Fig. 3.1. Erythrocyte kinetics in health: The erythron contains three major pools: erythrocyte precursors (mostly in marrow), blood erythrocytes, and splenic erythrocytes. After stimulation by Epo, colony-forming unit–erythroid cells (CFU-E) differentiate into rubriblasts (Rb) and the precursors proliferate (via mitosis) and mature until erythrocytes (E) are formed. An orderly maturation process produces a pyramidal distribution of erythroid cell populations (only the top half is shown). After release to the blood, erythrocytes circulate in the vascular system to transport O_2 to tissues. A reserve pool of erythrocytes is sequestered in the spleen of most mammals. Senescent erythrocytes are destroyed by macrophages.

Mϕ, macrophage; Mr, metarubricyte; Pr, prorubricyte; Rc, rubricytes; and Rt, reticulocyte.

Fig. 3.2. Hemoglobin synthesis and degradation.

- Hgb synthesis in erythrocyte precursors: The synthesis of Hgb has three major stages: (1) a series of porphyrin reactions, (2) incorporation of Fe^{2+} into protoporphyrin IX to form heme, and (3) binding of four ferriheme and four globin molecules to form hemoglobin.
- Hemoglobin degradation in macrophages: In health, senescent erythrocytes are engulfed by macrophages and heme is split from globin chains. Heme is degraded to bilirubin, Fe^{2+}, and carbon monoxide (CO). The globin chains are degraded to amino acids.

Fig. 3.3. Bilirubin metabolism.

- In health, erythrocyte destruction in macrophages of spleen, liver, or marrow results in Bu formation. Small and usually clinically insignificant amounts of Bu are formed from heme degradation associated with ineffective erythropoiesis and degradation of other heme-containing molecules (catalase, peroxidase, and cytochromes). As Bu leaves a macrophage, it forms a noncovalent association with albumin and is transported to hepatocytes. Bu is relatively water insoluble prior to binding to albumin (Alb).
- When Bu enters the liver and its protein-permeable sinuses, it probably binds to hepatocyte membrane receptors of the organic anion transport polypeptide (OATP) family, enters hepatocytes without albumin, and binds to Y-protein (ligandin, also called glutathione transferase) or Z-protein (fatty acid–binding protein). Bu probably enters hepatocytes by a passive but facilitated process; binding proteins enhance the process by reducing the efflux of Bu back to the sinusoidal plasma.
- Within hepatocytes, Bu diffuses to the endoplasmic reticulum, where it is conjugated with glucuronide (> 60 % with glucose in horses) to form bilirubin monoglucoronide or bilirubin diglucuronide, collectively called Bc. It then diffuses to the canalicular membrane.
- Bc is transported from the hepatocytes into the canaliculi (the rate-limiting step in bilirubin excretion) by an energy-dependent transport system for organic anions other than bile acids. The primary transporter is probably the multidrug resistance protein 2 (MRP2), also called the canalicular multispecific organic anion transporter (cMOAT).
- Bc in bile enters the intestine and is degraded to urobilinogen (colorless), which can be passively absorbed in the intestine and then enter the hepatocytes for excretion in the bile, or bypass the liver and be excreted in the urine. Urobilinogen can also be degraded to stercobilinogen (dark brown) and excreted in feces.
- If Bc escapes the hepatocytes and enters the blood, it can pass through a glomerulus and be excreted in the urine. Because albumin does not pass through the glomerular filtration barrier of most mammals, Bu/Alb does not enter urine in those animals.

Bu/Alb, Bu associated with albumin; Mϕ, macrophage; Sb, stercobilinogen; Ub, urobilinogen; and UDP-G, uridine diphosphoglucoronide.

Fig. 3.4. Fe kinetics in healthy animals.

- Absorption: Diets of domestic mammals may contain Fe^{2+} or Fe^{3+}. Ingested Fe^{3+} is converted to Fe^{2+} by ferric reductase (duodenal cytochrome *b* or Dcyt *b*), a surface enzyme, prior to entering enterocytes via divalent metal transporter 1 (DMT1). If there are low intracellular concentrations of hepcidin, there is increased synthesis of ferroportin, which, in conjunction with hephaestin, a copper-containing Fe oxidase related to ceruloplasmin, transports the Fe^{2+} to plasma transferrin in the form of Fe^{3+}. Apoferritin in mucosal epithelial cells binds to Fe^{3+} to form mucosal ferritin, which appears to be lost into the intestine when mucosal cells are sloughed. In most mammals, the rate of intestinal absorption is influenced by the need for Fe by the body; that is, if Fe is needed, more Fe is absorbed. In healthy adults, the amount of Fe absorbed per day is a very small percentage of total body Fe stores.
- Transport: Nearly all Fe in plasma is bound to apotransferrin, a transport protein (β-globulin) produced by hepatocytes. When Fe is bound to apotransferrin, the complex is called transferrin. Transferrin carries Fe^{3+} to and from tissues (for use by cells or for storage). In health, about one-third of transferrin's Fe-binding sites are occupied by Fe. Many cells have transferrin receptors but especially marrow erythroid cells and hepatocytes.
- Use in erythroid cells: After transferrin binds to and enters erythroid precursors, Fe^{3+} dissociates from apotransferrin and binds to cytoplasmic apoferritin (to form ferritin) or is incorporated into heme (Fe^{2+}) and then hemoglobin. Most apotransferrin escapes degradation and is returned to plasma. In health, about 50–70 % of total body Fe is within erythrocytes.
- Storage: Fe^{3+} is stored in two protein-Fe complexes: ferritin (plasma and tissue) and hemosiderin (tissue macrophages). In health, about 25–40 % of total body Fe is within storage forms. Young animals (especially neonates) have low amounts of stored Fe.
  - Ferritin consists of apoferritin complexed with Fe^{3+} and is a relatively soluble, mobile source of Fe^{3+}. There are several forms of apoferritin because of various combinations of H or L subunits. Plasma ferritin is a glycosylated polymer that is relatively Fe poor. Tissue ferritin, which is nonglycosylated and relatively Fe rich, is produced by many cells, primarily macrophages, hepatocytes, intestinal mucosal epithelial cells, and erythroid precursors. Synthesis of apoferritin by hepatocytes and macrophages is increased by inflammation (apoferritin is a positive acute-phase protein) and when Fe storage is increased.
  - Hemosiderin is a relatively insoluble, poorly mobile source of Fe^{3+} and represents the major storage form of Fe. Hemosiderin is a complex of protein and Fe oxides that is found primarily in lysosomes of macrophages in the spleen, liver, and marrow of most mammals. A healthy cat's marrow does not have enough hemosiderin to be detected by routine staining methods.
- Tissue forms: A relatively small quantity of Fe is present in myoglobin, catalase, peroxidases, and cytochromes.

Fig. 3.5. Major biochemical reactions in erythrocytes.

- Anaerobic glycolysis (also known as the Embden-Meyerhof pathway) provides the biochemical skeleton for erythrocyte metabolism and generates ATP and NADH. Because of the split of F-1,6-DP into two molecules of G-3-P for two parallel pathways, glycolysis consumes two molecules of ATP and produces four molecules of ATP. PFK is the rate-limiting enzyme, and its activity is enhanced by alkalemia and reduced by acidemia. PK catalyzes the last reaction, resulting in a net ATP production via anaerobic glycolysis. NADH is used to reduce $Hgb\text{-}Fe^{3+}$.
- The hexose monophosphate shunt (also known as the pentose shunt and the pentose phosphate pathway) generates NADPH that is used to keep GSH in a reduced state in a reaction catalyzed by GR (with cofactor FAD). GSH and NADPH are the major reducing agents in erythrocytes and are used to maintain Hgb and other proteins in a functional reduced state. G6PD is the rate-limiting enzyme of the shunt. R-5-P, which is also formed from inosine in pig erythrocytes, can undergo a series of reactions yielding F-6-P and G-3-P for glycolysis.
- Cb_5R uses FAD and NADH to catalyze the conversion of two molecules of $Fe^{3+}Cb_5$ to two molecules of $Fe^{2+}Cb_5$. The $Fe^{2+}Cb_5$ reduces $Hgb\text{-}Fe^{3+}$ to Hgb in a nonenzymatic reaction. Because of the second reaction, Cb_5R is sometimes called methemoglobin reductase (or NADH methemoglobin

reductase)

- NADPH D also catalyzes conversion of Hgb-Fe^{3+} to Hgb and thus is another methemoglobin reductase. However, this is a very minor reaction in physiologic states and requires an electron acceptor. Therapeutically, MB can be used as the electron acceptor and is converted to LMB; LMB then reacts nonenzymatically to reduce Hgb-Fe^{3+} to Hgb.

Fig. 3.5. *continued*

- The diphosphoglycerate (DPG) shunt (also known as the Rapaport-Luebering cycle) provides 2,3-DPG at the expense of ATP production; 2,3-DPG decreases Hgb affinity for O$_2$ and thus promotes O$_2$ delivery to tissues. Species vary in the 2,3-DPG content of their erythrocytes: Concentrations are high in dogs and horses but very low in cattle and cats.[250]

1,3-DPG, 1,3-diphosphoglycerate; 2,3-DPG, 2,3-diphosphoglycerate; 2-PG, 2-phosphoglycerate; 3-PG, 3-phosphoglycerate; 6-PG, 6-phosphogluconate; ADP, adenosine diphosphate; ATP, adenosine triphosphate; Cb_5R, cytochrome-b_5 reductase; DPG, diphosphoglycerate; F-1,6-DP, fructose-1,6-diphosphate; F-6-P, fructose-6-phosphate; Fe^{2+}Cb_5, ferrocytochrome b_5; Fe^{3+}Cb_5, ferricytochrome b_5; G-3-P, glyceraldehyde-3-phosphate; G-6-P, glucose-6-phosphate; G6PD, glucose-6-phosphate dehydrogenase; GSH, glutathione, reduced; GS-SG, glutathione disulfide; Hgb, deoxyhemoglobin; Hgb-Fe^{3+}, methemoglobin; Hgb-O$_2$, oxyhemoglobin; HK, hexokinase; HMP, hexose monophosphate; LMB, leukomethylene blue; MB, methylene blue; NAD$^+$, nicotinamide adenine dinucleotide; NADH, reduced nicotinamide adenine dinucleotide; NADP$^+$, nicotinamide adenine dinucleotide phosphate; NADPH D, NADPH dehydrogenase; NADPH, reduced nicotinamide adenine dinucleotide phosphate; PEP, phosphoenolpyruvate; PFK, 6-phosphofructokinase; PGD, phosphogluconate dehydrogenase; PK, pyruvate kinase; PO$_4$, phosphate; Prot-SH, protein with reduced sulfhydryl groups; Prot-S-S-Prot, protein with disulfide bridges; and R-5-P, ribulose-5-phosphate.

Fig. 3.6. Erythrocyte cytograms (ADVIA) from a healthy cat and an anemic dog: The volume (V) and Hgb concentration (HC) of each erythrocyte (RBC) is graphically displayed in an RBC VHC (volume hemoglobin concentration) grid (tic-tac-toe display). In these cytograms, most erythrocytes are within section *v*, indicating they are normochromic normocytes. Macrocytes are displayed in regions *i*, *ii*, and *iii*; microcytes are in regions *vii*, *viii*, *ix*; hypochromic erythrocytes are in regions *i*, *iv*, and *vii*; and hyperchromic erythrocytes are in regions *iii*, *vi*, and *ix*. Shifts in the erythrocyte distribution represent changes in erythrocyte populations.

- A healthy cat: There are no erythrocyte population shifts from the central normochromic normocyte region (region *v*). Erythrocyte indices from this nonanemic cat were WRIs.

- A dog with regenerative anemia (Hct = 28 % and reticulocytes = 266 × 10^3/μL): A population of erythrocytes extends into region *i*. This pattern is typical of regeneration because immature erythrocytes are hypochromic macrocytes. The shift may be noted prior to changes in MCV, MCHC, and CHCM because those values reflect the mean values of the erythrocytes. This anemic dog had a mildly increased MCV, mildly decreased MCHC and CHCM, and moderately increased RDW and HDW.

Differences in grid placement reflect different expected erythrocyte indices for cats compared to dogs.

Fig. 3.7. An approach to problem-solving anemias: After anemia has been detected or confirmed, the presence or absence of a regenerative response is determined by assessing RC or CRP or detecting increased polychromasia (see Anemia, sect. II.A). If it is a regenerative anemia, then the anemia is probably due to either blood loss or hemolysis. If the anemia is nonregenerative and has been present for several days, then it is due to reduced or ineffective erythropoiesis.

Fig. 3.8. Erythrocyte kinetics of acute blood loss.

A. Immediately after whole blood is lost, Hct and [total protein] should not change because erythrocytes and plasma are lost proportionately. However, blood volume is decreased.

B. Hypovolemia stimulates thirst to replenish ECF volume and induces movement of ECF from the extravascular space to the intravascular space, thus expanding blood volume. The fluid shift dilutes erythrocytes (and plasma proteins), and thus anemia (and hypoproteinemia) develops. The degree of anemia depends on the quantity and duration of hemorrhage and the time period from the onset of hemorrhage. Splenic contraction will diminish the severity of the anemia because splenic blood is rich in erythrocytes (especially in horses and dogs).

Fig. 3.9. Erythrocyte kinetics of chronic blood loss that results in Fe deficiency.

A. Initially, there is a continual loss of small quantities of blood over weeks to months. Anemia does not develop as long as compensatory increased erythropoiesis (using stored Fe) replaces lost erythrocytes.

B. After prolonged blood loss, Fe deficiency develops (decreased total body Fe). When Fe deficiency is severe enough, effective erythropoiesis decreases sufficiently so that the compensation for blood loss is inadequate, and thus anemia ensues. Concurrently, the erythrocyte life span decreases because of increased erythrocyte membrane fragility. Fe deficiency affects many organs and is present before anemia occurs.

C. Microcytosis and hypochromasia result from the defective heme synthesis caused by Fe deficiency. Hypochromasia develops because the amount of Fe that is available is inadequate for incorporation into heme for Hgb formation. While attempting to reach optimal cytoplasmic Hgb concentration, erythroid precursors are thought to undergo additional mitoses, so microcytes are formed (microcytic normochromic anemia). With severe Fe depletion, precursors are eventually unable to reach optimal cytoplasmic Hgb concentration, and then hypochromic cells are formed (microcytic hypochromic anemia).

Fig. 3.10. Hemolytic icterus: Accelerated destruction of erythrocytes in macrophages of spleen (also liver and marrow) increases the production and delivery of Bu to the hepatocytes. If the rate of Bu formation exceeds the liver's ability to clear Bu from plasma, hyperbilirubinemia with increased [Bu] develops. Increased delivery of Bu to hepatocytes also increases Bc formation and biliary excretion. If Bc formation exceeds Bc transport to canaliculi, then excess hepatocellular Bc diffuses to sinusoidal plasma. The increased [Bc] in plasma will increase the Bc excretion in urine (bilirubinuria). If there is persistently increased plasma [Bc], there may be increased formation of Bδ (see the Bilirubin Concentration section in Chapter 13).

Bu/Alb, Bu associated with albumin; Mφ, macrophage; Sb, stercobilinogen; Ub, urobilinogen; and UDP-G, uridine diphosphoglucoronide.

Fig. 3.11. Pathogenesis of hemoglobinemia and hemoglobinuria during intravascular hemolysis.

- The normal physiological processes that conserve Fe released during intravascular hemolysis can be divided into primary and secondary systems. These systems are not saturated during health, and thus hemoglobinemia is not seen in health. In pathologic states, saturation of the primary and secondary systems leads to hemoglobinemia and hemoglobinuria (loss of Hgb and Fe).

- The primary system of Fe conservation (the most important, which may become saturated or overwhelmed)
  - Intravascular erythrocyte damage or death causes release of Hgb to plasma. The unstable Hgb tetramer splits into dimers and immediately binds to Hpt (if available). The Hpt/Hgb dimer complexes are cleared from plasma primarily by hepatocytes,[251] which degrade Hgb dimers to Bu, Fe^{3+}, and amino acids.[252,253] There is evidence that macrophages have a receptor for the Hpt/Hgb complex and thus may also be involved in clearing the complexes from plasma.[254]
  - Plasma [Hgb] or [Hgb/Hpt] may be high enough to cause the plasma to be pink, and thus hemoglobinemia is recognized. Plasma will appear pink when plasma [Hgb] as low as 50 mg/dL.

- The secondary system of Fe conservation (becomes more important after plasma [Hpt] decreases)
  - Continued or more severe intravascular erythrocyte damage or death causes release of more Hgb to plasma. The plasma Hgb may oxidize to form methemoglobin, which dissociates and releases metheme and globin. Metheme binds to Hpx to form a metheme/Hpx complex.[251]
  - Metheme/Hpx complexes enter hepatocytes, where the complexes are degraded to Bu, amino acids, and Fe^{3+}.[251]
  - In people and nonhuman primates, metheme also binds to albumin, and the metheme/albumin complex enters hepatocytes so that Fe can be

conserved. However, albumin molecules of dogs, cattle, horses, and other domestic mammals do not bind metheme, and thus this pathway is not functional in those animals.[255]
- Overflow (which occurs when Fe conservation systems are saturated and results in marked hemoglobinemia and concurrent hemoglobinuria)
  - If the rate of hemolysis exceeds the ability of Hgb-binding proteins to conserve Fe, free Hgb dimers accumulate in plasma (hemoglobinemia). The transport maximum of Hpt for Hgb dimers is near 150 mg/dL.
  - Hgb dimers pass through the glomerular filtration barrier and are excreted in the urine (hemoglobinuria). Proximal tubule epithelial cells have some ability to resorb Hgb dimers and degrade them to Bu for conjugation and urinary excretion.

Hpt, haptoglobin; and Hpx, hemopexin.

Fig. 3.12. Pathogenesis of immune hemolysis.
- Erythrocytes coated with ESAIg and/or C3 undergo extravascular hemolysis in macrophages.
- Erythrocytes coated with ESAIg and/or C3 are converted to spherocytes by macrophages removing the erythrocyte membrane. Spherocytes undergo either extravascular or intravascular hemolysis because of their rigidity and fragility, respectively.
- Some ESAIg may bind complement (especially IgM), which activates the complement cascade and leads to intravascular hemolysis via the membrane attack complex (C5b-9). Damaged cells will be removed from circulation by macrophages.

ESAIg, erythrocyte surface-associated immunoglobulin; C3, third component of complement; and C5b-9, complex of complement factors 5b through 9.

Fig. 3.13. An approach to problem-solving erythrocytoses: After erythrocytosis has been detected or confirmed, the animal is examined for evidence of the most common causes: hemoconcentration or splenic contraction. If they are not found and the erythrocytosis is persistent, then diagnostic plans are formulated to pursue identification of secondary or primary erythrocytotic disorders. However, the erythrocytosis may be idiopathic.

Fig. 3.14. Pathogeneses of the erythrocytoses.
- Erythrocytosis of hemoconcentration: Dehydration (decreased total body water) results in a decreased ECF volume and thus a decreased plasma volume. With a decreased plasma volume but no change in number of blood erythrocytes, the [RBC] is increased. Loss of plasma H_2O because of increased vascular permeability in endotoxic shock also causes an erythrocytosis via hemoconcentration.
- Erythrocytosis due to splenic contraction (physiologic erythrocytosis): The fight-or-flight response or exercise causes the release of epinephrine. Epinephrine administration causes the contraction of splenic smooth muscle and thus the release of splenic blood to peripheral blood vessels. Splenic blood with a high Hct (70–80 %) is mixed with peripheral blood (Hct = 40–50 %) and causes erythrocytosis.
- Secondary erythrocytosis: Increased Epo activity stimulates erythropoiesis to cause erythroid hyperplasia and erythrocytosis associated with an increase in total body erythrocyte mass. Increased Epo production may be appropriate (if stimulated by tissue hypoxia) or inappropriate (if not stimulated by tissue hypoxia). Several disease states can cause a secondary erythrocytosis (see Erythrocytosis and Polycythemia, sects. II.C and D).
- Primary erythrocytosis: There is a proliferation of erythroid cells in the absence of increased Epo production that causes an erythrocytosis and increases total body erythrocyte mass. This may be neoplastic or nonneoplastic. Nonneoplastic primary erythrocytosis caused by defective Epo receptors has been reported in people. If erythrocytosis is concurrent with a neoplastic proliferation of leukocytes and megakaryocytes, then polycythemia vera is present.

Fig. 3.15. Agglutination reaction of a positive canine Coombs' test. Washed canine erythrocytes are incubated with rabbit anti-(dog immunoglobulin). If the dog's washed erythrocytes are coated with dog immunoglobulin, the antiserum will cause agglutination of the dog's erythrocytes when the concentration of rabbit anti-(dog immunoglobulin) is appropriate. Tests may also detect erythrocyte-bound complement proteins by using anti-(dog complement) immunoglobulin.