CIRCULATORY SYSTEM

THE BLOOD AND LYMPHATICS

HEMATOPOIETIC SYSTEM INTRODUCTION

Blood supplies cells with water, electrolytes, nutrients, and hormones and removes waste products. The cellular elements supply oxygen (RBCs), protect against foreign organisms and antigens (WBCs), and initiate coagulation (platelets). Because of the diversity of the hematopoietic system, its diseases are best discussed from a functional perspective. Function may be classified as either normal responses to abnormal situations (eg, leukocytosis and left shift in response to inflammation) or primary abnormalities of the hematopoietic system (eg, pancytopenia from marrow failure). Furthermore, abnormalities may be quantitative (ie, too many or too few cells) or qualitative (ie, abnormalities in function).

RED BLOOD CELLS

The function of RBCs is to carry oxygen to the tissues at pressures sufficient to permit its rapid diffusion. This is accomplished through the following mechanisms: a carrier molecule, hemoglobin (Hgb); a vehicle (RBC) capable of bringing the intact Hgb to the cellular level; and a metabolism geared to protect both the RBC and the Hgb from damage. Interference with synthesis or release of Hgb, production or survival of RBCs, or metabolism causes disease.

Hgb is a complex molecule, formed of four heme units attached to four globins (two α and two β globins). Iron is added in the last step by the ferrochelatase enzyme. Interference with the normal production of heme or globin leads to anemia. Causes include copper or iron deficiency and lead poisoning. Hemoglobinopathies such as thalassemias and sickle cell anemia, important genetic diseases of people, have not been seen in other animals. In these diseases, the production of globins (α or β or both) does not balance heme production, and the Hgb is not functional. The only known hemoglobinopathy of animals is porphyria. Although described in several species, it is most important as a cause of photosensitivity in cattle (*see* p 976).

Red cell mass, and thus oxygen-carrying capacity, remains constant over time in healthy animals. Mature RBCs have a finite life span; their production and destruction must be carefully balanced, or disease ensues.

Erythropoiesis is regulated by erythropoietin, which increases in the presence of hypoxia and regulates RBC production. In most species, the kidney is both the sensor organ and the major site of erythropoietin production, so chronic renal failure is associated with anemia. Erythropoietin acts on the marrow in concert with other humoral mediators to increase the number of stem cells entering RBC production, to shorten maturation time, and to cause early release of reticulocytes. Other factors that affect erythropoiesis are the supply of nutrients (eg, iron, folate, or vitamin B_{12}) and cell-cell interactions between erythroid precursors, lymphoid cells, and other components of the hematopoietic microenvironment. Factors that may suppress erythropoiesis include chronic debilitating diseases and endocrine disorders (such as hypothyroidism or hyperestrogenism).

Two mechanisms exist for removal of senescent RBCs; both conserve the principal constituents of the cell for reuse. Removal of aged RBCs normally occurs by phagocytosis by the fixed macrophages of the spleen. As the RBC ages it may change antigenically, acquiring senescent antigens and losing its flexibility due to impaired ATP production. Both of these changes increase the risk that the cell will become trapped in the spleen and removed by macrophages. After phagocytosis and subsequent disruption of the cell membrane, Hgb is converted to heme and globin. Iron is released from the heme moiety and either stored in the macrophage as ferritin or hemosiderin or released into the circulation for transport back to the marrow. The remaining heme is converted to bilirubin, which is released by the macrophages into the systemic circulation, where it complexes with albumin for transport to the hepatocytes; there, it is conjugated and excreted into the bile. In extravascular hemolytic anemias, RBCs have a shortened life span, and the same mechanisms occur at an increased rate.

Approximately 1% of normal aging RBCs are hemolyzed in the circulation, and free Hgb is released. This is quickly converted to Hgb dimers that bind to haptoglobin and are transported to the liver, where they are metabolized in the same manner as products from RBCs removed by phagocytosis. In intravascular hemolytic anemia, more RBCs are destroyed in the circulation (hemoglobinemia) than can be bound to haptoglobin. The excess Hgb and, therefore, iron are excreted in the urine (hemoglobinuria).

The principal metabolic pathway of RBC is glycolysis, and the main energy source in most species is glucose. Glucose enters the RBC by an insulin-independent mechanism, and most is metabolized to produce ATP and reduced nicotinamide adenine dinucleotide (NADH). The energy of ATP is used to maintain RBC membrane pumps so as to preserve shape and flexibility. The reducing potential of the NADH is utilized via the methemoglobin reductase pathway to maintain the iron in Hgb in its reduced form (Fe^{2+}).

The glucose not used in glycolysis is metabolized via a second pathway, the hexose monophosphate (HMP) shunt. No energy is produced via the HMP shunt; its principal effect is to maintain reducing potential in the form of reduced nicotinamide adenine dinucleotide phosphate (NADPH). In conjunction with the glutathione reductase/peroxidase system, NADPH maintains the sulfhydryl groups of globin in their reduced state.

Some disorders are the direct result of abnormal RBC metabolism and interference with glycolysis. Inherited deficiency of pyruvate kinase, a key glycolytic enzyme, causes ATP deficiency, which leads to reduced RBC life span and hemolytic anemia. Excessive oxidant stress may overload the protective HMP shunt or methemoglobin reductase pathways, causing Heinz body hemolysis or methemoglobin formation, respectively. Hemolytic anemia caused by a drug, such as acetaminophen in cats, is an example of this mechanism. (*See also* ANEMIA, p 7.)

A decreased RBC mass (anemia) may be caused by blood loss, hemolysis, or decreased production. In acute blood loss anemia, RBCs are lost, but mortality is usually related to loss of circulating volume rather than to loss of RBC. Iron is the limiting factor in chronic blood loss. Hemolysis may be caused by toxins, infectious agents, congenital abnormalities, or antibodies directed against RBC membrane antigens. Decreased RBC production may result from primary marrow diseases (eg, aplastic anemia, hematopoietic malignancy, or myelofibrosis) or from other causes such as renal failure, drugs, toxins, or antibodies directed against RBC precursors. Malignancy of RBCs or their precursors may be acute (eg, erythroleukemia) or chronic (eg, polycythemia vera). Animals with erythroleukemia are anemic despite having a marrow filled with rubriblasts, whereas those with polycythemia vera have erythrocytosis.

WHITE BLOOD CELLS

Phagocytes: The principal function of phagocytes is to defend against invading microorganisms by ingesting and destroying them, thus contributing to cellular inflammatory responses. There are two types of phagocytes: mononuclear phagocytes and granulocytes. Mononuclear phagocytes arise primarily from the marrow and are released into the blood as monocytes. They may circulate for hours to a few days before entering the tissues and differentiating to become macrophages. Granulocytes have a segmented nucleus and are classified according to their staining characteristics as neutrophils, eosinophils, or basophils. Neutrophils circulate for only a few hours before travelling to the tissues.

Five distinct stages in the process of phagocytosis have been identified: 1) attraction of phagocytes (chemotaxis) to microorganisms, antigen-antibody complexes, and other mediators of inflammation; 2) attachment to the organism; 3) ingestion; 4) fusion of cell lysosomes with ingested microorganisms and bacterial killing; and 5) digestion. In addition, many phagocytes have other specialized functions. Monocytes form a link to the specific immune system by processing antigen for presentation to lymphocytes and by producing substances such as interleukin-1, which initiates fever and lymphocyte activation and stimulates early hematopoietic progenitors.

Eosinophils, while having a role as phagocytes, also have more specific functions that include providing a defense against metazoan parasites and modulating the inflammatory process. They respond chemotactically to histamine, immune complexes, and eosinophil chemotactic factor of anaphylaxis, a substance released by degranulating mast cells. Basophils are not true phagocytes but contain large amounts of histamine and other mediators of inflammation. Eosinophilia and basophilia may be seen in response to systemic allergic reactions and invasion of tissues by parasites.

As with the RBCs, the production and circulating numbers of phagocytes are tightly regulated and controlled by various humoral factors, including colony-stimulating factors and interleukins. Unlike the RBCs, which remain circulating in the blood, the phagocytes use this compartment as a pathway to the tissues. Consequently, the number of phagocytes in the blood reflects circumstances in the tissues (eg, inflammation) as well as the proliferative function of the bone marrow. The sensitivity with

which phagocytes reflect these conditions varies from species to species. Abnormal response, such as neutropenia from marrow failure, infections, drugs, or toxins, is likely to result in secondary bacterial infections. Some cases of "idiopathic" neutropenia in dogs may have an immune-mediated cause. Finally, phagocyte precursors may undergo malignant transformation, which results in acute or chronic myelogenous leukemia.

Lymphocytes: Lymphocytes are responsible for both humoral and cellular immunity. Cells of the two branches of the immune system cannot be differentiated morphologically, but they differ in their dynamics of production and circulation. Lymphocyte production in mammals originates in the bone marrow. Some of the lymphocytes destined to be involved in cellular immunity migrate to the thymus and differentiate further under the influence of thymic hormones. These become T cells and are responsible for a variety of helper or cytotoxic immunologic functions. Most circulating lymphocytes are T cells, but T cells are also present in the spleen and lymph nodes. The B cells migrate directly to organs without undergoing modification in the thymus and are responsible for humoral immunity (antibody production).

Thus, lymphoid organs have populations of both B and T lymphocytes. In the lymph nodes, follicular centers are primarily B cells, and parafollicular zones are primarily T cells. In the spleen, most of the lymphocytes of the red pulp are B cells, whereas those of the periarteriolar lymphoid sheaths are T cells. Close association of T cells and B cells within lymphoid organs is essential to immune function.

Lymphocyte function in the cellular immune system features both afferent (receptor) and efferent (effector) components. Long-lived T cells of the peripheral blood are the receptors. In response to antigens to which they have been previously sensitized, they leave the circulation and undergo blast transformation to form activated T cells, which in turn cause other T cells to undergo blast transformation, both locally and systemically. Stimulated T cells produce lymphokines with a wide range of activities, such as attraction and activation of neutrophils, macrophages, and lymphocytes.

The humoral immune system is composed of B cells that produce antibodies of several classes. When sensitized B cells encounter antigen, they divide and differentiate into plasma cells that produce antibody. Therefore, each initially stimulated B cell produces a clone of plasma cells, all producing the same specific antibody.

Antibody molecules (immunoglobulins) fall into several classes, each with its own functional characteristics. For example, IgA is the principal antibody of respiratory and intestinal secretions, IgM is the first antibody produced in response to a newly recognized antigen, IgG is the principal antibody of the circulating blood, and IgE is the principal antibody involved in allergic reactions.

Antibodies perform their function by combining with the specific antigens that stimulated their production. Antigen-antibody complexes may be chemotactic for phagocytes, or they may activate complement, a process that produces both cell lysis and substances chemotactic for neutrophils and macrophages. In this manner, the humoral immune system is related to, and interacts with, the nonspecific immune system.

The humoral immune system also is related to both the nonspecific immune system and the cellular immune system in other ways. Both "helper" (CD4) and "cytotoxic" (CD8) T-cell classes have been described. Helper T cells recognize processed antigen and activate the humoral immune response. Cytotoxic T cells, after sensitization by antigen, are effector cells, which are especially important in antiviral immunity. Natural killer cells, which are a class of lymphocyte distinct from T cells and B cells, destroy foreign cells (eg, neoplastic cells) even without prior sensitization. Antigen processing by macrophages precedes recognition of an antigen by lymphocytes. These complex processes are involved in routine surveillance against neoplastic cells and recognition of "self."

Lymphocyte response in disease may be appropriate (activation of the immune system) or inappropriate (immune-mediated disease and lymphoproliferative malignancies). (*See also* THE BIOLOGY OF THE IMMUNE SYSTEM, p 811.) Immune-mediated disease results from failure of the immune system to recognize host tissues as self. For example, in immune-mediated hemolytic anemia, antibodies are produced against the host's own RBCs. Another inappropriate response of the immune system is allergy. In allergic individuals, IgE antibodies to allergens are bound to the surface of basophils and mast cells. When exposure to the allergen occurs, antigen-antibody complexes are formed, and degranulation of the mast cells and basophils releases vasoactive amines. Reaction to this may be mild (as in urticaria or atopy) or life-threatening (as in anaphylaxis).

Lymphocytosis occurs in some species, especially the cat, as a response to epinephrine secretion. Atypical lymphocytes may be seen in the blood in response to antigenic stimulation (eg, vaccination). Persistent lymphocytosis in cattle infected with bovine leukemia virus is a benign polyclonal increase in lymphocyte numbers. Lymphoproliferative malignancies include lymphomas and acute lymphoblastic and chronic lymphocytic leukemias. Lymphopenia may occur most commonly as a response to glucocorticoid secretion.

PLATELETS

Platelets form the initial hemostatic plug whenever hemorrhage occurs. They also are the source of phospholipid, which is needed for the interaction of coagulation factors to form a fibrin clot. Platelets are produced in the bone marrow from megakaryocytes, under the influence of thrombopoietin. Platelet production begins with invagination of the megakaryocyte cell membrane and the formation of cytoplasmic channels and islands. The cytoplasmic islands produce platelets by fragmentation from the megakaryocyte.

Mature circulating platelets are packed with dense granules containing ATP, adenosine diphosphate (ADP), and calcium, as well as serotonin, lysosomes, glycogen, mitochondria, and an intracellular canalicular system. The mitochondria and glycogen are involved in energy production, and the canalicular system serves both as a transport system for granule components and as a source of phospholipid, which is found in high concentration in the membrane lining of the canals.

When vessel walls are damaged, collagen and tissue factor are exposed, and circulating platelets adhere via von Willebrand factor and undergo a change in shape with the accompanying release of ADP. Local platelet aggregation is stimulated by ADP, with the ultimate formation of the primary platelet plug. The local accumulation of fibrin and platelets is known as a hemostatic plug. The fibrin clot that then forms is consolidated by the action of platelet contractile proteins.

Platelet disorders are either quantitative (thrombocytopenia or thrombocytosis) or qualitative (thrombocytopathy). Thrombocytopenia is one of the most common bleeding disorders of animals. In general, platelet counts must fall to <30,000/µL before the risk of hemorrhage increases. Consumption, destruction, or sequestration of platelets causes thrombocytopenia associated with increased production by the bone marrow. Consumptive thrombocytopenia occurs with massive hemorrhage or with disseminated intravascular coagulation, secondary to a variety of diseases. Destruction occurs in immune-mediated thrombocytopenia, in which platelets become coated with antiplatelet antibodies and are removed from the circulation by the fixed phagocyte system. Excessive sequestration of platelets by an enlarged spleen (hypersplenism) may occur in conditions such as myeloproliferative diseases.

Decreased production of platelets in the marrow may be caused by drugs, toxins, or by primary marrow disorders such as aplasia, fibrosis, or hematopoietic malignancy. In primary marrow disorders, more than one hematopoietic cell line is often decreased, resulting in pancytopenia.

Thrombocytosis is rare and often idiopathic. It may be associated with primary marrow disease such as in megakaryocytic leukemia. It is often associated with chronic blood loss and iron deficiency because of increased platelet production in the marrow reacting to continued consumption and loss.

Thrombocytopathies comprise a poorly defined group of diseases in which platelet numbers are normal but their function is impaired. Von Willebrand disease is characterized primarily by a defect in platelet adhesion to the endothelium. The platelets themselves are normal. Other hereditary disorders of platelet function have been described but are relatively rare. Probably the most common platelet function defect is the irreversible inhibition of thromboxane (which is necessary for platelet aggregation) caused by aspirin administration.

ANEMIA

Anemia is defined as an absolute decrease in the red cell mass as measured by RBC count, hemoglobin concentration, and/or PCV. It can develop from loss, destruction, or lack of production of RBCs. Anemia is classified as regenerative or nonregenerative.

With regenerative anemia, the bone marrow responds appropriately to the decreased red cell mass by increasing RBC production and releasing reticulocytes. With nonregenerative anemia, the bone marrow responds inadequately to the increased need for RBCs. Anemia caused by hemorrhage or hemolysis is typically regenerative. Anemia caused by decreased erythropoietin or an abnormality in the bone marrow is nonregenerative.

Clinical Findings: Clinical signs in anemic animals depend on the degree of anemia, the duration (acute or chronic), and the underlying cause. Acute anemia can result in shock and even death if more than a third of the blood volume is lost rapidly and not replaced. In acute blood loss, the animal usually presents with tachycardia, pale mucous membranes, bounding or weak peripheral pulses, and hypotension. The cause of the blood loss may be overt, eg, trauma. If no evidence of external bleeding is found, a source of internal or occult blood loss must be sought, eg, a ruptured splenic tumor, other neoplasia, coagulopathy, GI ulceration, or parasites. If hemolysis is present, the animal may be icteric. Animals with chronic anemia have had time to accommodate, and their clinical presentation is usually more indolent with vague signs of lethargy, weakness, and anorexia. These animals may have similar physical examination findings such as pale mucous membranes and weak peripheral pulses. The lack of expected clinical signs may alert the clinician to the time frame involved. Splenomegaly, abdominal distention, and/or heart murmur may be present, depending on the underlying cause of anemia.

Diagnosis: A complete history is an important part of the evaluation of an anemic animal. Questions might include duration of clinical signs, history of exposure to toxins (eg, rodenticides, heavy metals, toxic plants), drug treatments, vaccinations, travel history, and any prior illnesses.

A CBC, including a platelet and a reticulocyte count, will provide information on the severity of anemia and degree of bone marrow response, and also allow for evaluation of other cell lines. A blood smear should be evaluated for abnormalities in RBC morphology or size and for RBC parasites. The RBC indices (measures of size and hemoglobin concentration) are calculated by automated cell counters calibrated for the species in question. RBC size is expressed by the mean corpuscular volume (MCV) in femtoliters and can reflect the degree of regeneration. Macrocytosis (an increase in the MCV) usually correlates with a regenerative anemia. Macrocytosis can be a heritable condition in Poodles without anemia and may be seen in anemic cats infected with feline leukemia virus. Microcytosis (a decrease in the MCV) is the hallmark of iron-deficiency anemia. The hemoglobin concentration of each RBC, measured in g/dL, is defined as the mean corpuscular hemoglobin concentration (MCHC). Terms used for description of abnormalities with MCHC include normochromia and hypochromia. Abnormalities in RBC morphology, such as basophilic stippling, can indicate lead intoxication. Heinz body formation indicates oxidative injury to the RBCs, secondary to toxin exposure (*see* TABLE 1). Cats are more susceptible to Heinz body formation than other species, and cats without anemia can have a small number of Heinz bodies. The presence of schistocytes or spherocytes may also help identify the pathophysiology associated with the cause of anemia.

The reticulocyte count is usually reported as a percent of the RBC mass. This value should be corrected for the degree of anemia to evaluate the degree of regeneration. An absolute reticulocyte count (measured by RBCs/µL × reticulocyte percentage) of >50,000/µL in cats or >60,000/µL in dogs is considered regenerative. To correct the percent reticulocytes, the formula (*see* below) can be applied. A corrected reticulocyte percent >1% indicates regeneration in dogs and cats. After acute blood loss or hemolytic crisis, reticulocytosis usually takes 3–4 days to become evident.

A serum chemistry panel and urinalysis evaluate organ function. If GI blood loss is suspected, an examination of the feces for occult blood and parasites can be useful. Radiographs can help identify occult disease, such as a penny (zinc toxicity) in the stomach of a puppy with hemolytic anemia. Bruising or bleeding may be signs of a coagulopathy and indicate the need for a coagulation profile. Presence of petechiae or ecchymotic hemorrhage suggest significant thrombocytopenia or thrombocytopathy. If hemolytic disease is suspected, blood can be evaluated for autoagglutination, or a direct Coombs' test might be indicated. A test for autoagglutination can be done by placing a

$$\text{corrected reticulocyte \%} = (\text{observed reticulocyte \%}) \times \frac{\text{PCV of the patient}}{\text{normal PCV for that species}}$$

TABLE 1 TOXIC CAUSES OF ANEMIA

Pathogenic Mechanism	Drugs	Plants, Foods	Toxins, Chemicals	Heavy Metals
Oxidation	Acetaminophen, benzocaine, dapsone, nitrofurans, primaquine, propofol, quinacrine	Fava beans, oak, onions, propylene glycol, red maple	Crude oil, naphthalene	Copper, zinc
Blood loss	Aspirin, naproxen	Bracken fern, sweet clover	Dicoumarol	
Immune-mediated hemolysis	Cephalosporins, levamisole, penicillin, propylthiouracil, sulfonamides		Pirimicarb	
Hemolysis	Fenbendazole, heparin		Indole	Lead, selenium
Decreased marrow production	Amphotericin, azidothymidine, cephalosporins, chloramphenicol, estrogen, fenbendazole, griseofulvin, meclofenamic acid, phenobarbital, phenothiazine, phenylbutazone, propylthiouracil, quinidine, recombinant human erythropoietin, sulfonamides, thiacetarsamide	Bracken fern	Benzene, trichloro ethylene	Lead

drop of saline on a slide with a fresh drop of the animal's blood; the slide should be gently rotated to mix the drops together, then evaluated grossly and microscopically for macro- and microagglutination. If autoagglutination is present, there is no need to perform a Coombs' test. Serology or PCR for infectious agents such as feline leukemia virus, *Ehrlichia*, equine infectious anemia virus, and *Babesia* may also help define the cause of anemia (*see* TABLE 2).

Bone marrow evaluation by aspiration and/or biopsy (*see* p 14) is indicated in any animal with an unexplained, nonregenerative anemia. If the CBC reveals a decrease in more than one cell line, possibly indicating hypoplastic marrow, a biopsy would be indicated along with an aspirate. Biopsies and aspirates are complementary: biopsies are better to evaluate the architecture and degree of cellularity of the marrow, and aspirates allow for better evaluation of cellular morphology. Aspirates also allow for an evaluation of orderly maturation of the red and white blood cell lines, the ratio of myeloid to erythroid precursors (M:E ratio), and the number of platelet precursors. Iron stores can also be evaluated by Prussian blue staining. An M:E ratio of <1 indicates that red cell production is greater than white cell production; with an M:E ratio >1, the opposite is likely. The M:E ratio is always interpreted in light of a recent CBC, because changes in the ratio could also be due to suppression of one cell line compared with the other.

REGENERATIVE ANEMIAS

BLOOD LOSS ANEMIA

Acute blood loss can lead to shock and even death if >30%–40% of blood is lost and the hypovolemia that develops is not treated aggressively with IV fluids or compatible blood (*see* p 17), or both. Causes of acute loss can be known (eg, trauma, surgery) or occult. Coagulopathies, bleeding tumors, gastric ulceration, and external or internal parasites should be excluded as causes. GI parasites, such as *Haemonchus* in ruminants and hookworms in dogs, can lead to severe blood loss, especially in young animals. Low-grade, chronic blood loss eventually results in iron-deficiency anemia, although some degree of reticulocytosis may persist even after iron stores become depleted. The hallmark of an iron-deficiency anemia is microcytic, hypochromic anemia. This chronic blood loss can be due to some type of parasitism in young animals (eg, fleas, lice, intestinal parasitism), but in older animals, bleeding from GI ulcers or tumors is more common.

TABLE 2 INFECTIOUS CAUSES OF ANEMIA

Infectious Agent	Species Affected	Hemolytic	Marrow Affected
	BACTERIA		
Clostridium perfringens A	Cattle, sheep	Yes	No
Clostridium haemolyticum	Cattle, sheep	Yes	No
Leptospira interrogans	Cattle, pigs, sheep	Yes	No
Mycoplasma spp	Cats	±	Rarely
Haemobartonella spp	Cattle, cats	±	No
	VIRUSES		
Equine infectious anemia virus	Horses	±	Rarely
Feline leukemia virus	Cats	±	Yes
Feline immunodeficiency virus	Cats	No	Yes
	RICKETTSIA		
Mycoplasma spp	Cattle, goats, llamas, pigs, sheep[a]	Yes (piglets only)	No
Anaplasma spp	Cattle, goats, sheep	Yes	No
Ehrlichia spp	Dogs	Yes	Yes
	PROTOZOA		
Babesia spp	Cattle, cats, dogs, horses, sheep	Yes	No
Theileria spp[b]	Cattle, goats, sheep	±	No
Cytauxzoon spp	Cats	No	Yes
Trypanosoma spp	Cattle, horses, pigs	Yes	No
Sarcocystis cruzi	Cattle	Yes	No

[a] In adults, only clinically relevant in splenectomized or critically ill animals.

[b] Pathogenic species of *Theileria* are found in Africa, the Mediterranean, the Middle East, Asia, and Europe. Species found in North America are nonpathogenic.

HEMOLYTIC ANEMIA

Hemolytic anemias are typically regenerative and result from lysis of RBCs in either the intra- or extravascular space. Intravascular hemolysis results in hemoglobinemia and hemoglobinuria, whereas extravascular hemolysis does not. Both types of hemolysis can result in icterus. In dogs, the most common cause of hemolytic anemia is immune mediated (60%–75%), although toxins, RBC trauma, infections, and RBC membrane defects can also cause hemolysis.

Immune-mediated Hemolytic Anemia: Immune-mediated hemolytic anemia (IMHA, *see* p 826) can be primary or secondary to neoplasia, infectious agents, drugs, or vaccinations. In IMHA, the immune system no longer recognizes RBCs

as self and develops antibodies to circulating RBCs, leading to RBC destruction by macrophages and complement. In some cases, antibodies are directed against RBC precursors in the marrow, resulting in nonregenerative anemia. Animals with IMHA are usually icteric, sometimes febrile, and may have splenomegaly. Hematologic hallmarks of IMHA are regenerative anemia, hyperbilirubinemia, spherocytosis, autoagglutination, or a positive Coombs' test.

Another methodology to evaluate dogs for anti-RBC antibodies is flow cytometry. Flow cytometry allows for detection and quantitation of red cell surface–bound IgG and IgM. Flow cytometry was found to be 88%–100% specific for diagnosing dogs with anti-RBC antibodies. One report suggests using flow cytometry to assess response to treatment for dogs, because there is a decrease in surface anti-RBC antibodies before reticulocytosis or increase in RBC count. Flow cytometry may not be readily available to all veterinary hospitals.

Animals with IMHA can show mild, indolent signs or be in an acute crisis. It is important to tailor treatment to the animal's signs, including treating any underlying infections. Transfusion with packed RBCs is usually required. The goal of therapy is to stop the destruction of RBCs by treating with immunosuppressive drugs; supportive care is also a priority. Prednisone or prednisolone at a dosage of 1–2 mg/kg, bid, is considered first-line therapy, with azathioprine at 2 mg/kg/day (azathioprine is contraindicated in cats and may be replaced by chlorambucil) or cyclosporine at 5–10 mg/kg/day considered as a possible second agent. In one study, low-dose aspirin at 0.5 mg/kg/day improved survival times in dogs treated with azathioprine and prednisone. The veterinary literature is ambiguous on choice of second agent or when to introduce a second agent. Other immunosuppressive agents that have been used include mycophenolate and leflunomide.

In the acute hemolytic crisis, drugs such as cyclosporine (10 mg/kg/day, initially) or human intravenous immunoglobin (IVIG, 0.5–1.5 g/kg as a single dose) may also have benefit because of rapid onset of action.

Pulmonary thromboembolism is a risk in dogs with IMHA. These dogs are often hypercoagulable, which can be documented with thromboelastography. Dogs documented to be in a hypercoagulable state should be anticoagulated with heparin, which may be used in combination with antiplatelet therapy (aspirin 0.5 mg/kg/day with or without clopidogrel 1–2 mg/kg/day) if the platelet count is >40,000/µL. The dosing range for heparin is wide and variable, and dosage also depends on whether fractionated or unfractionated heparin is used. Heparin therapy can be monitored using activated partial thromboplastin time (APTT) or antifactor Xa concentrations (low-molecular-weight heparin).

Mortality rates for IMHA range from 20%–75%, depending on the severity of clinical signs. Negative prognostic indicators may include a rapid drop in PCV, high bilirubin concentration, moderate to marked leukocytosis (28,000 to >40,000 cells/µL), increased BUN, petechiae, intravascular hemolysis, autoagglutination, disseminated intravascular coagulation, and thromboembolic complications. Moderate to marked leukocytosis has been reported to be associated with tissue necrosis, most likely secondary to tissue hypoxia or thromboembolic disease. Referral to tertiary care facilities may improve survival.

Alloimmune Hemolysis: Neonatal isoerythrolysis (NI) is an immune-mediated hemolytic disease seen in newborn horses, mules, cattle, pigs, cats, and rarely dogs. NI is caused by ingestion of maternal colostrum containing antibodies to one of the neonate's blood group antigens. The maternal antibodies develop to specific foreign blood group antigens during previous pregnancies, unmatched transfusions, and from *Babesia* and *Anaplasma* vaccinations in cattle. Cats are unique in that blood type B cats have naturally occurring anti-A antibodies without prior exposure, and their kittens that are type A develop hemolysis after nursing. In horses, the antigens usually involved are A, C, and Q; NI is most commonly seen in Thoroughbreds and mules. Neonates with NI are normal at birth but develop severe hemolytic anemia within 2–3 days and become weak and icteric. Diagnosis is confirmed by screening maternal serum, plasma, or colostrum against the paternal or neonatal RBCs. Treatment consists of stopping any colostrum while giving supportive care with transfusions. If necessary, neonates can be transfused with triple-washed maternal RBCs. NI can be avoided by withholding maternal colostrum and giving colostrum from a maternal source free of the antibodies. The newborn's RBCs can be mixed with maternal serum to look for agglutination before the newborn is allowed to receive maternal colostrum.

Microangiopathic Hemolysis: Microangiopathic hemolysis is caused by RBC damage secondary to turbulent flow through abnormal vessels. In dogs, it can be seen secondary to severe heartworm infection, vascular tumors (hemangiosarcoma), splenic torsions, and disseminated intravascular coagulation; in other species, causes include hemolytic uremic syndrome in calves, equine infectious anemia, African swine fever, and chronic classical swine fever. Schistocytes are common in blood smears from these animals. Treatment involves correction of the underlying disease process.

Metabolic Causes of Hemolysis: Hypophosphatemia (*see* p 1000) causes postparturient hemoglobinuria and hemolysis in cattle, sheep, and goats. It can occur 2–6 wk after parturition. Hypophosphatemia with secondary hemolysis is seen in dogs and cats secondary to diabetes mellitus, hepatic lipidosis, and refeeding syndrome. Treatment with either oral or IV phosphorus is indicated, depending on the degree of hypophosphatemia. Cattle that drink too much water (water intoxication) are at risk of developing hemolysis secondary to hypotonic plasma. This is seen in calves 2–10 mo old and causes respiratory distress and hemoglobinuria. Clinical signs can progress to convulsions and coma. Hemolytic anemia, hyponatremia and hypochloremia, decreased serum osmolality, and low urine specific gravity in a calf would support the diagnosis of water intoxication. Treatment consists of hypertonic fluids (2.5% saline) and diuretics (eg, mannitol).

Toxins: Toxins and drugs can cause anemia by many mechanisms. Those implicated most frequently in animals and their pathogenic mechanisms are listed (*see* TABLE 1).

Infections: Many infectious agents—bacterial, viral, rickettsial, and protozoal—can cause anemia by direct damage to RBCs, leading to hemolysis, or by direct effects on precursors in the bone marrow (*see* TABLE 2).

Heritable Diseases: Several heritable RBC disorders cause anemia. Pyruvate kinase deficiencies are seen in Basenjis, Beagles, West Highland White Terriers, Cairn Terriers, and other breeds, as well as Abyssinian and Somali cats. Phosphofructokinase deficiency occurs in English Springer Spaniels. Deficiencies in these enzymes lead to shortened RBC life span and regenerative anemia. In dogs with phosphofructokinase deficiency, the hemolytic crises are set off by alkalosis secondary to excessive excitement or exercise. If such situations are minimized, these dogs may have a normal life expectancy. There is no treatment for pyruvate kinase deficiency, and affected dogs will have a shortened life span due to myelofibrosis and osteosclerosis of the bone marrow. Affected cats will have chronic intermittent hemolytic anemia, which is sometimes helped by splenectomy and steroids. Unlike dogs, cats have not been reported to develop osteosclerosis. A hereditary hemoglobinopathy, porphyria (*see* p 986), leads to build-up of porphyrins in the body and has been described in cattle, cats, and pigs. It is most prevalent in Holstein cattle and can lead to a hemolytic crisis. Affected calves fail to thrive and are photosensitive. Diagnosis is made by finding increased levels of porphyrins in bone marrow, urine, or plasma. Teeth of affected animals fluoresce under ultraviolet light.

NONREGENERATIVE ANEMIAS

NUTRITIONAL DEFICIENCIES

Nutritional deficiency anemias develop when micronutrients needed for RBC formation are not present in adequate amounts. Anemia develops gradually and may initially be regenerative but ultimately becomes nonregenerative. Starvation causes anemia by a combination of vitamin and mineral deficiencies as well as a negative energy and protein balance. Deficiencies most likely to cause anemia are iron, copper, cobalamin (B_{12}), B_6, riboflavin, niacin, vitamin E, and vitamin C (important only in primates and guinea pigs).

Iron deficiency is the most common deficiency seen in dogs and piglets but occurs less commonly in horses, cats, and ruminants. Iron deficiency is rarely nutritional in origin—it most commonly occurs secondary to blood loss (*see* p 9). Young animals have minimal iron stores, and milk contains very little iron. This can be especially important for piglets that grow rapidly and are often raised indoors with no access to iron. Oral iron supplementation is indicated as treatment for iron deficiency; any source of blood loss must be eliminated.

Copper deficiency can develop in ruminants fed forage grown in copper-deficient soil. Copper is necessary for the

metabolism of iron. Copper deficiency may occur secondary to high dietary molybdenum or sulfate in cattle and can develop in pigs fed whey diets. Low blood copper concentrations or low copper concentrations in liver biopsies (more definitive) are diagnostic. Treatment is oral or injectable copper supplementation.

B vitamin deficiencies are rare. Certain drugs (anticonvulsants, drugs that interfere with folate metabolism) have been associated with development of folate or cobalamin deficiency, leading to a normocytic, normochromic, nonregenerative anemia. Cobalamin malabsorption has been reported in Giant Schnauzers (their enterocytes are unable to absorb cobalamin). These dogs respond to parenteral supplementation with cobalamin. Ruminants also develop a secondary cobalamin deficiency when grazing on cobalt-deficient pasture. Treatment with oral cobalt or parenteral cobalamin is indicated.

ANEMIA OF CHRONIC DISEASE

Anemia of chronic disease can be characterized as mild to moderate, nonregenerative, normochromic, and normocytic. It is the most common form of anemia seen in animals. The anemia can be secondary to chronic inflammation or infection, neoplasia, liver disease, hyper- or hypoadrenocorticism, or hypothyroidism. The anemia is mediated by cytokines produced by inflammatory cells, which lead to decreases in iron availability, RBC survival, and the marrow's ability to regenerate. Treatment should be directed at the underlying disease and often results in resolution of the anemia. The anemia may be reduced by treatment with recombinant human erythropoietin, but the risk of antibody formation to endogenous erythropoietin may outweigh benefit. Darbepoetin appears to have less impact to induce reactive antibodies.

RENAL DISEASE

Chronic renal disease is a common cause of nonregenerative anemia in animals. Erythropoietin is normally produced by the peritubular endothelial cells in the renal cortex. Animals with renal disease produce less erythropoietin, leading to anemia. Recombinant human erythropoietin (44–132 U/kg, three times/wk, with most animals starting at 88 U/kg) has been used for treatment. PCV is monitored weekly until the desired improvement is reached (this will vary with the initial degree of anemia), after which the dosage is decreased. Animals receiving recombinant human erythropoietin require supplemental iron to support RBC production. (*See also* HEMATINICS, p 2540.) Darbepoeitin also has been found to be valuable in management of anemia associated with chronic kidney disease.

PRIMARY BONE MARROW DISEASES

Primary bone marrow disease or failure from any cause can lead to nonregenerative anemia and pancytopenia. With diffuse marrow involvement, granulocytes are affected first, followed by platelets and finally RBCs.

Aplastic anemia has been reported in dogs, cats, ruminants, horses, and pigs with pancytopenia and a hypoplastic marrow, replaced by fat. Most cases are idiopathic, but reported causes include infection (feline leukemia virus, *Ehrlichia*, parvovirus), drug therapy (methimazole, chemotherapeutic agents, antibiotics [trimethoprim-sulfa, chloramphemicol], fenbendazole), toxin ingestion (estrogen), and total body irradiation (*see* TABLES 1 and 2). There may also be an immune-mediated component to this disease. Diagnosis is confirmed by bone marrow aspiration and core biopsy. Treatment consists of eliminating the underlying cause and providing supportive measures such as broad-spectrum antibiotics and transfusions. Immunosuppressive agents such as prednisone, cyclosporine, mycophenolate, or azathioprine may be considered. Recombinant human erythropoietin and granulocyte colony-stimulating factor (5 mcg/kg/day, PO) can be used until the marrow recovers. If the disease is idiopathic or if marrow recovery is unlikely (eg, phenylbutazone toxicity in dogs), bone marrow transplantation is beneficial if a suitable donor is available (investigational and limited availability).

In **pure red cell aplasia** (PRCA), only the erythroid line is affected. It is characterized by a nonregenerative anemia with severe depletion of red cell precursors in the bone marrow. It has been reported in dogs and cats and may be primary or secondary. Primary cases are most commonly immune mediated and may respond to immunosuppressive therapy. Supportive care, including transfusion, may be indicated when anemia is severe. Feline leukemia–positive cats can have PRCA. Recombinant human erythropoietin has been reported to cause PRCA in dogs and

horses. Discontinuation of therapy may eventually lead to RBC recovery in some animals.

Primary leukemias are uncommon to rare in domestic species but have been reported in dogs, cats, cattle, goats, sheep, pigs, and horses. Retroviruses are a cause in some cattle, cats, primates, and chickens. Leukemias can develop in myeloid or lymphoid cell lines and are further classified as acute or chronic. Most affected animals have nonregenerative anemia, neutropenia, and thrombocytopenia, with circulating blasts usually present. Acute leukemias, characterized by infiltration of the marrow with blasts, generally respond poorly to chemotherapy. In animals that do respond, remission times are usually short. In acute lymphoblastic leukemia in dogs, the response rate to chemotherapy is ~30%, with a median survival of 4 mo. Acute myeloblastic leukemias are less common and even less responsive to treatment than acute lymphoblastic leukemia. In acute leukemias, the cell lineage is often difficult to identify morphologically, so cytochemical stains or immunologic evaluation of cell surface markers may be necessary for definitive diagnosis. Chronic leukemias, characterized by an overproduction of one hematopoietic cell line, are less likely to cause anemia and more responsive to treatment.

Myelodysplasia (myelodysplastic syndrome, MDS) is considered a preleukemic syndrome characterized by ineffective hematopoiesis, resulting in a nonregenerative anemia or other cytopenias. MDS has been described in dogs, cats, and people. The disease can be primary or secondary and is commonly seen in cats with feline leukemia. Primary syndromes probably arise from mutations in stem cells. Secondary syndromes are caused by other neoplasia or drug therapy. Some cats and dogs respond to treatment with recombinant human erythropoietin and prednisone. Supportive care with transfusions may be helpful. Survival is variable because MDS can progress to leukemia; many animals are euthanized or die of sepsis, bleeding, or anemia.

Myelofibrosis causes bone marrow failure secondary to replacement of normal marrow elements with fibrous tissue. It has been seen in dogs, cats, people, and goats. It can be a primary disorder or secondary to malignancies, immune-mediated hemolytic anemia, whole body irradiation, and congenital anemias (eg, pyruvate kinase deficiency). Diagnosis can be made by bone marrow biopsy. Treatment varies with the underlying cause but usually consists of immunosuppressive therapy.

Bone Marrow Aspiration and Biopsy: Bone marrow aspiration and biopsy are techniques used to evaluate the bone marrow in domestic animal species. The basic technique involves introducing a hollow needle into the bone marrow to obtain a sample for evaluation. Bone marrow aspiration provides a sample for cytologic evaluation, and bone marrow biopsy provides a sample for histopathologic evaluation.

Specific clinical indications to evaluate bone marrow include but are not limited to investigation of nonregenerative anemia, thrombocytopenia, leukopenia, bicytopenia, pancytopenia, abnormal circulating cells of any type, monoclonal gammopathy, suspected osteomyelitis, suspected bone neoplasia, infectious disease affecting the bone, and clinical staging of neoplastic processes such as lymphoma and mast cell disease.

The conventional anatomic sites used for bone marrow aspiration include the iliac crest, the trochanteric fossa of the femur, the tibial crest, and the greater tubercle of the humerus. Some clinicians have also used the rib (costochondral junction) or sternabrae. The humerus is the most common site for bone marrow biopsy.

For bone marrow aspiration, cats and dogs generally require sedation, although some cats may require general anesthesia. General anesthesia is required for bone marrow biopsy. The animal is positioned in lateral recumbency when using the trochanteric fossa or the greater tubercle, and in sternal recumbency when using the iliac crest. The area to be accessed is shaved and aseptically prepared. The site, including the periosteum, is infiltrated with local anesthetic. A #11 scalpel blade is then used to make a stab incision through the skin.

The equipment used for these procedures may differ slightly. Most bone marrow aspiration needles (eg, Rosenthal, Illinois) are very similar and have a removable stylet. With the stylet in place, the needle is advanced with a back and forth screw-like motion until the needle is well seated in the bone. The stylet is then removed, and a syringe (6–12 mL) attached for aspiration. If the animal feels any discomfort, this is when it will occur. Only a small sample is required, ie, enough to fill the syringe hub, which should be put onto slides for cytologic evaluation. The needle can then be removed.

Bone marrow biopsy generally requires a Jamshidi needle, which is rigid and hollow with a stylet. The Jamshidi needle has a cutting edge at its end, designed to obtain a core marrow sample (although it can also be used to obtain a bone marrow aspirate). The needle is driven into the bone with a back and forth, screw-like motion. Once the needle is well seated, the cap is unscrewed and the stylet removed. An aspirate can be obtained similar to the technique described above. To obtain a core sample for histopathology, the needle is advanced further (about ¼ in.) into the marrow and twisted in one direction. The needle is then moved in a wide, circular motion to try to dislodge a core sample. The needle is removed by twisting in the opposite direction in which it was advanced. A blunt stylet is then passed retrograde to remove the core sample. The core can be gently rolled onto a slide for cytology and then placed into formalin for histopathology.

BLOOD GROUPS AND BLOOD TRANSFUSIONS

Blood groups are determined by genetically controlled, polymorphic, antigenic components of the RBC membrane. The allelic products of a particular genetic locus are classified as a blood group system. Some of these systems are highly complex, with many alleles defined at a locus; others consist of a single defined antigen. Blood group systems, in general, are independent of each other, and their inheritance conforms to Mendelian dominance. For polymorphic blood group systems, an animal usually inherits one allele from each parent and thus expresses no more than two blood group antigens of a system. An exception is in cattle, in which multiple alleles, or "phenogroups," are inherited. Normally, an individual does not have antibodies against any of the antigens present on its own or against other blood group antigens of that species' systems unless they have been induced by transfusion, pregnancy, or immunization. In some species (people, sheep, cattle, pigs, horses, cats, and dogs), so-called "naturally occurring" isoantibodies, not induced by transfusion or pregnancy, may be present in variable but detectable titers. For example, Group B cats have naturally occurring anti-A antibody. Also, circulating antibodies to animal blood group antigens may be induced by transfusion. With random blood transfusions in dogs, there is a 30%–40% chance of sensitization of the recipient, primarily to blood group antigen DEA 1, but risk is also present for development of antibody to any other antigen lacked by the recipient. In horses, transplacental immunization of the mare by an incompatible fetal antigen inherited from the sire may occur. Immunization also may result when some homologous blood products are used as vaccines (eg, anaplasmosis in cattle). In dogs, prior pregnancy does not result in sensitization of the bitch to foreign blood group antigens.

The number of major recognized blood group systems (*see* TABLE 3) varies among domestic species, with cattle being the most complex and cats the simplest. Animal

TABLE 3 MAJOR BLOOD GROUPS OF CLINICAL INTEREST

Species	Blood Group
Canine	DEA 1.1 and 7
Feline	A, B, *mic*
Equine	A, C, Q
Bovine	B, J
Ovine	B, R

blood groups are typed to aid in the matching of donors and recipients and, especially in horses, to identify breeding pairs potentially at risk of causing hemolytic disease in their offspring. Because expression of blood group antigens is genetically controlled and the modes of inheritance are understood, these systems also have been used to substantiate pedigrees in cattle and horses; however, in most cases, DNA testing has replaced blood typing for paternity testing.

BLOOD TYPING

Antisera used to identify blood groups (typing reagents) usually are produced as isoimmune sera. Their in vitro serologic characteristics vary with the species. Many reagents are hemagglutinins; others are hemolytic and require complement to complete the serologic reaction, such as in cattle (because RBCs do not readily agglutinate) and horses (because RBC rouleaux are a problem). Other typing reagents, neither hemagglutinating nor hemolytic, combine with RBC antigens in an "incomplete" reaction because they lack additional combining sites to agglutinate other RBCs; addition of species-specific antiglobulin is required for agglutination.

The diversity of blood groups in animals and the lack of commercially available blood-typing reagents to all antigens make complete typing difficult but should not preclude the clinical use of transfusions. In horses and dogs, the blood group antigens most commonly implicated in transfusion incompatibilities are known; by selecting donor animals that lack these groups, or that match the recipient, the risk of sensitization of the recipient to the most important antigens can be minimized. For dogs and cats, commercially available, point-of-care testing for major antigens is available in either gel or card testing kits. Reagents are available for only some antigens, generally those that are most likely to sensitize a recipient, or those for which naturally occurring antibodies, primarily in cats, might be present. For example, dogs have more than 12 blood group systems but generally are typed for only one (DEA 1.1). An additional blood group antigen (dal) was discovered when a dal-negative, previously transfused Dalmatian reacted to many potential donors, and only a few Dalmatians were found to be compatible. It is a common antigen in most dogs but is lacking in some Dalmatians. Because multiple blood group antigens are present, it is likely that an animal receiving a transfusion might be exposed to some antigens that are not present on its RBCs.

CROSSMATCHING

Previously sensitized recipients or those with naturally occurring antibodies can be detected by crossmatching, which is done to preclude administration of incompatible blood. In the USA, >99% of cats are of blood group A, so the risk of incompatible transfusion is low. However, certain breeds, including Abyssinian, Birman, British Shorthair, Devon Rex, Himalayan, Persian, Scottish Fold, and Somali, have a higher frequency of blood group B. Any incompatible transfusion in cats results in rapid destruction of transfused cells, so typing and crossmatching should be done before any transfusion. The *mic* antigen is present in some cats, and naturally occurring antibodies are present in cats that lack the *mic* antigen. For that reason, crossmatching should be performed for cats before the first transfusion, even if they will receive A or B matched blood.

The direct crossmatch procedure, with appropriate controls, is effective for all species. The **major crossmatch** detects antibodies already present in recipient plasma that could cause a hemolytic reaction when donor RBCs are transfused; it will not detect the potential for sensitization to develop. Anticoagulant (calcium disodium edetate or citrate) is added to blood samples from donor and recipient; the donor RBCs are washed 3 times with 0.9% saline, and a 4% RBC suspension in saline is made from the washed cells. The major crossmatch consists of combining equal volumes (0.1 mL) of the donor RBC suspension and recipient plasma. The control tube contains recipient RBCs and recipient plasma. The samples are incubated, centrifuged, and evaluated for hemolysis or agglutination. Hemolysis is evaluated by comparing the color of the supernatant in the test sample with that of the control sample. Each sample is then gently shaken until all cells in the "button" at the bottom of the tube have returned to suspension. Again, the degree of cell clumping of the test sample is compared with that of the control sample. The test is negative, or compatible, when the plasma is clear and the RBCs are readily suspended. A positive, or incompatible, test can have hemolysis or hemagglutination or both. All tests judged macroscopically to be negative for hemagglutination should be confirmed microscopically at low power. Some newer

crossmatching systems that use a gel technique are becoming available. This is particularly important in horses, because their RBCs tend to form rouleaux.

The **minor crossmatch** is the reverse of the major crossmatch, ie, recipient cells are combined with donor plasma. The minor crossmatch is important only in species such as cats with clinically significant naturally occurring isoantibodies or if the donor has been previously transfused or, in horses, those with previous pregnancies.

BLOOD TRANSFUSIONS

Frequently, the need for blood transfusions is acute, as in acute hemolysis or hemorrhage; transfusions are also appropriate in treatment of acute or chronic anemias. Animals with hemostatic disorders often require repeated transfusions of whole blood, red cells, plasma, or platelets. Blood transfusions must be given with care, because they have the potential to further compromise the recipient.

Whole blood frequently is not the ideal product to be administered. If the need is to replace the oxygen-carrying capability of the blood, then packed RBCs are more appropriate; if replacement of circulatory volume is needed, crystalloid or colloid solutions may be used, with packed RBCs added as needed. Platelet numbers rise rapidly after hemorrhage, so replacement is rarely needed. Plasma proteins equilibrate from the interstitial space, so plasma is not needed except in massive hemorrhage (>1 blood volume in 24 hr). Animals that require coagulation factors benefit most from administration of fresh-frozen plasma or cryoprecipitate if the need is specifically for factor VIII, von Willebrand factor, or fibrinogen. Platelet-rich plasma or platelet concentrates may be of value in thrombocytopenia, although immune-mediated thrombocytopenia usually does not respond to administration of platelets because they are removed rapidly by the spleen.

The decision to transfuse RBCs is determined by clinical signs, not by any pre-selected PCV. Animals with acute anemia show signs of weakness, tachycardia, and tachypnea at a higher PCV than animals with chronic anemia. The amount of RBCs required to relieve clinical signs will generally increase the PCV above 20%. Domestic animals have blood volumes of 7%–9% of their body weight; cats have a slightly lower volume of ~6.5%. By determining the recipient's blood volume and knowing the animal's PCV, the required replacement RBC volume can be calculated. For example, a 25-kg dog has a total blood volume of ~2,000 mL; with a PCV of 15%, the RBC volume is 300 mL; if the PCV is to be increased to 20%, that equals an RBC volume of 400 mL. Therefore, 100 mL of RBCs or 200 mL of whole blood (with PCV of 50%) would be required to increase the recipient's PCV to the desired level. These calculations assume no ongoing losses of RBCs through hemorrhage or hemolysis. Obviously, the post-transfusion PCV is the most important measure of adequacy of red cell dose. No more than 20% of a donor animal's blood should be collected at one time.

Collection, storage, and transfusion of blood must be done aseptically. The anticoagulant of choice is citrate phosphate dextrose adenine (CPDA-1). Commercial blood bags containing the appropriate amount of anticoagulant for a "unit" (500 mL) are available. Heparin should not be used as an anticoagulant, because it has a longer half-life in the recipient and causes platelet activation; also, heparinized blood cannot be stored.

Blood collected in CPDA-1 with added RBC preservation or nutrient solutions may be safely stored at 4°C for 4 wk. If the blood will not be used immediately, the plasma can be removed and stored frozen for later use as a source of coagulation factors or albumin for acute reversible hypoalbuminemia. Plasma must be frozen at –20° to –30°C within 6 hr of collection to assure that levels of factor VIII are adequate and will remain so for 1 yr. Chronic hypoproteinemia is not helped by plasma, because the total body deficit of albumin is so large that it could not be improved by the small amount contained in plasma. Colloid solutions such as hetastarch are more effective for treatment of hypoalbuminemia. Human albumin has been used in dogs; however, the risk of sensitization and allergic reactions is significant.

Risks of Transfusion: The most serious risk of transfusion is acute hemolysis. Fortunately, this is rare in domestic animals. Dogs rarely have clinically significant preformed antibodies, so only those that have received repeated transfusions are at risk. The most common hemolytic reaction in dogs that have received multiple transfusions is delayed hemolysis, seen clinically as shortened survival of transfused RBCs and a positive Coombs' test. Even crossmatch-compatible RBCs given to horses or cattle survive only 2–4 days. Nonimmune causes of hemolysis include improper collection or separation of blood,

freezing or overwarming of RBCs, and infusing under pressure through a small needle.

Other complications include sepsis from contaminated blood, hypocalcemia from too much citrate, and hypervolemia (especially in animals with preexisting heart disease or in very small animals). Urticaria, fever, or vomiting are seen occasionally. Transfusions can also spread disease from donor to recipient, such as RBC parasites (eg, *Mycoplasma* in cats or *Babesia* in dogs) and viruses (eg, retroviruses in cats, horses, or cattle). Other diseases, such as those caused by rickettsia or other bacteria, can also be spread if the donor is bacteremic. Donors should be tested periodically for infectious diseases that are prevalent locally. Flea and tick prevention is also important to prevent vector-borne infections in donors.

BLOOD SUBSTITUTES

(Hemoglobin-based oxygen carrier solutions)

Because of problems associated with finding compatible donors and disease transmission by transfusion, the search for a red cell substitute has been ongoing for >50 yr. An ideal substitute would carry and deliver oxygen like red cells, be easy to produce in large quantities, be nonantigenic, and persist in the circulation at least long enough for resuscitation.

One hemoglobin-based oxygen carrier of bovine origin is currently licensed for use in dogs. The hemoglobin is collected aseptically, filtered to remove all red cell stromal elements, and polymerized to allow the product to persist in the circulation for a half-life of ~36 hr. This product has been shown to carry and deliver oxygen efficiently, can be used immediately without need for typing or crossmatching, and has a 3-yr shelf life at room temperature. Because the structure of the hemoglobin molecule is similar between species, bovine hemoglobin is minimally antigenic. Although currently licensed for use only in dogs, it has been used in cats, horses, llamas, birds, and people. Its colloidal effects are especially useful in resuscitation after trauma with acute blood loss. Because the cost of hemoglobin solution is often higher, and duration of effect is shorter than that of blood, the main value of hemoglobin is in emergency situations when blood is not immediately available. Volume overload is a potential risk if hemoglobin is given too rapidly. Another concern with hemoglobin solutions is that nitric oxide is scavenged and removed by the product. This paradoxically might cause vasoconstriction and decrease oxygen delivery to ischemic tissues.

BLOOD PARASITES

ANAPLASMOSIS

Anaplasmosis, formerly known as gall sickness, traditionally refers to a disease of ruminants caused by obligate intraerythrocytic bacteria of the order Rickettsiales, family Anaplasmataceae, genus *Anaplasma*. Cattle, sheep, goats, buffalo, and some wild ruminants can be infected with the erythrocytic *Anaplasma*. Anaplasmosis occurs in tropical and subtropical regions worldwide (~40°N to 32°S), including South and Central America, the USA, southern Europe, Africa, Asia, and Australia.

The *Anaplasma* genus also includes *A phagocytophilum* (compiled from species previously known as *Ehrlichia phagocytophila*, *E equi*, and human granulocytic ehrlichiosis agent, *see* p 803), *A bovis* (formerly *E bovis*), and *A platys* (formerly *E platys*), all of which invade blood cells other than erythrocytes of their respective mammalian hosts. Bovine anaplasmosis is of economic significance in the cattle industry.

Etiology and Pathogenesis: Clinical bovine anaplasmosis is usually caused by *A marginale*. An *A marginale* with an appendage has been called *A caudatum*, but it is not considered to be a separate species. Cattle are also infected with *A centrale*, which generally results in mild disease. *A ovis* may cause mild to severe disease in sheep, deer, and goats. *A phagocytophilum* has recently been reported to infect cattle; however, natural infection is rare and it does not cause clinical disease.

Transmission and Epidemiology: Up to 17 different tick vector species (including *Dermacentor*, *Rhipicephalus*, *Ixodes*, *Hyalomma*, and *Argas*) have been reported to transmit *Anaplasma* spp. Not all of these are likely significant vectors in the field, and it has been shown that strains of *A marginale* also coevolve with particular tick strains. *Rhipicephalus* (*Boophilus*) spp are major vectors in Australia and Africa, and *Dermacentor* spp have been incriminated as the main vectors in the USA. After feeding on an infected animal, intrastadial or trans-stadial transmission may occur. Transovarial transmission may also occur, although this is rare, even in the single-host *Rhipicephalus* spp. A replicative cycle occurs in the infected tick. Mechanical transmission via biting dipterans occurs in some regions. Transplacental transmission has been reported and is usually associated with acute infection of the dam in the second or third trimester of gestation. Anaplasmosis may also be spread through the use of contaminated needles or dehorning or other surgical instruments.

There is a strong correlation between age of cattle and severity of disease. Calves are much more resistant to disease (although not infection) than older cattle. This resistance is not due to colostral antibody from immune dams. In endemic areas where cattle first become infected with *A marginale* early in life, losses due to anaplasmosis are minimal. After recovery from the acute phase of infection, cattle remain chronically infected carriers but are generally immune to further clinical disease. However, these chronically infected cattle may relapse to anaplasmosis when immunosuppressed (eg, by corticosteroids), when infected with other pathogens, or after splenectomy. Carriers serve as a reservoir for further transmission. Serious losses occur when mature cattle with no previous exposure are moved into endemic areas or under endemically unstable situations when transmission rates are insufficient to ensure that all cattle are infected before reaching the more susceptible adult age.

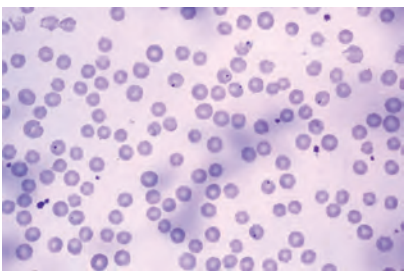
Clinical Findings: In animals <1 yr old anaplasmosis is usually subclinical, in yearlings and 2-yr-olds it is moderately severe, and in older cattle it is severe and often fatal. Anaplasmosis is characterized by progressive anemia due to extravascular destruction of infected and uninfected erythrocytes. The prepatent period of *A marginale* is directly related to the infective dose and typically ranges from 15–36 days (although it may be as long as 100 days). After the prepatent period, peracute (most severe but rare), acute, or chronic anaplasmosis may follow. Rickettsemia approximately doubles every 24 hr during the exponential growth phase. Generally, 10%–30% of erythrocytes are infected at peak rickettsemia, although this figure may be as high as 65%. RBC count, PCV, and hemoglobin values are all severely reduced. Macrocytic anemia with circulating reticulocytes may be present late in the disease.

Animals with peracute infections succumb within a few hours of the onset of clinical signs. Acutely infected animals lose condition rapidly. Milk production falls. Inappetence, loss of coordination, breathlessness when exerted, and a rapid bounding pulse are usually evident in the late stages. The urine may be brown but, in contrast to babesiosis, hemoglobinuria does not occur. A transient febrile response, with the body temperature rarely exceeding 106°F (41°C) occurs at about the time of peak rickettsemia. Mucous membranes appear pale and then yellow. Pregnant cows may abort. Surviving cattle convalesce over several weeks, during which hematologic parameters gradually return to normal.

Bos indicus breeds of cattle appear to possess a greater resistance to *A marginale* infection than *B taurus* breeds, but variation of resistance of individuals within breeds of both species occurs. Differences in virulence between *Anaplasma* strains and the level and duration of the rickettsemia also play a role in severity of clinical manifestations.

Lesions: Lesions are typical of those found in animals with anemia due to erythrophagocytosis. The carcasses of cattle that die from anaplasmosis are generally markedly anemic and jaundiced. Blood is thin and watery. The spleen is characteristically enlarged and soft, with prominent follicles. The liver may be mottled and yellow-orange. The gallbladder is often distended and contains thick brown or green bile. Hepatic and mediastinal lymph nodes appear brown. There are serous effusions in body cavities, pulmonary edema, petechial hemorrhages in the epi- and endocardium, and often evidence of severe GI stasis. Widespread phagocytosis of erythrocytes is evident on microscopic examination of the reticuloendothelial organs. A significant proportion of erythrocytes are usually found to be parasitized after death due to acute infection.

Diagnosis: *A marginale*, together with the hemoprotozoa *Babesia bovis* and *B bigemina*, are the causative agents of tick fever in cattle. These three species have similar geographic distributions, except that anaplasmosis occurs in the absence of babesiosis in the USA. Microscopic examination of Giemsa-stained thin and thick blood films is critical to distinguish anaplasmosis from babesiosis (*see* p 21) and other conditions that result in anemia and jaundice, such as leptospirosis (*see* p 646) and theileriosis (*see* p 33). Blood in anticoagulant should also be obtained for hematologic testing. In Giemsa-stained thin blood films, *Anaplasma* spp appear as dense, homogeneously staining blue-purple inclusions 0.3–1 µm in diameter. *A marginale* inclusions are usually located toward the margin of the infected erythrocyte, whereas *A centrale* inclusion bodies are located more centrally. *A caudatum* cannot be distinguished from *A marginale* using Giemsa-stained blood films. Special staining techniques are used to identify this species based on observation of characteristic appendages associated with the bacteria. *A caudatum* has been reported only in North America and could possibly be a morphologic form of *A marginale* and not a separate species. Inclusion bodies contain 1–8 initial bodies 0.3–0.4 µm in diameter, which are the individual rickettsiae. The percentage of infected erythrocytes varies with the stage and severity of disease; maximum rickettsemias in excess of 50% can occur with *A marginale*. Microscopically, the infection becomes visible 2–6 wk after transmission. During the course of infection, the rickettsemia can double each day for up to 10 days and then decreases. Severe anemia can persist for weeks after parasites cannot be detected in blood smears.



Anaplasma marginale in bovine blood, Wright-Giemsa, 100× oil immersion. Intracellular organisms appear as basophilic, spherical inclusions generally located near the margin of erythrocytes. Echinocytes are frequently present. *Courtesy of Ms. Sue Anderson, Tick Fever Centre, Wacol, Queensland, Australia.*

Chronically infected carriers may be identified with a fair degree of accuracy by serologic testing using the msp5 ELISA, complement fixation, or card agglutination tests. Nucleic acid–based detection methods are most useful, because species and strain differentiation tests may not detect carrier levels.

At necropsy, thin blood films of liver, kidney, spleen, lungs, and peripheral blood should be prepared for microscopic examination.

Treatment, Control, and Prevention: Tetracycline antibiotics and imidocarb are currently used for treatment. Cattle may be sterilized by treatment with these drugs and remain immune to severe anaplasmosis subsequently for at least 8 mo.

Prompt administration of tetracycline drugs (tetracycline, chlortetracycline, oxytetracycline, rolitetracycline, doxycycline, minocycline) in the early stages of acute disease (eg, PCV >15%) usually ensures survival. A commonly used treatment consists of a single IM injection of long-acting oxytetracycline at a dosage of 20 mg/kg. Blood transfusion to partially restore the PCV greatly improves the survival rate of more severely affected cattle. The carrier state may be eliminated by administration of a long-acting oxytetracycline preparation (20 mg/kg, IM, at least two injections with a 1-wk interval). Withholding periods for tetracyclines apply in most countries. Injection into the neck muscle rather than the rump is preferred.

Imidocarb is also highly efficacious against *A marginale* as a single injection (as the dihydrochloride salt at 1.5 mg/kg, SC, or as imidocarb dipropionate at 3 mg/kg). Elimination of the carrier state requires the use of higher repeated doses of imidocarb (eg, 5 mg/kg, IM or SC, two injections of the dihydrochloride salt 2 wk apart). Imidocarb is a suspected carcinogen with long withholding periods and is not approved for use in the USA or Europe.

In South Africa, Australia, Israel, and South America, infection with live *A centrale* (originating from South Africa) is used as a vaccine to provide cattle with partial protection against the disease caused by *A marginale*. *A centrale* (single dose) vaccine produces severe reactions in a small proportion of cattle. In the USA, where live vaccines cannot be used,