DUNCAN & PRASSE'S VETERINARY LABORATORY MEDICINE: CLINICAL PATHOLOGY

Fifth Edition

CHAPTER 1

ERYTHROCYTES

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BASIC CONCEPTS OF ERYTHROCYTE FUNCTION, METABOLISM, PRODUCTION, AND BREAKDOWN

I. THE ERYTHRON

A. This widely dispersed mass of erythroid cells includes circulating erythrocytes and bone marrow precursor, progenitor, and stem cells.

B. Its function is oxygen transport, which is mediated by hemoglobin.

C. Hemoglobin is transported in erythrocytes whose membrane, shape, cytoskeleton, and metabolic processes ensure survival of the cell against the stresses of circulation and various injurious substances.

D. Hemoglobin consists of heme and globin, and each complete hemoglobin molecule is a tetramer.

1. Each heme moiety contains an iron atom in the 2^+ valence state (Fe^{2+}).

2. A globin chain of specific amino acid sequence is attached to each heme group.

3. The complete hemoglobin molecule is a tetramer, containing four heme units and four globin chains. The globin chains are identical pairs (dimers), designated as α-chains or δ-chains.

II. HEME SYNTHESIS

A. Heme synthesis is unidirectional and irreversible. It is controlled at the first step by the enzyme δ-aminolevulinic acid synthase, whose synthesis is controlled by negative feedback from heme concentration within the erythrocyte.

1. Lead inhibits most of the steps in heme synthesis to some degree. Lead also inhibits the delivery of iron to the site of ferrochelatase activity.

2. Chloramphenicol may inhibit heme synthesis.

B. Porphyrins and their precursors are the intermediates of heme biosynthesis.

1. Certain enzyme deficiencies in the synthetic pathway can lead to excessive accumulation of porphyrins and their precursors.

2. These excesses of porphyrins and their precursors are called porphyrias.

3. Porphyrias vary in the intermediate products that accumulate and in their clinical manifestations.

4. These excess porphyrins escape the erythrocyte and may be deposited in the tissues or excreted in the urine and other body fluids.

C. After formation of protoporphyrin, iron is inserted into the molecule by ferrochelatase, and heme is formed.

III. GLOBIN SYNTHESIS

A. Each hemoglobin molecule is comprised of four globin chains, each of which binds to a heme group.
- **1.** The hemoglobin type depends on the type of globin chains, which are determined by amino acid sequences.
  - **a.** Embryonic, fetal, and adult hemoglobins are found in various animals.
  - **b.** The presence and number of each hemoglobin type vary with the species.
- **2.** Heme and globin synthesis are balanced (increase in one results in an increase in the other).

B. Abnormalities in globin synthesis (i.e., hemoglobinopathies) have not been described in domestic animals.

IV. IRON METABOLISM

Body iron metabolism/content is based on an extremely efficient system of conservation and recycling that is regulated by the rate of duodenal absorption rather than excretion. Hepcidin is a recently identified 25 amino acid peptide (bioactive form) produced within the liver and transported within the blood by α-2-macroglobulin. It has been found to play a key role in mediation of iron metabolism. In short, increased hepcidin is accompanied by a decrease in iron availability, whereas decreased Hepcidin is associated with an increase in iron availability. Hepcidin is a component of the type II acute phase response induced by interleukin-6 and controls plasma iron concentration by inhibiting iron export by ferroportin from enterocytes and macrophages. Absorption is regulated by the amount of storage iron (large iron stores decrease absorption) and rate of erythropoiesis (accelerated erythropoiesis increases absorption). Less than 0.05% of the total body iron is acquired or lost each day.

A. Iron is transported in blood bound to the δ-globulin, transferrin.
- **1.** Iron bound to transferrin is measured as serum iron (SI). This is an unreliable measure of total body iron stores.
  - **a.** Conditions with decreased SI
    - **(1)** Iron deficiency
    - **(2)** Acute and chronic inflammation or disease (including anemia of inflammatory disease)
    - **(3)** Hypoproteinemia
    - **(4)** Hypothyroidism
    - **(5)** Renal disease
  - **b.** Conditions with increased SI
    - **(1)** Hemolytic anemia
    - **(2)** Accidental lysis of erythrocytes during sampling (hemolysis)
    - **(3)** Glucocorticoid excess in the dog and horse. In contrast, SI is decreased in cattle with glucocorticoid excess.
    - **(4)** Iron overload, which may be an acquired (e.g., iron toxicity) or hereditary (e.g., hemochromatosis in Salers cattle) condition. Iron overload in some birds (e.g., mynahs and toucans) also may be hereditary.
    - **(5)** Nonregenerative anemia
  - **c.** SI can be expressed as a percentage of total iron-binding capacity (TIBC, see below) and reported as the percent saturation.
- **2.** TIBC is an indirect measurement of the amount of iron that transferrin will bind. An immunologic method is available to quantitate transferrin, but is not used commonly.
  - **a.** Only one-third of transferrin binding sites usually are occupied by iron. This is expressed as percent saturation.
  - **b.** TIBC is increased in iron deficiency in most species except the dog.

3. Transferrin can bind more iron than is normally present. Therefore, the numeric difference between TIBC and SI is the amount of iron-binding capacity remaining on transferrin or the unbound iron-binding capacity (UIBC).

B. Hepcidin has been found to be the main regulator of iron homeostasis; it is produced in the liver and acts systemically in iron overloading (increased) or in response to anemia or hypoxia (decreased). Hephaestin (an intestinal ceruloplasmin analog) and ceruloplasmin (synthesized in the liver) are both copper-containing proteins involved in iron transport. Ceruloplasmin also is an acute phase inflammatory reactant. Ferroportin 1 and divalent metal transporter 1 (DMT1) are necessary for transfer of iron from intestinal epithelium and macrophages to serum transferrin. Hepcidin induces the internalization and degradation of ferroportin, thereby inhibiting iron transport.

C. Iron is incorporated into hemoglobin during the last step of heme synthesis. Lack of intracellular iron causes an increase in erythrocyte protoporphyrin concentration.

D. Iron is stored in macrophages as ferritin and hemosiderin.

E. Ferritin is a water-soluble iron-protein complex.

1. Ferritin is the more labile storage form of iron.

2. Small amounts circulate that can be measured as serum ferritin, which is an indirect measurement of the storage iron pool. A species-specific immunoassay is required.

a. Serum ferritin concentration is decreased in iron deficiency.

b. Serum ferritin concentration is increased in the following:

(1) Hemolytic anemia

(2) Iron overload

(3) Acute and chronic inflammation

(4) Liver disease

(5) Some neoplastic disorders (e.g., lymphoma, malignant histiocytosis)

(6) Malnutrition (cattle)

F. Hemosiderin is a more stable, but less available, storage form of iron that is comprised of native and denatured ferritin and protein. It is not water-soluble and is stainable within tissues by Perl's or Prussian blue techniques.

G. Abnormalities in serum iron are related to absorptive failures, nutritional deficiencies, iron loss via hemorrhage, and aberrant iron metabolism with diversion to macrophages at the expense of hematopoietic cells (which occurs in chronic disease processes and inflammation).

V. ERYTHROCYTE METABOLISM

Metabolism is limited after the reticulocyte stage because mature erythrocytes lack mitochondria for oxidative metabolism. Biochemical pathways found in mature erythrocytes are listed in Figure 1.1 with their functions and associated abnormalities.

A. Embden-Meyerhof pathway

1. By this anaerobic pathway, glycolysis generates adenosine triphosphate (ATP) and NADH. ATP is essential for membrane function and integrity, whereas NADH is used to reduce methemoglobin.

2. Important enzymes in this pathway include pyruvate kinase (PK) and phosphofructokinase (PFK). Enzyme deficiencies in this pathway can lead to hemolytic anemia (e.g., PK and PFK deficiency anemias of dogs).

3. PK deficiency impairs ATP production, resulting in a macrocytic hypochromic anemia with 15% to 50% reticulocytes, myelofibrosis, hemochromatosis, decreased erythrocyte lifespan, and accumulation of phosphoenolpyruvate (PEP) and 2,3 diphosphoglyceric acid (DPG). PK deficiency has been reported in dogs (Basenji, West Highland White Terrier, Cairn Terrier, American Eskimo Dog, Miniature Poodle, Pug, Chihuahua, and Beagle) and cats (Abyssinian and Somali).

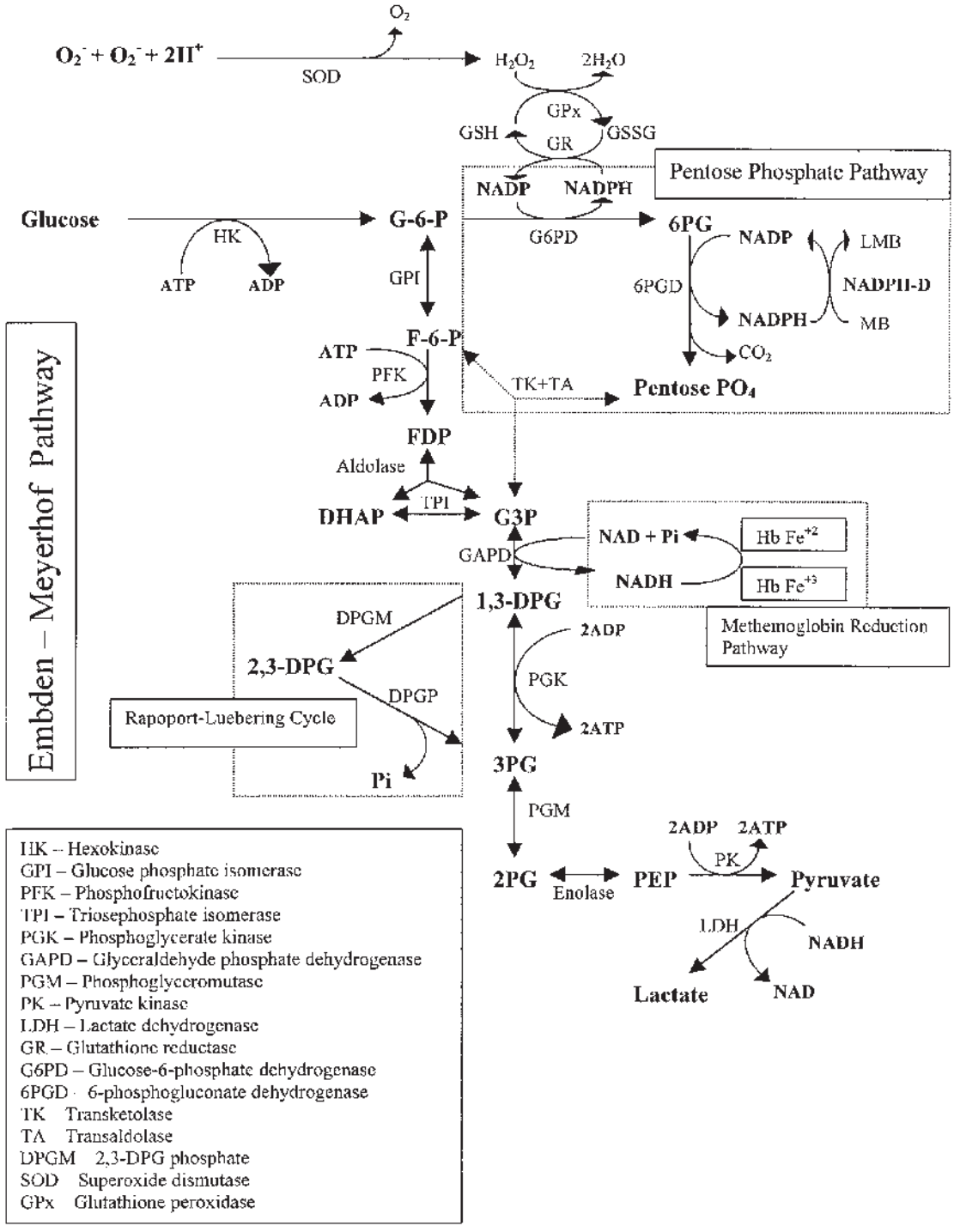


FIGURE 1.1. A schematic diagram showing the major erythrocyte metabolic pathways that provide energy and protect from oxidative injury.

4. PFK deficiency results in decreased erythrocytic 2,3 DPG concentration, hematocrit (Hct) that is within the reference interval or decreased, persistent reticulocytosis, and alkalemia leading to hemolysis. This enzyme deficiency is reported in dogs (English Springer Spaniels, Cocker Spaniels, and some mixed-breed dogs).

B. Pentose phosphate pathway (Hexose-monophosphate pathway)

1. Glucose-6-phosphate dehydrogenase is the rate-limiting enzyme in this anaerobic pathway.

2. This pathway produces NADPH, which is a major reducing agent in the erythrocyte. NADPH serves as a co-factor for the reduction of oxidized glutathione. Reduced glutathione neutralizes oxidants that can denature hemoglobin.

3. A deficiency or defect in glucose-6-phosphate dehydrogenase results in hemolytic anemia under conditions of mild oxidative stress (e.g., glucose-6-phosphate dehydrogenase deficiency in the horse with eccentrocytes and Heinz bodies).

C. Methemoglobin reductase pathway

1. Hemoglobin is maintained in the reduced state (i.e., oxyhemoglobin; Fe^{2+}) necessary for transport of oxygen by this pathway.

2. Enzyme deficiency results in methemoglobin accumulation. Methemoglobin (Fe^{3+}) cannot transport oxygen, and cyanosis results. With substantially increased methemoglobin concentration, the blood and mucous membranes may appear brown.

3. NADH and NADPH methemoglobin reductases also are present. The former predominates in normal conditions and the latter is activated by redox dyes (e.g., methylene blue).

4. Methemoglobin reductase deficiency results in cyanosis, methemoglobinemia, pO_2 within the reference interval, and exercise intolerance. This deficiency has been reported in dogs (American Eskimo Dog, Poodle, Cocker Spaniel-Poodle cross, Chihuahua, and Borzoi).

D. Rapoport-Luebering pathway

1. This pathway allows formation of 2,3 diphosphoglycerate (2,3 DPG), which has a regulatory role in oxygen transport. Increased 2,3 DPG favors oxygen release to tissues by lowering the oxygen affinity of hemoglobin.

2. Depending upon the species, some anemic animals usually have increased 2,3 DPG concentrations and deliver more oxygen to tissues with a lesser amount of hemoglobin (a compensatory mechanism).

3. Animal erythrocytes vary in the concentration of 2,3 DPG and its reactivity with hemoglobin. Dog, horse, and pig erythrocytes have high concentrations and reactivity, whereas cat and ruminant erythrocytes have low concentrations and reactivity.

VI. ERYTHROKINETICS

A. Stem cells, progenitor cells, and precursor cells (Figure 1.2)

1. Pluripotential and multipotential stem cells (CFU-GEMM or CD34+ cells)

a. These cells have the capacity for self-renewal and differentiate into progenitor cells.

b. Differentiation is controlled by growth-promoting stimuli produced by marrow stromal cells. A variety of growth factors and cytokines are involved (SCF, IL-3, IL-9, IL-11, and erythropoietin).

c. When a stem cell differentiates, it loses some of its ability to self-replicate and also loses some of its potentiality.

2. Progenitor cells

a. Some early progenitor cells have the capability of differentiating into more than one cell line (e.g., CFU-GEMM has the potential to differentiate into granulocytes, erythrocytes, monocytes, or megakaryocytes).

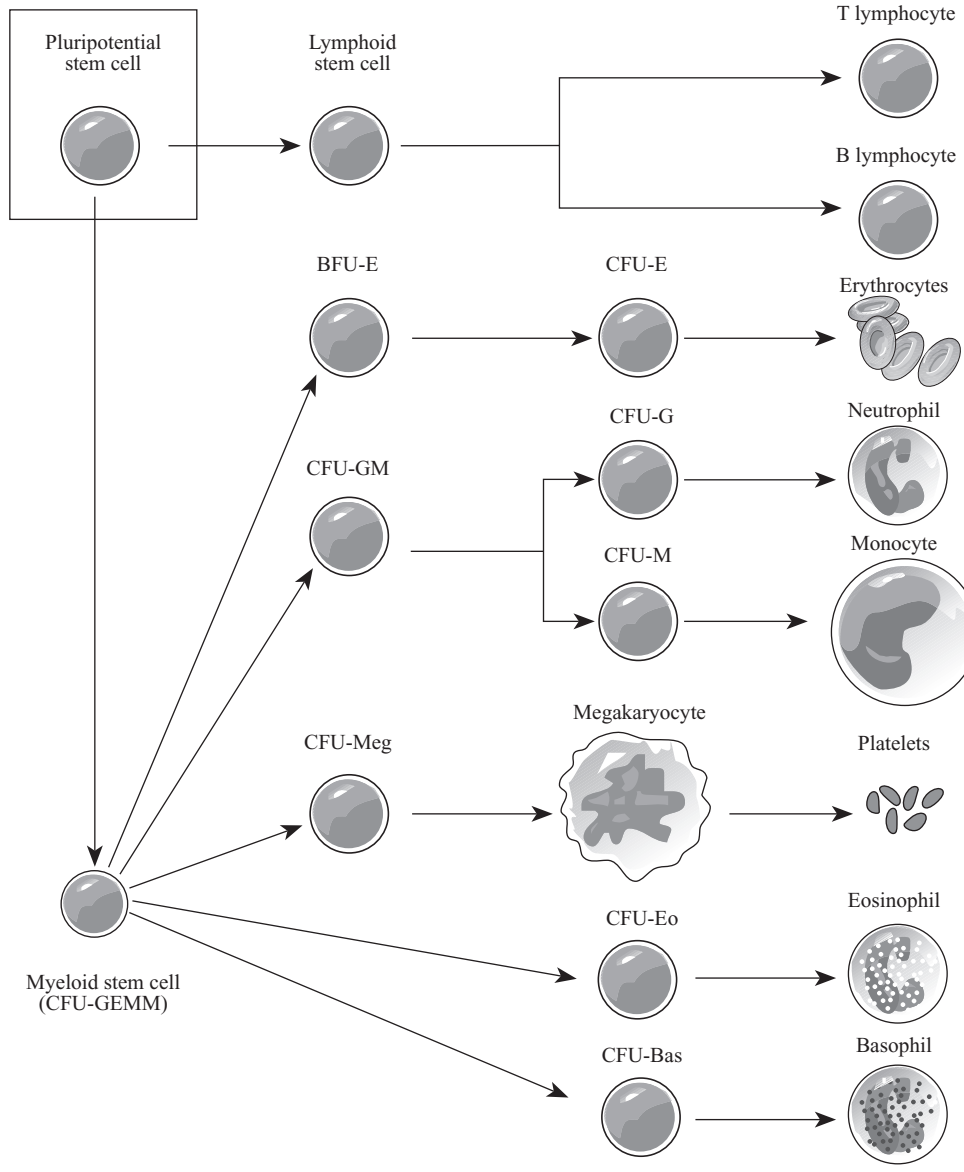


FIGURE 1.2. A model of hematopoiesis. The pluripotential stem cell gives rise to lymphoid and myeloid multipotential stem cells. The lymphoid stem cell differentiates into T- and B-lymphocytes. The myeloid stem cell (CFU-GEMM) forms progenitor cells that include erythroid burst-forming units (BFU-E), which differentiate into erythroid colony-forming units (CFU-E); granulocyte/monocyte colony-forming units (CFU-GM), which differentiate into granulocyte colony-forming units (CFU-G) and monocyte colony-forming units (CFU-M); megakaryocytic colony-forming units (CFU-Meg); eosinophil colony-forming units (CFU-Eo); and basophil colony-forming units (CFU-Bas). These colony-forming units differentiate into precursor cells, then mature cells, of the various cell lines.

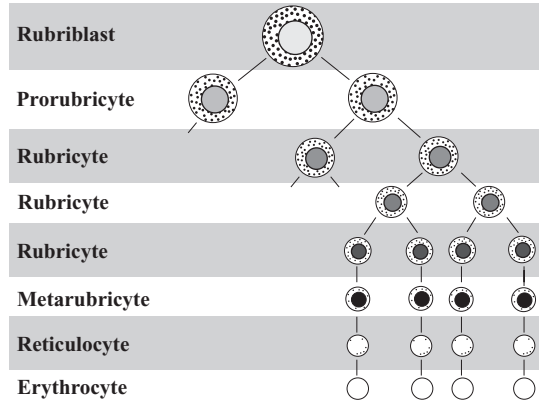


FIGURE 1.3. Sequence of erythropoiesis.

b. Other progenitor cells are unipotential (e.g., CFU-E can only differentiate into erythroid cells).

c. Progenitor cells have limited capacity for self renewal and differentiate into precursor cells of the various cell lines.

d. Progenitor cells are not recognizable morphologically with Romanowsky stains, but resemble small lymphocytes.

3. Precursor cells

a. Precursor cells have no capacity for self-renewal but proliferate while differentiating into the mature, functional cells.

b. These are the first cells that can be recognized as members of a particular cell line.

B. Erythropoiesis (Figure 1.3)

1. In mammals, erythropoiesis occurs extravascularly in bone marrow parenchyma. In avian species, erythropoiesis occurs within the vascular sinuses of the bone marrow (intravascular or intrasinusoidal development).

2. Characteristic morphologic changes take place during maturation from the rubriblast to the mature erythrocyte (Figure 1.4).

a. Cells become smaller.

b. Nuclei become smaller and their chromatin is more aggregated:

(1) Cell division stops in the late rubricyte stage when a critical intracellular concentration of hemoglobin is reached.

(2) The nucleus is extruded at the metarubricyte state, and a reticulocyte is formed in mammals. In contrast, avian reticulocytes and mature erythrocytes retain their nuclei.

c. Cytoplasmic color changes from blue to orange as hemoglobin is formed and RNA is lost.

3. In mammals, reticulocytes and erythrocytes migrate into the venous sinus of the bone marrow through transient apertures in endothelial cell cytoplasm.

a. Reticulocytes of most species remain in the bone marrow for two to three days before release and ultimately mature in the peripheral blood or spleen.

b. In health, the reticulocytes of cattle and horses mature in the bone marrow; mature erythrocytes are released.

4. The time from stimulation of the erythropoietic progenitor cell until reticulocytes are released is approximately five days.

5. Starting with the rubriblast, three to five divisions produce eight to 32 differentiated cells.

6. The bone marrow has the capacity to increase erythropoiesis.

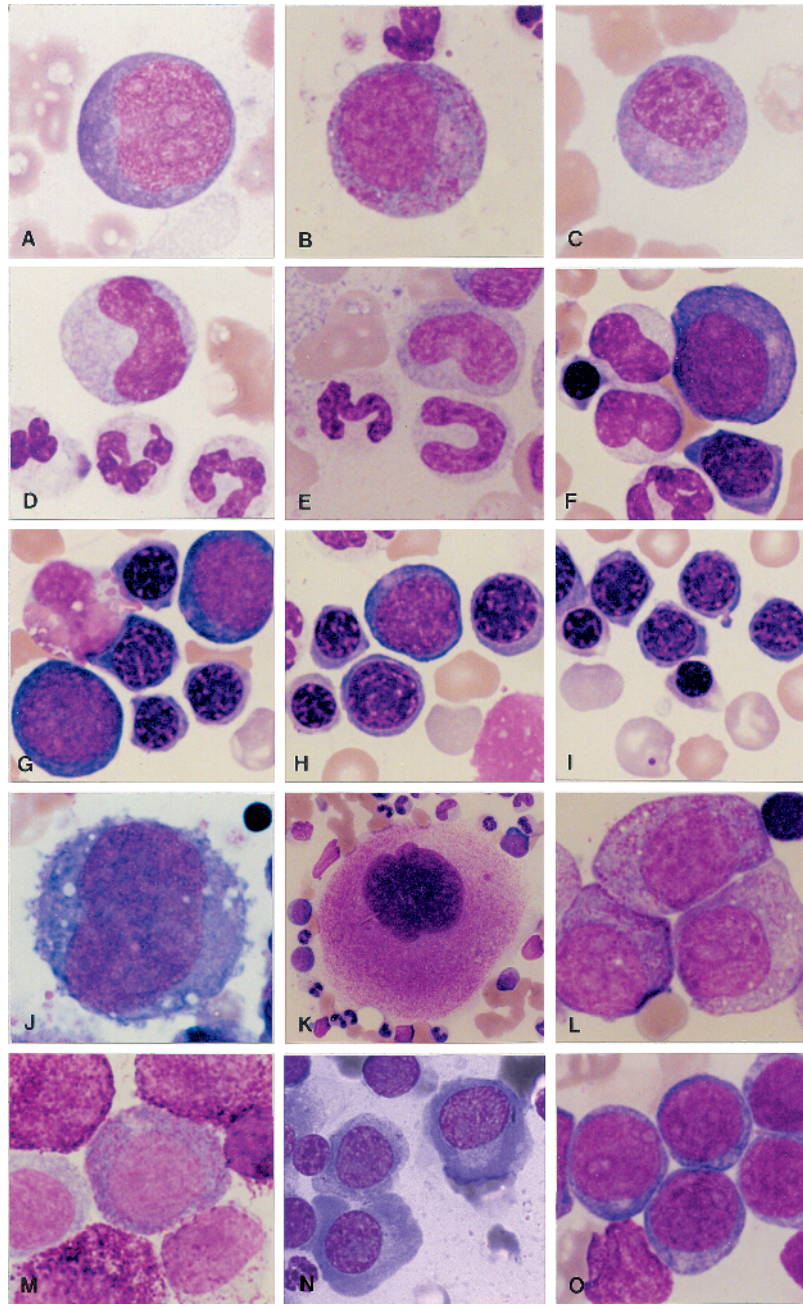


FIGURE 1.4. Normal hematopoietic cells and leukemic cells in bone marrow. A. myeloblast; B. promyelocyte; C. neutrophil myelocyte; D. neutrophil metamyelocyte and segmenters; E. neutrophil metamyelocyte, band, and segmenter; F. rubriblast, rubricyte, metarubricyte, two neutrophil metamyelocytes, and a neutrophil segmenter; G. two prorubricytes, four rubricytes, and an eosinophil; H. five rubricytes; I. five rubricytes, a metarubricyte, and a polychromatophilic erythrocyte with a Howell-Jolly body; J. immature megakaryocyte with blue, granular cytoplasm; K. mature megakaryocyte with granular, pink cytoplasm (low magnification); L. promyelocytes in canine myeloblastic leukemia; M. poorly differentitied mast cells in feline mast cell leukemia; N. plasma cells in canine plasma cell myeloma; O. lymphoblasts in canine acute lymphocytic leukemia (Wright-Leishman stain).

a. Erythrocyte production can be increased up to seven times the normal rate in humans, providing the necessary stimulation and nutrients are present. This capacity to increase production varies with the animal species. It is greatest in birds and dogs and least in cattle and horses.

b. An increase in the number of erythrocytes delivered to the blood occurs primarily via increased stem cell input and, to a lesser extent, by a shortened maturation time.

c. Erythrocytes may be delivered to the circulation faster by earlier reticulocyte release and skipped cell divisions. These processes do not increase the total number of erythrocytes produced and are of temporary benefit.

7. Regulation of erythropoiesis

a. Erythropoietin (Epo)

(1) The majority of Epo is produced by peritubular interstitial cells of the kidney in response to hypoxia, but the liver may account for 10% to 15% of Epo production by specific hepatocytes and Ito cells.

(2) Actions of Epo

(a) Inhibition of apoptosis of newly formed progenitor cells and prorubricytes, allowing them to differentiate into mature erythrocytes.

(b) Stimulation of hemoglobin synthesis in already dividing erythroid cells.

(c) Switching of hemoglobin synthesis in sheep from one adult type to another (i.e., HbA to HbC).

b. Interleukin-3 (IL-3) and colony-stimulating factors (GM-CSF and G-CSF).

(1) IL-3 is produced by activated T-lymphocytes; GM-CSF by activated T-lymphocytes, macrophages, endothelial cells, and fibroblasts; and G-CSF by macrophages, monocytes, neutrophils, endothelial cells, and fibroblasts.

(2) In concert with Epo, these factors stimulate the multiplication of a primitive erythroid progenitor cell, BFU-E, and its differentiation into the CFU-E progenitor cell.

(3) The BFU-E progenitor cell is rather insensitive to Epo stimulation alone.

c. Androgens increase Epo release. In contrast, estrogens and corticosteroids decrease Epo release, but their effect is probably not clinically significant.

d. Thyroid and pituitary hormones alter the tissue demands for oxygen, thereby changing the requirement for erythropoiesis.

VII. ERYTHROCYTE DESTRUCTION

A. The average erythrocyte lifespan in circulation varies with the species: cow, 160 days; sheep, 150 days; horse, 145 days; dog, 110 days; pig, 86 days; cat, 70 days; bird, approximately 35 days. Thus, ruminant blood smears have infrequent reticulocytosis in health, while avian blood smears may have 4% to 5% reticulocytes in health. In certain disease states, anemia may develop more quickly in birds and cats than in large animals because of the normally short erythrocyte lifespan.

B. Aging of erythrocytes is accompanied by changes in enzyme content and cell membrane structure that make the cells less capable of survival and subject to removal by the spleen.

C. In health, senescent erythrocytes are removed from circulation by two routes.

1. Phagocytosis by macrophages is the major route of senescent erythrocyte removal (Figure 1.5).

a. Within the phagosome, the erythrocyte releases its hemoglobin, which is split into heme and globin.

b. Globin is broken down to its constituent amino acids, which are reutilized.

c. After releasing the iron, heme is cleaved by heme oxygenase, forming carbon monoxide and biliverdin.

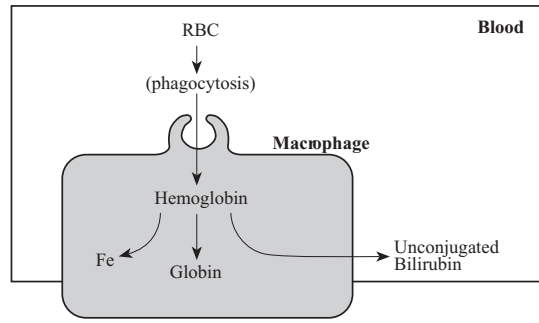


FIGURE 1.5. Pathway of phagocytic (extravascular) destruction of erythrocytes.

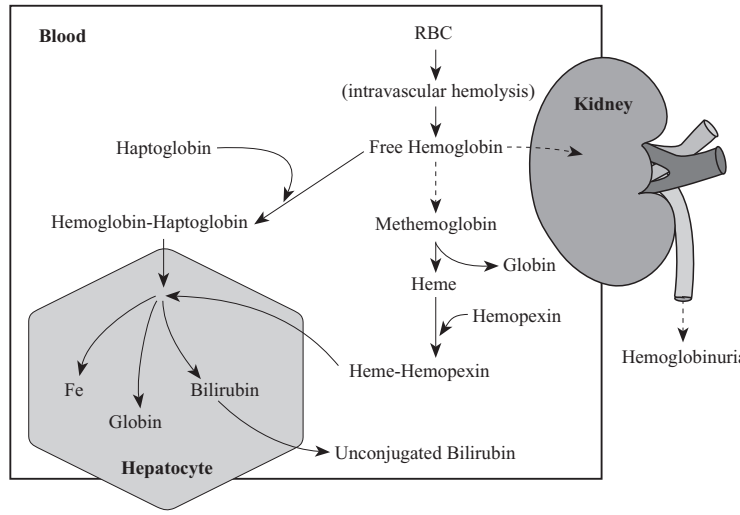


FIGURE 1.6. Pathway of intravascular destruction of erythrocytes.

d. Biliverdin is reduced by biliverdin reductase to bilirubin, which is excreted into the blood, where it binds with albumin for transport to the liver. Birds lack biliverdin reductase; therefore, they form biliverdin as an end product and not bilirubin. Biliverdin is green, which gives the characteristic color to bruises in avian tissue.

2. Intravascular lysis with release of hemoglobin into plasma is a minor route of senescent erythrocyte removal (Figure 1.6).

a. Free hemoglobin in the plasma binds to the α_2-globulin, haptoglobin. The hemoglobin-haptoglobin complex is cleared from plasma by the liver, preventing loss of hemoglobin in the urine. Enough haptoglobin usually is present to bind 150 mg/dL of hemoglobin. Plasma appears pink to red when 50 to 100 mg/dL of hemoglobin is present; therefore, discoloration of plasma precedes hemoglobinuria. In health, plasma discoloration is not observed.

b. If intravascular lysis is excessive, the serum haptoglobin may become saturated. The free hemoglobin then dissociates into dimers, which can pass the glomerular filter. This does not occur in health.

c. With time, free hemoglobin in the plasma is oxidized to methemoglobin, which dissociates to free ferriheme, which complexes with the β-globulin, hemopexin.

d. The heme-hemopexin complexes are cleared by the liver, again preventing hemoglobin loss in the urine.

e. Hemoglobin that passes into the glomerular filtrate is absorbed by the proximal tubules and catabolized to iron, bilirubin, and globin.

f. Unabsorbed hemoglobin passes into the urine, causing hemoglobinuria.

g. Tubular epithelial cells containing hemosiderin may slough into the urine, producing hemosiderinuria.

3. Similar routes of destruction occur in hemolytic anemia, but either extravascular or intravascular hemolysis will predominate.

MEANS OF EVALUATING ERYTHROCYTES

I. HEMATOCRIT (HCT), HEMOGLOBIN (HB) CONCENTRATION, AND RED BLOOD CELL (RBC) COUNT ARE INDICATORS OF CIRCULATING RBC MASS. COMPUTER GRAPHICS OF AUTOMATED HEMATOLOGY ANALYZERS ARE SENSITIVE IN DETECTING CHANGES IN ERYTHROCYTE VOLUME OR HB CONCENTRATIONS.

A. Hct is the percent of blood comprised of erythrocytes.

1. Centrifugal methods give a packed-cell volume (PCV), a very accurate measurement with small inherent error (±1%).

a. Plasma obtained by this method can be used for other routine determinations.

(1) Plasma protein concentration using refractometry

(2) Plasma fibrinogen concentration using heat precipitation and refractometry

(3) Plasma color and transparency

(a) Normal plasma is clear and colorless (dog and cat) to light yellow (horse and cow).

(b) Icteric plasma is yellow and clear.

(c) Hemoglobinemic plasma is pink to red and clear.

(d) Lipemic plasma is whitish to pink and opaque.

b. The buffy coat zone, a white layer between the RBCs and plasma, is comprised of leukocytes and platelets. Measurement of its width has been used to estimate white blood cell counts.

c. Microfilaria may be detected by microscopic examination of the plasma just above the buffy coat layer.

2. Most automated cell counters, designed for human blood, calculate the Hct after determining the RBC count and the mean corpuscular volume of the erythrocyte population. The formula for this calculation is Hct % = (RBC/μL) × MCV (fL). The potential for error is greater than the PCV method, because only dogs have an erythrocyte volume that is comparable to human RBCs. Other domestic mammals have smaller erythrocytes than humans. However, newer and more advanced hematology analyzers can be modified easily to identify blood cells of many different species. Birds have elliptical, nucleated erythrocytes, which may interfere with automated RBC counts.

B. Hb concentration

1. Colorimetric determination by the cyanmethemoglobin technique or the newer cyanide-free hemoglobinhydroxylamine complex method is used most frequently. Avian erythrocytes must be lysed and the specimen must be centrifuged to remove free nuclei before Hb concentration can be accurately determined and indices calculated.

a. The coefficient of variation is approximately ±5%.

b. Heinz bodies, hemolysis, lipemia, and treatment with Oxyglobin® may cause false high values.

2. Some automated instruments directly measure optical density of oxyhemoglobin.

3. Hb concentration provides the most direct indication of oxygen transport capacity of the blood and should be approximately one-third the Hct if erythrocytes are of normal size.

4. Determination of Hb concentration provides no clinical advantage over the Hct other than allowing the calculation of mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC) (see below). However, Hb concentration may be slightly more accurate in determining changes in circulating RBC mass when compared to Hct.
5. Newer hematology analyzers also generate a Hb concentration histogram.

C. RBC count
1. RBC counts, performed with a hemocytometer, have a large degree of error. Thus, hemacytometer-derived RBC counts are of limited value, except in avian species.
2. Automatic counters, if standardized for mammalian blood, allow for more accurate RBC counts. Automated counters are not well validated for avian blood, because all nucleated cells (RBCs, WBCs, and thrombocytes) are counted.
3. The primary value of the RBC count is that it allows determination of MCV and MCH (see below).

D. Factors affecting Hct, Hb concentration, and RBC count
1. Change in circulating RBC mass affects all three parameters.
   a. Low values occur in anemia. Decreases in the three parameters may be disproportionate if cell size and/or Hb content/cell also are altered.
   b. Increased RBC mass (absolute polycythemia) causes high values.
   c. Spuriously high values occur with dehydration and excitement-induced splenic contraction.
   d. Sighthound breeds such as the Greyhound, Saluki, Whippet, and Afghan hound normally have higher Hct/PCV values than other breeds, with values often found into the mid-60s.
2. Change in plasma volume affects all three parameters; therefore, interpretation must always be made with knowledge of the patient's hydration status (Cases 6, 9, 18, 24).
   a. Dehydration or fluid shifts to visceral organs cause increased values.
   b. Overhydration with parenteral fluids causes a reduction in values, simulating anemia.

II. RED BLOOD CELL INDICES. RBC INDICES ARE HELPFUL IN THE CLASSIFICATION OF CERTAIN ANEMIAS.

A. Mean corpuscular volume (MCV) represents the red cell volume in femtoliters (fL)
1. (PCV × 10) ÷ RBC count (millions) = MCV (femtoliters)
2. The MCV is determined directly by automatic cell counters.
3. Factors affecting MCV values
   a. Reticulocytosis is the most common cause of macrocytosis (increased MCV) (Cases 1, 2). Reticulocytes, particularly early forms, are large cells.
   b. Immature animals of most species have small erythrocytes and microcytosis (low MCV). This also could reflect iron deficiency, which is more common in young animals.
   c. Iron deficiency causes microcytosis (Case 5). An extra cell division occurs before the critical cytoplasmic concentration of hemoglobin is reached that is necessary to stop DNA synthesis and cell division. Smaller cells subsequently are produced.
   d. Microcytosis occurs in dogs with portosystemic venous shunts (Case 13).
   e. Healthy Asian breeds of dogs (Akita, Chow Chow, Shar Pei, and Shiba Inu) often have microcytic erythrocytes.
   f. Greyhounds normally have a higher MCV than non-greyhounds that may be due to their erythrocyte's significantly shortened life span (approximately 55 days).
   g. Interference with nucleic acid synthesis causes an inhibition of cell division and thereby larger cells. Macrocytic anemia from an inherited, selective intestinal malabsorption of cobalamin (vitamin B_{12}) has been documented in Giant Schnauzers.
   h. Congenital macrocytosis occurs in Poodles.

i. Hereditary stomatocytosis with macrocytosis has been observed in Alaskan Malamutes, Drentse-Partrijshond, and Miniature Schnauzers.
j. FeLV-infected cats often have macrocytic erythrocytes, possibly due to asynchronous maturation.
k. Erythrocyte agglutination can cause a false increase in the MCV.

B. Mean corpuscular hemoglobin (MCH) represents how much Hb is present within an average erythrocyte in picograms (pg)

1. (Hb concentration × 10) ÷ RBC count (millions) = MCH (picograms)
2. Factors affect the MCH and MCHC in a similar way (below); therefore, the MCH offers little additional hematologic information concerning the patient.
3. MCH is influenced by MCV. For example, smaller erythrocytes contain less Hb; therefore, they have a decreased MCH.
4. In some cases of iron deficiency anemia, MCH may decrease before MCHC decreases.
5. This index is not generally used in the classification of anemias. If the MCHC and MCH differ, interpretation of the hemoglobin concentration should be based upon the MCHC, because the latter value corrects for cell volume.

C. Mean corpuscular hemoglobin concentration (MCHC) represents the average Hb concentration per average erythrocyte in grams of Hb/100 mL of erythrocytes

1. Hb concentration (pg) × 100] ÷ Hct (%) = MCHC (g/dL)
2. The MCHC is the most accurate of the RBC indices because its calculation does not necessarily require the RBC count. However, if the Hct is a calculated value (as is the case in automated hematology analyzers), the accuracy of the MCHC may decrease.
3. The MCHC is used in the classification of anemias.
4. Factors affecting the MCHC
a. Increased MCHC is usually the result of *in vitro* or *in vivo* hemolysis or treatment with Oxyglobin®. Both intra- and extracellular Hb are measured in the hemoglobin procedure, but the formula assumes all Hb is intracellular, giving a false high value (Case 3).
b. A true increase in MCHC does not normally occur; increased concentrations of hemoglobin cannot be produced within the cell.
c. Reticulocytes do not have their full component of hemoglobin; therefore, the MCHC may be decreased in reticulocytosis (Cases 1, 2).
d. Hypochromia (i.e., low MCHC) occurs in some cases of iron deficiency (Case 5).
(1) Iron-deficient cats may not have hypochromic erythrocytes.
(2) The MCHC may not be low in iron-deficient dogs when measured on some electronic counters.

D. Red cell distribution width (RDW)

1. This erythrocyte parameter can be determined by some automated cell counters.
2. The RDW is the coefficient of variation of the red cell volume distribution and is calculated by the formula RDW = (SD_{MCV} ÷ MCV) × 100. It is an index of the degree of anisocytosis or variation in size of the erythrocytes.
3. Anemias with significant microcytosis or macrocytosis have an increased RDW. Reticulocytosis may result in an increased RDW.

III. PERIPHERAL BLOOD SMEAR

A. Staining and examination of the smear

1. New methylene blue (NMB). A drop of NMB between a coverslip and an air-dried blood smear gives a rapid, meaningful stain but is a nonpermanent preparation. Acidic groups stain blue (i.e., nuclear DNA and RNA, cytoplasmic RNA, and basophil granules). Depending upon the distance

between the acidic groups, a blue or purple color may result. Eosinophil granules are unstained. The reticulum of reticulocytes stains blue, but reticulocytes are best stained by mixing equal parts of NMB and blood, holding at room temperature for 10 minutes, and then making the smear.

2. Romanowsky stains (e.g., Wright's stain, Diff-Quik®, Hemacolor®, etc.). These polychromic preparations stain certain acidic groups blue (RNA) to purple (mast cell and basophil granules and nuclear DNA), whereas basic groups stain red to orange (proteins, eosinophil granules).

3. Systematic evaluation of the stained blood smear

a. Low magnification. Select a thin area of the smear where the cells are evenly distributed, and look for the following features:

(1) RBC rouleaux formation (described below)

(2) RBC agglutination (described below)

(3) Platelet aggregation, especially at the feathered edge of the smear (Chapter 4)

(4) Relative number of leukocytes

b. High-dry magnification. Confirm observations made at low magnification, and:

(1) Note the concentration of leukocytes and obtain an impression as to whether the white blood cell (WBC) count is decreased, within the reference interval, or increased.

(2) Calculate a differential leukocyte count. This usually can be done at high-dry magnification, but certain cells may require oil immersion magnification for identification.

(3) Look for nucleated erythrocytes (nRBCs) and polychromasia (reticulocytes).

c. Oil-immersion magnification

(1) Examine the erythrocyte morphology (described below).

(2) Conduct the differential leukocyte count at this magnification if difficulty is encountered at high-dry magnification.

(3) Examine leukocyte morphology (Chapter 2).

(4) Estimate the adequacy of platelet numbers and evaluate their morphology (Chapter 4).

B. Erythrocyte morphology (Figure 1.7)

1. Normal morphology

a. Canine erythrocytes average 7 µm in diameter, are uniform in size, and have central pallor (biconcave disk).

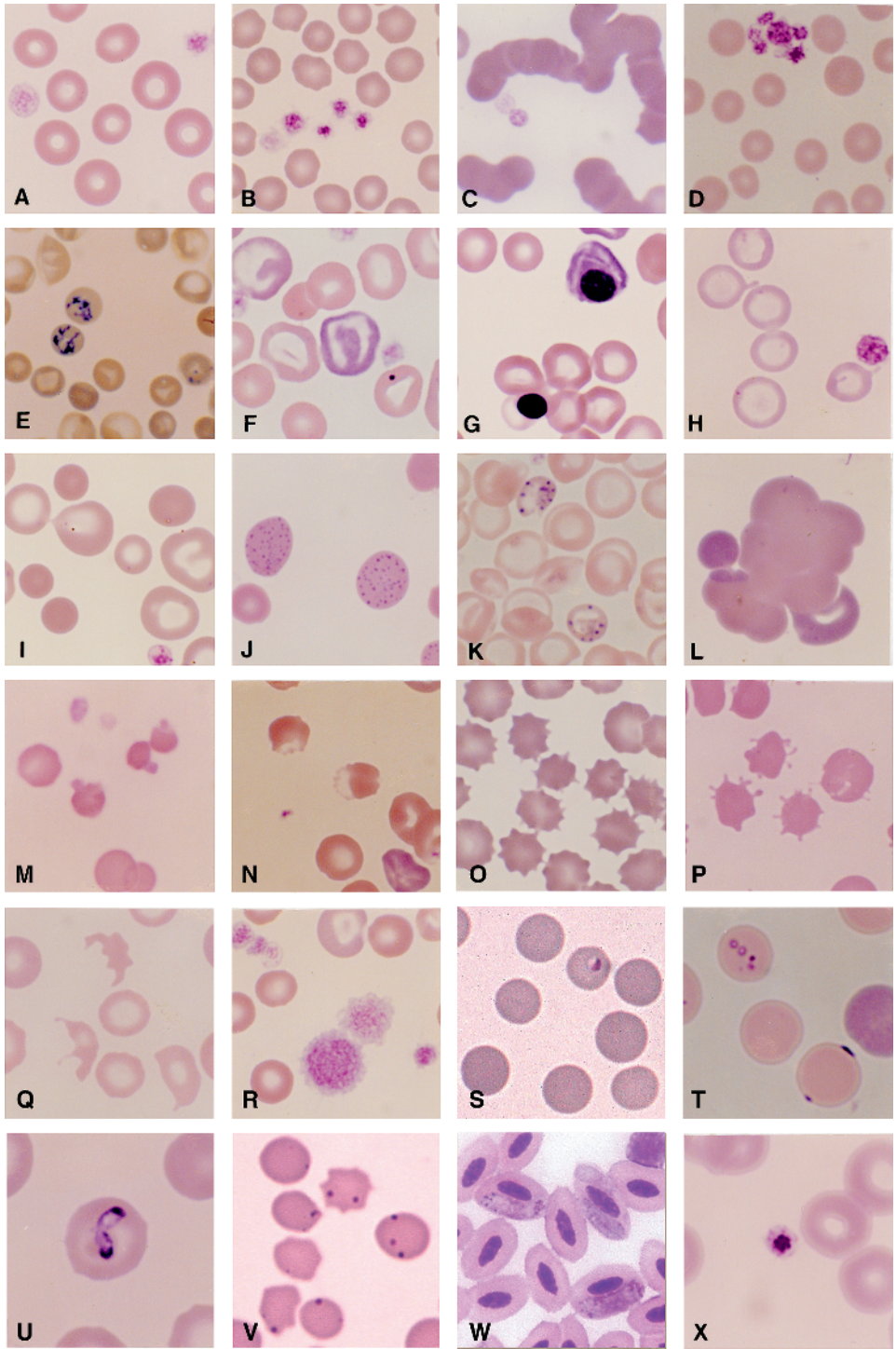
b. Feline erythrocytes average 5.8 µm in diameter, have mild anisocytosis (i.e., variation in size), and exhibit very slight central pallor. Crenation is commonly observed. Howell-Jolly bodies (nuclear remnants) occur in up to 1% of the erythrocytes. Rouleaux formation also may be present.

c. Bovine erythrocytes average 5.5 µm in diameter. Anisocytosis is common, and central pallor is usually slight. Crenation is common.

d. Equine erythrocytes average 5.7 µm in diameter and lack central pallor. Rouleaux formation is common.

e. Porcine erythrocytes average 6 µm in diameter and often exhibit poikilocytosis.

FIGURE 1.7. Erythrocyte and platelet morphology. A. canine erythrocytes and platelets; B. feline erythrocytes and platelets; C. equine erythrocytes in rouleaux and platelets; D. bovine erythrocytes and platelets; E. canine reticulocytes (new methylene blue stain); F. polychromasia, leptocytes, and Howell-Jolly body (dog); G. metarubricytes (dog); H. hypochromasia (iron deficiency, dog); I. spherocytes (immune-mediated anemia, dog); J. basophilic stippling (regenerative anemia, cow); K. basophilic stippling (lead toxicosis, dog); L. autoagglutination (immune-mediated anemia, dog); M. Heinz bodies (red maple toxicosis, horse); N. Heinz bodies in erythrocyte ghosts (acetaminophen toxicosis, cat); O. eccentrocytes (onion toxicosis, dog); P. keratocyte (dog); Q. echinocytes (dog); R. acanthocytes (dog); S. schistocytes (dog); T. macroplatelet (shift platelet; dog); U. *Mycoplasma haemofelis* (formerly *Hemobartonella felis*; cat); V. *Babesia canis* (dog); W. *Anaplasma marginale* (cow); X. *Anaplasma platys* (formerly *Ehrlichia platys;* dog) (Wright-Leishman stain unless indicated).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f. Ovine erythrocytes are similar to those of the cow but smaller. The average diameter is 4.5 μm.

g. Caprine erythrocytes are the smallest blood cells of the domestic animals. They are usually less than 4 μm in diameter. Anisocytosis and poikilocytosis are common.

h. Camelidae (camel, llama, alpaca, etc.) erythrocytes are thin (approximately 1.1 μm) and ellipsoidal in shape with an average diameter of 6.5 μm, and usually have high erythrocyte counts.

i. Avian erythrocytes are oval and nucleated with an average size 12 × 6 μm.

2. Rouleaux formations are groups of erythrocytes resembling stacks of coins. The degree of rouleaux tends to positively correlate with the erythrocyte sedimentation rate (ESR), and is usually associated with an altered surface membrane charge (zeta potential). The intensity of this charge can be a species characteristic or the result of disease. Rouleaux is common in horses that have a decreased membrane charge in health. In certain diseases, normal membrane surface charge may be partially masked by excess protein (hyperfibrinogenemia, hyperglobulinemia) that decreases the repelling negative surface charges of the erythrocytes. Rouleaux and an increased ESR will be observed. Microscopically, rouleaux can be distinguished from autoagglutination by its dispersion in wet mounts when blood is diluted with physiologic saline solution.

a. Marked rouleaux formation is common in equine blood in health, but may be absent in the blood of severely anemic or cachectic horses.

b. Moderate and mild rouleaux may be present in feline and canine blood in health, respectively. Marked rouleaux may be observed during inflammatory and neoplastic diseases.

c. Rouleaux formation is rare in ruminant blood in health and disease.

3. Agglutination is a grape-like aggregation of erythrocytes occurring in some blood specimens of animals with immune- (antibody-) mediated anemia. Occasionally, it may be observed grossly (on the sides of the blood collection tube) or microscopically (in an unstained wet mount or on a stained blood smear). Agglutination is present if the erythrocytes remain clumped when blood is diluted 50:50 to 10:90 with physiologic saline solution and viewed microscopically as a wet mount. In anemic animals, agglutination is an indicator of an antibody-mediated effect, but the absence of agglutination does not exclude immune-mediated anemia.

4. Anisocytosis is variation in the size of erythrocytes because of the presence of macrocytes and/or microcytes among normocytes (normally-sized erythrocytes; Cases 1, 2, 5).

5. Macrocytes are large erythrocytes. Reticulocytes are usually macrocytic and polychromatophilic (light blue-gray color when using Wright's stain). Normochromic macrocytes may occur in certain conditions (e.g., macrocytosis of Poodles, FeLV infections, preleukemia of cats and dogs, erythroid aplasia of cats, and vitamin B_{12} deficiency of Giant Schnauzers). If significant numbers of macrocytes are present, the MCV may be increased.

6. Microcytes are small erythrocytes. They may be observed in iron and pyridoxine deficiency anemias in association with a low MCV. Microcytes can include cell remnants in Heinz body and fragmentation anemias. Microcytes also are associated with portosystemic shunts (PSS) and hyponatremia. Finally, microcytes may be observed in healthy Asian breeds of dogs (Akita, Chow Chow, Shar Pei, and Shiba Inu).

7. Spherocytes, associated with immune-mediated anemias, have a decreased MCV as a result of a decreased membrane surface area. Spherocytes are globoid because the remaining smaller cell membrane must enclose a normal amount of hemoglobin. Because spherocytes do not flatten well on the blood smear, they appear smaller than normochromic, biconcave disk erythrocytes.

8. Polychromasia refers to the blue-gray erythrocytes with residual RNA that are generally large (macrocytic) and seen on routinely stained blood smears. Polychromatophilic erythrocytes (as observed with Romanowsky stains) are synonymous with reticulocytes (as observed with NMB stain). Increased numbers of these cells are associated with increased erythropoietic activity and an attempted regeneration in response to anemia (Cases 1, 2, 5, 25). The degree of regeneration depends on the number of polychromatic erythrocytes (reticulocytes) relative to the degree of

anemia. A few polychromic cells are normal in the dog and cat, less common in cattle, and not usually seen in horses (in health or anemia).

9. Hypochromia is decreased cytoplasmic staining intensity and increased central pallor of the erythrocyte caused by insufficient Hb within the red cell. The most common cause of hypochromia is iron deficiency, but it can also occur with lead toxicosis via inhibition of hemoglobin synthesis. Hypochromia in avian blood smears has been observed with lead toxicosis and inflammation.

10. Poikilocyte is a general term for an abnormally shaped erythrocyte. Blood smears should be submitted with CBC specimens to prevent artifactual alterations in cellular shape if there will be a substantial lag time between blood collection and analysis. Poikilocytes may be seen in young healthy calves and goats (due to structural Hb switching), as well as pigs of any age. Poikilocytes are considered an abnormality in other species where they can arise from trauma to the erythrocyte membrane associated with turbulent blood flow or intravascular fibrin deposition. Poikilocytes may be removed prematurely from circulation, leading to hemolytic anemia. Specific types of poikilocytes include the following:

a. Echinocytes are spiculated erythrocytes with many evenly spaced, uniform projections. Type I echinocytes contain spicules on the periphery of the erythrocyte. These are crenated erythrocytes that are an *in vitro* artifact associated with changes in temperature, pH, drying, or other interactions between the blood and smear preparation. Type II and III echinocytes (Burr cells) have spicules covering the entire surface of the rounded erythrocyte which are attributed to altered/fluxing electrolytes with expansion of the outer layer of the cell membrane. They also have been observed in uremia, electrolyte depletion, lymphoma, doxorubicin toxicity, and glomerulonephritis.

b. Keratocytes (helmet cells) are erythrocytes with one or two projections that form a ruptured vesicle. These abnormalities often result from oxidative damage to the erythrocyte membrane, as listed for Heinz body formation.

c. Schistocytes (schizocytes) are irregular erythrocyte fragments that result from shearing by intravascular fibrin or by turbulent blood flow within the vasculature (Case 11). Schistocytes are associated with disseminated intravascular coagulation (DIC), hemangiosarcoma, glomerulonephritis, congestive heart failure, myelofibrosis, chronic doxorubicin toxicosis, and vasculitis, to name a few conditions.

d. Acanthocytes are spiculated erythrocytes with two or more irregular, often blunted, projections. These cells are thought to form as a result of altered lipid:cholesterol ratios in the erythrocyte membrane. In animals, acanthocytes are associated with hemangiosarcoma (especially involving the liver), glomerulonephritis, lymphoma, and liver diseases.

e. Fusocytes are elongated erythrocytes that are seen in healthy Angora goats.

f. Elliptocytes are oval cells that are seen in healthy camelids. A rare hereditary disease in dogs with 4.1 band deficiency of the erythrocyte membrane cytoskeleton has been reported to have elliptocytosis. Occasionally, elliptocytes may be observed in iron deficiency.

g. Dacryocytes are teardrop-shaped erythrocytes that may result from the inability of the erythrocyte to return to its pre-existing shape after deforming in the blood vessels (decreased deformability). This change may be related to alterations in cytoskeleton proteins. If the "tails" of the dacryocytes are all in the same direction, this may be an artifact of blood smear preparation. Dacryocytes are observed in blood smears of llamas with iron deficiency anemia.

h. Leptocytes are thin cells with an increased membrane:volume ratio; they may appear folded due to the excess membrane. Leptocytes have been associated with portosystemic shunts (Case 13). Polychromatophilic erythrocytes (reticulocytes) may appear as leptocytes due to increased cell membrane.

i. Target cells (codocytes) are a type of leptocyte that are bell-shaped, but resemble a target on smears due to the distribution of Hb centrally and peripherally in the cell. Formation of target cells may occur by increasing the amount of membrane via lipid and cholesterol insertion or

by decreasing cytoplasmic volume as in hypochromia. Target cells may be associated with liver disease, iron deficiency anemia, and reticulocytosis.

j. Stomatocytes are a type of leptocyte that are bowl-shaped with oval areas of central pallor on blood smears. This change in shape results from expansion of the inner layer of the cell membrane. These cells are observed in hereditary stomatocytosis of Alaskan Malamutes, Drentse Partrijshond, and Miniature Schnauzers. Stomatocytes also can be artifacts in the thick area of the smear.

k. Spherocytes are small dark microcytes that lack central pallor and have a reduced amount of membrane per unit volume. They are readily detected only in the dog because of the normal abundance of central pallor. Spherocytes are observed most frequently in immune-mediated hemolytic anemias (Case 2), but may also be seen following transfusions and in some stages of Heinz body anemia. They result from partial phagocytic removal of antibody-coated membrane or "pitting" of Heinz bodies. Spherocytes are prematurely removed from circulation by splenic macrophages because of their reduced ability to deform (i.e., loss of cell flexibility) and to traverse the splenic microvasculature.

11. Basophilic stippling represents punctate aggregation of residual RNA in Romanowsky-stained (Wright- or Diff-Quik®-stained) cells. This often occurs in anemic sheep and cattle, and occasionally in feline anemia. It has the same significance as polychromasia (regenerative anemia), and can be an appropriate response during anemia. Basophilic stippling also may be an indication of lead toxicosis when accompanied by metarubricytosis with minimal polychromasia (an inappropriate response) in an animal with RBC indicators within reference values.

12. Howell-Jolly bodies are basophilic nuclear remnants within the cytoplasm of erythrocytes. These structures are observed more frequently in accelerated erythropoiesis or post-splenectomy.

13. The Heinz body is a round structure that protrudes from the membrane of the erythrocyte or appears as a small refractile spot in the cytoplasm. Heinz bodies are comprised of denatured, precipitated Hb caused by oxidation. They are often attached to the inner cell membrane (Case 3). Because Heinz bodies are derived from hemoglobin, they are the same color as the remainder of the cytoplasm and can be indistinct with Romanowsky staining. Following NMB staining, Heinz bodies appear as dark basophilic bodies. Heinz bodies alter the cell membrane and decrease erythrocyte deformability when traversing capillaries. They may result in intravascular hemolysis. The Heinz body itself may be removed by splenic macrophages, leaving a spherocyte. Cats are more susceptible to Heinz body formation (sometimes called erythrocyte refractile bodies or ER bodies in this species). Feline Hb has a larger number (8 to ten) of sulfhydryl groups that increase susceptibility to oxidation. Furthermore, the feline spleen is inefficient in removing these structures. Up to 10% of feline erythrocytes may contain Heinz (ER) bodies in health. In birds, Heinz bodies are smaller and more numerous within erythrocytes.

14. Eccentrocytes (hemi-ghost erythrocytes) are erythrocytes with the hemoglobin condensed in one portion of the cell, leaving a clear or blister-like area in the remaining portion of the cell. They are the result of oxidative injury with lipid peroxidation and cross-linking of the cell membrane (Case 3).

15. Nucleated erythrocytes (nRBCs) in the stained blood smear include metarubricytes, rubricytes, and earlier stages of erythroid development. The term "metarubricytosis" refers to the presence of any nRBCs and represents a premature release of these cells into the circulation. The release of nRBCs in disease may be classified as an appropriate or inappropriate response.

a. The release of nRBCs is an expected or appropriate response with an intense increase in erythropoiesis in strongly regenerative anemias. The nRBCs are accompanied by reticulocytosis (Cases 1, 2, 5). Erythropoietin also may stimulate release of nRBCs during hypoxia, unrelated to anemia.

b. For Camelidae, llamas often have high numbers of nRBCs in regenerative anemias accompanied by absence of or mild polychromasia.

c. Metarubricytes may be inappropriately released in lead toxicosis, iron deficiency, copper deficiency, hemangiosarcoma, extramedullary hematopoiesis, myelophthisis, intervertebral disc syndrome, hereditary macrocytosis of Poodles, endotoxemia, bone marrow trauma, bone marrow necrosis, metastatic neoplasia of the marrow cavity, myelofibrosis, FeLV infection, myelodysplastic syndrome, and leukemia, especially erythremic myelosis in cats.

d. nRBCs may be seen in high numbers in healthy pigs, especially in piglets less than three weeks of age.

e. Nucleated erythrocytes normally occur in birds.

f. Nucleated erythrocytes have been reported in normal Miniature Schnauzers.

16. Erythroplastids are anucleate fragments of erythrocyte cytoplasm occasionally found in avian blood smears, while hematogones are free erythrocyte nuclei.

17. Parasites can occur within the erythrocyte (intracellular) or within depressions on the membrane surface (epicellular), or within the plasma (extracellular):

a. Intracellular parasites include *Hemoproteus* spp., *Leukocytozoon* spp., and *Plasmodium* spp. (birds); *Cytauxzoon felis, Babesia cati, B. felis* (cats); *Anaplasma marginale, A. centrale* (cattle); *Babesia bovis, B. bigemina* (cattle); *Theileria mutans, T. annulata* (cattle); *Theileria cervi,* (deer, elk); *Babesia canis, B. gibsoni* (dogs); *Babesia equi, B. caballi* (horses); and *Babesia ovis, B. motasi* (sheep).

b. Common epicellular parasites include *Trypanosoma johnbakeri* (birds), *Mycoplasma haemofelis* (formerly *Hemobartonella felis)* (cats), *M. haemocanis* (formerly *H. canis)* (dogs), *Mycoplasma haemosuis (formerly Eperythrozoon suis)* (pigs), *Eperythrozoon wenyoni* (cattle), and *Eperythrozoon* sp. (llamas).

c. Common extracellular parasites include filarids and trypanosomes.

(1) Microfilariae: *Dipetalonema reconditum* (dogs), *Dirofilaria immitis* (dogs, rarely cats), and *Setaria* sp. (horse).

(2) Trypanosomes: *Trypanosoma theileri, T. congolense, T. vivax* (cattle); *Trypanosoma cruzi,* (dogs); and *Trypanosoma brucei, T. evansi* (horses).

18. Distemper inclusions in canine erythrocytes are irregular to round to ring-shaped and stain magenta with Romanowsky and Diff-Quik® stains (the inclusions may stain blue with other rapid blood stains). They also may be observed in leukocytes. Distemper inclusions are transient.

IV. BLOOD RETICULOCYTE EVALUATION

A. Reticulocytes are immature anuclear erythrocytes in mammals that contain residual RNA and mitochondria, aggregated into a reticular pattern when stained with supravital stains (e.g., NMB stain) (Figure 1.7). Reticulocytes correspond to polychromatophilic erythrocytes observed in Romanowsky-stained preparations. Reticulocytes released prematurely in response to an anemia are larger and are called shift reticulocytes. Reticulocytes in birds are nucleated and occasionally may appear more rounded.

B. Quantitation of reticulocytes in circulation, by calculation of absolute reticulocyte numbers, is the best indicator of the bone marrow erythroid response to anemia. Newer hematology analyzers have the ability to segregate reticulocytes into age groups (time from release from bone marrow).

C. Species characteristics of reticulocytes

1. The dog has small numbers (up to 1%) of aggregate type reticulocytes (containing blue-stained aggregates with supravital stains) in health (Figure 1.7).

2. Two types of reticulocytes occur in the cat.

a. Aggregate reticulocytes are similar to those of other species and account for up to 0.4% of the erythrocytes in health. This type of reticulocyte is enumerated hematologically. Increased

aggregate reticulocytes reflect the current bone marrow response to anemia (i.e., increased erythropoiesis or regeneration). Reticulocytosis is less dramatic than in the dog.

b. Punctate reticulocytes (containing small, blue-stained dots) are derived from aged aggregate reticulocytes and persist for at least two weeks. Like aggregate reticulocytes, they are increased with increased erythropoiesis. Because punctate reticulocytes circulate for weeks and persist after the aggregate reticulocyte count has returned to the reference interval, their enumeration is of little benefit in assessing the current regenerative response. Punctate reticulocytes indicate a bone marrow response to anemia occurring as much as three to four weeks previously.

3. Reticulocytes are absent from the blood of ruminants in health because of the long erythrocyte lifespan, but increase modestly in responding anemias.

4. Reticulocytes are absent in the blood of horses in both health and regenerative anemias.

5. Reticulocytosis is a prominent feature of healthy suckling pigs, but is less dramatic in adults (1%). Reticulocyte counts increase in regenerative anemias.

6. Reticulocyte percentage in the blood of healthy birds is higher (4% to 5%) than mammals because of a short erythrocyte lifespan (approximately 35 days). Polychromasia is more prominent in younger birds than in adults but usually does not exceed 5%.

D. Means of reticulocyte enumeration

1. Reticulocyte percentage. Reticulocytes are counted in a NMB-stained blood smear and are expressed as the percentage of the total erythrocyte population. This parameter can overestimate the bone marrow response because:

a. Reticulocytes released from the bone marrow into the blood of anemic animals are mixed with fewer mature erythrocytes. Thus, a higher relative percentage of reticulocytes results.

b. Larger, more immature reticulocytes are released earlier (shift reticulocytes) in response to anemia. These reticulocytes persist longer in the blood because it takes them more time to mature. This results in a higher percentage of reticulocytes.

2. Corrected reticulocyte percentage

a. Formula: Observed reticulocyte percentage × (patient's Hct percentage ÷ "normal" Hct percentage) = corrected reticulocyte percentage

The "normal" hematocrit (Hct) is considered to be 45% in the dog and 37% in the cat.

b. This parame'ter corrects for (1a) above but not for (1b).

c. Corrected reticulocyte percentages greater than 1% in the dog and greater than 0.4% in the cat indicate bone marrow response to the anemic state. The corrected reticulocyte percentage has not been validated for use in other animal species.

3. Absolute reticulocyte count

a. Formula: Reticulocyte percentage (converted to a decimal figure) × (total RBC count/μL) = absolute reticulocyte count/μL

b. This parameter corrects for item 1a (above) but does not correct for the effect described in item 1b (above).

c. An absolute reticulocyte count greater than 80,000/μL in the dog and greater than 60,000 /μL in the cat indicates a regenerative response. Depending on the reduction in Hct, a very low Hct should have a proportionately higher absolute reticulocyte count.

d. The following are estimated degrees of regeneration for dogs:

(1) None = 60,000/μL
(2) Slight = 150,000/μL
(3) Moderate = 300,000/μL
(4) Marked = more than 500,000/μL

e. The following are estimated degrees of regeneration (aggregate reticulocytes) for cats:

(1) None = less than 15,000/μL
(2) Slight = 50,000/μL
(3) Moderate = 100,000/μL
(4) Marked = more than 200,000/μL

f. Although not often used, similar estimates of regeneration for feline punctate reticulocytes include the following:
- **(1)** None = less than 200,000/μL
- **(2)** Slight = 500,000/μL
- **(3)** Moderate = 1,000,000/μL
- **(4)** Marked = 1,500,000/μL

4. Reticulocyte production index (RPI). This correction index is used in humans and also has been used in the dog and cat to correct for items 1a and 1b above. This parameter remains controversial because definitive correlation studies have not been done in animals.

a. RPI = observed reticulocytes (percentage) × [observed Hct (percentage) ÷ 45 (normal Hct)] × [1 + blood maturation time]

b. Blood maturation times of reticulocytes at various Hcts are 45% = 1 day, 35% = 1.5 days, 25% = 2 days, and 15% = 2.5 days.

c. RPI values equal the increase in erythrocyte production (e.g., RPI of 3 equals three times normal erythrocyte production). An RPI greater than 2 equals a regenerative response.

E. Interpretation of reticulocyte parameters

1. Absolute increases in reticulocytes indicate a responding bone marrow (regenerative anemia) and that the cause of the anemia is extra-marrow (i.e., hemorrhage or hemolysis).

2. Reticulocytosis is more intense in hemolytic than in external hemorrhagic anemias. Iron from disrupted erythrocytes is more readily available for erythropoiesis than the iron that is stored as hemosiderin.

3. Reticulocytosis does not become clearly evident until 48 to 72 hours after the occurrence of anemia. With normally responsive bone marrow, maximum reticulocytosis should be achieved within seven days. However, reticulocytosis may be delayed in animals with various systemic diseases and in aged animals.

4. Dogs have a greater reticulocyte response than cats.

5. Significant reticulocytosis does not develop in cattle with acute responding anemias until the Hct is very low.

6. Reticulocytosis does not occur in the horse with any type of anemia.

7. Healthy suckling pigs have high reticulocyte counts.

8. Healthy birds often have a higher reticulocyte count (approximately 4% to 5%) than mammals due to their short erythrocyte life span (25 to 40 days).

9. The lack of a reticulocyte response following anemia suggests that the bone marrow is not responding (nonregenerative anemia). This may be due to insufficient time for reticulocytosis to occur, a deficiency in the existing reticulocyte response, or defective erythropoiesis.

V. BONE MARROW EXAMINATION

A. Indications for examination

1. Nonregenerative or nonresponding anemia

2. Persistent neutropenia

3. Unexplained thrombocytopenia

4. Suspicion of hematopoietic neoplasia

5. Suspicion of osteomyelitis, infiltrative, or proliferative bone marrow disease

6. Fever of unknown origin

B. Technique

1. Bone marrow aspirates can be obtained from the iliac crest, trochanteric fossa, sternum, humerus, or rib of mammals using a special bone marrow or 18-gauge spinal needle. Aspirates are

obtained from the sternal ridge (keel) or tibiotarsus in birds. Other prominent long bones of birds are pneumatized and lack significant hematopoietic tissue.

2. Particle smears are preferred because they are less likely to have blood contamination.

3. Core biopsies, obtained with special needles, may be taken from the above locations and provide a better indication of overall cellularity than cytologic smears alone.

C. Examination of the stained bone marrow smear

1. Observe the relative number, size, and cellularity of the particles, including the proportion of adipocytes (approximately 50%) and hematopoietic progenitor cells (approximately 50%). The number and cellularity of particles is an estimation of the overall cellularity of the bone marrow; however, low-cellularity aspirates may occur with incomplete sampling of the bone marrow. Histologic examination of a bone marrow core biopsy is more accurate in assessing marrow cellularity and in detecting stromal reactions (e.g., myelofibrosis).

2. Note the adequacy of megakaryocyte numbers, maturity, and morphology. Immature megakaryocytes have blue, granular cytoplasm, whereas mature megakaryocytes have pink, granular cytoplasm.

3. The ratio of myeloid:nucleated erythroid cells (M:E ratio) may be estimated.

a. The M:E ratio is usually determined to assess the erythropoietic response to anemia. The WBC count or, more specifically, the neutrophil count, is needed for proper interpretation of the ratio.

b. If the WBC count is within the reference interval, any change in the M:E ratio is due to changes in the erythroid series.

c. A high M:E ratio in an anemic animal with a WBC count within the reference interval suggests erythroid hypoplasia.

d. A high M:E ratio in an anemic animal with an increased WBC count is more difficult to interpret because the increased ratio could result from myeloid (granulocytic) hyperplasia and/or erythroid hypoplasia.

e. A low M:E ratio in an anemic animal with a WBC count within or above the reference interval suggests early erythroid regeneration of ineffective erythropoiesis.

4. The relative percentages of the various stages of each series should be observed to assess maturation (Figure 1.5). In health, approximately 80% of the myeloid series should be metamyelocytes, bands, and segmenters (nonproliferating, maturation, storage pool); 90% of the erythroid series should be rubricytes and metarubricytes. A high percentage of immature forms suggests hyperplasia, neoplasia, or maturation abnormality of the respective series. In the myeloid series, depletion of the storage pool (which contains the more mature cells) can cause a shift toward immaturity.

5. Abnormal cells should be identified and described.

6. Perl's or Prussian blue staining for iron in bone marrow macrophages may be useful to distinguish iron deficiency anemia (decreased iron stores) from the anemia of chronic disease (increased iron stores). Perl's or Prussian blue staining detects hemosiderin (insoluble iron) but does not detect ferritin (soluble iron that is removed during staining). Hemosiderin usually is not observed in feline bone marrow.

VI. ANTIGEN AND ANTIBODY DETECTION

A. General concepts concerning RBC antigens and antibodies

1. In domestic animals, anti-erythrocyte antibodies are a consequence of transfusions, cross-placental transfer during pregnancy in the horse, vaccination with blood origin products, autoimmune phenomena, and natural occurrence in the cat.

2. Antigen identification is helpful in parentage testing, identifying potentially compatible blood donors (e.g., DEA-1.1-, 1.2-, and 1.3-negative dogs are best as blood donors), and identifying

matings likely to lead to isoimmunization and subsequent hemolytic disease of newborn animals (neonatal isoerythrolysis) (e.g., Aa-negative mares bred to Aa-positive stallions are at greater risk with the second foal).

3. Agglutination and hemolytic tests with specific antisera for each antigen are used for identification. Some antigens (blood group subtypes) are more important than others in determining animals at risk of hemolytic disorders. Blood from animals with the following blood antigen types has the greatest potential to cause sensitization and subsequent reactions:

a. In the dog, DEA (dog erythrocyte antigen)-1.1 (Aa_1), DEA-1.2 (Aa_2), and DEA-1.3 (Aa_3) are highly immunogenic and will sensitize recipients. Anti-DEA 1.1 and anti-DEA 1.2 are not naturally occurring, and their presence in blood requires prior exposure to these antigens. Anti-DEA 1.1 and anti-DEA 1.2 antibodies result in hemolytic reactions. Naturally occurring anti-DEA-3 (Ba) antibodies result in early erythrocyte removal or hemolysis. Anti-DEA 5 (Da) and anti-DEA-7 (Tr) antibodies occur naturally and result in increased erythrocyte removal.

b. Antibodies in mare colostrum to Aa and Qa blood types are most frequently involved in neonatal isoerythrolysis.

c. Cattle have marked variability of erythrocyte antigens with 70 blood group factors.

d. Type A and B antibodies occur naturally in the cat. Type B cats with anti-A antibody may have life-threatening hemolytic reactions when transfused with type A blood. Type A cats with anti-B antibodies have early erythrocyte removal when transfused with type B blood.

B. Detection of anti-erythrocyte antibodies

1. Blood cross matching

a. These tests are used to detect antibodies and determine when blood may be safely transfused.

b. The major cross match tests erythrocytes of the donor against serum of the recipient. This is a test to detect antibodies in the recipient that will react with donor cells. Incompatibilities in the major cross match are clinically significant because of the systemic reaction caused by the massive lysis of transfused cells.

c. The minor cross match tests serum of the donor against erythrocytes of the recipient. This is a test to detect antibody in donor blood. Incompatibilities are not life threatening because the transfused antibody will be diluted in the recipient.

d. Incompatible cross matches usually indicate prior sensitization.

(1) Naturally occurring antibodies usually are not present in sufficient concentration in animals to cause transfusion reactions, except in A-negative (Type-B) cats, which have naturally occurring anti-A antibodies.

(2) An incompatible cross match, therefore, indicates prior sensitization, except in the cat.

e. An incompatible cross match is indicated by agglutination in most species; however, hemolysis predominates in the horse and cow.

2. Antiglobulin (Coombs') tests

a. Antiglobulin (Coombs') tests are used to confirm immune-mediated hemolysis of erythrocytes.

b. The direct antiglobulin test (DAT) detects antibody and/or complement attached to the membrane of the patient's washed erythrocytes.

(1) The antiglobulin reagent (Coombs' serum) may be prepared against any antibody type (IgG, IgM) or complement (C3) or may be a mixture (IgG and C_3). Reagents that test for one antibody or complement are monovalent. Reagents that test for multiple antibodies and/or complement are polyvalent.

(2) Species-specific Coombs' serum must be used (e.g., rabbit anti-canine IgG for the dog).

c. The indirect antiglobulin test detects anti-erythrocyte antibody in the serum of the patient. In this test, the patient's serum is tested against washed erythrocytes from the sire, offspring, or a prospective blood donor animal. Supernatant (whey) from colostral milk may be used instead of serum in postpartum mares or cows to detect potential reactions against the offspring's cells.

d. A newer cell-enzyme-linked immunosorbent assay (ELISA)-based method (direct enzyme-linked antiglobulin test or DELAT test) has been described and used successfully to detect antibodies in DAT-negative canine autoimmune hemolytic anemia (AIHA). IgG, IgM, IgA, and C_3 have been detected coating the RBC membrane in dogs; however, this test has been used primarily as a research tool.

ANEMIA: DIAGNOSIS AND CLASSIFICATION

Anemia is an absolute decrease in the Hct, Hb concentration, and/or RBC count. Relative anemia may occur when the plasma volume is expanded (e.g., excessive parenteral fluid administration, pregnancy, neonates) or when blood specimens are improperly obtained from intravenous fluid lines (pseudoanemia).

I. DETERMINATION OF THE CAUSE OF ANEMIA. AS WITH ANY DISEASE, THE DIAGNOSIS OF ANEMIA IS MADE FROM HISTORICAL INFORMATION, PHYSICAL FINDINGS, AND LABORATORY FINDINGS.

A. History. The following findings may be important:

1. Drug administration or vaccination
2. Exposure to toxic chemicals or plants
3. Familial or herd occurrence of disease
4. Recent transfusions or colostral ingestion
5. Age at onset of clinical signs
6. History of prior blood disorder
7. Diet
8. Breed, signalment
9. Prior pregnancy of dam
10. Reproductive status (intact versus neutered)

B. Physical findings

1. Clinical signs suggesting the presence of anemia are related to decreased oxygen transport capacity of the blood and physiologic adjustments to increase the efficiency of the erythron and reduce the workload on the heart. Typical clinical signs include the following:
   - **a.** Pale mucous membranes
   - **b.** Weakness, loss of stamina, exercise intolerance
   - **c.** Tachycardia and polypnea, particularly after exercise
   - **d.** Syncope, depression
   - **d.** Increased sensitivity to cold
   - **e.** Heart murmur caused by reduced viscosity and increased turbulence of blood flow
   - **f.** Weak or fluttering pulse
   - **g.** Shock, if one-third of the blood volume is lost rapidly
2. Icterus, hemoglobinuria, hemorrhage, melena, petechiae, or fever may be observed, depending on the pathophysiologic mechanism involved.
3. Clinical signs are less obvious if the onset of anemia is gradual and the animal has adapted to the decreased erythrocyte mass and lowered oxygen transport capability. In this scenario the anemia upon presentation can be profound (Hct of less than 10%).

C. Laboratory findings. Laboratory confirmation is necessary because anemias are not always accompanied by typical clinical signs. Mild anemia often is diagnosed from the laboratory data of a sick animal when its presence was not previously suspected.

1. The Hct is the easiest, most accurate method to detect anemia. The Hct value should be interpreted with knowledge of the patient's hydration status and with consideration of any possible influence of splenic contraction.
2. Hb concentration and RBC count may be used to further classify the anemia but usually are not needed to confirm its presence.
3. Other laboratory procedures may be used to further characterize the anemia and arrive at a specific diagnosis. These procedures are discussed below and earlier in this chapter.

II. CLASSIFICATION

The cause of the anemia should be identified when possible because the term "anemia" per se does not constitute a definitive diagnosis. Classification schemes are used for definitive diagnosis, although a single classification may not be entirely satisfactory.

A. Classification according to size (MCV) and Hb concentration (MCHC) of the erythrocyte
   1. The MCV categorizes the anemia as normocytic, macrocytic, or microcytic. The average erythrocyte volume is within the reference interval, increased, or decreased, respectively.
   2. The MCHC categorizes the anemia as normochromic, hypochromic, or hyperchromic. The Hb concentration is within the reference interval if the erythrocytes are normochromic. Hb concentration is decreased if the erythrocytes are hypochromic. Hyperchromasia is most often the result of erythrocyte hemolysis or Oxyglobin® administration, because erythrocytes do not overproduce hemoglobin. In rare circumstances, spherocytosis may be associated with hyperchromasia because of decreased erythrocyte volume.

B. Classification according to bone marrow response
   1. Regenerative anemia
      a. The bone marrow actively responds to the anemia by increasing production of erythrocytes.
      b. Findings that denote regeneration of erythrocytes
         (1) Polychromasia
         (2) Reticulocytosis with anisocytosis and increased RDW
         (3) Macrocytosis (increased MCV) and hypochromasia (decreased MCH and MCHC) associated with reticulocytosis
         (4) Basophilic stippling of erythrocytes in ruminants
         (5) Hypercellular bone marrow with a decreased M:E ratio due to erythroid hyperplasia
      c. Species that are capable of the highest maximal reticulocyte response exhibit the most intense regeneration; the Hct returns to the reference interval more rapidly. In decreasing order, the ability of a species to mount a regenerative response is bird, dog, cat, cow, horse.
      d. The presence of regeneration suggests an extramarrow etiology, implying loss (e.g., hemorrhage) or lysis (e.g., Heinz bodies, immune-mediated, or fragmentation) of erythrocytes of sufficient duration (two to three days) for a regenerative response to be evident in the blood.
      e. Bone marrow examination is rarely necessary to detect erythrocyte regeneration; however, erythroid hyperplasia should be evident if aspirates are taken.
      f. Regeneration is difficult to detect in the horse because reticulocytes are not released into the blood. An increased MCV and RDW may suggest a regenerative response. Bone marrow examination may be helpful but is not always conclusive; erythroid hyperplasia and increased numbers of reticulocytes may be observed in the aspirates.
      g. Examples of regenerative anemias include hemolysis, hemorrhage, or regeneration after the cause of a nonregenerative anemia has been resolved.
   2. Nonregenerative anemia
      a. Nonregenerative anemia suggests the lack of an erythroid response in the bone marrow. Lack of response could be the result of inadequate time for erythropoiesis to occur as well as

conditions such as chronic inflammation, renal disease, and endocrine disorders. Horses do not release reticulocytes into blood; therefore, all anemias appear nonregenerative in this species.
b. Polychromasia, reticulocytosis, and basophilic stippling (ruminants) are inadequate to absent.
c. During the first two to three days after the onset of peracute or acute hemorrhage or hemolysis, the anemia may appear nonregenerative.
d. Bone marrow examination occasionally may reveal the pathophysiologic mechanism or etiology of some cases of nonregenerative anemia.
e. Examples of nonregenerative anemia include: anemia of inflammatory disease (AID), renal failure, iron deficiency anemia, aplastic anemia, pure red cell aplasia, and endocrine disorders.
f. The cause of anemia may be multifactorial, which could affect the regenerative response. For example, acute hemorrhage may be minimally regenerative or nonregenerative if the underlying cause is an inflammatory condition resulting in AID.

C. Classification according to major pathophysiologic mechanisms
1. Blood loss (hemorrhagic anemia)
2. Accelerated erythrocyte destruction (decreased erythrocytic life span) by intra- or extravascular hemolysis
3. Reduced or defective erythropoiesis

ANEMIA FROM BLOOD LOSS (HEMORRHAGIC ANEMIA) (TABLE 1.1)

I. CHARACTERISTICS OF ACUTE BLOOD LOSS (CASES 1, 25)

A. Clinical findings
1. Direct visual evidence of hemorrhage usually is present, but occult hemorrhage may occur. When laboratory findings suggest hemorrhagic anemia and direct evidence of hemorrhage cannot be found, sources of occult bleeding such as gastrointestinal hemorrhage should be considered. Thrombocytopenia and clotting test abnormalities indicate the potential for hemorrhage. Thrombocytopenia alone rarely results in hemorrhagic anemia. Autotransfusion may accompany hemorrhage into body cavities.

TABLE 1.1.
CAUSES OF BLOOD LOSS.

Acute hemorrhage	Chronic hemorrhage
GI ulcers	GI ulcers, neoplasms
Hemostasis defects	Hematuria
Bracken fern toxicosis	Hemophilia
Disseminated intravascular coagulation	Neoplasia
Factor X deficiency	Gastrointestinal neoplasms
Hemophilia A and B	Vascular neoplasms
Rodenticide toxicosis	Parasitism
Sweet clover toxicosis	Ancylostomiasis
Neoplasia	Coccidiosis
Splenic hemangiosarcoma	Fleas, ticks, lice
Splenic hemangioma	Hemonchosis
Thrombocytopenia	Strongylosis
Trauma	Vitamin K deficiency
Surgery	

2. Clinical signs depend upon the amount of blood lost, period of time during which bleeding occurred, and site of hemorrhage. Hemorrhage from multiple sites or delayed onset of hemorrhage at sites of vascular intervention suggest clotting abnormalities (Chapter 4).

B. Laboratory findings

1. The Hct initially is within the reference interval because all blood components (i.e., cells and plasma) are lost in similar proportions. The animal may be in hypovolemic shock if more than 33% of the blood volume is lost rapidly.
2. Splenic contraction delivers high-Hct splenic blood (Hct = 80%) into the circulation, temporarily increasing the Hct.
3. Blood volume subsequently is restored by the addition of interstitial fluid beginning two to three hours after the onset of hemorrhage and proceeding for 48 to 72 hours. This fluid shift causes dilution of the erythrocyte mass and laboratory signs of anemia (i.e., reduced Hct, RBC count, and Hb concentration) become evident. Hypoproteinemia (decreased plasma protein concentration) also may be observed.
4. Platelet numbers usually increase during the first few hours after hemorrhage. Persistent thrombocytosis may suggest continued blood loss.
5. Neutrophilic leukocytosis commonly occurs approximately three hours post-hemorrhage.
6. Signs of increased erythrocyte production (e.g., polychromasia, reticulocytosis) become evident by 48 to 72 hours and reach maximum values approximately seven days after the onset of hemorrhage. Erythroid hyperplasia is evident in bone marrow aspirates and precedes changes in the blood.
7. Plasma protein concentration begins to increase within two to three days and returns to the reference interval before Hct, RBC count, and Hb concentrations normalize.
8. The entire hemogram returns to reference intervals in one to two weeks in the dog following a single, acute, hemorrhagic episode. If reticulocytosis persists longer than two to three weeks, continuous bleeding should be suspected.
9. Thrombocytopenia and subsequent hemorrhage may occur with primary bone marrow failure; the anemia in these cases appears nonregenerative.

II. CHARACTERISTICS OF CHRONIC BLOOD LOSS (CASE 5)

A. Clinical findings

1. Anemia develops slowly and hypovolemia does not occur.
2. The Hct can reach low values before clinical signs of anemia become obvious because the slow onset of anemia allows for physiologic adaptations.

B. Laboratory findings

1. A regenerative response occurs but is usually less intense than with acute blood loss.
2. Hypoproteinemia usually is observed.
3. Persistent thrombocytosis may be evident.
4. Iron deficiency anemia, characterized by microcytosis and hypochromasia, may develop over time as body iron stores are depleted.

III. DIFFERENTIAL FEATURES OF ANEMIAS CAUSED BY EXTERNAL AND INTERNAL HEMORRHAGE

A. External blood loss, including gastrointestinal bleeding, prevents reutilization of certain components such as iron and plasma protein. These may be reabsorbed and recycled following internal hemorrhage.

B. Internal hemorrhage may be less severe and more intensely regenerative. Following internal hemorrhage, some erythrocytes are reabsorbed by lymphatic vessels (autotransfusion), particularly when hemorrhage occurs into body cavities. Remaining erythrocytes are lysed or phagocytosed. The iron and amino acids from catabolized proteins are recycled.

ANEMIA FROM ACCELERATED ERYTHROCYTE DESTRUCTION (HEMOLYTIC ANEMIA) (TABLE 1.2)

I. CHARACTERISTICS OF HEMOLYTIC ANEMIA (CASES 2, 3)

A. Clinical findings

1. Clinical signs of hemorrhage are absent.

2. In acute hemolytic anemia, clinical signs related to the severity of anemia may be dramatic because compensatory mechanisms develop more slowly.

3. Icterus may be seen in acute, severe hemolytic anemia.

4. Hemoglobinuria and hemoglobinemia (red plasma) are seen if significant intravascular hemolysis occurs.

5. Extravascular hemolysis is much more common than intravascular hemolysis.

B. Laboratory findings

1. Reticulocyte counts characteristically are higher in hemolytic anemias than in external hemorrhagic anemias. Iron from hemolyzed erythrocytes is more readily available for erythropoiesis than is storage iron or hemosiderin. When iron-containing blood is lost externally, body storage iron must be mobilized for increased erythropoiesis, delaying regeneration.

2. Plasma protein concentration is within the reference interval or increased (hyperproteinemia). Hemoglobinemia may be present with intravascular hemolysis, resulting in artificial hyperchromasia (increased MCH and MCHC) and hyperproteinemia.

3. Neutrophilic leukocytosis and monocytosis may occur.

4. Evidence of Hb degradation (e.g., hyperbilirubinemia, hemoglobinuria) may be present.

5. Abnormal erythrocyte morphology (e.g., Heinz bodies, erythrocytic parasites, spherocytes, or poikilocytes) (Figure 1.7) may suggest the mechanism of hemolysis.

II. DIFFERENTIATION OF THE CAUSES OF HEMOLYTIC ANEMIAS

A helpful approach in diagnosing hemolytic anemias is to identify the site and mechanism of erythrocyte destruction. Hemolysis can occur outside of the blood vessels following phagocytosis of erythrocytes by macrophages (extravascular hemolysis), or hemolysis can occur within blood vessels (intravascular hemolysis). Hemolysis can occur both extra- and intravascularly; however, erythrocyte destruction usually predominates at one of these sites.

A. Extravascular hemolysis (phagocytosis) (Case 2) Erythrocytes are sequestered in spleen or liver, where they are phagocytized or lysed. Hemoglobin is catabolized at the site of destruction.

1. Mechanisms of extravascular hemolysis

a. Antibody and/or C_3b mediated (Case 2)

(1) Antibody binds to an erythrocyte membrane antigen or other antigen (including haptens) tightly adsorbed to the erythrocyte membrane. Nonerythrocyte antigen-antibody complexes may be non-specifically adsorbed to the cell membrane. Glycophorins, band 3, and spectrin are the membrane antigens that usually are recognized by antibodies.

(2) C_3b is fixed into the erythrocyte membrane by the antigen-antibody reaction. Adsorbed immune complexes may fix C_3b and then elute from the cell, leaving only C_3b deposited on

TABLE I.2.
CAUSES OF ACCELERATED ERYTHROCYTE DESTRUCTION.

Intravascular hemolysis*	Extravascular (phagocytic) hemolysis*
Bacteria	RBC parasites
Clostridium hemolyticum	*Anaplasma* spp.
Cl. novyi	*Cytauxzoon* spp.
Cl perfringens	*Mycoplasma* (formerly *Eperythrozoon*) spp.
E. coli (hemolytic uremic syndrome)	*Mycoplasma* (formerly *Hemobartonella*) spp.
Leptospira spp.	*Theileria* spp.
RBC parasites	*Trypanosoma* spp.
Babesia spp.	Immune-mediated
Chemicals and plants	Autoimmune hemolytic anemia (dogs, cats)
Oxidants	Equine infectious anemia virus
Acetaminophen	*Anaplasma* spp. (formerly *Ehrlichia*)
Benzocaine	Feline leukemia virus
Brassica spp.	Lupus erythematosus
Copper, molybdenum deficiency	Hemangiosarcoma
Onions	Hematopoietic neoplasia
Phenothiazine	Penicillin
Phenazopyridine	RBC parasites
Propylene glycol	*Sarcocystis* spp.
Red maple	Intrinsic erythrocytic defects
Rye grass	Erythrocytic porphyria
Vitamin K	Hereditary stomatocytosis
Cephalosporins	Pyruvate kinase deficiency (dogs)
Ricin (castor bean)	Fragmentation
Snake venoms	Disseminated intravascular coagulation
Zinc	Heartworms
Immune-mediated	Hemangiosarcoma
Autoimmune hemolytic anemia (horses, cattle)	Vasculitis
Hemolytic disease of newborn (neonatal isoerythrolysis)	Hemophagocytic syndrome
Incompatible transfusions	Hypersplenism
Hypo-osmolality	Malignant histiocytosis
Selenium deficiency (cattle)	
Cold hemoglobinuria	
Hypotonic fluids	
Water intoxication	
Fragmentation	
Vena caval syndrome	
Hypophosphatemia	
Hyperalimentation	
Postparturient hemoglobinuria	
G-6-PD deficiency	
GSH deficiency (sheep)	
Hepatic failure (horses)	
Phosphofructokinase deficiency (dogs)	

*Many of the conditions listed in this table have both intravascular and phagocytic components, but are listed by the predominant type of hemolysis.

the membrane. The erythrocyte is an innocent bystander but is subsequently targeted for phagocytic removal.

(3) Macrophages have receptors for both the Fc component of antibody and C_3b. These receptors facilitate the recognition and attachment of macrophages to erythrocyte membranes that are coated with antibody and/or C_3b. Affected erythrocytes are completely or partially phagocytosed. Spherocytes are formed by partial phagocytosis with subsequent resealing of the erythrocyte's membrane. Because more membrane is removed than cell contents, the spherocyte appears small and round, and lacks central pallor (Figure 1.7).

(4) Immune-mediated hemolytic anemias may be caused by the following mechanisms when erythrocytes, and rarely erythroid precursors, are destroyed by a Type II hypersensitivity response.

(a) Unknown mechanisms, termed idiopathic autoimmune hemolytic anemia (AIHA)

(b) Infectious agents such as FeLV, EIA virus, *Ehrlichia* spp., or *Mycoplasma haemofelis* (formerly *Hemobartonella felis*)

i) These agents may alter the erythrocyte membrane, exposing hidden antigens to which the host produces antibody.

ii) Some pathogens form immune complexes that adsorb to the cell and fix complement (C_3b).

iii) Cross-reacting antibody may be formed in response to infection.

(c) Some drugs, such as penicillin, adsorb to the erythrocyte membrane and act as haptens in the production of anti-drug antibodies.

(d) Alterations in the immune system

i) Disturbances in T-cell function may disrupt immune regulation. T-cells from AIHA or AIHA-related sibling dogs have a greater degree of reactivity.

ii) Coombs' positive anemia has been observed in some lymphoid malignancies, protozoal diseases, rickettsial diseases, *Mycoplasma haemofelis (formerly Hemobartonella felis)*, FIV, FIP, and other chronic inflammatory diseases.

(e) Immune-mediated anemia also may occur as a paraneoplastic syndrome with lymphoma and plasma cell myeloma.

(5) The direct antiglobulin or Coombs' test detects warm-active IgG alone, IgG plus C_3, C_3 alone, and, rarely, cold-active IgM on the erythrocyte cell membrane. Cold-reactive IgM may fix complement in the absence of IgG. This cold agglutinin disease is often associated with autoagglutination, intravascular hemolysis, acute onset, and severe clinical signs. A newer and more sensitive direct-enzyme linked antiglobulin test (DELAT) has identified multiple immunoglobulin types (IgG, IgM, IgA) and complement components (C_3) on the surface of the erythrocyte membrane. A synergistic effect between the types of antibody and complement may occur.

(6) Warm-active IgM occasionally fixes complement to C_9; severe intravascular hemolysis occurs with activation of membrane attack complex.

(7) Cold autoagglutinins that bind to the erythrocyte membrane below 10°C to 15°C are usually not significant and are observed in blood specimens from many healthy animals.

b. Decreased erythrocyte deformability

(1) Changes in the erythrocyte membrane, increase in internal viscosity, or decrease in surface area to volume ratio predisposes affected erythrocytes to early removal from circulation. These less deformable erythrocytes are sequestered in the spleen and phagocytosed by macrophages.

(2) Examples of decreased erythrocyte deformability (Figure 1.7)

(a) Schistocytes of microangiopathic anemia

(b) Spherocytes of immune-mediated anemia

(c) Parasitized erythrocytes

(d) Eccentrocytes or Heinz body-containing erythrocytes

c. Reduced glycolysis and ATP content of the erythrocyte

(1) Affected erythrocytes are predisposed to removal from the vasculature by splenic macrophages.

(2) Reduction in glycolysis occurs with normal aging.

(3) This reduction is accelerated in hereditary pyruvate kinase and phosphofructokinase deficiency anemias.

d. Increased macrophage phagocytic activity

(1) When there is excessive macrophage phagocytic activity, normal erythrocytes also may be phagocytosed.

(2) Increased phagocytic activity is associated with conditions causing splenomegaly. Splenomegaly promotes excessive sequestration of erythrocytes and their exposure to macrophages.

(3) In humans, this condition has been called "hypersplenism."

(4) Increased macrophage phagocytic activity also occurs in hemophagocytic syndrome and malignant histiocytosis, resulting in cytopenias.

2. Clinical and laboratory characteristics of extravascular (phagocytic) hemolysis

a. The clinical course of disease is usually chronic with an insidious onset.

b. A regenerative response is associated with normal or increased plasma protein concentration.

c. Hemoglobinemia and hemoglobinuria are absent.

d. Hyperbilirubinemia occurs if the magnitude of the hemolysis is sufficient to exceed uptake, conjugation, and excretion of bilirubin by the liver. Unconjugated bilirubin usually predominates in early disease, but conjugated bilirubin may be prominent at a later time.

e. The bone marrow response may compensate for the destruction of erythrocytes in cases of low-grade hemolysis. In such instances, the Hct remains in the reference interval. This situation is referred to as a "compensated hemolytic anemia."

f. Neutrophilia, monocytosis, and thrombocytosis commonly accompany extravascular hemolysis.

g. Splenomegaly may result from increased macrophage activity and extramedullary hematopoiesis.

h. Low-grade extravascular hemolysis occurs in many anemias that are primarily nonhemolytic (e.g., anemia of chronic renal disease, iron-lack anemia). This is referred to as the "hemolytic component" of these other types of anemia.

3. Aids in identification of the specific cause of extravascular hemolysis

a. History of a particular breed and/or of affected littermates may suggest a hereditary cause of hemolysis. Examples include the following:

(1) Phosphofructokinase deficiency of American Cocker Spaniel, English Springer Spaniel, and mixed breed dogs with Spaniel heritage.

(2) Pyruvate kinase deficiency in dogs (Basenji, Beagle, Chihuahua, Dachshund, Pug, Miniature Poodle, West Highland White, American Eskimo Dog, and Cairn Terrier) and cats (Abyssinian, Somali, and domestic short haired).

(3) Possible hereditary predisposition to hemolytic anemia also may exist in other dog breeds (Border Collie, Cocker Spaniel, English Springer Spaniel, German Shepherd, Irish Setter, Old English Sheepdog, Poodle, and Whippet).

b. Additional laboratory findings

(1) Positive direct antiglobulin (Coombs') test against a specific immunoglobulin or C_3 on patient's erythrocytes in immune-mediated anemia or a positive DELAT test even if the direct antiglobulin test is negative.

(2) Abnormal erythrocyte morphology occurs in a variety of anemias (Figure 1.7). Erythrocytic parasites, spherocytes, schistocytes, and keratocytes suggest the potential for excessive phagocytosis of erythrocytes.

B. Intravascular hemolysis (Case 3). Erythrocytes are destroyed within the circulation, releasing hemoglobin into the plasma where it is either removed by the liver or excreted by the kidneys.

1. Mechanisms of intravascular hemolysis. The erythrocyte membrane must be significantly disrupted to allow escape of the Hb molecule into the plasma. Most of the mechanisms of intravascular hemolysis are extrinsic or extracorpuscular defects (i.e., the erythrocyte itself is initially normal).

a. Complement-mediated lysis

(1) Complement (C_3) is deposited onto the erythrocyte membrane by surface antigen-antibody reactions. If complement is activated to C_9, the membrane attack complex is formed, producing a large enough membrane defect for the escape of Hb.

(2) Complement-mediated lysis occurs most commonly in immune-mediated anemias when IgM is involved. IgM is very effective in fixing complement. In contrast, IgG fixes complement poorly but may occasionally cause complement-mediated lysis.

(3) IgM-mediated complement lysis is the mechanism in most cases of hemolytic disease of newborn foals (neonatal isoerythrolysis) and transfusion reactions (in cats and large animals). Occasionally, a similar mechanism involving IgM may occur in autoimmune hemolytic anemia.

(4) Complement fixation to C_3 promotes phagocytosis but not intravascular lysis.

b. Physical injury

(1) Traumatic disruption of the erythrocyte membrane can occur from the shearing effect of intravascular fibrin strands.

(2) Because fibrin usually is formed in small blood vessels, this type of anemia is called "microangiopathic anemia."

(3) Examples of microangiopathic anemia include disseminated intravascular coagulation, vasculitis, hemangiosarcoma, and heartworm disease.

(4) Schistocytes are fragmented erythrocytes whose membranes have been altered by trauma (Figure 1.7). Their presence suggests shearing of erythrocyte membranes and the presence of microangiopathic anemia.

c. Oxidative injury (Case 3)

(1) Oxidants affect the erythrocyte in three ways:

(a) Denaturation of Hb with Heinz body formation

(b) Oxidation and cross-linking of membrane proteins with eccentrocyte formation

(c) Oxidation of hemoglobin iron (Fe^{2+}) with the formation of methemoglobin (Fe^{3+}). This interferes with oxygen transport but does not cause anemia.

(2) Heinz body formation and oxidation of cell membranes can cause sufficient cellular damage for hemoglobin to escape from the cytoplasm.

(3) If intravascular lysis does not occur, these altered cells are removed prematurely from circulation by phagocytosis.

(4) The erythrocyte is protected from daily exposure to oxidants via two major pathways.

(a) Reduced glutathione, which neutralizes oxidants, is produced and maintained in the reduced state by the pentose phosphate (hexose monophosphate) pathway. Deficiency of enzymes and intermediates (e.g., glucose-6-phosphate dehydrogenase) in this pathway can lead to membrane oxidation and Heinz body formation in the presence of excessive exogenous oxidants.

(b) Iron is maintained in the reduced state by methemoglobin reductase; accumulation of methemoglobin is minimized. Methemoglobin reductase deficiency has been reported in dogs and one horse.

(5) In most cases, the offending oxidant is drug- or diet-derived. Either the oxidant or its intermediate metabolites directly oxidize or interfere with formation of reduced glutathione.

(6) Heinz bodies or eccentrocytes suggest oxidative damage (Figure 1.7).

d. Osmotic lysis

(1) Hemolysis may be associated with hypophosphatemia, especially in patients with diabetes.

(2) Membrane alterations insufficient to allow leakage of Hb may alter permeability to the extent that excess water is drawn into the normally hypertonic cell and lysis occurs.

(3) Hypotonic intravenous fluids cause osmotic lysis.

(4) Cold hemoglobinuria of cattle is thought to occur by this mechanism.

(5) Many of the causes listed under extravascular hemolysis (Table 1.2) may alter the membrane of some cells to the degree that osmotic lysis occurs prior to phagocytosis.

e. Membrane alterations by other mechanisms

(1) Castor beans contain ricin, which causes direct membrane lysis.

(2) Snake venoms have lytic properties.

(3) Bacterial toxins, such as the phospholipase of *Clostridium novyi*, directly attack membrane lipids.

(4) *Babesia* spp. multiply in the erythrocyte and disrupt the membrane upon exiting the cell.

f. Intravascular causes of hemolysis do not lyse all affected erythrocytes; some altered cells may remain in circulation and are subsequently removed by phagocytosis.

2. Clinical and laboratory characteristics of intravascular hemolytic anemia

a. Intravascular hemolysis usually presents as a peracute or acute disease.

b. History may reveal exposure to causative drugs or plants, recent transfusion of incompatible blood, or recent ingestion of colostrum.

c. A regenerative response occurs, but it may not be evident in early stages because two to three days are required before significant reticulocytosis occurs.

d. Hemoglobinemia (free Hb in the plasma) is the principal feature of intravascular hemolysis. Hemoglobinemia is usually detected by the following:

(1) Red discoloration of plasma

(2) Increased MCHC and MCH

(3) Decreased serum haptoglobin and hemopexin concentrations

e. Hemoglobinuria may occur 12 to 24 hours following hemolysis if the concentration of free hemoglobin saturates the available haptoglobin and hemopexin and exceeds the capacity of the renal tubular epithelial cells to absorb and metabolize any hemoglobin that passes the glomerular filter.

f. Hemosiderinuria occurs if there is sufficient renal tubular epithelial cell absorption and metabolism to form detectable hemosiderin.

g. Hyperbilirubinemia

(1) Bilirubin is not formed until 8 to 10 hours after the onset of the hemolytic episode.

(2) Hyperbilirubinemia will occur if bilirubin formation is of sufficient magnitude to exceed the capacity of the liver to remove bilirubin from plasma, conjugate it, and excrete it into bile.

(3) Unconjugated bilirubin is the predominant form early in the disease. Conjugated bilirubin becomes more prominent with time and occasionally may be the major form present. Conjugated hyperbilirubinemia is accompanied by bilirubinuria (Chapters 7 and 9).

h. Additional laboratory findings may include schistocytes, keratocytes, Heinz bodies, eccentrocytes, erythrocytic parasites (Figure 1.7), positive direct antiglobulin (Coombs') test on patient's erythrocytes, or antibody titer to or culture of potentially causative organisms.

ANEMIA FROM REDUCED OR DEFECTIVE ERYTHROPOIESIS (TABLE 1.3)

Anemias caused by reduced or defective erythropoiesis are nonregenerative. They are characterized by an abnormal bone marrow that cannot maintain effective erythropoiesis. The clinical course is usually long

TABLE 1.3.
CAUSES OF REDUCED OR DEFECTIVE ERYTHROPOIESIS.

Reduced erythropoiesis	Defective erythropoiesis
Anemia of chronic disorders	Abnormal maturation
Chronic inflammation	Congenital dyserythropoiesis of Herefords
Neoplasia	Dyserythropoiesis of English Springer Spaniels
Cytotoxic bone marrow damage	Erythremic myelosis
Bracken fern	Erythroleukemia
Cytotoxic cancer drugs	Macrocytosis of Poodles
Estrogen	Myelodysplastic syndrome
Furazolidone	Disorders of heme synthesis
Phenylbutazone	Chloramphenicol toxicity
Radiation	Copper deficiency
Erythropoietin lack	Iron deficiency
Chronic renal disease	Lead poisoning
Hypoadrenocorticism	Molybdenum poisoning
Hypoandrogenism	Pyridoxine deficiency
Hypopituitarism	Disorders of nucleic acid synthesis
Hypothyroidism	B_{12} deficiency, malabsorption
Immune-mediated	Folic acid deficiency
Pure red cell aplasia	
Infections	
Anaplasma (formerly *Ehrlichia*) spp.	
Feline leukemia virus	
Feline panleukopenia virus	
Parvovirus	
Trichostrongyles (non-bloodsucking)	
Myelophthisis	
Lymphocytic leukemia	
Metastatic neoplasia	
Myelofibrosis	
Myeloproliferative disorders	
Osteopetrosis, osteosclerosis	

and the onset insidious. Nonregenerative anemias, such as the anemia of inflammatory disease, are observed commonly in veterinary medicine.

I. GENERAL CONSIDERATIONS

A. Mechanisms

1. Ability of the bone marrow to sustain erythrocytic mass requires the following items to be adequate:

- **a.** Precursor cells (i.e., multi- and unipotential stem cells)
- **b.** Nutrients (e.g., iron and B vitamins)
- **c.** Stimulation (e.g., Epo, IL-3, G-CSF, GM-CSF)
- **d.** Microenvironment

2. Primary and secondary bone marrow failure

a. Primary bone marrow failure from intramarrow disease results in inadequate production of stem and progenitor cells.

b. Secondary bone marrow failure occurs from extramarrow causes such as lack of nutrients or growth factors (Epo, colony stimulating factors, or cytokines).

3. Bone marrow failure may be selective for the erythroid series (e.g., pure red cell aplasia) or may affect granulocytes and/or platelet-producing megakaryocytes leading to bicytopenia or pancytopenia. In pancytopenia (aplastic anemia), a nonregenerative anemia occurs concurrently with granulocytopenia and thrombocytopenia.

B. Bone marrow response

1. When the number of precursor cells or erythropoietic stimulation is inadequate, the erythroid marrow is normo- to hypocellular.

2. Maturation abnormalities are associated with a hypercellular marrow and ineffective erythropoiesis (i.e., failure of the erythrocytes produced to mature normally or to be delivered to the blood). Maturation abnormalities are especially prominent in nutritional deficiencies, myelodysplastic syndrome (MDS), leukemias, and hereditary dyserythropoiesis. Abnormal cells such as microcytes, macrocytes, or nRBCs usually are observed in the stained blood film.

3. All degrees of bone marrow failure can occur, from suboptimal response of the erythroid marrow following hemorrhage or hemolysis to complete aplasia.

II. DIFFERENTIATION OF ANEMIAS CAUSED BY REDUCED OR DEFECTIVE ERYTHROPOIESIS

This practical approach to the diagnosis of nonregenerative anemias is based on erythrocyte morphology, blood neutrophil and platelet numbers, and bone marrow cellularity. These anemias can be divided into the following hematologic patterns.

A. Normocytic, normochromic anemia with normal to increased neutrophil and platelet counts, and an increased M:E ratio caused by hypocellular erythroid marrow (this is expected but not always readily apparent). These general types of anemia include the following:

1. Anemia of erythropoietin lack caused by certain diseases:

a. Chronic renal disease (Cases 15, 19)

(1) The degree of anemia is roughly proportional to the severity of the uremia.

(2) Causes for the development of anemia

(a) Erythropoietin deficiency caused by destruction of the Epo-secreting peritubular interstitial cells

(b) Hemolysis caused by factors in uremic plasma

(c) Gastrointestinal hemorrhage from abnormal platelet function and vascular lesions

(d) Inhibitors of erythropoiesis in uremic plasma

b. Endocrinopathies (e.g., hypoadrenocorticism, hypoandrogenism, and hypopituitarism)

(1) Some of these hormones (e.g., androgens) may enhance the action of erythropoietin.

(2) In other cases, the exact mechanism of anemia is unknown.

2. Anemia of inflammatory disease (AID) (Cases 7, 8, 10, 11)

a. AID (anemia of chronic disease [ACD], anemia of chronic disorders) occurs in chronic infectious, inflammatory, or neoplastic disorders. The onset of anemia may be as short as three to 10 days.

b. AID is mediated by hepcidin, a peptide made within and released by the liver in response to inflammation and induced by the proinflammatory cytokine interleukin-6.

c. Diminished marrow responsiveness to erythropoietin, blunted erythropoietin release, and impaired availability of iron to the erythron are all involved in the pathogenesis of anemia.

d. Erythrocyte life span is shortened.

e. Laboratory findings in AID:

(1) Decreased to normal serum iron concentration
(2) Decreased to normal total iron-binding capacity
(3) Normal to increased serum ferritin concentration
(4) Normal to increased bone marrow macrophage iron stores
(5) Mild to moderate anemia (Hct 20% to 30%) that is usually nonprogressive
(6) Normocytic, normochromic erythrocyte indices
(7) Microcytosis and hypochromia rarely occur. Sequestration of iron must be markedly prolonged to cause microcytosis and hypochromia in AID.
(8) Increased serum copper and zinc concentrations
(9) Variable serum erythropoietin concentration
(10) Increased serum hepcidin concentration

f. Signs of inflammatory or neoplastic disease often dominate the clinical picture, obscuring signs of anemia.

g. If the primary disease process is alleviated, then recovery from AID follows.

3. Feline leukemia virus (FeLV)-associated nonregenerative anemia

a. Erythroid stem and progenitor cells are selectively killed by FeLV.

b. The anemia may be macrocytic due to asynchronous maturation.

4. Pure red cell aplasia (PRCA)

a. Anemia is characterized by a selective loss of erythroid precursors in the bone marrow.

b. PRCA appears to be immune-mediated because it may respond to treatment with corticosteroids and/or lymphocytotoxic drugs.

c. Some cases of PRCA have been positive by Coombs' testing and have been designated as "nonregenerative autoimmune hemolytic anemia."

d. Rarely, PRCA may be observed secondary to lymphoma.

5. Unknown mechanisms of nonregenerative anemia

a. Trichostrongyle infection (non-blood sucking) in cattle and sheep

b. Liver disease

c. Vitamin E deficiency

(1) Dietary deficiency of vitamin E may produce a nonregenerative anemia in swine.
(2) The erythroid marrow is hyperplastic with evidence of dyserythropoiesis.

B. Normocytic, normochromic anemia with neutropenia (except in the case of myeloproliferative disorders) and/or thrombocytopenia, variable M:E ratio. Generalized bone marrow hypocellularity and/or proliferation of abnormal cells may be present. These types of anemia include the following:

1. Aplastic anemia or pancytopenia (Case 4)

a. This is a disease of the multipotential stem cell or bone marrow microenvironment that leads to pancytopenia and an acellular, fatty bone marrow.

b. Concomitant deficiencies in erythropoiesis, granulopoiesis, and thrombopoiesis occur. Leukopenia and thrombocytopenia usually precede the development of anemia because of the shorter life span of leukocytes and platelets.

c. Causes of aplastic anemia

(1) Predictable or idiosyncratic drug reactions that can vary by species (e.g., late estrogen toxicosis in dogs, chloramphenicol toxicosis in cats, phenylbutazone, trimethoprim-sulfadiazine, albendazole)
(2) Chemical exposure and plant toxicosis (e.g., bracken fern in ruminants and horses)
(3) Irradiation
(4) Cytotoxic T-cells or antibody (suggested to be involved in some human aplastic anemias)
(5) Infectious agents (e.g., FeLV in cats, ehrlichiosis in dogs)

2. Myelophthisic anemia

a. In myelophthisic anemia, the bone marrow is physically replaced by an abnormal proliferation of stromal, inflammatory, or neoplastic cells. Examples of disease leading to myelophthisic anemia:

(1) Myeloproliferative disorders (e.g., hematopoietic malignancies, leukemias)
(2) Myelofibrosis
(3) Osteosclerosis
(4) Diffuse granulomatous osteomyelitis
(5) Metastatic cancer

b. Bone marrow aspirates may yield very few cells in some conditions (e.g., myelofibrosis) and many cells in other instances (myeloproliferative disorders). Core biopsies are preferred to diagnose stromal reactions.

c. Leukoerythroblastic reactions (metarubricytosis without reticulocytosis and a neutrophilic left shift in the absence of inflammation) may occur from disruption of marrow architecture with disorderly release of myeloid and erythroid precursor cells.

d. A mild regenerative response, caused by isolated erythroid foci, may be observed in the early stages of myelophthisic anemia.

e. Dacryocytes (teardrop-shaped poikilocytes) are present in human blood smears, but have not been recognized consistently in animal blood smears.

f. Myelophthisic anemia caused by myeloproliferative disorders often have a leukocytosis with a leukemic blood picture.

3. Nonregenerative anemias caused by infectious agents

a. Acute ehrlichiosis may present as pancytopenia. In chronic ehrlichiosis, only mild thrombocytopenia may be evident on the hemogram.

b. FeLV infection occasionally may cause concomitant anemia and leukopenia, resulting in pancytopenia. FeLV-induced anemias may be macrocytic.

c. Feline and canine parvoviral infections destroy hematopoietic cells, lymphoid cells, and gastrointestinal crypt epithelial cells. Neutropenia is often evident on the leukogram. Despite the destruction of hematopoietic cells by parvovirus, the anemia is usually masked by relative polycythemia from dehydration secondary to vomiting, diarrhea, and lack of fluid intake.

d. Canine parvoviral infection of sufficient duration can produce concurrent neutropenia and nonregenerative anemia.

C. Microcytic, hypochromic anemia with variable neutrophil and platelet counts, and usually a hypercellular marrow with a variable M:E ratio. Causes of this type of anemia include the following:

1. Iron deficiency (Case 5)

a. The most common cause of iron deficiency is chronic hemorrhage, usually with external blood loss.

b. Young, rapidly growing animals consuming an all-milk diet may have transient dietary iron deficiency resulting in mild anemia.

c. Iron deficiency anemia is associated with ineffective erythropoiesis and a hyperplastic marrow early in the disease process. With chronicity, the bone marrow becomes hypoplastic and both microcytosis and hypochromia are more evident.

d. Laboratory findings in iron deficiency anemia

(1) Decreased serum iron concentration
(2) Variable total iron-binding capacity, but TIBC is often within the reference interval or increased
(3) Low percent saturation of transferrin
(4) Decrease or absence of marrow macrophage iron stores
(5) Decreased serum ferritin concentration
(6) Increased free erythrocyte protoporphyrin
(7) Microcytosis. Microcytosis develops because the critical concentration of hemoglobin necessary to stop cell division is not reached, and an extra division occurs. Microcytosis often precedes hypochromasia.
(8) Hypochromasia is present in most species, but may not be observed in the cat.

(9) Poikilocytes (e.g., schistocytes, keratocytes). Poikilocytes are thought to result from oxidation of membrane proteins.
(10) Hypercellular bone marrow in early disease with a disproportionate number of late rubricytes and metarubricytes due to extra cell divisions.
(11) Low serum Hepcidin concentration.

2. Pyridoxine deficiency. This vitamin is a cofactor in heme synthesis. Pyridoxine deficiency leads to a failure to utilize iron and an iron-lack-type anemia.
3. Copper deficiency. Copper-containing ceruloplasmin and hephaestin are important in iron absorption and transfer between intestine, macrophages, and transferrin. Therefore, copper deficiency leads to iron deficiency.
4. Dyserythropoiesis in English Springer Spaniels. This disease is associated with polymyopathy and cardiac disease and is characterized by a microcytic, nonregenerative anemia with metarubricytosis and dysplastic change of nucleated erythroid cells.
5. Microcytosis without anemia occurs in the Asian dog breeds including the Akita, Chow Chow, Shar Pei, and Shiba Inu. Microcytosis also has been reported in a dog with hereditary elliptocytosis.
6. Microcytosis with mild anemia is a common finding in portosystemic shunts (PSS) in the dog (Case 13). Alterations in iron metabolism appear similar to those in the anemia of inflammatory disease. Normal to decreased serum iron concentration and serum TIBC occur in nearly half of affected dogs. Serum ferritin concentrations are normal to increased in conjunction with stainable marrow and hepatic iron stores. One-third of cats with PSS exhibit microcytosis, but anemia usually is not observed.
7. Members of the Camelidae family, including llamas, have elliptical erythrocytes. In iron deficiency, these erythrocytes are microcytic and exhibit irregular or eccentric areas of hypochromasia representing irregular Hb distribution within the cell.
8. Drugs or chemicals, including chloramphenicol and lead toxicoses, can block synthesis of heme.

D. Macrocytic, normochromic anemia with variable neutrophil and platelet counts. M:E ratio is usually low because of hypercellular erythroid marrow. Causes of macrocytic, normochromic anemia include the following:

1. Ruminants grazing cobalt-deficient or molybdenum-rich pastures
2. Vitamin B_{12} and folic acid deficiencies
a. This type of anemia has not been produced experimentally in animals, but macrocytic anemias that responded to these vitamins have been described (e.g., Giant Schnauzers).
b. Megaloblastoid erythroid precursors are observed in the bone marrow.
c. Enlarged, hypersegmented neutrophils may be observed in the blood smear.
d. The hypercellular bone marrow indicates ineffective erythropoiesis.
3. Erythremic myelosis or erythroleukemia (Chapter 3)
4. Congenital dyserythropoiesis and progressive alopecia of polled Hereford calves. This disease is characterized by a macrocytic, nonregenerative anemia with ineffective erythropoiesis.
5. FeLV infection. Cats may present with a macrocytic anemia, but the erythroid marrow is usually hypocellular.
6. Macrocytosis of Poodles. This hereditary condition is uncommon. Neither anemia nor reticulocytosis occurs. Erythrocyte counts typically are within the low end of the reference interval and MCVs often are very high (over 100 fL).

POLYCYTHEMIA

Polycythemia is an increase in the Hct, RBC count, and Hb concentration.

I. SPURIOUS OR RELATIVE POLYCYTHEMIA. THE TOTAL RBC MASS IS NORMAL. CAUSES OF RELATIVE POLYCYTHEMIA INCLUDE THE FOLLOWING:

A. Dehydration (Cases 6, 9, 18, 24)
- **1.** A decrease in plasma volume causes a relative increase in the Hct, RBC count, Hb concentration, and plasma protein concentration.
- **2.** Determination of dehydration is based on physical examination and not on laboratory tests.
- **3.** Mechanisms of relative polycythemia
  - **a.** Water loss caused by vomiting, diarrhea, excessive diuresis, water deprivation, perspiration, or febrile dehydration
  - **b.** Internal fluid loss in shock via increased vascular permeability
  - **c.** Loss of fluid by effusion into body cavities
- **4.** The Hct of sick animals may fluctuate 2% to 5% daily as a consequence of changes in the patient's hydration status.

B. Redistribution of erythrocytes
- **1.** Excitement causes epinephrine release and splenic contraction. Splenic contraction delivers high-Hct splenic blood (Hct = 80%) into the general circulation.
- **2.** This effect is common in the horse and cat.

II. ABSOLUTE POLYCYTHEMIA

Increased erythropoiesis expands the total RBC mass. Plasma volume and plasma protein concentration are within the reference interval.

A. Primary absolute polycythemia (polycythemia vera or primary erythrocytosis) is a myeloproliferative disorder of stem cells.
- **1.** Clinical pathology findings include the following:
  - **a.** Erythropoietin concentration is within the reference interval or decreased.
  - **b.** PO_2 is within the reference interval.
  - **c.** Thrombocytosis and leukocytosis occasionally accompany the erythrocytosis.

B. Secondary absolute polycythemia is caused by increased Epo secretion.
- **1.** Appropriate, compensatory Epo secretion occurs during chronic hypoxia (low PO_2) which occurs in the following instances:
  - **a.** High altitude
  - **b.** Chronic pulmonary disease
  - **c.** Cardiovascular anomalies with right to left shunting of blood
- **2.** Inappropriate Epo secretion (normal PO_2, no hypoxia) occurs in some cases of hydronephrosis or renal cysts, Epo-secreting neoplasms (embryonal nephroma, renal carcinoma, uterine leiomyoma, cerebellar hemangioma, hepatoma, other endocrine neoplasms), and certain endocrinopathies (Case 27).

C. Animals with severe polycythemia may present for seizures because of blood sludging and CNS ischemia.

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