mature neutrophilia and lymphopenia. This is a stress leukogram, and may support the possibility of hyperadrenocorticism as part of the disease process.

Biochemical profile

The serum glucose concentration is markedly increased. This is well beyond the level encountered due to excitement (sympathetic activation) or stress (glucocorticoid release), and should immediately suggest diabetes mellitus.

The BUN is disproportionately increased relative to the mild increase in serum creatinine concentration. The BUN : creatinine ratio is 50.6, which should suggest gastrointestinal hemorrhage, leading to an increase in hepatic urea production. Nevertheless, some degree of azotemia (prerenal, renal, or postrenal) is probably also present (refer to discussion of urinalysis below). The serum phosphorus is moderately increased, and may be associated with the impaired glomerular filtration and the azotemia. Because the serum total calcium concentration is also decreased, one should consider dietary imbalance or renal disease as causes of secondary hyperparathyroidism. See discussion of ionized Ca below.

The serum total protein and albumin concentrations are normal. Unless there is a concomitant cause for hypoproteinemia, the absence of hyperproteinemia decreases the probability for hemoconcentration and prerenal azotemia due to dehydration.

The serum cholesterol is moderately increased. This may be related to cholestasis, as indicated by the moderate increase in serum to biliruhin concentration and serum ALP and GGT activities. However, the degree of increase in cholesterol is sufficient to warrant consideration of abnormalities in lipoprotein metabolism owing to hepatic disease or an endocrine abnormality. Likewise, the degree of increase in ALP and GGT activities suggests other means for their induction beyond cholestasis, such as hyperadrenocorticism. Marked increases in serum ALT and AST activities indicate hepatocellular damage, which may have contributed to the increases in ALP and GGT activities. The serum CK activity is essentially normal, and rules out the potential contribution of muscle damage to serum AST and ALT increases. Hepatic lipidosis associated with diabetes should be considered as a cause of hepatocellular injury and cholestasis.

A marginal increase in serum amylase and marked increase in serum lipase activities may indicate the presence of pancreatitis. However, concurrent azotemia may impair renal extraction of these enzymes from the serum, leading to increases in their activities.

Serum Na, K, and Cl concentrations are decreased. One should consider typical causes for electrolyte depletion, including pathologic losses from the gastrointestinal and urinary systems, as well as a shift to third space. The marked hyperglycemia should initiate consideration of diabetic ketoacidosis with subsequent urinary electrolyte loss. However, although the anion gap is increased, the serum total CO_2 is normal. It is possible that there are concurrent causes for metabolic acidosis (ketoacidosis) and metabolic alkalosis (vomiting and/or gastrointestinal stasis).

Urinalysis

The urinary specific gravity is in the isosthenuric range, despite azotemia and hyperphosphatemia. This may be the result of renal disease, or impaired concentrating ability due to electrolyte depletion and loss of the medullary concentration gradient. There is significant proteinuria, pyuria, hematuria, and bacteriuria which most likely indicate a bacterial infection and inflammatory response in the urinary tract. In the absence of tubular casts or information regarding enzymuria or urinary fractional excretion of electrolytes, it is difficult to specify the anatomic location of this disorder. There is significant glucosuria corresponding to the marked hyperglycemia rioted earlier. The absence of ketones on the dipstick speaks against the possibility of prominent ketoacidosis (and ketonuria) noted above. However, this test does not detectg one of the ketones, β-hydroxybutyric acid. One can anticipate that detectable ketosis would develop if untreated.

Coagulation data

The coagulation profile indicates a slightly prolonged APTT and mildly increased FDP concentration. This may be the result of liver disease (although one may expect a change in PT prior to one in the APTT), or incipient DIC (although platelet concentration is usually decreased with DIC). If liver disease was severe enough to impair coagulation factor synthesis, one would first expect to see hypoalburninemia and/or hypocholesterolemia. It is not possible to draw conclusions with these borderline abnormalities.

Endocrine data

Low free T4, low total T4, and low endogenous TSH are diagnostic for secondary hypothyroidism. Secondary hypothyroidism as a result of decreased endogenous TSH is commonly associated with diabetes mellitus.

Summary

Diabetes mellitus and secondary hypothyroidism.

Case 63

Signalment: 3-year-old MC English springer spaniel
History: Anorexia, occasional vomiting
Physical examination: Lethargic, thin, approximately 8% dehydrated

Hematology		Reference Interval
PCV (%)	**32**	37–55
Hgb (g/dL)	**11.1**	12–18
RBC ($\times 10^6/\mu 1$)	**4.47**	5.5–8.5
MCV (fL)	72	60–72
MCHC (g/dL)	35	34–38
Retics ($\times 10^3/\mu L$)	ND*	<60
NCC ($\times 10^3/\mu 1$)	9.8	6–17
Segs ($\times 10^3/\mu 1$)	5.6	3–11.5
Monos ($\times 10^3/\mu 1$)	0.8	0.1–1.3
Lymphs ($\times 10^3/\mu 1$)	2.2	1.0–4.8
Eos ($\times 10^3/\mu 1$)	1.2	0.1–1.2
Platelets ($\times 10^3/\mu 1$)	Adequate	200–500
TP (P) (g/dL)	**8.5**	6–8

*Not Determined

Biochemical Profile		Reference Interval
Gluc (mg/dL)	83	65–122
BUN (mg/dL)	**47 *(16.8)***	7–28 *(2.5–10.0 mmol/L)*
Creat (mg/dL)	1.6	0.9–1.7
Ca (mg/dL)	**13.8 *(3.45)***	9.0–11.2 *(2.25–2.80 mmol/L)*
Phos (mg/dL)	**6.2 *(2.0)***	2.8–6.1 *(0.9–2.0 mmol/L)*
TP (g/dL)	**7.5**	5.4–7.4
Alb (g/dL)	**5.0**	2.7–4.5
Glob (g/dL)	2.5	1.9–3.4
T. Bili (mg/dL)	0.2	0–0.4
Chol (mg/dL)	135	130–370
ALT (IU/L)	49	10–120
AST (IU/L)	19	16–40
ALP (IU/L)	98	35–280
Na (mEq/L)	**132**	145–158
K (mEq/L)	5.5	4.1–5.5
CL (mEq/L)	**97**	106–127
TCO_2 (mEq/L)	**10**	14–27
An. gap (mEq/L)	**30**	8–25
Amylase (IU/L)	1300	50–1250
Lipase (IU/L)	570	30–560

Endocrine Data		Reference Interval
ACTH stimulation		
serum cortisol (µg/dL)(pre)	**<0.1 *(<2.8)***	1–4 *(28–100 nmol/L)*
serum cortisol (µg/dL) (post)	**<0.1 *(<2.8)***	<10.5 *(<290 nmol/L)*

Urinalysis	
Urine specific gravity	1.020

Interpretive discussion

Hematology

A mild anemia is present. Reticulocyte concentration was not determined, thus the degree of regeneration is unknown. Increased polychromasia is not mentioned, suggesting that the anemia is nonregenerative; however, the MCV is at the upper end of the reference interval, suggesting the presence of large immature erythrocytes. Considering the degree of dehydration, anemia is likely more severe than is apparent.

While the leukogram is normal, a patient that is ill and vomiting would be expected to have a stress leukogram. The absence of a stress leukogram should prompt consideration of hypoadrenocorticism.

Plasma protein is increased, probably as a result of dehydration.

Biochemical profile

Azotemia is evidenced by increased BUN, creatinine, and phosphorus concentrations. While azotemia may be prerenal, since the dog is dehydrated, one would expect the urine specific gravity to be greater than 1.030, if this were the case. However, the serum sodium concentration is decreased, and ability to concentrate is impaired by medullary washout of sodium. Refer to the discussion on sodium and potassium for further interpretation.

Hypercalcemia, in light of hyponatremia and hyperkalemia, is likely due to hypoadrenocorticism. The pathophysiology may be related to decreased glucocorticoids and subsequent increased GI calcium uptake, calcium retention by the kidney, as related to sodium loss, or increased albumin-bound calcium. Other causes of hypercalcemia, such as hypercalcemia of malignancy, primary hyperparathyroidism, and vitamin D toxicosis are much less likely in this patient.

Mild hyperproteinemia, due to hyperalbuminemia, is due to dehydration.

Hyponatremia and high normal potassium should cause suspicion of Addison's disease. While these electrolyte abnormalities are not marked, and result in a Na:K ratio of 24, they should prompt an ACTH stimulation test. Hyponatremia and hyperkalemia in this patient, on the other hand, could be a result of renal disease. Hypochloridemia is consistent with hyponatremia. Low total CO_2 is consistent with metabolic acidosis, and the anion gap is increased due to increased unmeasured anions, which in this dehydrated hypovolemic patient are probably lactic acids.

Mild increase in serum amylase and lipase activities are probably secondary to decreased glomerular filtration.

Endocrine data

The immeasurably low cortisol concentration with a "flat-line" response to ACTH confirms hypoadrenocorticism

Summary

Hypoadrenocorticism

Case 64

Signalment: 8-month-old intact male dog
History: Suddenly collapsed during grooming; bloody diarrhea
Physical examination: Extreme weakness, bradycardia, and cool extremities

Hematology		Reference Interval
PCV (%)	42	37–55
Hgb (g/dL)	13.3	12–18
RBC ($\times 10^6/\mu L$)	6.6	5.5–8.5
MCV (fL)	64	60–72
MCHC (g/dL)	32	34–38
NCC ($\times 10^3/\mu L$)	12.0	6–17
Segs ($\times 10^3/\mu L$)	7.2	3–11.5
Monos ($\times 10^3/\mu L$)	0.6	0.1–0.3
Lymphs ($\times 10^3/\mu L$)	3.6	1–4.8
Eos ($\times 10^3/\mu L$)	0.6	0.1–1.2
Platelets ($\times 10^3/\mu L$)	410	200–500
TP (P) (g/dL)	6.9	6–8
Hemopathology: Normal		

Biochemical Profile		Reference Interval
Gluc (mg/dL)	87	65–122
BUN (mg/dL)	**63 *(22.5)***	7–28 *(2.5–10.0 mmol/L)*
Creat (mg/dL)	1.6	0.9–1.7
Ca (mg/dL)	10.3	9.0–11.2
Phos (mg/dL)	5.6	2.8–6.1
TP (g/dL)	6.8	5.7–7.4
Alb (g/dL)	3.9	2.7–4.5
Glob (g/dL)	2.9	1.9–3.4
T. Bili (mg/dL)	0.3	0–0.4
Chol (mg/dL)	230	130–370
ALT (IU/L)	80	10–120
AST (IU/L)	32	16–40
ALP (IU/L)	90	35–280
Na (mEq/L)	**127**	145–158
K (mEq/L)	**7.5**	4.1–5.5
CL (mEq/L)	**99**	106–127
TCO_2 (mEq/L)	**12**	14–27
An. gap (mEq/L)	2.3	8–25

Urinalysis			
Color	Yellow	**Urine Sediment**	
Transparency	Cloudy	WBCs/hpf	0–1
Sp. Gr.	**1.019**	RBCs/hpf	2–3
Protein	Negative	Epith cells/hpf	1–2 transitional
Gluc	Negative	Casts/lpf	0
Bilirubin	Negative	Crystals	0
Blood	Negative	Bacteria	0
pH	6.0		

Endocrine Data		Reference Interval
ACTH stimulation:		
serum cortisol (µg/dL)(pre)	1.1	1–4
serum cortisol (µg/dL)(post)	**1.3 *(36)***	10–20 *(276–552 nmol/L)*

Interpretive discussion

Hematology

The CBC reveals no significant abnormalities.

Biochemical profile

This dog is azotemic. Since urine concentration is not adequate (i.e., it is not >1.030), this may be a renal azotemia, but prerenal azotemia with inadequate renal concentrating ability may occur in hypoadrenocorticism. The hypotension and dehydration that accompany hypoadrenocortism may result in azoternia while hyponatremia and solute diuresis may result in medullary washout which, in turn, limits renal concentrating ability. The result is azotemia with a urine specific gravity indicating inadequate renal concentrating ability.

Hyponatremia and hyperkalemia, in combination with the abnormal response to ACTH stimulation, confirms the diagnosis of hypoadrenocorticism (see discussion of the ACTH stimulation test below). While a Na:K ratio <23:1 is suggestive of hypoadrenocorticism, hyponatremia and hyperkalemia are not specific for this disease. Oliguric or anuric renal failure are common causes of hyponatremia and hyperkalemia and should be considered when these abnormalities are observed, but the response to ACTH stimulation should he adequate to distinguish these diseases.

Hypochloremia is common in animals with hypoadrenocorticism. In renal tubules, Cl is reabsorbed with Na in both the proximal tubule and the loop of Henle. After hyponatremia develops, the concentration of Na in the ultrafiltrate is decreased, and this, in turn, decreases the amount of Na available for reabsorption in these portions of the nephron. The decreased Na absorption results in decreased Cl absorption and hypochloremia.

Decreased serum total CO_2 concentration suggests metabolic acidosis. Metabolic acidosis is common in hypoadrenocorticism and results from decreased tissue perfusion secondary to hypotension and from decreased renal tubular excretion of H+ secondary to mineralocorticoid deficiency.

Urinalysis

Except for the evidence of inadequate urine concentrating ability (see the discussion of azotemia above), the urinalysis is normal.

Endocrine data

The inadequate response to ACTH stimulation in combination with hyponatremia and hyperkalemia confirms hypoadrenocorticism. Dogs with hypoadrenocorticism commonly have decreased basal plasma cortisol concentrations which fail to increase or increase only slightly after ACTH stimulation. If these values do increase after ACTH stimulation, they are usually well below normal post-ACTH stimulation values, especially in dogs with primary hypoadrenocorticism.

Summary

Hyponatremia, hyperkalemia, and a Na:K ratio of 17:1 strongly suggest hypoadrenocorticism. An inadequate response to ACTH stimulation confirms this disease. The azotemia with evidence of inadequate urine concentrating ability, while typical of primary renal failure, is more likely due to a combination of prerenal azotemia and decreased renal concentrating ability resulting from the effects of mineralocorticoid deficiency. It is typical of ill animals to have a stress leukogram (Iymphopenia); absence of stress leukogram in this ill animal is compatible with hypoadrenocorticism.

Case 65

Signalment: 6-year-old M canine
History: Lethargic, quit eating
Physical examination: Depressed, weak pulse, apparent weakness

Hematology		Reference Interval
PCV (%)	46.0	37–55
Hgb (g/dL)	16.2	12–18
PBC ($\times 10^6/\mu L$)	7.10	5.5–8.5
MCV (f1)	65.0	60–72
MCHC (g/d1)	35.0	34–38
NCC ($\times 10^3/\mu L$)	**20.4**	6–17
Segs ($\times 10^3/\mu L$)	11.4	3–11.5
Monos ($\times 10^3/\mu L$)	**1.8**	0.1–1.3
Lymphs ($\times 10^3/\mu L$)	**5.5**	1–4.8
Eos ($\times 10^3/\mu L$)	**1.6**	0.1–1.2
Platelets ($\times 10^3/\mu L$)	574	200–500
TP (P) (g/dL)	**5.9**	6–8

Biochemical Profile		Reference Interval
Gluc (mg/dL)	**79**	65–122
BUN (mg/dL)	**95 *(33.9)***	7–28 *(2.5–10.0 mmol/L)*
Creat (mg/dL)	**3.8 *(334)***	0.9–1.7 *(80–150 µmol/L)*
Ca (mg/dL)	**14.3 *(3.57)***	9.0–11.2 *(2.25–2.80 mmol/L)*
Phos (mg/dL)	**9.9 *(3.2)***	*2.8–6.1 (0.9–2.0 mmol/L)*
TP (g/dL)	5.8	5.4–7.4
Alb (g/dL)	3.0	2.7–4.5
Glob (g/dL)	2.8	1.9–3.4
T. Bili (mg/dL	0.3	0–0.4
Chol (mg/dL)	130	130–370
ALT (IU/L)	62	10–120
AST (IU/L)	**108**	16–40
ALP (IU/L)	38	35–280
GGT (IU/L)	3	0–6
Na (mEq/L)	**124**	145–158
K (mEq/L)	**7.1**	4.1–5.5
CL (mEq/L)	**8.9**	106–127
TCO_2 (mEq/L)	**10.1**	14–27
An. gap (mEq/L)	**32**	8–25
Amylase (IU/L)	**1490**	50–1250
Lipase (IU/L)	130	30–560

Blood Gas Data (arterial)		Reference Interval
pH	**7.213**	7.33–7.45
PO_2 (mmHg)	101.0	67–92
PCO_2 (mmHg)	27.6	24–39
HCO_3(meq/L)	**10.4**	14–24
ionized Ca++ (mg/dL)	**6.40**	4.5–5.6

Urinalysis			
Color	Yellow	**Urine Sediment**	
Transparency	Cloudy	WBCs/hpf	1–4
Sp. Gr.	**1.018**	RBCs/hpf	1–2
Protein	Negative	Epith cells/hpf	1–2
Gluc	Negative	Casts/lpf	Negative
Bilirubin	Trace	Crystals	Negative
Blood	Negative	Bacteria	Negative
pH	6.0	Other	
UPC	0.93		

Endocrine Data		Reference Interval
ACTH stimulation:		
serum cortisol (µg/dL)(pre)	**0.04 *(1.1)***	1–4 *(28–110 nmol/L)*
Serum cortisol (µg/dL)(post)	**0.09 *(2.5)***	<20 *(<552 nmol/L)*

CASES

Interpretive discussion

Hematology

There are no erythrocyte abnormalities. There is a lymphocytosis, which should prompt brief consideration of lymphoma (note the hypercalcemia), or which could be explained by a corticosteroid deficiency. Whenever an ill animal does not have a stress leukogram, one should consider the possibility of hypoadrenocorticism.

Biochemical profile

The BUN, serum creatinine, and phosphorus concentrations are moderately increased. These findings indicate decreased glomerular filtration rate. However, one cannot differentiate the nature of the azotemia (prerenal, renal, or postrenal) based on these findings alone. Refer to the discussion of urinalysis results for further interpretation.

The serum total calcium concentration is moderately increased. The most common causes for this would be malignancy-associated hypercalcemia, hypoadrenocorticism, or renal failure. One might also consider primary hyperparathyroidism and vitamin D toxicosis.

The serum total protein, albumin, and globulin concentrations are normal. The absence of hemoconcentration decreases the probability for prerenal azotemia associated with dehydration.

There are no significant changes in indices of liver disease, with the exception of a mild increase in serum AST activity. This may be due to mild hepatocellular damage or muscle damage, but is small enough that further consideration may not be necessary.

There are significant decrease in the serum concentrations of Na and Cl, as well as a significant increase in serum K concentration. The Na:K ratio is 17.5, which is strongly suggestive of hypoadrenocorticism. The presence of a metabolic acidosis (low total CO_2) is consistent with that possibility, and the anion gap may be increased owing to accumulation of unmeasured anions such as lactic acids or phosphates.

Blood gas data

The blood gas data indicate an uncompensated metabolic acidosis (decreased pH and HCO_3, normal pCO_2). The ionized calcium concentration is increased, further supporting a finding of hypercalcemia. One should consider the possibilities of either primary hypoadrenocorticism or renal disease resulting in a functional deficit in response to corticosteroids and calcium retention.

Urinalysis

The urinary specific gravity revels only marginal concentrating ability, which may result from either renal disease, or loss of the medullary concentration gradient due to electrolyte depletion. This is a common finding in hypoadrenocorticism that should prompt further diagnostics to rule out primary renal disease. The absence of nonregenerative anemia is evidence counter to chronic renal disease. The dipstick protein was negative, and the UPC is <1.0, supporting no significant urinary protein loss.

Endocrine data

The pre and post ACTH cortisol concentrations are both low, and there is an inadequate response. This confirms hypoadrenocorticism.

Summary

Hypoadrenocorticism with typical azotemia secondary to hypovolemia. While there is no biochemical evidence of hemoconcentration, hypovolemia is a consistent event in the pathogenesis of azotemia associated with hypoadrenocorticism.

Case 66

Signalment Six-month-old FS Norwegian elkhound
History Poor appetite, small, and has not grown well
Physical examination Quiet, unhappy thin dog

Hematology		Reference Interval
PCV (%)	35.0	34–55
Hgb (g/dL)	11.8	11–18
RBC (10^6/μL)	5.6	5.5–8.5
MCV (fL)	63	60–72
MCHC (g/dL)	34	34–38
NCC (×10^3/μL)	7.7	6–17
Segs (×10^3/μL)	3.6	3–11.5
Bands (×10^3/μL)	0	0–0.3
Monos (×10^3/μL)	1.2	0.1–1.3
Lymphs (×10^3/μL)	2.4	1–4.8
Eos (×10^3/μL)	0.5	0.1–1.2
Platelets (×10^3/ μL)	299	200–500
TP (P) (g/dL)	**8.5**	6–8

Biochemical Profile		Reference Interval
Gluc (mg/dL)	67	65–122
BUN (mg/dL)	**54**	7–28
Creat (mg/dL)	0.9	0.9–1.7
Ca (mg/dL)	**12.7**	9.0–11.2
Phos (mg/dL)	**10.2**	2.8–6.1
TP (g/dL)	**7.8**	5.4–7.4
Alb (g/dL)	**4.9**	2.7–4.5
Glob (g/dL)	2.9	1.9–3.4
T. Bili (mg/dL)	0.2	0–0.4
Chol (mg/dL)	211	130–370
ALT (IU/L)	92	10–120
AST (IU/L)	22	16–40
ALP (IU/L)	155	35–280
Na (mEq/L)	**130**	145–158
K (mEq/L)	**7.7**	4.1–5.5
CL (mEq/L)	**98**	106–127
Na:K ratio	**17**	>25
TCO_2 (mEq/L)	**11**	14–27
An. gap (mEq/L)	**28.7**	8–25
Lipase (IU/L)	175	<500
Amylase	**1895**	220–800

Urine Analysis	
Urine s.g. voided	**1.022**

Endocrine tests requested		Reference Interval
ACTH stimulation		
serum cortisol (μg/dL)(Pre–basal)	0.4	1–4.5
serum cortisol (μg/dL)(Post)	0.5	5.5–20

Interpretive discussion

Hematology

The PCV, hemoglobin, and total RBC count are within reference limits but plasma protein is increased indicating dehydration. If the animal is dehydrated, it is likely that it is mildly anemic. There are no abnormalities in leukogram. Ill animals usually have a stress leukogram.

Biochemical profile

The most significant abnormalities are Na, K, Na:K ratio, and Cl. There are three possible differential diagnoses. The most likely differentials are Addison's disease or chronic renal failure, possibly with a ruptured urinary bladder third. Chronic renal failure is not as probable considering that the creatinine is normal and the animal is capable of concentrating urine. The BUN:Ct ratio is 50, therefore one should suspect dehydration or GI bleeding. Dehydration is determined to be present by increases in both albumin and total serum and plasma protein and can be further confirmed by physical examination. Creatinine will increase in dehydration due to decreased excretion; however, the BUN increases earlier because there will be decreased excretion and increased reabsorption from tubules. The slow transit time of glomerular filtrate through the tubules due to dehydration allows for increased reabsorption of BUN, hence it increases more than creatinine. The urine specific gravity would be expected to be >1.035 if the dog is dehydrated and has normal renal function. The inability to concentrate beyond 1.020 is likely attributable to medullary washout due to the low sodium. Ruptured urinary bladder is unlikely since the dog is urinating and there is no history of trauma. To confirm Addison's disease one should perform an ACTH stimulation test following the determination of baseline cortisol concentrations.

Further support for Addisonian is: hypercalcemia. Hypercalcemia is seen in one third of dogs with hypoadrenocoriticism. However, hypercalcemia can also be seen in a small percentage of dogs with renal failure. It would not be expected in a dog with ruptured bladder. The presence of hypercalcemia in this dog helps prioritize Addison's before renal failure, ruptured urinary bladder and other differentials. Hyperphosphatemia is attributed to decreased glomerular filtration rate due to dehydration in this case.

The serum glucose concentration is at the low end of the reference interval. Hypoglycemia is sometimes seen in patients with hypoadrenocorticism, likely due to a lack of glucocorticoids as well as mineralocorticoids.

The decreased bicarbonate (TCO_2) is indicative of a metabolic acidosis. Amylase is increased and this is attributed due to decreased glomerular filtration rate. Amylase and lipase are excreted through the urine and any cause of decreased GFR may result in one or both enzymes being increased.

Urine specific gravity of 1.022 in a dog with dehydration indicates inadequate concentrating ability which could be due to renal disease or medullary washout of sodium. The latter is more likely as azotemia is considered to be prerenal and medullary washout fits with Addison's and chronic hyponatremia. The two most important solutes that produce a concentration gradient in the medullary interstitium are UN and sodium. The decreased sodium in the medullary interstitium means glomerular filtrate (forming urine) can only be partially concentrated.

Special testing

ACTH stimulation confirmed hypoadrenocorticism. The basal sample is less than 1 µg/dL which strongly implicates Addison's especially given a Na:K ratio of 17. The post sample of 0.5 is not an increase over basal and is therefore flatline which confirms hypoadrenocorticism in this dog.

Summary

Hypoadrenocorticism (Addison's disease), with prerenal azotemia and probable medullary washout.

The most likely lesion is lymphyocytic adenalitis which destroys all three cortical zones of both adrenal glands. Regeneration will not occur therefore recommend treatment with glucocorticoids and mineralocorticoids for life. Dog was treated successfully, gained weight, and lived for 7 years.

Case 67

Signalment: 11-year-old spayed female beagle
History: Polyuria, polydipsia, polyphagia, and bilateral symmetrical alopecia for 5 months
Physical examination: "Pot-bellied," comedones in inguinal region, panting

Hematology		Reference Interval
PCV (%)	50	37–55
NCC ($\times 10^3/\mu l$)	**22.6**	6–17
Segs ($\times 10^3/\mu l$)	**20.0**	3–11.5
Monos ($\times 10^3/\mu l$)	**2.3**	0.1–1.3
Lymphs ($\times 10^3/\mu l$)	**0**	1–4.8
Eos ($\times 10^3/\mu l$)	0	0.1–1.2
NRBC ($\times 10^3/\mu l$)	**0.3**	0
Platelets ($\times 10^3/\mu l$)	Adequate	200–500
TP (P) (g/dL)	7.6	6–8

Biochemical Profile		Reference Interval
Gluc (mg/dL)	**140** ***(7.7)***	65–122 *(3.5–6.7 mmol/L)*
BUN (mg/dL)	**6** ***(2.1)***	7–28 *(2.5–10.0 mmol/L)*
Creat (mg/dL)	1.0	0.9–1.7
Ca (mg/dL)	10.2	9.0–11.2
Phos (mg/dL)	**2.7** ***(0.9)***	2.8–6.1 *(0.9–2.0)*
TP (g/dL)	7.2	5.4–7.4
Alb (g/dL)	4.1	2.7–4.5
Glob (g/dL)	3.1	1.9–3.4
T. Bili (mg/dL)	0.2	0–0.4
Chol (mg/dL)	**460** ***(12.0)***	130–370 *(3.4–9.6)*
ALT (IU/L)	**400**	10–120
ALP (IU/L)	**4500**	35–280
Na (mEq/L)	**159**	145–158
K (mEq/L)	**3.9**	4.1–5.5
CL (mEq/L)	127	106–127
TCO_2 (mEq/L)	20	14–27
An. gap (mEq/L)	16	8–25

Urinalysis	
Specific Gravity	**1.005**

Endocrine Data		Reference Interval
ACTH stimulation		
serum cortisol (µg/dL)(pre)	**12** ***(331)***	1–4 *(28–110)*
serum cortisol (µg/dL)(post)	15.5	<20
Low dose dexamethasone suppression test		
serum cortisol (µg/dL)(Pre)	**9.0** ***(248)***	1–4 *(28–110)*
serum cortisol (µg/dL)(3 hour post)	**8.0** ***(221)***	<1.5 *(41)*
serum cortisol (µg/dL)(8 hour post)	**6.0** ***(166)***	<1.5 *(41)*
High dose dexamethasone suppression test		
serum cortisol (µg/dL)(Pre)	**10** ***(276)***	1–4 *(28–110)*
serum cortisol (µg/dL)(post)	**8** ***(221)***	<1.5 *(41)*
endogenous ACTH (pg/mL)	**10** ***(2.2)***	20–100 *(4.4–22.0)*

Interpretive discussion

Hematology

Mature neutrophilia, monocytosis, and lymphopenia are typically seen with increased endogenous or exogenous corticosteroids. Increased concentration of nucleated RBCs are seen with a variety of conditions; in this case they are likely secondary to hyperadrenocorticism.

Biochemical profile

Mild hyperglycemia is consistent with increased endogenous or exogenous corticosteroids. Glucocorticoids increase gluconeogenesis and decrease peripheral utilization of glucose by antagonizing the effects of insulin.

The BUN concentration is below the reference interval. While decreased BUN may be associated with liver failure or inadequate protein intake, diuresis will also result in increased urinary loss of urea nitrogen. In this case, diuresis is probably stimulated by glucocorticoids.

Hypercholesterolemia is associated with numerous conditions, including hypothyroidism, diabetes mellitus, hyperadrenocorticism, and cholestasis. In this patient, the increase is probably due to hyperadrenocorticism.

Alanine aminotransferase activity is mildly increased, indicating glucocorticoid-induced increase in ALT production or hepatocellular damage. Hepatocellular damage is an important feature of steroid hepatopathy, which may be occurring in this dog. Alkaline phosphatase activity is markedly increased. While cholestasis may result in an increase of this magnitude, bilirubin is not increased, suggesting that the increase is likely due to corticosteroid induction of alkaline phosphatase. Activities of this magnitude are almost always related to steroid effect. Determination of steroid-induced alkaline phosphatase isoenzyme would be helpful.

Mild hypernatremia and hypokalemia are commonly seen in approximately 50% of dogs with hyperadrenocorticism.

Urinalysis

Urine specific gravity is low, and is likely due to hyperadrenocorticism. Glucocorticoids are thought to interfere with ADH receptors, resulting in isosthenuria or hyposthenuria, and polyuria and polydipsia.

Endocrine data

ACTH stimulation: The baseline cortisol concentration is well above normal and the post-stimulation cortisol concentration is within the reference interval. While most dogs with hyperadrenocorticism have normal basal cortisol concentrations, this increase is very suggestive of hyperadrenocorticism. While dogs with pituitary dependent hyperplasia (PDH) have hyperplastic adrenals and dogs with functional adrenocortical tumors have the potential to respond to ACTH stimulation by increasing cortisol production and release, not all do so. Cortisol increases above the reference interval following ACTH stimulation in approximately 85% of dogs with pituitary dependent disease, and in approximately 50% of dogs with adrenal tumors. In summary, while ACTH stimulation is a useful screening test for PDH and adrenal tumors, cortisol concentrations do not exceed the high end of the reference interval in many dogs. Thus, this dog may have pituitary dependent disease or an adrenal tumor, based on the ACTH stimulation results.

Low and High Dose Dexamethasone Suppression: Dexamethasone screening tests are diagnostically useful because in patients with pituitary dependent disease, the abnormal pituitary is somewhat resistant to the negative feedback action of cortisol. Moreover, while dexamethasone may inhibit endogenous ACTH production in dogs with adrenal tumors, endogenous ACTH production is probably already maximally suppressed, and at any rate, these tumors usually autonomously secrete cortisol, independent of ACTH. In normal dogs, endogenous ACTH is suppressed by dexamethasone, resulting in a rapid decline in plasma cortisol concentrations which remain suppressed for up to 48 hours. Thus, since this dog's cortisol concentration did not decrease, either pituitary dependent disease resulting in adrenocortical hyperplasia, or adrenal neoplasia, is present.

Endogenous ACTH: Endogenous ACTH is below the reference interval in this dog, indicating that the dog has a functional adrenal tumor, rather than pituitary disease.

Summary

Hyperadrenocorticism due to functional adrenal tumor. On abdominal radiographs, a calcified mass cranial to the right kidney was observed. On ultrasound examination, a large right adrenal mass was seen. The left adrenal was not detectable. A CT scan of the brain was normal.

Case 68

Signalment: 4-year-old MC golden retriever
History: Polyuria, polydipsia for several months, on medication for flea allergy dermatitis
Physical examination: Exudative, erythematous plaques in inguinal area, "pot-bellied" appearance

Hematology		Reference Interval
PCV (%)	**40**	37–55
NCC ($\times 10^3/\mu L$)	**25.9**	6–17
Segs ($\times 10^3/\mu L$)	**23.4**	3–11.5
Monos ($\times 10^3/\mu L$)	**2.0**	0.1–1.3
Lymphs ($\times 10^3/\mu L$)	**0.4**	1–4.8
Eos ($\times 10^3/\mu L$)	0.1	0.1–1.2
Platelets ($\times 10^3/\mu L$)	Adequate	200–500
TP (P) (g/dL)	7.5	6–8

Biochemical Profile		Reference Interval
Gluc (mg/dL)	**140 (7.7)**	65–112 *(3.5–6.7 mmol/L)*
BUN (mg/dL)	18	7–28
Creat (mg/dL)	1.2	0.9–1.7
Ca (mg/dL)	10.5	9.0–11.2
Phos (mg/dL)	4.0	2.8–6.1
TP (g/dL)	7.0	5.4–7.4
Alb (g/dL)	4.0	2.7–4.5
Glob (g/dL)	3.0	1.9–3.4
T. Bili (mg/dL)	0.2	0–0.4
Chol (mg/dL)	350	130–370
ALT (IU/L)	110	10–120
AST (IU/L)	30	16–40
ALP (IU/L)	**5500**	35–280
GGT (IU/L)	**260**	0–6
Na (mEq/L)	**148**	145–158
K (mEq/L)	5.0	4.1–5.5
CL (mEq/L)	112	106–127
TCO_2 (mEq/L)	16	14–27
An. gap (mEq/L)	25	8–25

Urinalysis		Urine Sediment	
Color	Yellow	WBCs/hpf	2
Transparency	Cloudy	RBCs/hpf	2
Sp. Gr.	**1.002**	Epith cells/hpf	0
Protein	Negative	Casts/lpf	0
Gluc	Negative	Crystals	0
Bilirubin	Negative	Bacteria	4+
Blood	Negative		
pH	6.5		

Endocrine Data		Reference Interval
ACTH stimulation		
serum cortisol (µg/dL)(pre)	1.2	1–4
serum cortisol (µg/dL)(post)	1.2 *(33)*	>10.5; <20 *(>290; <550 nmol/L)*
Low dose dexamethasone suppression test		
serum cortisol (µg/dL)(pre)	2.0	1–4
serum cortisol (µg/dL)(3-hour post)	**2.0 *(55)***	<1.5 *(<41 nmol/L)*
serum cortisol (µg/dL)(8-hour post)	**1.7 *(47)***	<1.5 *(<41 nmol/L)*

Interpretive discussion

Hematology

Mature neutrophilia, monocytosis, and lymphopenia are indicative of a corticosteroid (stress) leukogram.

Biochemical profile

Mild hyperglycemia is consistent with increased endogenous or exogenous corticosteroids.

Alkaline phosphatase activity is markedly increased. While cholestasis may result in an increase of this magnitude, bilirubin is not increased, suggesting that the increase is likely due to corticosteroid induction of alkaline phosphatase. Determination of steroid-induced alkaline phosphatase isoenzyme would be helpful.

Gamma glutamyl transferase activity is also markedly increased, and with the lack of increase in ALT and AST activities, as well as bilirubin concentration, corticosteroid induction is likely.

The combination of mild hyperglycemia and increased ALP and GGT activities, with no other evidence of cholestasis, should trigger further endocrine testing.

Urinalysis

Low urine specific gravity (often hyposthenuria) is commonly seen in patients with hyperadrenocorticism. Glucocorticoids are thought to interfere with ADH receptors, resulting in isosthenuria or hyposthenuria, and polyuria and polydipsia. Bacteriuria without significant pyuria may also occur with hyperadrenocorticism.

Endocrine data

ACTH stimulation: Patients with iatrogenic hyperadrenocorticism have a "flat-line" response to ACTH stimulation (much like a patient with hypoadrenocorticism) due to feedback to the pituitary and secondary adrenal atrophy. While some corticosteroid drugs cross-react on the cortisol assay, the post-ACTH response will not be higher than the pre-ACTH response.

Low Dose Dexamethasone Suppression: LDDS is not helpful in diagnosing iatrogenic hyperadrenocorticism. The pituitary is already responding to feedback from iatrogenic corticosteroids, and adrenal glands are atrophied.

Summary

Iatrogenic Cushing disease which resulted from Vetalog injections for flea allergy dermatitis. Fleas were eliminated, and the dog was slowly withdrawn from corticosteroids by treating on alternate days with decreasing doses over several months.

Case 69

Signalment: 10-year-old spayed female Airedale
History: "Leaking" urine, polydipsia, limping
Physical examination: Ruptured anterior cruciate ligament, "pot-bellied," mild truncal alopecia

Hematology		Reference Interval
PCV (%)	**58**	37–55
NCC ($\times 10^3/\mu L$)	**24.4**	6–17
Segs ($\times 10^3/\mu L$)	**21.5**	3–11.5
Monos ($\times 10^3/\mu L$)	**2.4**	0.1–1.3
Lymphs ($\times 10^3/\mu L$)	**0**	1–4.8
NRBC ($\times 10^3/\mu L$)	**0.5**	0
Platelets ($\times 10^3/\mu L$)	Adequate	200–500

Biochemical Profile		Reference Interval
Gluc (mg/dL)	**130 *(7.1)***	65–122 *(3.5–6.7 mmol/L)*
BUN (mg/dL)	18	7–28
Creat (mg/dL)	1.2	0.9–1.7
Ca (mg/dL)	10.2	9.0–11.2
Phos (mg/dL)	4.9	2.8–6.1
TP (g/dL)	5.7	5.7–7.4
Alb (g/dL)	2.7	2.7–4.5
Glob (g/dL)	3.0	1.9–3.4
T. Bili	0.3	0–0.4
Chol (mg/dL)	350	130–370
ALT (IU/L)	65	10–120
AST (IU/L)	**60**	16–40
ALP (IU/L)	**4000**	35–280

Urinalysis	
Sp. Gr.	**1.008**
Bacteria	Many

Endocrine Data		Reference Interval
ACTH stimulation:		
serum cortisol (µg/dL)(pre)	**8 *(221)***	1–4 *(28–110 nmol/L)*
serum cortisol (µg/dL)(post)	**20 *(552)***	<20 *(<552 nmol/L)*
Low dose dexamethasone suppression test		
serum cortisol (µg/dL)(pre)	**6 *(166)***	1–4 *(28–110 nmol/L)*
serum cortisol (µg/dL)(3-hour post)	0.9	<1.5
serum cortisol (µg/dL)(8-hour post)	**1.7 *(47)***	<1.5 *(<41 nmol/L)*
High dose dexamethasone suppression test		
serum cortisol (µg/dL)(Pre)	**9 *(248)***	1–4 *(28–110 nmol/L)*
serum cortisol (µg/dL)(post)	**3 *(83)***	<1.5 *(<41 nmol/L)*
endogenous ACTH (pg/mL)	**350 *(77)***	20–100 *(4.4–22.0 pmol/L)*

Interpretive discussion

Hematology

The PCV is mildly increased, with increased nucleated erythrocyte concentration. Possibilities for this combination might include hypoxia or other causes of increased erythropoietin concentration. Dogs with hyperadrenocorticism will sometimes have increased erythropoiesis. Additionally, corticosteroids may inhibit removal of NRBC by macrophages in spleen. Mature neutrophilia, monoctosis, and lymphopenia are indicative of a stress leukogram.

Biochemical profile

Mild hyperglycemia is consistent with a stress leukogram, and may be a result of increased endogenous or exogenous glucocorticoids.

Alkaline phosphatase is markedly increased, AST is mildly increased, and cholesterol is borderline high. No other abnormalities are present. Increased alkaline phosphatase activity and mild hypercholesterolemia may be secondary to cholestasis; however, serum bilirubin is not increased. Alkaline phosphatase activity may also increase secondary to steroid induction. This is most likely given the magnitude of increase. The slight increase in serum AST activity may be due to mild steroid hepatopathy or steroid induction.

Urinalysis

Urine specific gravity is quite low, and while it is not necessarily abnormal, it is consistent with decreased urinary concentrating ability in dogs with hyperadrenocorticism, related to decreased responsiveness to ADH. Bacteriuria without pyuria may be seen in dogs with hyperadrenocorticism.

Physical exam, history, stress leukogram, hyperglycemia, and increased serum alkaline phosphatase activity should trigger screening tests for hyperadrenocorticism.

Endocrine data

ACTH stimulation: Baseline cortisol is above normal and poststimulation is "borderline." Stimulation of above 20 is consistent with hyperadrenocorticism. Eighty-five percent of dogs with pituitary dependent hyperplasia stimulate, as do approximately 50% of dogs with adrenal tumors. Thus, the ACTH stimulation is not diagnostic in this dog, but is suspicious.

Low dose dexamethasone suppression: Baseline cortisol is above normal. The dog suppressed at 3 hours, with escape from suppression at 8 hours. In normal dogs, endogenous ACTH is suppressed by dexamethasone, resulting in a rapid decline in plasma cortisol concentrations which remain suppressed for up to 48 hours. Most dogs with adrenal tumors show no suppression at 3 or 8 hours. If a dog suppresses at 3 hours, but does not remain suppressed at 8 hours, it is likely that the dog has PDH, rather than an adrenal tumor. This "escape" is thought to be due to rapid clearance of dexamethasone.

High dose dexamethasone suppression: Baseline cortisol is above normal. The dog did not suppress to the range for normal dogs. Dogs with adrenal disease do not suppress, and most dogs with pituitary dependent adrenal hyperplasia (PPH) do suppress. Very high dose steroids will suppress ACTH production, and hence cortisol secretion, even with PPD. However, most dogs with pituitary macroadenomas do not suppress; an endogenous ACTH serum concentration is indicated.

Endogenous ACTH: Dogs with PDH have normal to increased endogenous ACTH, while dogs with adrenal tumors have decreased endogenous ACTH. Thus, this dog has pituitary dependent disease.

Summary

A large pituitary macroadenoma was present in this dog. Note that multiple endocrine tests were required to make this diagnosis.

Case 70

Signalment Eight-year-old MC, poodle
History Hair loss, PUPD
Physical exam Hair loss along abdomen and legs, pendulous abdomen

Hematology		Reference Interval
PCV (%)	42.0	37–55
Hgb (g/dL)	13.8	12–18
RBC (10^6/μL)	5.8	5.5–8.5
MCV (fL)	72.0	60–72
MCHC (g/dL)	33	34–38
NCC (×10^3/μL)	**23.4**	6–17
Segs (×10^3/μL)	**20.1**	3–11.5
Bands (×10^3/μL)	0	0–0.3
Monos (×10^3/μL)	2.7	0.1–1.3
Lymphs (×10^3/μL)	**0.6**	1–4.8
Eos (×10^3/μL)	0	0.1–1.2
Platelets (×10^3/ μL)	455	200–500
TP (P) (g/dL)	6.5	6–8
Hemopathology: few nucleated red blood cells noted		

Biochemical Profile		Reference Interval
Gluc (mg/dL)	**289**	65–122
BUN (mg/dL)	22	7–28
Creat (mg/dL)	0.8	0.9–1.7
Ca (mg/dL)	10.1	9.0–11.2
Phos (mg/dL)	5.2	2.8–6.1
TP (g/dL)	6.7	5.4–7.4
Alb (g/dL)	3.3	2.7–4.5
Glob (g/dL)	3.4	1.9–3.4
T. Bili (mg/dL)	0.2	0–0.4
Chol (mg/dL)	**411**	130–370
ALT (IU/L)	**420**	10–120
AST (IU/L)	**122**	16–40
ALP (IU/L)	**6855**	35–280
Na (mEq/L)	146	145–158
K (mEq/L)	4.3	4.1–5.5
CL (mEq/L)	115	106–127
Na : K ratio	34	>25
TCO_2 (mEq/L)	20	14–27
An. gap (mEq/L)	15.3	8–25
Lipase (IU/L)	175	<500
Amylase	441	220–800

Urinalysis			
Color	yellow	**Urine Sediment**	
Transparency	hazy	WBCs/hpf	**20–30**
Specific Gravity	**1.008**	RBCs/hpf	**50–100**
Protein	**2+**	Epithelial cells/hpf	few
Glucose	**1+**	Casts/lpf	neg
Ketones	neg		
Bilirubin	neg	Crystals	triple phosphate
Blood	**3+**	Bacteria	**1+**

Endocrine data	Cortisol (μg/dL)	Reference Interval
Basal cortisol	**3.6**	1–4
ACTH stim	**28**	8–16
Basal cortisol	**4.1**	1–4
LDDS 8 h post	**4.4**	<1.4
Basal cortisol		**3.8**
HDDS 4 h	**4.6**	<1.4
HDDS 8 h	**2.2**	<1.4
Endogenous ACTH pg/mL	**264**	10–80
Two weeks later		
Basal cortisol	**0.3**	1–4
ACTH stim	**0.4**	8–16

Interpretive discussion

Hematology

A few nucleated red blood cells are present in the absence of anemia. This may indicate a disruption in the endothelial barrier in centers of hematopoiesis, or is possibly a result of immunosuppression and lack of removal of nuclei by macrophages. Increased NRBCs can be seen with hemangiosarcoma, some leukemias, lead toxicity, hyperadrenocorticism (HAC) and DIC. The leukogram is characteristic of a stress or steroid response: mature neutorphilia, lymphopenia, eosinopenia and monocytosis.

Biochemical profile

Marked increase in ALP with only mild increases in ALT and AST indicate possible cholestasis and or hyperadrenocorticism. An ALP over 5000 IU/L without bilirubinemia and only mild increases in ALT and AST is most consistent with HAC. Further support for this diagnosis is history of alopecia and PUPD coupled with dilute urine, cystitis and nucleated red blood cells. Over 90% of Cushingoid dogs will have mild to marked ALP. If ALP is not increased it is very unlikely that a dog has HAC. Cholesterol is increased which in this dog could be cholestasis, hypothyroidism, or HAC. Increases in ALT and AST are attributed to glycogen (steroid) hepatopathy induced by hyperadrenocorticism. Hyperglycemia is moderate, which is consistent with hyperadrenocorticism.

The urine analysis has abundant evidence for infectious cystitis; numerous leukocytes and bacteria are present. Collection method is not specified, therefore inflammation could be present anywhere in the urogenital tract. Proteinuria may be due to the inflammatory response and increased capillary permeability. Absence of casts and no azotemia support cystitis over pyleonephritis. Dilute urine is likely a result of a failure to concentrate due to glucocorticoid interference with ADH, subsequent polyuria, and responsive polydipsia. Cystitis is fairly common in dogs with hyperadrenocorticism.

At this point, laboratory evaluation of the endocrine system should be done. Initially, a low dose dexamethasone suppression test (LDDS) should be performed. If LLDS indicates hyperadrenocorticism, one should then perform an endogenous ACTH to distinguish pituitary dependent hyperadrenocorticism (PD HAC) from an adrenal tumor.

Endocrine testing

In this patient, the ACTH stimulation was done and although the basal cortisol is normal the post stim sample is >22 µg/dL which is excessive and supports hyperadrenocorticism. One must now differentiate pituitary dependent disease from an adrenal tumor. The basal concentration of cortisol in the LDDS is just above reference interval but the 8 hour value is well above 1.4 µg/dL therefore there was failure to suppress and Cushing's disease is ruled in when all the other data fits, as in this dog. (False positive rate for LDDS, however, is as high as 50% therefore all the other lab and historical and physical exam results need to fit with HAC.) Unfortunately, a 4 hour sample was not collected which could have proven helpful to distinguish pituitary and adrenal dependent HAC. If the 4 hour sample exhibited suppression (<1.4) with the present value for the 8 hour sample of no suppression it would have indicated a rebound which is consistent with pituitary dependent Cushing's disease, the more common cause of HAC. The HDDS is confusing or at least not very helpful. The basal cortisol is normal, the 4 hour sample clearly failed to suppress and the 8 hour sample is above 1.4, but it is almost 50% less than the basal and the 4 hour samples. The interpretation is the HDDS failed to suppress, indicating AT or PD HAC (suppression would indicate the dog had PD HAC). Although the endocrine testing to this point indicates an adrenal tumor is possible, adrenal tumors only account for 10–20% of dogs with HAC. Furthermore there seemed to be some suppression by the HDDS but even using a decrease of cortisol by 50% from basal it still did not clearly suppress. Another way to define suppression is if the 8 hour sample is less than 50% of basal even if it is still greater than 1.4 µg/dL. In this dog the 8 hour sample is 57% of basal, and the 8 hour sample is 47% of the 4 hour sample. Because distinction of AT and PD is needed for treatment and the HDDS was equivocal, abdominal ultrasonography (US) and an endogenous ACTH concentration were performed. Abdominal US did not identify an adrenal tumor and the endogenous ACTH clearly indicates this dog has PD HAC.

Summary

The e ACTH is markedly increased, and therefore the dog has a pituitary neoplasm secreting ACTH. The dog was given mitotane. The ACTH stim 2 weeks post diagnosis indicates a flat line response. The dog was clinically normal, and the electrolytes were normal, and therefore this indicated degeneration to necrosis of zona fasiculata by the mitotane. When dosed correctly the cortex will eventually regenerate. Under the stimulation of the ACTH secreting pituitary tumor the adrenal cortex will regenerate and is the reason repeated ACTH stims will be required during maintenance therapy. The results of the ACTH stim are the same pattern seen with hypoadrenocorticism, spontaneous disease, or from mitotane or steroid therapy.

Case 71

Signalment: 6-year-old male dog
History: Change in temperament from docile to irritable. Severe constipation for several days.
Physical examination: No abnormalities detected

Hematology		Reference Interval
PCV (%)	44	37–55
Hgb (g/dL)	14.5	12–18
RBC ($\times 10^6/\mu L$)	6.7	5.5–8.5
MCV (fL)	66	60–72
MCHC (g/dL)	33	34–38
NCC ($\times 10^3/\mu L$)	15.6	6–17
Segs ($\times 10^3/\mu L$)	12.7	3–11.5
Monos ($\times 10^3/\mu L$)	0.2	0.1–1.3
Lymphs ($\times 10^3/\mu L$)	2.4	1–4.8
Eos ($\times 10^3/\mu L$)	0.3	0.1–1.2
Platelets ($\times 10^3/\mu L$)	440	200–500
TP (P) (g/dL)	6.8	6–8
Hemopathology: Normal		

Biochemical Profile		Reference Interval
Gluc (mg/dL)	80	65–122
BUN (mg/dL)	28	7–28
Creat (mg/dL)	1.5	0.9–1.7
Ca (mg/dL)	**14.3 *(3.57)***	9.0–11.2 *(2.25–2.80 mmol/L)*
Phos (mg/dL)	**1.7 *(0.5)***	2.8–6.1 *(0.9–2.0 mmol/L)*
TP (g/dL)	6.1	5.4–7.4
Alb (g/dL)	3.4	2.7–4.5
Glob (g/dL)	2.7	1.9–3.4
T. Bili (mg/dL)	0.4	0–0.4
Chol (mg/dL)	235	130–370
ALT (IU/L)	100	10–120
AST (IU/L)	33	16–40
ALP (IU/L)	**285**	35–280
Na (mEq/L)	145	145–158
K (mEq/L)	5.3	4.1–5.5
CL (mEq/L)	115	106–127
TCO_2(mEq/L)	21	14–27
An. gap (mEq/L)	14	8–25

Urinalysis (catheterized)			
Color	Yellow	**Urine Sediment**	
Transparency	Clear	WBCs/hpf	0–2
Sp. Gr.	**1.011**	RBCs/hpf	0
Protein	Negative	Epith cells/hpf	0
Gluc	Negative	Casts/lpf	0
Bilirubin	Negative	Crystals	0
Blood	Negative	Bacteria	0
pH	6.5		

Endocrine Data		Reference Interval
Intact parathormone	**22**	2–13 (pmol/L)
PTHrp	Undetectable	<0.2 (pmol/L)

Interpretive discussion

Hematology

In light of normal results for other erythrocyte measurements, the decreased MCHC is marginal and not important.

The mild mature neutrophilia, in the absence of lymphopenia, suggests normal variability or very mild inflammation. This dog's irritability may have predisposed it to epinephrine release when the venipuncture was performed, although excitement leukograms are quite rare in dogs.

Biochemical profile

Hypercalcemia and hypophosphatemia can occur with primary hyperparathyroidism and pseudohyperparathyroidism (hypercalcemia of malignancy). In this case, the increased intact parathormone (iPTH) and normal parathormone-related protein (PTHrp) concentrations are most suggestive of primary hyperparathyroidism (see discussion of hormone assays below). Other causes of hypercalcemia include vitamin D toxicosis, excessive bone resorption, and renal failure (5–10% of these cases in dogs), but serum phosphorus concentration is typically normal to increased in these cases.

The slightly increased serum alkaline phosphatase activity is not significant. There is no evidence suggesting either cholestasis or increased corticosteroid levels. Since this dog has an abnormality of calcium and phosphorus metabolism, it is possible that altered bone metabolism is occurring. Although the net effect in this animal is probably bone demineralization, increased osteoblastic activity, as part of effort to regenerate bone, may have resulted in this slight increase in activity.

Urinalysis

Low urine specific gravity may reflect this dog's hydration status and, therefore, may be normal in this patient. Hypercalcemia can, however, interfere with renal concentrating ability and can result in decreased urine specific gravity with subsequent polyuria and polydipsia. Nephrocalcinosis, other toxic effects of calcium on renal tubules, and interference with the action of antidiuretic hormone are possible mechanisms for decreased concentrating ability in hypercalcemic animals. The absence of polyuria and polydipsia in this dog suggests that calcium interference with renal concentrating ability is not a major factor.

Endocrine data

Increased intact parathormone (iPTH) concentration and undetectable PTH-related protein (PTHrp) concentration indicate that this dog has primary hyperparathyroidism, rather than hypercalcemia of malignancy. The iPTH concentrations are increased due to overproduction of PTH by hyperplastic or neoplastic parathyroid glands. Parathormone-related protein is synthesized by malignant cells of neoplasms such as lymphoma and apocrine gland adenocarcinoma of the anal sac, but not by the parathyroid glands, and concentrations of PTHrp are, therefore, not increased in animals with primary hyperparathyroidism.

Summary

The combination of hypercalcemia, hypophosphatemia, increased iPTH concentration, and undetectable PTHrp concentration indicate primary hyperparathyroidism in this case. A mass in the neck region compatible in location with the parathyroid gland was found during a more thorough physical examination. Surgical removal and histopathologic examination revealed a parathyroid adenoma. This dog's clinical signs and serum calcium and phosphorus concentrations returned to normal after surgery. Irritability is unusual in hypercalcemic dogs; dullness is more common.

Case 72

Signalment Eleven-year old FS Australian cattle dog
History Poor appetite
Physical examination Quiet, adequate body condition

Hematology		Reference Interval
PCV (%)	53	39–58
Hgb (g/dL)	19.7	13.8–20.3
RBC (10^6/µL)	7.67	5.7–8.0
MCV (fL)	75	61–75
MCHC (g/dL)	34.3	30.8–35.4
NCC (×103/µL)	6.91	4.4–11.6
Segs (×103/µL)	4.9	2.8–9.1
Bands (×103/µL)	0	0–0.3
Monos (×103/µL)	0.9	0.07–1.0
Lymphs (×103/µL)	2.4	0.6–3.3
Eos (×103/µL)	0.2	0–1.2
Platelets (×103/ µL)	366	200–500
TP (P) (g/dL)	7.4	6.1–7.5

Biochemical Profile		Reference Interval
Gluc (mg/dL)	91	70–131
BUN (mg/dL)	14	6–26
Creat (mg/dL)	0.7	0.7–1.5
Ca (mg/dL)	**12.3**	9.3–11.5
Phos (mg/dL)	3.3	2.5–5.6
Magnesium (mg/dL)	2.0	1.8–2.5
TP (g/dL)	6.7	5.2–7.4
Alb (g/dL)	3.9	3–3.9
Glob (g/dL)	2.8	1.7–3.8
T. Bili (mg/dL)	0.1	0–0.3
Chol (mg/dL)	274	124–344
ALS (IU/L)	72	12–54
ALP (IU/L)	62	16–140
GGT (IU/L)	5	0–6
CK (IU/L)	176	43–234
Na (mEq/L)	145	140–156
K (mEq/L)	4.4	4–5.3
CL (mEq/L)	111	108–122
Na:K ratio	32.6	>25
TCO_2 (mEq/L)	22	18–26
An. gap (mEq/L)	16.1	11.2–19
Lipase (IU/L)	210	12–147
Amylase	600	236–1337

Urine Analysis	
Urine s.g. voided	1.007

Interpretive discussion

Hematology

Unremarkable; absence of a stress leukogram may prove informative.

Biochemical profile

Mild hypercalcemia is the only abnormality. The increased total serum calcium explains the dilute hyposthenuria, as hypercalcemia affects the function of ADH on tubules. Serum phosphorus is normal which is somewhat helpful to shorten the list of differentials for hypercalcemia. Recommend rechecking calcium, total and ionized, and if both are increased pursue possible causes of hypercalcemia. The calcium is likely to be increased again given the urine specific gravity. The two most likely differentials are hypercalcemia of malignancy and primary hyperparathyroidism because the serum phosphorus is normal and there are no other significant biochemical abnormalities. Unlikely differentials are hypoadrenocorticism, renal failure, vitamin D toxicity, and granulomatous diseases, all of which usually have increased serum phosphorus and produce other biochemical disturbances. The only two diseases that produce hypercalcemia and hypophosphatemia in dogs are hypercalcemia of malignancy and primary hyperparathyroidism.

Summary and follow-up

Both total serum calcium and ionized calcium were increased on a recheck. On physical examination, no evidence of lymphoma or perirectal apocrine gland adenocarcinoma was found. Serum was sent for PTH and PTH-rp.

Endocrine Data		Reference Interval
PTH (pmol/L)	**35.5**	3–17
PTHrp (pmol/L)	0	0–0.9
iCa	**1.65**	1.25–1.45

These results confirm hypercalcemia and rule in primary hyperparathyroidism.

The neck region was explored and a small mass was found in the region of one thyroid lobe. During surgery a STAT methodology to measure PTH pre and post removal of any mass was employed and the results were:

Additional Endocrine Data		Reference Interval
PTH Sample 1, pre, Turbo intact PTh (pg/mL)	**98**	11.2–72.8
PTH Sample 2, post, Turbo intact PTh (pg/mL)	**9.0**	

The decrease in PTH in the second sample, after the parathyroid mass was removed is dramatic (less than 50% of previous sample) indicating that the offending lesion was removed.

Comment

The serum concentration of PTH was increased in this dog when it was hypercalcemic which makes the diagnosis of primary hyperparathyroidism (HPTH) quite simple. However, increased PTH is only present in about 25% of dogs and the remaining dogs (75%) with primary HPTH will have a concentration of PTH within reference interval. Moreover, 45% of dogs with primary hyperparathyroidsm will have serum PTH concentrations in the low to middle range. Increased concentrations of PTH are the exception in dogs, but if PTH is detectable in an animal that is hypercalcemic and not azotemic then this combination is inappropriately abnormal because PTH should be decreased or undetectable in response to nonparathyroid induced hypercalcemia. If PTH is within the reference interval, it is inappropriately high in the face of hypercalcemia, and therefore diagnostic for primary HPTH. It indicates the parathyroid gland is secreting PTH at a time when secretion should be suppressed. It is critical to measure PTHrp concurrently as many dogs with hypercalcemia of malignancy will have measureable PTH.

Ultrasonography of the neck region is just as accurate at identifying a parathyroid mass as is measuring serum PTH and if positive will localize the side of the neck to look for the adenoma during surgery. More than one mass is possible, especially in Keeshonds.

Case 73

Signalment Eleven-year-old FS mixed breed dog
History Weight loss, poor appetite and lethargy
Physical examination Thin body condition, depressed attitude

Hematology		Reference Interval
Packed cell volume (%)	**32**	39–58
Hemoglobin (g/dL)	**10.8**	13.8–20.3
RBC (10^6/μL)	**4.27**	5.7–8.01
MCV (fL)	74	61–75
MCHC (g/dL)	34	30.8–35.4
Total nucleated cell count ($\times10^3$/μL)	10.2	4.4–11.6
Segmented neutrophils ($\times10^3$/μL)	7.9	2.84–9.11
Band neutrophils ($\times10^3$/μL)	0	0–0.3
Monocytes ($\times10^3$/μL)	1.9	0.075–1.0
Lymphocytes ($\times10^3$/μL)	**0.4**	0.59–3.3
Eosinophils ($\times10^3$/μL)	0	0.03–1.2
Platelets ($\times10^3$/ μL)	386	190–468
Plasma protein (g/dL)	7.1	6.1–7.5

Biochemical Profile		Reference Interval
Gluc (mg/dL)	98	70–131
BUN (mg/dL)	**74**	6–26
Creat (mg/dL)	**3.7**	0.7–1.5
Ca (mg/dL)	**17.3**	9.3–11.5
Phos (mg/dL)	**7.3**	2.5–5.6
Magnesium (mg/dL)	**2.0**	**1.8–2.5**
TP (g/dL)	6.9	5.2–7.4
Alb (g/dL)	3.9	3–3.9
Glob (g/dL)	3.0	1.7–3.8
T. Bili (mg/dL)	0.1	0–0.3
Chol (mg/dL)	254	124–344
ALS (IU/L)	**372**	12–54
ASt (IU/L)	**388**	42–175
ALP (IU/L)	**662**	16–140
GGT (IU/L)	**15**	0–6
CK (IU/L)	111	43–234
Na (mEq/L)	141	140–156
K (mEq/L)	4.9	4–5.3
CL (mEq/L)	110	108–122
Na:K ratio	28.7	>25
TCO_2 (mEq/L)	**12**	18–26
An. gap (mEq/L)	**23.9**	11.2–19
Lipase (IU/L)	**510**	12–147
Amylase	**1724**	236–1337

Urinalysis cystocentesis			
Color	yellow	**Urine Sediment**	
Transparency	clear	WBCs/hpf	0–3
Specific Gravity	**1.010**	RBCs/hpf	**10–30**
Protein	**1+**	Epithelial cells/hpf	none
Glucose		Casts/lpf	neg
Ketones	neg	Crystals	none
Bilirubin	neg		
Blood	**2+**		

Interpretive discussion

Hematology

There is a mild anemia. Although a reticulocyte count is not present the indices are normocytic normochromic which indicate it may be nonregenerative. The azotemia could explain this anemia if the azotemia is due to chronic renal disease. Anemia of inflammatory disease is another possible cause of the anemia in this dog. There is a stress leukogram as evidenced by the lymphopenia, eosinopenia, and monocytosis.

Biochemical profile

The dog has renal failure based on mild azotemia combined with isosthenuria. However, any time the serum calcium is increased the kidneys may not be able to concentrate urine adequately (interference with ADH) which in this case clouds the interpretation that primary renal disease is present. If this dog is dehydrated the azotemia may all or partially be due to prerenal and the dilute urine in the face of dehydration is caused by hypercalcemia. Hypercalcemia is severe, hyperphosphatemia is mild to moderate. The Ca × P product is 126, which indicates mineralization of soft tissues is occurring. Mineralization may have caused the renal failure or at least enhanced it. The diagnostic dilemma is to determine which came first, the renal failure or the hypercalcemia. It is often difficult to distinguish and there may be two diseases occurring, renal failure and a disease other than renal disease that caused hypercalcemia (e.g., a malignancy, vitamin D toxicity, etc.). In this dog it seems more likely the hypercalcemia came first or that there is a second disease causing hypercalcemia. This is based on mild hyperphosphatemia and marked hypercalcemia. Rules to help make this distinction are: the greater the serum P the more likely the primary disease is renal and the lower the serum P the more likely there is hypercalcemia of malignancy (HCM). The higher the serum calcium the more likely there is a malignancy and the lower the calcium the more likely renal disease is causing hypercalcemia. The greater the

azotemia the more likely it is primary renal and the lower the azotemia the more likely the renal problems are caused by the mineralization or it is pre renal. The easiest way to distinguish is to either find the malignancy or identify the type of renal failure (chronic, acute, glomerular, pyelonephritis etc.). In this dog it seems clear the primary disease is one that is causing hypercalcemia because the azotemia is mild, the hypercalcemia is severe and hyperphosphatemia is mild. This distinction can be more difficult in other cases. A reasonable plan is to search for cancer and give the dog fluids to see if the azotemia can be reversed. Measuring ionized calcium may also be diagnostically helpful. If the ionized calcium is within the reference interval then primary renal failure is more likely, but if ionized calcium is increased it could still be either primary renal or a malignancy.

The urine is not concentrated, which could be primary renal or secondary to hypercalcemia. There is some blood likely due to cystocentesis, which may also be responsible for the 1+ protein. The rest of the analysis is unremarkable.

The hepatic leakage enzymes are increased (ALT and AST) and the enzymes associated with cholestasis are increased (ALP, GGT). There are many possible causes of cholestasis in this dog, one of which is an infiltrative disease in the liver such as lymphoma, another possiblity is pancreatitis. The increases in lipase and amylase are mild and seem more likely due to decreased GFR (azotemia) and therefore decreased excretion than pancreatitis. If the dog had pancreatitis it may have hypocalcemia, tender abdomen and be overweight with an acute onset of problems, none of which this dog has. The dog has a metabolic acidosis probably caused by dehydration and or renal disease.

A reasonable plan is to search for lymphoma (palpate lymph nodes, search for an anterior thoracic mass, abdominal organ evaluation, etc.) and do a rectal and perirectal exam to evaluate if a carcinoma of the anal sacs is present. If a malignant tumor is found there is no need to perform PTH and PTH rp assays.

Summary

A mass was found in the pelvic vault, and aspirational cytology indicated it was a tumor of the anal sacs. These are invariably malignant, but repeated excisions and or chemotherapy can extend a dog's life for months or years. These owners declined treatments. Intravenous and subcutaneous fluid therapy decreased the azotemia, but it did not return to normal. An autopsy was not performed so it is not known if or what type of renal disease was present and whether mineralization played a role. The anemia was due to anemia of chronic disease (cancer) and or concurrent renal disease. The most common substance secreted by tumors is PTHrp, which stimulates phosphaturia and results in hypophosphatemia, absolute or as in this dog relative for the degree of azotemia. Primary hyperparathyroidism could cause the hypercalcemia and phosphaturia, but rarely is there concurrent azotemia with primary hyperparathyroidism.

Case 74

Signalment: 9-year-old intact male dog
History: One seizure. Occasional tremors observed.
Physical examination: Physical abnormalities, but had seizure during examination

Hematology		Reference Interval
PCV (%)	44	37–55
Hgb (g/dL)	15.2	12–18
RBC ($\times 10^6/\mu L$)	7.1	5.5–8.5
MCV (fL)	62	60–72
MCHC (g/dL)	35	34–38
NCC ($\times 10^3/\mu L$)	**20.2**	6–17
Segs ($\times 10^3/\mu L$)	**17.2**	3–11.5
Monos ($\times 10^3/\mu L$)	**2.4**	0.1–1.3
Lymphs ($\times 10^3/\mu L$)	**0.6**	1–4.8
Platelets ($\times 10^3/\mu L$)	470	200–500
TP (P) (g/dL)	7.2	6–8
Hemopathology: Normal		

Biochemical Profile		Reference Interval
Gluc (mg/dL)	**138 *(7.6)***	65–122 *(3.5–6.7 mmol/L)*
BUN (mg/dL)	14	7–28
Creat (mg/dL)	**0.5**	0.9–1.7
Ca (mg/dL)	**4.0 *(1.0)***	9.0–11.2 *(2.25–2.80 mmol/L)*
Phos (mg/dL)	**7.0 *(2.3)***	2.8–6.1 *(0.9–2.9 mmol/L)*
TP (g/dL)	7.0	5.4–7.4
Alb (g/dL)	3.6	2.7–4.5
Glob (g/dL)	3.4	1.9–3.4
T. Bili (mg/dL)	0.4	0–0.4
Chol (mg/dL)	161	130–370
ALT (IU/L)	38	10–120
AST (IU/L)	18	16–40
ALP (IU/L)	176	35–280
Na (mEq/L)	145	145–158
K (mEq/L)	4.4	4.1–5.5
CL (mEq/L)	**103**	106–127
TCO_2 (mEq/L)	22	14–27
An. gap (mEq/L)	24	8–25

Urinalysis			
Color	Yellow	**Urine Sediment**	
Transparency	Clear	WBCs/hpf	0
Sp. Gr.	1.032	RBCs/hpf	0
Protein	Negative	Epith cells/hpf	0
Gluc	Negative	Casts/lpf	0
Bilirubin	Trace	Crystals	0
Blood	Negative	Bacteria	0
pH	6.0		

Endocrine Data		Reference Interval
iPTH (pmol/L)	**2**	2–13

Interpretive discussion

Hematology

Mature neutrophilia, lymphopenia, and monocytosis are typical of a stress leukogram.

Biochemical profile

The serum glucose concentration is in the range typical for glucocorticoid-induced hyperglycemia. Stress is the most likely cause in this case, particularly in light of the leukogram.

Decreased serum creatinine concentration is meaningless in most cases. This abnormality can result from diuresis, but, if this is the cause, the BUN concentration is usually also decreased. The absence of a history of polyuria and the normal BUN concentration make diuresis unlikely in this case.

Hypocalcemia and hyperphosphatemia can occur in renal failure, pancreatitis with prerenal azotemia, eating a diet containing excessive phosphorus, or hypoparathyroidism. Hypoparathyroidism is most likely in this case. Normal BUN concentration and decreased serum creatinine concentration indicate that renal function is normal. Clinical signs are not typical of pancreatitis, and there is no evidence of a prerenal azotemia. This dog may be receiving a diet with excessive phosphorus, but this is very unlikely if the dog is receiving a commercial diet. Hypoalbuminenmia is another cause of hypocalcemia, but the absence of hypoalbuminemia indicates that this is not a consideration. Vitamin D deficiency may also result in hypocalcemia, but hypophosphatemia rather than hyperphosphatemia is typical of such a deficiency. Hypoparathyroidism can be confirmed by measuring the serum intact parathormone concentration (see below).

Slight hypochloremia, in the absence of abnormalities in Na, K, or total CO_2, is probably insignificant.

Urinalysis

The urinalysis is normal.

Endocrine data

The serum intact parathyroid hormone (iPTH) concentration is at the low end of the reference interval. The normal response of the parathyroid glands to hypocalcemia is production of PTH. Low normal iPTH concentration in a hypocalcemic animal strongly suggests inability of the parathyroid glands to respond to hypocalcemia and, therefore, hypoparathyroidism. Other possible causes of hypocalcemia (discussed above) should result in high normal to increased iPTH concentrations.

Summary

The combination of hypocalcemia with low normal iPTH concentration indicate hypoparathyroidism. Other diseases can result in hypocalcemia and hyperphosphatemia, but iPTH concentration in these diseases is typically high normal to increased.

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Note: Page numbers in *italics* refer to Figures; those in **bold** to Tables.

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