SECTION I

Evaluation of Peripheral Blood Films and Hemic Cytology

1 Peripheral Blood of Mammals

Normal Hemic Cells

Rodents (Mice, Rats, Gerbils, Hamsters)

The hematology of rodents commonly seen in veterinary practices is similar to that of domestic mammals. Extensive reference values based on age, gender, diet, housing, supplier, and collection site are available for rodents used as laboratory animals (Leonard and Ruben, 1986; Moore, 2000a, b; Bolliger et al., 2010). These reference values should be used as a tool when evaluating the hematology of pet rodents and not as the sole guide to determine if values are abnormal because the parameters upon which these published reference intervals are based and laboratory instrument and methodology used likely will vary from those obtained for the patient (Appendix B: Tables B.1, B.2, and B.3).

It has been well established that factors such as site of sample collection, age, gender, strain, reproductive status, anesthesia, method of restraint, temperature, and stress may alter hematologic reference intervals in rodents (Wright et al., 1983; Suber and Kodell, 1985; Jackson et al., 1988; Turton et al., 1989; Drozdowicz et al., 1990; Robel et al., 1996; Alemán et al., 1998; Moore, 2000a; Nahas and Provost, 2002; Kampfmann et al., 2012). For example, male rodents tend to have higher erythrocyte concentrations than female rodents, but these differences are not clinically significant. Pregnant rats tend to have lower erythrocyte counts, hemoglobin concentrations, and hematocrits, but higher mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), reticulocyte percentage, and platelet counts than nonmated rats, requiring separate reference data (Liberati et al., 2004). Blood collected from the heart of rats has a significantly lower erythrocyte count, hemoglobin concentration, and hematocrit compared to samples taken from the retro-orbital venous sinus and tail (Suber and Kodell, 1985). External factors, such as exercise and environment, can also influence cell populations in peripheral blood (Robel et al., 1996; Kampfmann et al., 2012). Many studies have also shown that nutrition has an effect on hematologic variables in rats (Schwartz et al., 1973; Pickering and Pickering, 1984; Ogawa et al., 1985; Levin et al., 1993; Hubert et al., 2000; Yoshii et al., 2003; Moriyama et al., 2008; Miyata et al., 2009; Asanuma et al., 2011).

Erythrocytes

The Romanowsky-stained erythrocytes of true rodents (rats, *Rattus norvegicus*; mice, *Mus musculus*; gerbils, *Meriones unguiculatus*; and hamsters, *Mesocricetus auratus*) are round, anucleated, pink, biconcave disks with a central pale area and a mean diameter between 5 and 7 μm. The erythrocytes of these animals have a relatively short half-life (45–68 days) compared to the larger domestic mammals, such as dogs and cats, and as a result, their blood generally has a higher concentration of reticulocytes compared to other mammals; therefore, the presence of a greater degree of polychromasia and anisocytosis on the blood film is expected (Ringer and Dabich, 1979; Moore, 2000a, b; Everds, 2006) (Figure 1.1). Polychromatic cells represent 1–18% of the erythrocyte population in healthy rats and mice (Ringer and Dabich, 1979). In general, 1–5% reticulocytes are expected in adult non-anemic rodents. However, when evaluating the erythropoietic response in rodents, an actual reticulocyte count offers a better assessment compared to the relative percentages of these cells. For comparison, an absolute reticulocyte count between 150 000/μL and 300 000/μL is expected for non-anemic adult mice and rats. The presence of a low number (usually less than 2% of erythrocytes) of Howell–Jolly bodies, basophilic stippling, and nucleated red blood cells is also common in rodent blood films. Nucleated red blood cells may account for up to 2% of

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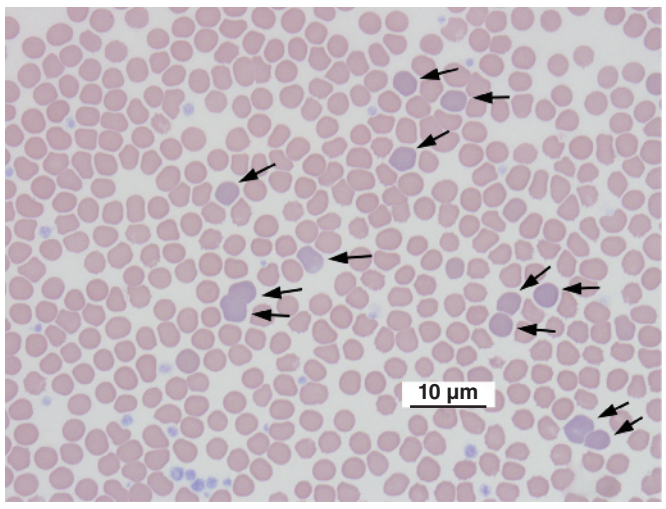


Fig. 1.1. Polychromatic erythrocytes (arrows) in the blood film of a mouse (*Mus musculus*), Wright–Giemsa stain.

erythrocytes in blood films of normal hamsters (Criswell et al., 2000; Car et al., 2006). Basophilic stippling and polychromasia are features of normal gerbil blood films. Rouleaux formation of erythrocytes is rarely seen in rodents, even with inflammatory disease.

Adult rats and mice normally have a high degree of reticulocytosis with means that average between 2% and 7% and the young have even higher numbers that range between 10% and 20%. In general, a normal hematocrit range for rodents ranges between 35% and 55% based on the normal hematocrit ranges of 38–51% for rats, 35–52% for mice (Bolliger et al., 2010), 35–50% for gerbils (Wagner and Farrar, 1987), and 36–55% for hamsters (Harkness and Wagner, 1989; Johnson-Delaney, 1995). The hemoglobin concentration of rodents generally ranges between 10 and 17 g/dL based on the normal hemoglobin ranges of 12–16 g/dL for rats, 10–17 g/dL for mice (Bolliger et al., 2010), 10–17 g/dL for gerbils (Wagner and Farrar, 1987), and 10–16 g/dL for hamsters (Harkness and Wagner, 1989; Johnson-Delaney, 1995). The mean corpuscular volume (MCV) of rodents generally ranges between 45 and 62 fL based on the normal MCV of 55–62 fL for rats (Moore, 2000a), 45–55 fL for mice, and 46–60 fL for gerbils (Mitruka and Rawnsley, 1981). The normal MCV of 65–78 fL for hamsters is higher than that of the other true rodents (Mitruka and Rawnsley, 1981). The red blood cell distribution width (RDW) obtained by calculation from automated hematology analyzers is a reliable indicator of variation in the size of red blood cells; however, the normal values are instrument-dependent. The MCHC of rodents generally ranges between 30 and 37 g/dL based on the normal MCHC of 30–34 g/dL for rats (Moore, 2000a), 30–38 g/dL for mice, 30–33 g/dL for gerbils (Mitruka and Rawnsley, 1981), and 28–37 g/dL for hamsters (Mitruka and Rawnsley, 1981). For best results in measuring hematologic analytes in rodents, the blood samples should be processed in a timely manner, preferably within 1 hour after collection (Ameri et al., 2011).

Leukocytes

Leukocytes of Mammals

The granulocytes of nondomestic mammals vary in appearance but can be classified as neutrophils or heterophils, eosinophils, and basophils (Hawkey, 1975; Hawkey et al., 1989; Campbell and Ellis, 2007). There are two types of neutrophils commonly found in normal blood samples of most exotic mammal species. These cells include segmented neutrophils and small numbers of band neutrophils. Band neutrophils are immature neutrophils and contain a smooth nucleus that has parallel sides and no constrictions in the nuclear membrane. Segmented neutrophils develop from band neutrophils. The nuclei of these cells have varying degrees of indentations and constrictions in the nuclear membrane, which causes the nucleus to fold into lobes of various shapes that are connected by filaments. Neutrophils contain numerous small granules that vary from colorless to pale-staining to dark-staining among different species of mammals. Cytochemical and ultrastructural features of cells often differ among species. For example, lysozyme activity is lacking in the neutrophils of hamsters and alkaline phosphatase activity is less in the neutrophils of mice (Parmley, 1988). Neutrophils of mammals are phagocytic and one of their primary functions is to destroy microorganisms. Circulating neutrophil concentration increases with inflammation especially when associated with invading microorganisms, such as bacteria.

The granules of eosinophils become intensely eosinophilic with maturation as a result of the changes in the basic protein content. The ultrastructure of the granules in mammalian eosinophils reveals a distinct crystalline shape (an electron-dense axial crystalloid that does not seem to be a constant feature of the eosinophils of other vertebrates) that varies with species; for instance, a trapezoidal pattern is found in the eosinophils of guinea pigs and true rodents and a needle-shaped pattern is found in rabbit eosinophils (Kelenyi and Nemeth, 1969; Parmley, 1988). Eosinophils contain large cytoplasmic granules that become increasingly eosinophilic in color as the cell matures as a result of the changes in the basic protein content of the granule. Mammalian eosinophils have phagocytic activity similar to that of neutrophils, but are less effective. Eosinophils are particularly numerous in the peripheral blood when antigens are continually being released, as occurs in parasitic disease (especially those involving larvae of helminths) and allergic reactions (especially those associated with mast

cell and basophil degranulation). In general, the presence of an eosinophilia is suggestive of one of these processes.

Mammalian basophils have characteristic cytoplasmic granules that are strongly basophilic in Romanowsky-stained blood films. Some species variation in the color of the granules does occur. For example, the granules present in guinea pig basophils often stain reddish-purple to black. Unlike basophils of lower vertebrates, those of mammals tend to have lobed nuclei. The ultrastructural appearance of the granules varies with species; for instance, a coiled threaded pattern is observed in basophil granules from primates and rabbits and a homogeneous pattern is observed in rodents (Parmley, 1988). Basophils participate in allergic and delayed hypersensitivity reactions.

Although rare, mast cells may occur in the peripheral blood and must be differentiated from basophils. Mast cells may be most commonly encountered with evaluating blood films of rodents if cardiocentesis is performed.

Mammalian monocytes generally are the largest leukocytes in peripheral blood films and do not vary grossly in appearance with species. The monocyte nucleus varies in shape (round or oval to lobed) and the moderately abundant cytoplasm is typically light blue-gray in color and may be vacuolated. The granules, when present, are very fine and appear azurophilic in Romanowsky-stained preparations. Monocytes engulf and degrade microorganisms, abnormal cells, and cell debris. Monocytes also regulate immune responses and myelopoiesis.

The appearance of mammalian lymphocytes varies depending upon the species, lymphocyte type, and degree of activation. Mammalian lymphocytes vary in size, color of cytoplasm (light to dark blue), and degree of nuclear chromatin condensation. Variability depends on the degree of antigenic stimulation and type of lymphocyte. The size of lymphocytes ranges from the size of an erythrocyte to the size of a neutrophil. The small lymphocytes are considered to be the inactive forms. Reactive lymphocytes have a slightly more abundant cytoplasm that stains basophilic and nuclei that have clefts or are irregular in shape. These cells are considered to be the B cells involved in immunoglobulin production (Weiser, 2012a). Large lymphocytes that have an increased amount of light-blue cytoplasm and azurophilic granules that vary in size are considered to be the T cells or natural killer cells (Weiser and Thrall, 2004).

In general, the leukocyte morphology of nondomestic mammals is a reliable indication of disease. The presence of immature cells, toxic neutrophils, and Döhle bodies is a more reliable criterion for infectious diseases than that of total leukocyte and differential counts, given the amount of information known regarding various strains and breeds.

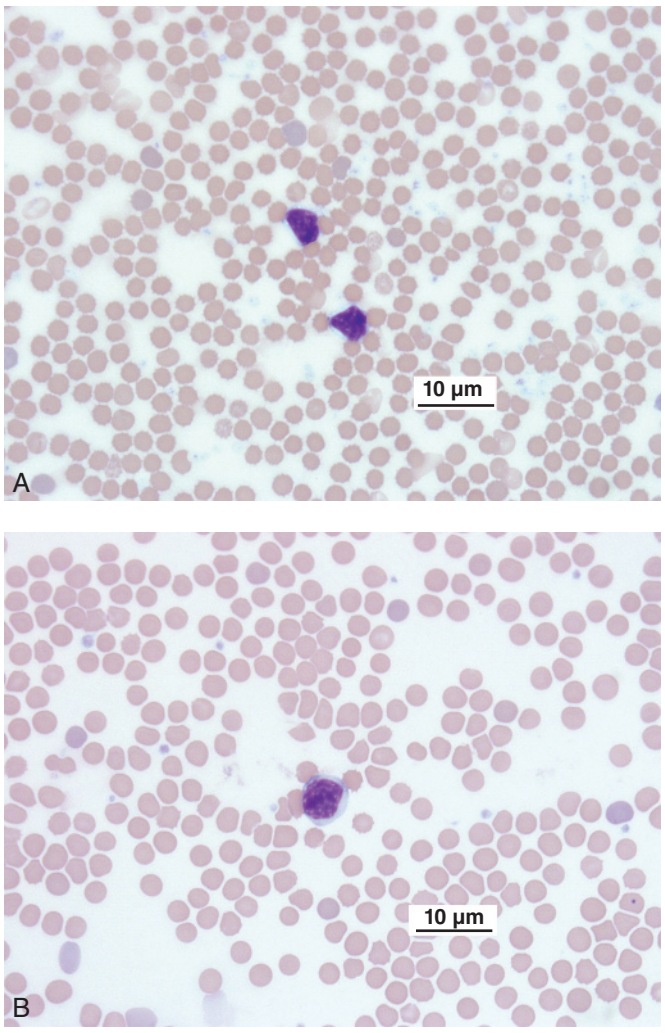


Fig. 1.2. (a) Small lymphocytes in the blood film of a mouse (*Mus musculus*), Wright–Giemsa stain; (b) large lymphocyte in the blood film of a mouse (*Mus musculus*), Wright–Giemsa stain.

Mice (*Mus musculus*) and Rats (*Rattus norvegicus*)

Lymphocytes are the predominant leukocytes in the blood of healthy mice and rats and they represent 70–80% and 60–75% of the leukocyte population, respectively (Bolliger et al., 2010; Campbell, 2012). The size of lymphocytes ranges from the size of erythrocytes to the size of neutrophils (Figures 1.2a and 1.2b). The cytoplasm of lymphocytes stains light blue, and azurophilic cytoplasmic granules are occasionally found in large lymphocytes.

Granulocytes of mice and rats often have nuclei without distinct lobes and typically exhibit a horseshoe, sausage, or ring (doughnut) shape (Campbell and Ellis, 2007; Bolliger et al., 2010) (Figures 1.3a and 1.3b). The ring shape results from a gradually increasing hole that develops in the nucleus during maturation of the granulocyte. Nuclear segmentation occurs as the ring breaks

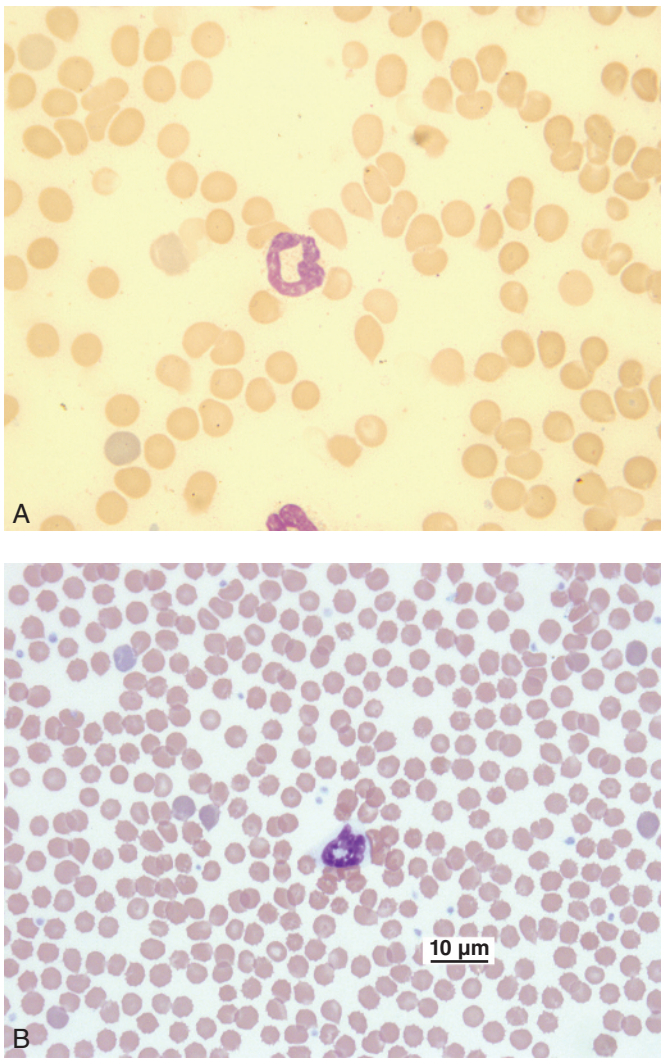


Fig. 1.3. (a) Neutrophil in the blood film of a domestic rat (*Rattus norvegicus*), Wright–Giemsa stain (1000×); (b) neutrophil in the blood film of a mouse (*Mus musculus*), Wright–Giemsa stain.

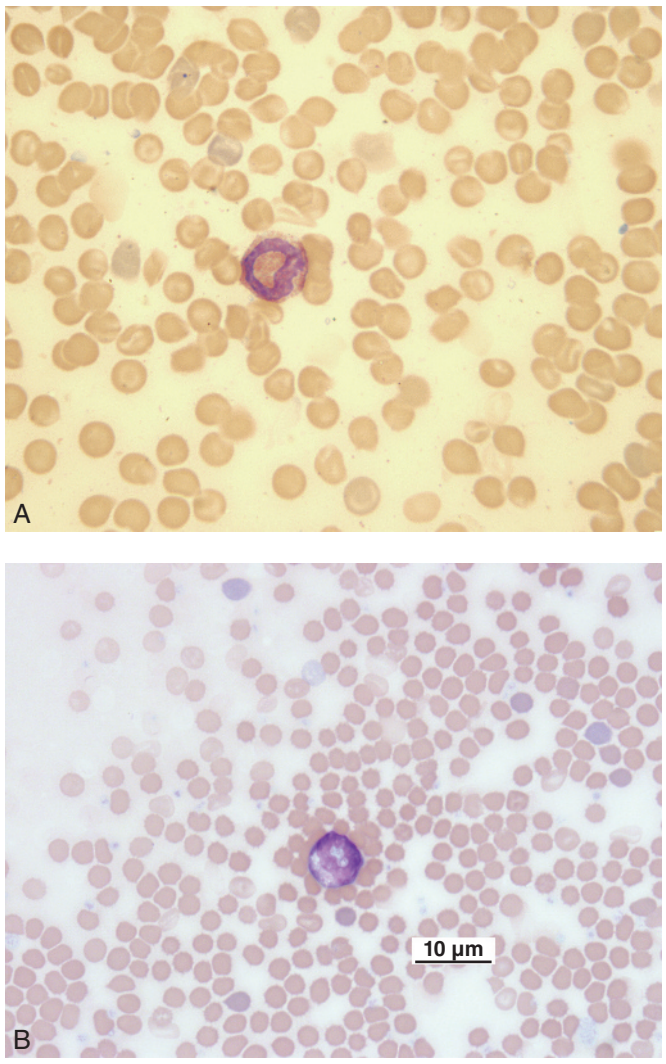


Fig. 1.4. (a) Eosinophil in the blood film of a domestic rat (*Rattus norvegicus*), Wright–Giemsa stain (1000×); (b) eosinophil in the blood film of a mouse (*Mus musculus*), Wright–Giemsa stain.

during maturation and begins to form constrictions; therefore, an increase in nuclear ring forms is suggestive of accelerated granulopoiesis.

Neutrophils represent 12–38% of the leukocyte differential in rats and 20–30% in mice. Neutrophils generally have a colorless cytoplasm but the cytoplasm of mice and rat neutrophils may contain dust-like red granules creating a diffusely pink appearance with Romanowsky stains. The nucleus of the typical rat neutrophil has few segments, but numerous indentations that make them appear hypersegmented. The nucleus of the mouse neutrophil is often segmented with fine connecting threads of chromatin. Rat neutrophils measure 11 µm in diameter.

Eosinophils generally comprise 0–7% of the leukocyte differential in the mouse and 1–4% in the rat. They have a ring- or U-shaped nucleus, a basophilic cytoplasm, and numerous round eosinophilic cytoplasmic granules that may be arranged in small clumps (Figures 1.4a and 1.4b). The granules found in the eosinophils of mice are large and uniform with indistinct margins, whereas those of rats are small and numerous.

Basophils are present in small numbers (0–1% of the leukocyte differential) in the blood of mice and rats. They often contain numerous large round purple cytoplasmic granules. Basophils with their lobed nuclei should be differentiated from mast cells with their nuclei without lobulation that may appear in peripheral blood, especially when cardiocentesis is performed. Basophil numbers appear higher in blood collected from the tail of mice and rats when excessive trauma is involved, such as laceration technique and compressing the tail to facilitate blood flow (Moore, 2000a).

Monocytes, measuring 17 µm in diameter, are the largest leukocytes found in the peripheral blood of rats

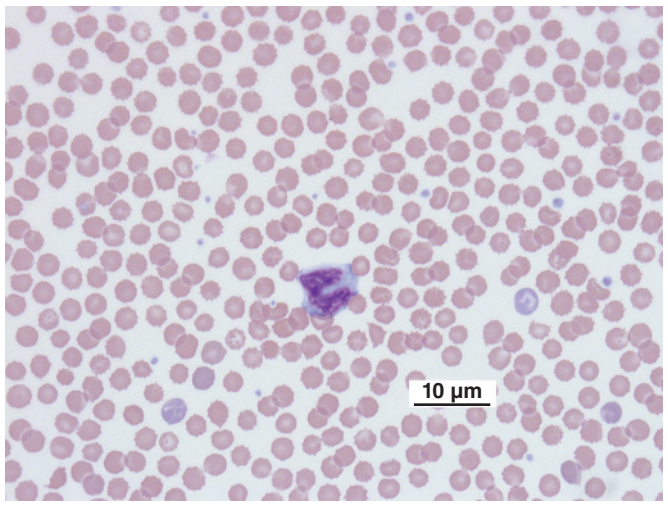


Fig. 1.5. Monocyte in the blood film of a mouse (*Mus musculus*), Wright–Giemsa stain.

and mice. They account for 1–6% of the leukocyte population in rats and 0–2% in mice. Monocytes have a variably shaped (round, indented, or lobulated) nucleus with the kidney-bean shape being the most common form (Figure 1.5). The abundant blue-gray cytoplasm often contains fine azurophilic granules and occasional vacuoles (Fredrickson and Harris, 2000).

Leukocyte concentrations of mice and rats not only demonstrate a distinct diurnal variation, but also vary markedly between strains and reproductive status (Wright et al., 1983). A distinct circadian rhythm affects peripheral leukocyte concentrations with an increase in circulating leukocyte concentration occurring during the light phase and a decrease during the dark phase. Pregnant rats tend to have higher leukocyte, segmented neutrophil, lymphocyte, and monocyte counts than nonmated rats, requiring separate reference data (Liberati et al., 2004). There is also an age-dependent variation in the neutrophil to lymphocyte ratio, with the lymphocyte concentration decreasing and neutrophil concentration increasing as a rodent ages. A distinct decrease in the total leukocyte count associated with a decrease in lymphocytes occurs in mice following the stress, such as occurs during transportation (Bean-Knudsen and Wagner, 1987; Drozdowicz et al., 1990). Thus, it is difficult to establish reference hematologic values for mice and rats because of the large number of strains and variations in blood collection methods, handling techniques, and environmental conditions.

Mongolian Gerbil (*Meriones unguiculatus*) and Hamsters (*Mesocricetus auratus*)

The hematologic features of hamsters and gerbils resemble those of mice and rats (Moore, 2000c, d). As with rats and mice, polychromasia/reticulocytosis and anisocytosis are normal findings in blood film from these rodents. Howell–Jolly bodies and nucleated red blood cells are commonly found, especially in the hamster; nucleated erythrocytes can represent up to 2% of the red blood cells in healthy adults (Harkness and Wagner, 1989). Stippled basophilia (remnant of cytoplasmic ribonucleoprotein) is a prominent feature of gerbil red blood cells (George et al., 1983). The red blood cell indices, such as MCV, hemoglobin concentration (Hgb), hematocrit (Hct), and MCHC, have been reported to be higher in adult male gerbils compared with adult females; however, the differences may not be clinically significant (Zimmerman et al., 2010c). The total erythrocyte count of male hamsters decreases by 25–30% following castration and will return to normal following testosterone supplementation (Smith et al., 2010). The red blood cell count and hemoglobin concentration increase with no change in the MCV in hibernating hamsters, which is likely associated with a near doubling of the red blood cell lifespan during this period (Reznik, 1975).

The neutrophils of some rodents were previously called pseudoeosinophils and later, heterophils, because their granules do not stain neutral with Romanowsky stains but are distinctly eosinophilic (Parmley, 1988). Because the neutrophils of hamsters and gerbils often contain round to rod-shaped acidophilic cytoplasmic granules, they are frequently called heterophils. The heterophils of gerbils often have a ring-shaped nucleus similar to those observed in rats and mice (Weeks and Glomski, 1978). Hamster eosinophils contain rod-shaped eosinophilic cytoplasmic granules compared to the more round granules of mice, rats, and gerbils. Eosinophils and basophils are rarely seen in the blood films of normal hamsters and gerbils. Whenever basophils are found, a nematodiasis should be suspected (Zimmerman et al., 2010c).

The normal total leukocyte counts of gerbils resemble those of mice rather than those of hamsters. The nocturnal habit of the hamster affects the white blood cells causing an increase in circulating heterophils (neutrophils) and thus the total leukocyte count when the animal is more active (Smith et al., 2010). The total leukocyte count of hibernating hamsters decreases with a shift to an even heterophil: lymphocyte ratio from that of non-hibernating hamsters where lymphocytes represent 60–80% of the leukocyte differential (Reznik, 1975). Gerbils normally exhibit a high degree of polychromasia, circulating reticulocytes, and stippled basophilia owing to the short red cell life span of 9–10 days. The normal hemogram of the gerbil is influenced by gender and age. Gerbils less than 8 weeks of age exhibit a macrocytosis, a panleukopenia, and an erythrocyte count that is half that of a normal adult, and male gerbils generally have higher MCV, Hb, packed cell volume (PCV), and MCHC as well as higher leukocyte counts with higher absolute lymphocytes compared to females (Heatley and Harris, 2009). Thus, the lymphocyte: neutrophil ratio of gerbils

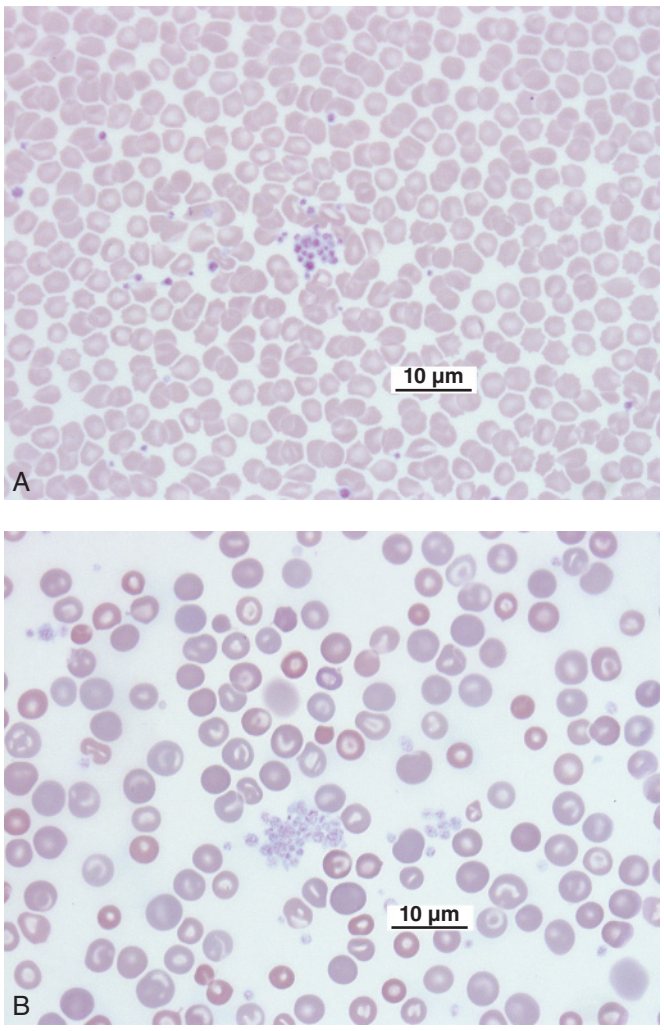


Fig. 1.6. (a) Platelets in the blood film of a ferret (*Mustela putorius furo*), Wright–Giemsa stain; (b) platelets in the blood film of an African hedgehog (*Atelerix albiventris*), Wright–Giemsa stain.

is generally considered to be 6.1:1 for males and 3.2:1 for females (Mays, 1969).

Platelets

Mammalian platelets are composed of cytoplasmic fragments that arise from megakaryocytes within the bone marrow and participate in hemostasis. Platelets are flat disks of the cytoplasm that contain cytoplasmic organelles (Figures 1.6a and 1.6b). They tend to be round, but can vary slightly in shape and size. The anucleated cytoplasm contains variable amounts of small purple granules on Romanowsky-stained blood films. Platelets are involved in the clotting process and are responsible for the initial hemostatic plug to prevent hemorrhage after vascular injury to the microcirculation. Because of this function, they are often found in clumps on

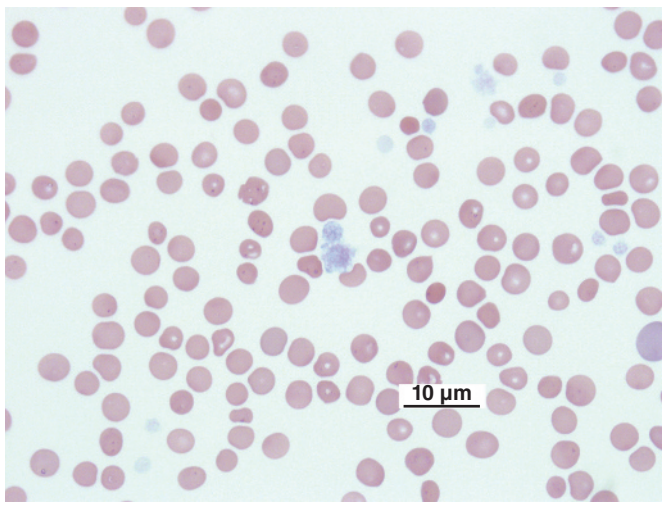


Fig. 1.7. Large platelets in the blood film of an African hedgehog (*Atelerix albiventris*), Wright–Giemsa stain.

blood films. Mammalian platelets are much smaller than erythrocytes in the same blood film. Platelets that are larger in size than erythrocytes are occasionally noted in the blood film. These cells are called macroplatelets or Shift platelets (Figure 1.7). These large platelets may indicate an accelerated thrombocytopoiesis with early release of immature forms into the circulating blood; therefore, they are an indication of platelet regeneration in some species.

Platelet numbers in the blood can be counted using automated methods or manual techniques using a hemacytometer. The number of platelets present in a blood film can be determined manually by counting the number of platelets per high-power field. A minimum of 5 platelets or range of 5–10 platelets per high-power field (1000× magnification or oil-immersion field) would be interpreted as an adequate number (Baker, 2004). Normal platelet concentrations for most mammals are greater than 100 000/mL of blood. If excessive platelet clumping is present, the platelet count may appear to be lower than normal. The presence of clumping and its artifactual effect on the platelet count can be confirmed by identifying clumps of platelets at the feathered edge of the smear.

Platelet concentrations in rodents tend to be high compared with those of larger domestic mammals and platelet concentrations greater than 1×10^6 per µL are common. The total platelet count of hamsters and gerbils is similar to that of other rodents with an expected range of $400–600 \times 10^3$/µL. A normal physiologic decrease in the total platelet count may occur as seen in hibernating hamsters (Reznik, 1975; Deveci et al., 2001).

Guinea Pigs (*Cavia porcellus*)

Erythrocytes

The Romanowsky-stained erythrocytes of guinea pigs (*Cavia porcellus*) are round, anucleated, pink,

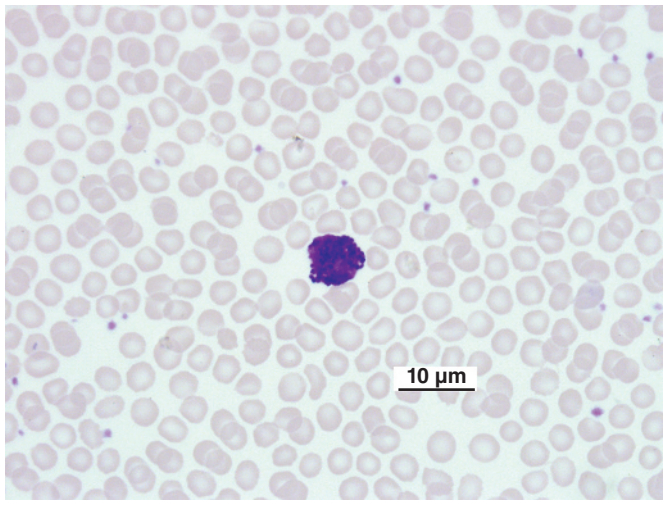


Fig. 1.8. Normal erythrocytes and a basophil in the blood film of a guinea pig (*Cavia porcellus*), Wright–Giemsa stain.

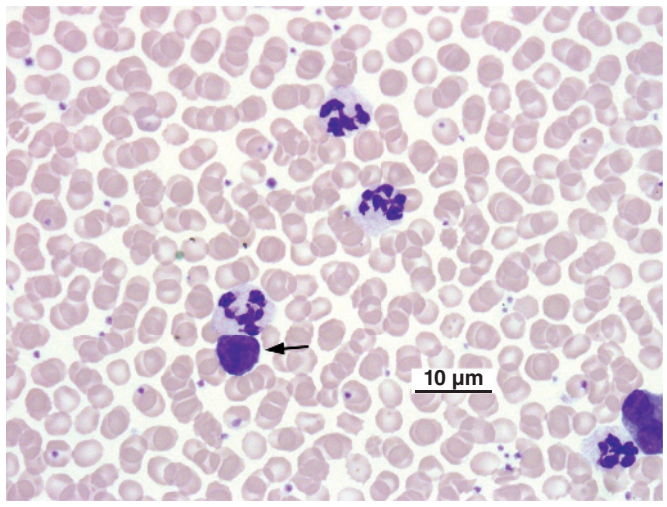


Fig. 1.9. Heterophils and lymphocyte (arrow) in the blood film of a guinea pig (*Cavia porcellus*), Wright–Giemsa stain.

biconcave disks with a central pale area and a mean diameter between 6.6 and 7.9 µm, larger than those of most other rodents (Moore, 2000e) (Figure 1.8). Polychromasia is commonly observed on guinea pig blood films, which like those of true rodents, is directly related to the short half-life of the erythrocytes. The normal degree of polychromasia is 1.5% in adult guinea pigs, but is much higher in young guinea pigs (4.5% in juveniles) (Zimmerman et al., 2010b). The red blood cell indices, such as the total erythrocyte count, PCV, and hemoglobin concentration, of guinea pigs are generally lower than those of true rodents (Marshall, 2008). Increased polychromasia/reticulocytosis and a macrocytosis characterize regenerative responses to anemia.

The normal erythrocyte parameters of guinea pigs are influenced by a variety of factors, such as age and gender. For example, 1-month-old or younger male guinea pigs tend to have lower erythrocyte concentrations and PCVs than older male guinea pigs (Jain, 1986). Male guinea pigs tend to have higher erythrocyte concentrations than females and females tend to have higher MCV values than males (Mitruka and Rawnsley, 1981; Jain, 1986).

Leukocytes

The guinea pig heterophil is analogous to the neutrophil of other species. Guinea pig heterophils measure 10–12 µm in diameter, have a pyknotic segmented nucleus, and contain cytoplasmic granules that stain eosinophilic that often cause them to be referred to as pseudoeosinophils in older references (Figures 1.9 and 1.11).

Guinea pig eosinophils (10–15 µm in diameter) tend to be slightly larger than the heterophils in the same

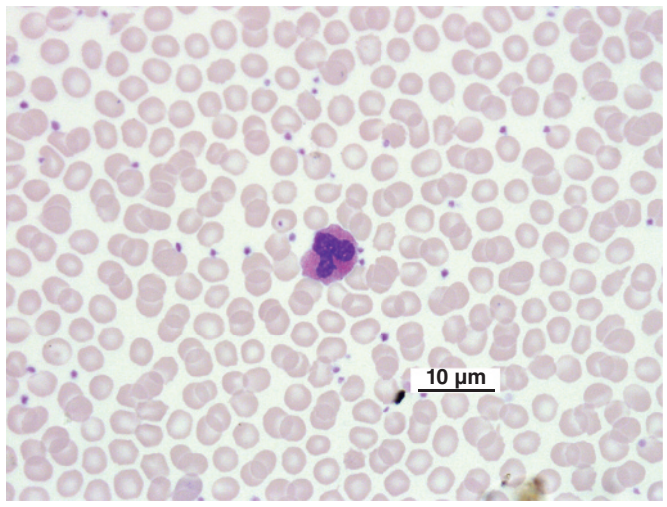


Fig. 1.10. Eosinophil in the blood film of a guinea pig (*Cavia porcellus*), Wright–Giemsa stain.

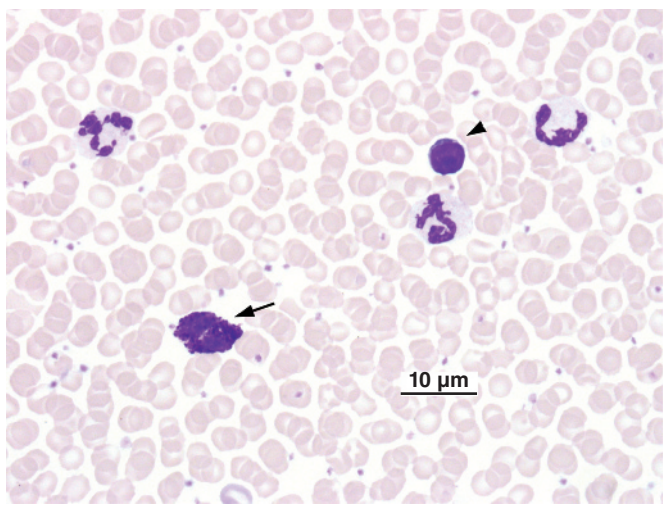


Fig. 1.11. Basophil (arrow), heterophils, and lymphocyte (arrowhead) in the blood film of a guinea pig (*Cavia porcellus*), Wright–Giemsa stain.

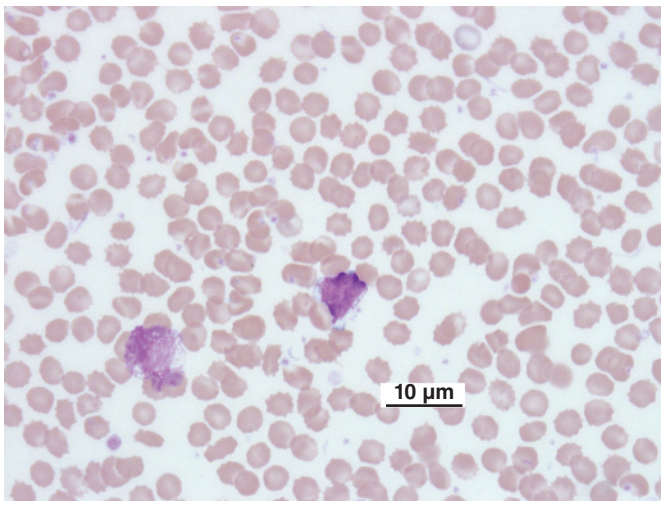


Fig. 1.12. Large lymphocyte with azurophilic granules in the blood film of a guinea pig (*Cavia porcellus*), Wright–Giemsa stain.

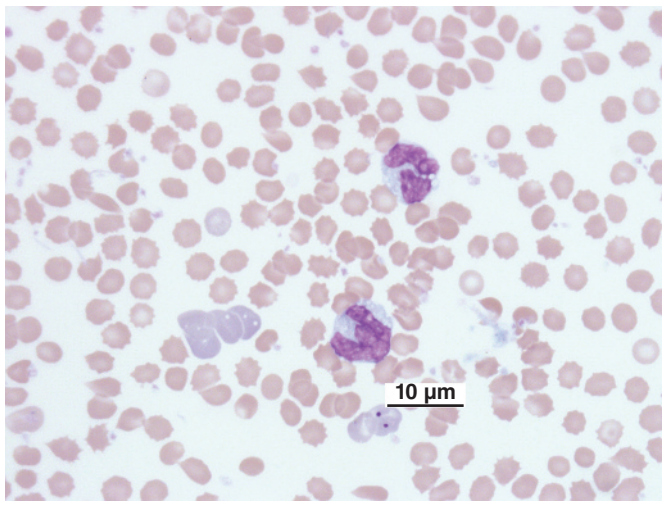


Fig. 1.13. Monocytes in the blood film of a guinea pig (*Cavia porcellus*), Wright–Giemsa stain.

blood film (Figure 1.10). They contain large round to rod-shaped bright red cytoplasmic granules. The granules of eosinophils are larger than the granules of heterophils, making eosinophils easy to differentiate from heterophils.

Guinea pig basophils are nearly the same size of heterophils. Their cytoplasm is densely packed with reddish-purple to black granules of variable sizes (Figures 1.8 and 1.11).

Lymphocytes are the predominant leukocytes in the differential of healthy guinea pigs (Figures 1.9, 1.11, and 1.12). Small lymphocytes (approximately the size of erythrocytes) are the most common form. They have a round nucleus with condensed nuclear chromatin that is surrounded by a narrow band of light blue cytoplasm with the Romanowsky stains. Large lymphocytes that are almost twice the size of small lymphocytes occur in fewer numbers. Large lymphocytes have a slightly smaller nucleus:cytoplasmic ratio, less condensed nucleus, and more abundant blue cytoplasm that often contains azurophilic granules (Figure 1.12).

Because guinea pigs are normally lymphocytic, the response in early inflammation reveals an increase in heterophils and decrease in lymphocytes with either a normal leukocyte count or a leukopenia. Often, the total platelet count is an important marker of inflammation in guinea pigs as well as other small mammals where a large increase in the platelet count (>1 000 000/µL) can be seen without a change in total white blood cell count (Riggs, 2009; Riggs and Mitchell, 2009).

Monocytes in guinea pig blood films are large mononuclear leukocytes with an abundant blue-gray cytoplasm that tends to be darker than that of large lymphocytes (Figure 1.13). The nuclear chromatin of monocytes is usually more dispersed compared to that of large lymphocytes.

Approximately 3–4% of the leukocytes in the peripheral blood of adult guinea pigs are large mononuclear cells that contain a single, large cytoplasmic inclusion referred to as a Kurloff body (Jain, 1986) (Figures 1.14a and 1.14b). These Foa-Kurloff cells are unique to cavies, such as guinea pigs and capybaras. The finely granular and occasionally vacuolated Kurloff bodies stain homogeneously red with Romanowsky stains and stain positive with toluidine blue and Periodic acid-Schiff (PAS) (Jain, 1993). Kurloff bodies displace the cell nucleus, measure 1–8 µm in diameter, and consist of mucopolysaccharide (Percy and Barthold, 2007; Marshall, 2008). They appear to be influenced by sex hormones and occur in low numbers in immature male guinea pigs. The exact function of these cells is not known, but many speculate that they may function as killer cells (Eremin, 1980; Debout et al., 1999; Moore, 2000e).

The normal leukogram of guinea pigs is influenced by a variety of factors, such as age and gender. For example, male guinea pigs tend to have more circulating monocytes compared to females (Mitruka and Rawnsley, 1981). Also, female guinea pigs tend to have higher total leukocyte counts than males until they reach the age of 4–6 months where the genders become more equal until they reach 12 months of age when males tend to have the higher counts (Jain, 1986).

The bone marrow evaluation of guinea pigs is the same as that of other rodents and domestic mammals. The normal myeloid:erythroid (M:E) ratio for these animals generally ranges between 1.2:1 and 1.6:1 (Marshall, 2008; Zimmerman, 2010b).

Chinchillas (*Chinchilla lanieger*)

The hematologic features of chinchillas resemble those of mice and rats. As with rats and mice, polychromasia is normal finding in blood film. The neutrophils

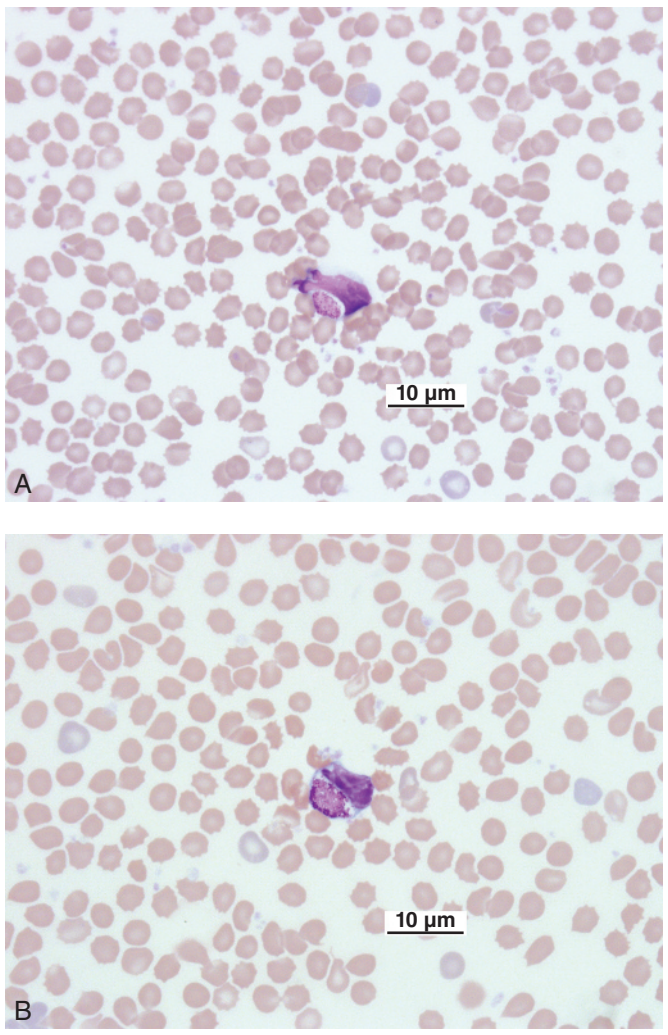


Fig. 1.14. (a) Lymphocyte with Kurloff body in the blood film of a guinea pig (*Cavia porcellus*), Wright–Giemsa stain. (b) Lymphocyte with Kurloff body in the blood film of a guinea pig (*Cavia porcellus*), Wright–Giemsa stain.

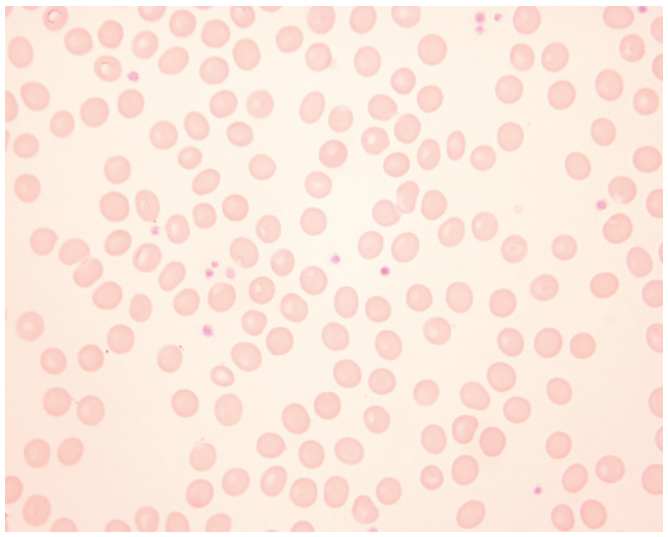


Fig. 1.15. Normal erythrocytes in the blood film of a chinchilla (*Chinchilla lanigera*), Wright–Giemsa stain (1000×).

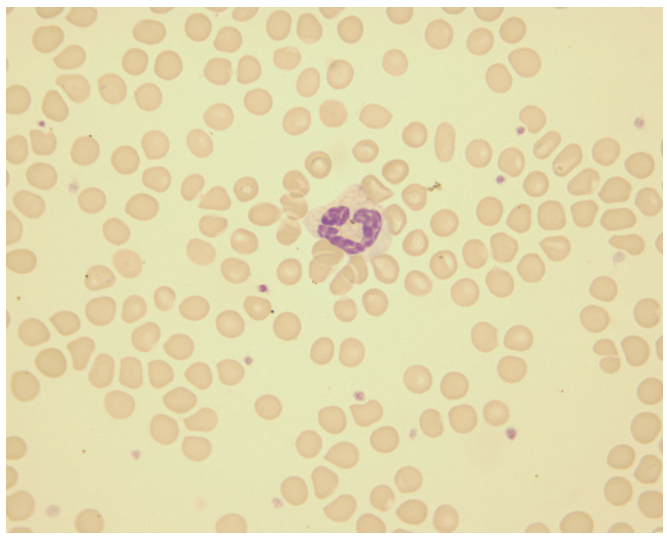


Fig. 1.16. Heterophil in the blood film of a chinchilla (*Chinchilla lanigera*), Wright–Giemsa stain (1000×).

of chinchillas are typically hyposegmented, often resembling neutrophils of dogs with the Pelger–Huët anomaly (Figure 1.16). Like the guinea pig, the chinchilla is normally lymphocytic; therefore, the hemic response in early inflammation often reveals an increase in heterophils and decrease in lymphocytes with either a normal leukocyte count or a leukopenia.

Rabbits (*Oryctolagus cuniculus*)

Erythrocytes

The Romanowsky-stained erythrocytes of rabbits are round, anucleated, pink, biconcave disks with an average diameter of 6.8 µm; however, the presence erythrocytes with a range of 5.0–7.8 µm makes reporting of a significant anisocytosis, a common finding in the hemogram of normal rabbits (Figure 1.17). The PCV of healthy rabbits generally range between 30% and 50%. Polychromatic erythrocytes and reticulocytes are common features of normal rabbit blood films. The estimated half-life of rabbit erythrocytes is between 45 and 70 days (Vacha, 1983; Zimmerman et al., 2010a). Polychromasia is typically observed in 2–4% of the erythrocyte population of healthy adult rabbits. The percentage of reticulocytes can be high (2.7-12.1%) in rabbits less than 2 months of age, but drops to 50% as much by 3 months of age and eventually leveling to 1.7-4.3% in adults (Jacobson, 1978). Nucleated erythrocytes and Howell-Jolly bodies are occasionally observed in blood films from healthy rabbits (Moore, 2000f).

A general anesthetic is often used in clinical practice to restrain rabbits for obtaining blood samples, but this practice does not appear to have an effect on the hematologic test results (Melillo, 2007). However, the normal erythrocyte parameters of rabbits can be

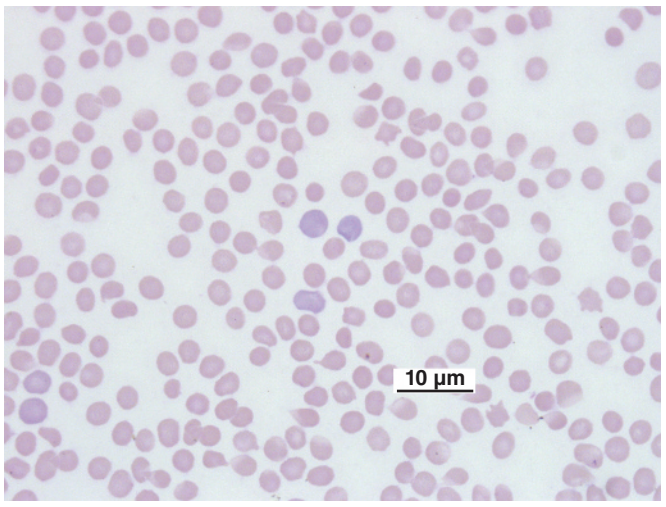


Fig. 1.17. Normal erythrocytes in the blood film of a rabbit (*Oryctolagus cuniculus*), Wright–Giemsa stain.

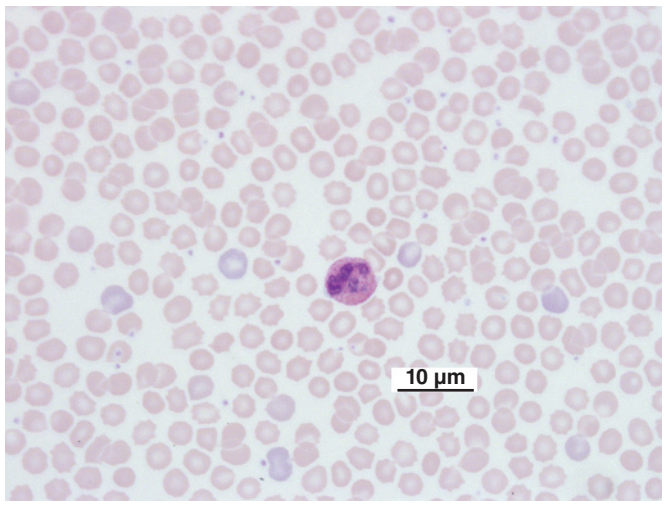


Fig. 1.18. Heterophil in the blood film of a rabbit (*Oryctolagus cuniculus*), Wright–Giemsa stain.

influenced by a variety of other factors, such as age, gender, and reproductive status. For example, rabbits less than 6 months of age have lower red blood cell counts and higher MCV and MCH values when compared to adults (Bartolotti et al., 1989; Marco et al., 2003). Male rabbits tend to have slightly higher erythrocyte counts and hemoglobin concentrations than females (Zimmerman et al., 2010a). The total erythrocyte count, hemoglobin concentration, and hematocrit values can be significantly lower in the pregnant rabbits in the third trimester compared to non-pregnant rabbits; however, the MCV value increases (Kim et al., 2002). For best results in measuring hematologic analytes in rabbits, the blood samples should be processed in a timely manner, preferably within 1 hour after collection (Ameri et al., 2011).

Anemia is commonly associated with a variety of diseases in rabbits. A regenerative response to an anemia in the rabbit patient is characterized by increased anisocytosis, polychromasia, nucleated erythrocytes, and presence of Howell-Jolly bodies. Infectious diseases often result in increases in the number of nucleated erythrocytes. Erythrocyte fragility studies used as a diagnostic aid in the detection of immune-mediated hemolytic anemia in rabbits is based upon the sodium chloride concentrations whereby the first detectable hemolysis in normal rabbits occurs at 0.5–0.3% NaCl (McLaughlin and Fish, 1994).

Leukocytes

The rabbit neutrophil is generally referred to as a heterophil because the cytoplasm typically stains diffusely pink with Romanowsky stains due to the fusion of many small acidophilic granules (primary granules) (Figures 1.18, 1.19, and 1.21). A variable number of larger eosinophilic granules are also present. The heterophils of rabbits and some rodents were previously

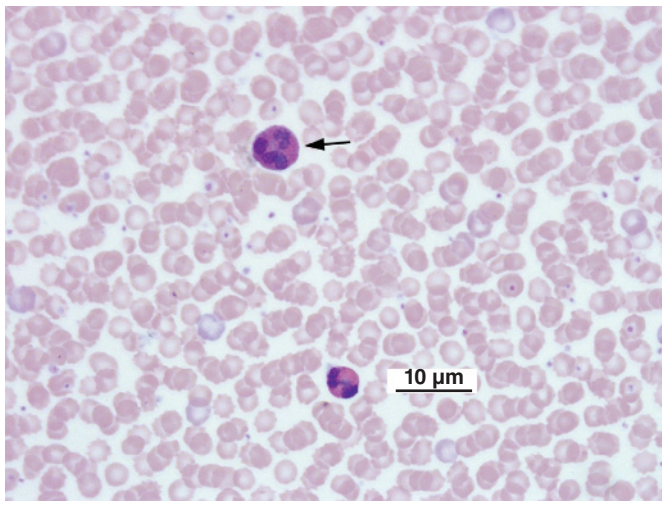


Fig. 1.19. Eosinophil (arrow) and heterophil in the blood film of a rabbit (*Oryctolagus cuniculus*), Wright–Giemsa stain.

called pseudoeosinophils because their granules (the larger secondary granules) do not stain neutral with Romanowsky stains but are distinctly eosinophilic. The rabbit heterophil normally measures between 10 and 15 µm in diameter. The polymorphic nucleus stains light blue to purple with Romanowsky stains. Rabbit heterophils are ultrastructurally, functionally, and biochemically equivalent to neutrophils from other domestic mammals and humans (Parmley, 1988). An occasional heterophil with characteristics of the Pelger–Huët anomaly may be observed in blood films from normal rabbits. Rabbit heterophils are easily distinguished from the eosinophils, which have large eosinophilic granules.

The eosinophils of rabbits measure between 12 and 16 µm in diameter; therefore, they are larger than the heterophils in the same blood film (Figure 1.19). Also,

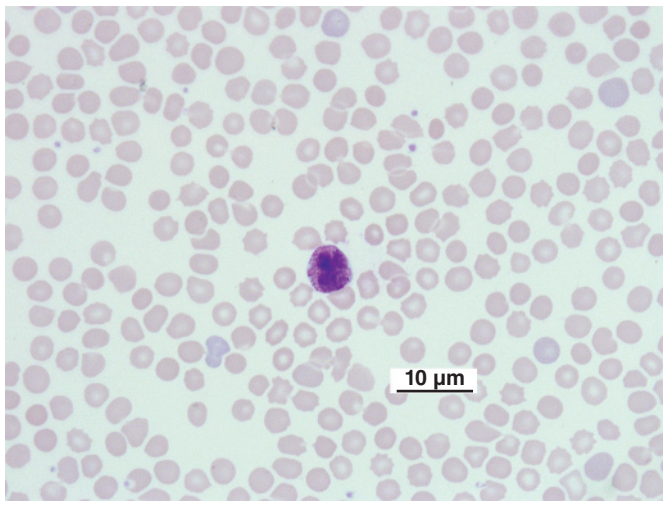


Fig. 1.20. Basophil in the blood film of a rabbit (*Oryctolagus cuniculus*), Wright–Giemsa stain.

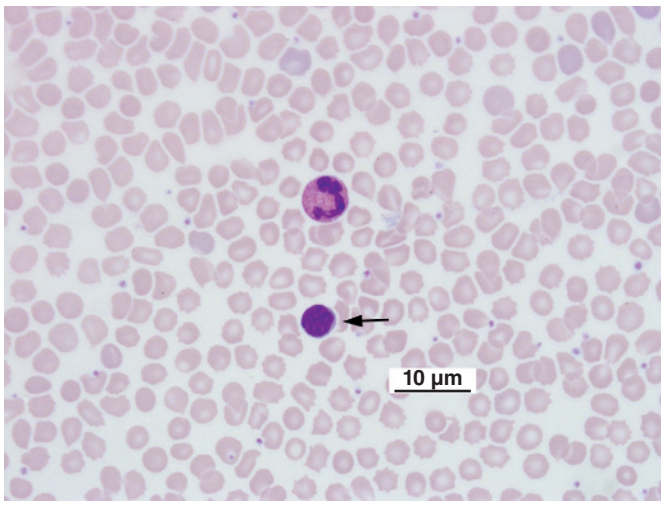


Fig. 1.21. Lymphocyte (arrow) and heterophil in the blood film of a rabbit (*Oryctolagus cuniculus*), Wright–Giemsa stain.

the cytoplasmic granules of the eosinophil are three to four times larger and more numerous than those of heterophils. Eosinophil granules typically stain intensely pink to pink-orange with Romanowsky stains creating a tinctorial quality that differs from that of heterophil granules. The nucleus of the eosinophil is often bi-lobed to U-shaped.

Rabbit basophils resemble those of domestic mammals. These are round cells filled with purple to black metachromatic cytoplasmic granules that often obscure the light purple nucleus in Romanowsky-stained blood films (Figure 1.20). Rabbits typically have more circulating basophils compared to other mammalian species. This is likely associated with fewer mast cells in the tissues of rabbits compared to those of other mammal (Jain and Zinkl, 1981). Basophils commonly represent 5% of the leukocytes in the differential white blood cell count of healthy rabbits, but they can also be as high as 30% in rabbits with no apparent abnormalities (Campbell and Ellis, 2007).

Rabbit lymphocytes are morphologically similar to those of other domestic mammals and humans (Figure 1.21). They have a round nucleus with condensed nuclear chromatin that is surrounded by a narrow band of blue cytoplasm. The majority of lymphocytes in healthy rabbit blood films are small (between 7 and 10 µm in diameter); however, large lymphocytes (between 10 and 15 µm in diameter) may also be present (Reagan et al., 2008). The large lymphocytes commonly demonstrate azurophilic granules in the cytoplasm.

Likewise, rabbit monocytes are morphologically similar to those found in other domestic mammals (Figure 1.22). These are large cells (15–18 µm in diameter) with a nuclear pattern that varies from lobulated to bean-shaped with a diffuse chromatin that stains light compared to that of lymphocytes. The monocyte

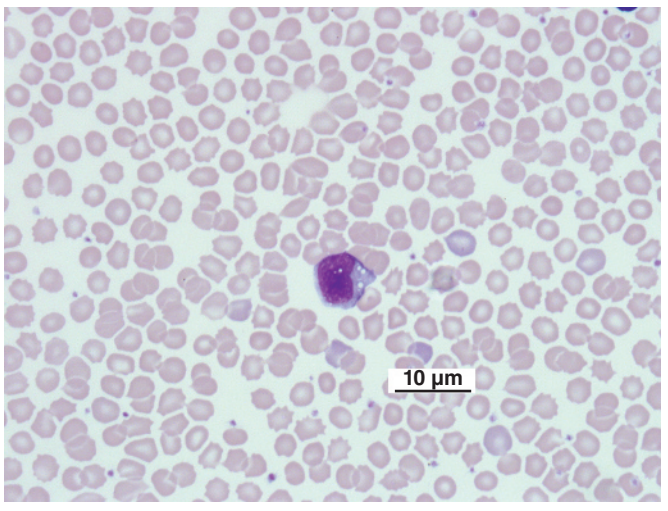


Fig. 1.22. Monocyte in the blood film of a rabbit (*Oryctolagus cuniculus*), Wright–Giemsa stain.

cytoplasm typically stains blue and may contain a few vacuoles.

The normal leukocyte concentration of rabbits is typically reported to range between 7000/µL and 9000/µL. Variations occur with age, restraint methods, pregnancy, and methods of blood collection that may alter the Heterophil:Lymphocyte (H:L) ratio. A bimodal increase in the leukocyte concentration is seen with increasing age, with the highest lymphocyte concentration occurring at 3 months of age then slowly declining, and highest neutrophil concentrations occurring in older animals (Moore, 2000). The normal H:L ratio of 33:60 at 2 months of age changes to 45:45 by 12 months of age. Therefore, rabbits younger than 12 months of age are expected to have lower H:L ratios than do older rabbits, which typically have equal numbers of heterophils

and lymphocytes. A stress response associated with restraint during blood collection procedures can result in a decrease in the total leukocyte count is as much as 15–30% (Drozdowicz et al., 1990).

A mature heterophilia and lymphopenia characterize glucocorticoid-mediated changes in the leukogram. Pregnant rabbits demonstrate a slight increase in total leukocyte counts during the first half of gestation due to an increase in lymphocyte numbers; however, a significant decrease can occur in the second half due to a decrease in lymphocytes and/or heterophil numbers (Kim, 2002). Rabbits generally do not develop a strong leukocytosis with bacterial infections, but will demonstrate a reversal of the H:L ratio; therefore, evaluation of the H:L ratio appears to be the more reliable indicator of inflammatory disorders than are total leukocyte concentrations. However, a reversal of the H:L ratio can also be associated with increases in serum cortisol concentrations (Toth and January, 1990).

Platelets

Rabbit platelets resemble those of other mammals. They appear as small pale blue cytoplasmic fragments that contain small clusters of azurophilic granules. The normal total platelet count of rabbits varies, but generally is considered to be between 200 000 and 1 000 000/µL (Harkness and Wagner, 1995).

Ferrets (*Mustela putorius*)

Erythrocytes

Romanowsky-stained erythrocytes of ferrets are round, anucleated, pink, biconcave disks with an average diameter of 5.94 µm for males and 6.32 µm for females; however, the normal range for both genders is 4.6–7.7 µm (Hillyer and Quesenberry, 1997; Marini et al., 2002; Siperstein, 2008) (Figure 1.23). In general, the hematology of ferrets resembles that of domestic carnivores except the hematocrit values and total red blood cell counts tend to be higher (Fox and Marini, 1998; Moore, 2000).

Ferrets are commonly anesthetized to restrain them for blood collection; however, the use of inhalant anesthetics such as Isoflurane, Enflurane, and Halothane can result in significant and rapid decreases in the red blood cell count, hematocrit, and hemoglobin concentration. As much as a 33% decrease in the hemoglobin concentration has been reported with the use of these inhalant anesthetics (Marini et al., 1994). Splenic sequestration and anesthetic-induced hypotension are possible causes for this response in ferrets. The erythron returns to normal within 45 minutes of recovery from the anesthetic. Either the use of manual restraint or injectable anesthesia such as Ketamine or rapid blood collection following anesthetic induction (less than 3 minutes) is required to avoid this effect in the erythron.

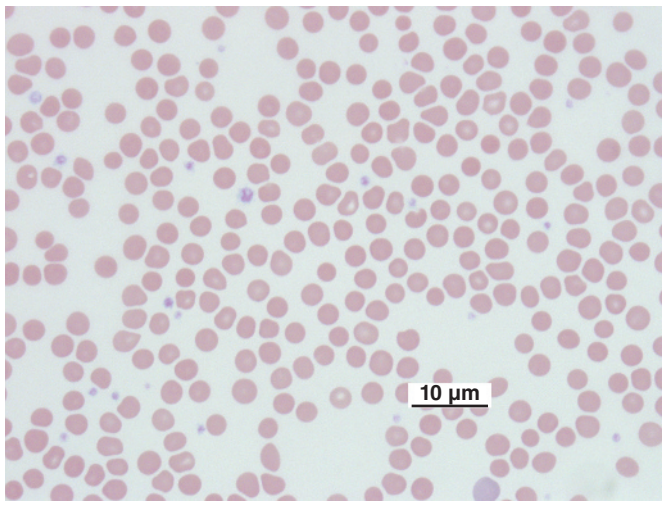


Fig. 1.23. Erythrocytes in the blood film of a ferret (*Mustela putorius furo*), Wright–Giemsa stain.

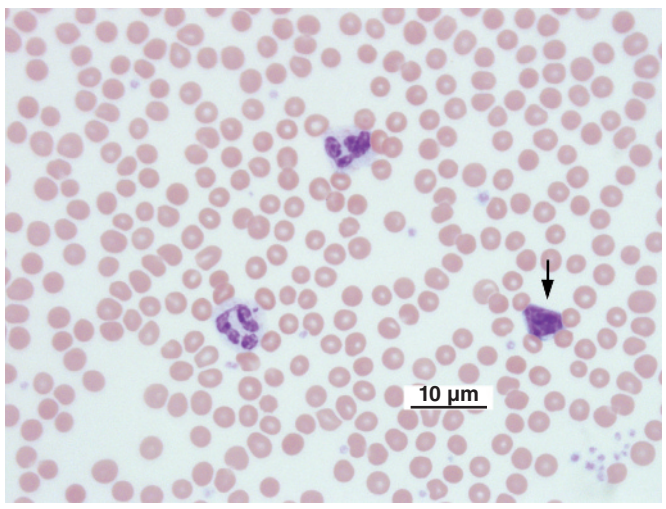


Fig. 1.24. Neutrophils and a lymphocyte (arrow) in the blood film of a ferret (*Mustela putorius furo*), Wright–Giemsa stain.

The hemogram of domestic ferrets is influenced by gender and age. Young male ferrets have lower red blood cell counts, hematocrits, and hemoglobin concentrations than do adult males and young females and females show a decrease in the hematocrit with increasing age (Fox, 1988).

Common causes of nonregenerative anemia in domestic ferrets include malignant neoplasia (such as lymphoma), systemic infections, and hyperestrogenism in intact females. Gastrointestinal ulcers are a common cause of blood loss anemia in the ferret.

Leukocytes

The morphology of ferret leukocytes is similar to that of dogs (Figures 1.24, 1.25, 1.26, 1.27, and 1.28). The ranges in size for the various ferret granulocytes are 10–13 µm for neutrophils in males and 9–10 µm in females;

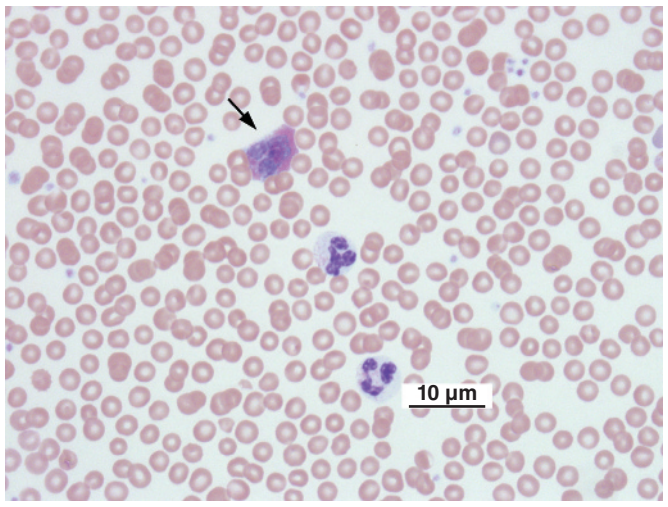


Fig. 1.25. Eosinophil (arrow) and neutrophils in the blood film of a ferret (*Mustela putorius furo*), Wright–Giemsa stain.

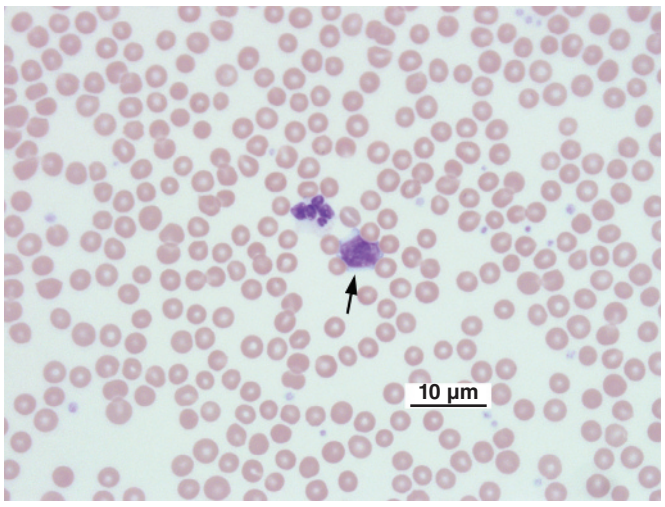


Fig. 1.26. Neutrophil and large lymphocyte (arrow) in the blood film of a ferret (*Mustela putorius furo*), Wright–Giemsa stain.

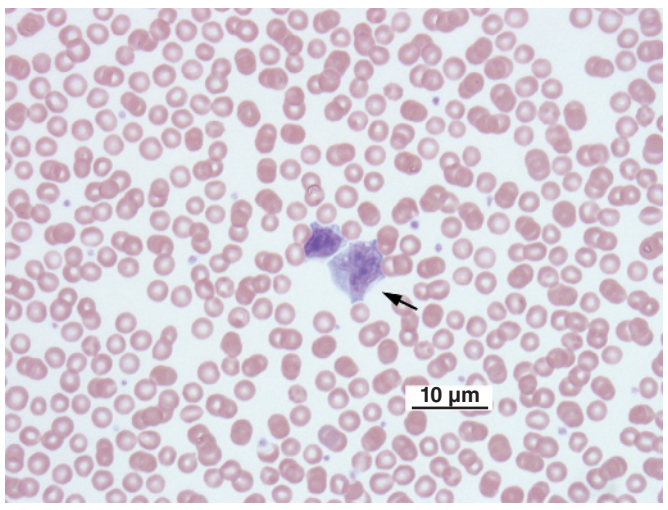


Fig. 1.27. Lymphocyte and monocyte (arrow) in the blood film of a ferret (*Mustela putorius furo*), Wright–Giemsa stain.

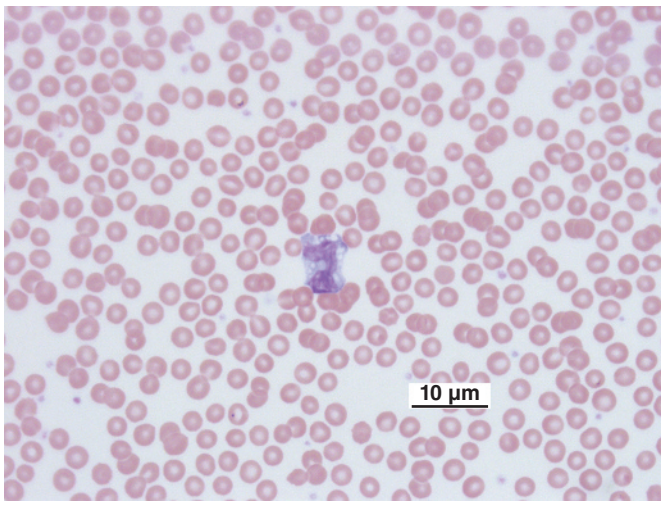


Fig. 1.28. Monocyte in the blood film of a ferret (*Mustela putorius furo*), Wright–Giemsa stain.

and 12 and 14 µm for eosinophils and basophils, respectively, regardless of gender (Thornton, 1979; Campbell and Ellis, 2007). The size of small lymphocytes ranges between 6 and 9 µm in male ferrets and 8 and 10 µm in females (Thornton, 1979). Large lymphocytes and monocytes measure 11–12 µm and 12–18 µm in both sexes, respectively (Thornton, 1979). Neutrophil concentrations are higher than lymphocyte concentrations in normal ferrets (Lee et al., 1982). Ferrets demonstrate an increase in neutrophil concentration and decrease in lymphocyte concentration as they age.

The total leukocyte count of healthy ferrets can be as low as 3000/µL. Ferrets are unable to develop a marked leukocytosis with inflammatory disease and concentrations greater than 20 000/µL are unusual and a left shift is rare (Kawasaki, 1994).

Platelets

Ferret platelets resemble those of other mammals. They appear as small pale blue cytoplasmic fragments that contain small clusters of azurophilic granules. The normal total platelet count of ferrets varies with the references, but generally is considered to be between 245 000/µL and 910 000/µL (Thornton, 1979; Besch-Williford, 1987; Kawasaki, 1994).

African Hedgehogs (*Atelerix albiventris*)

The hematology of African hedgehogs resembles that of domestic carnivores, such as the dog and cat. The morphology of the hemic cells is similar to that of other small mammals (Figures 1.29, 1.30, 1.31, 1.32, 1.33, 1.34, and 1.35). Likewise, interpretation of the hemic response is based upon that of other small mammals.

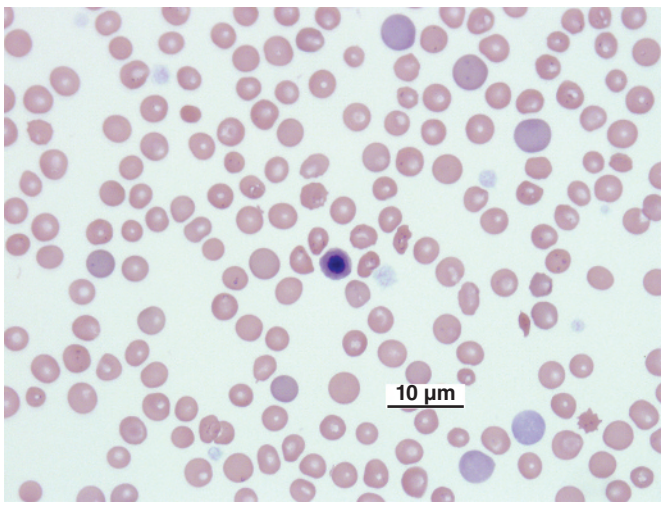


Fig. 1.29. Erythrocytes and nucleated erythrocyte in the blood film of an African hedgehog (*Atelerix albiventris*), Wright–Giemsa stain.

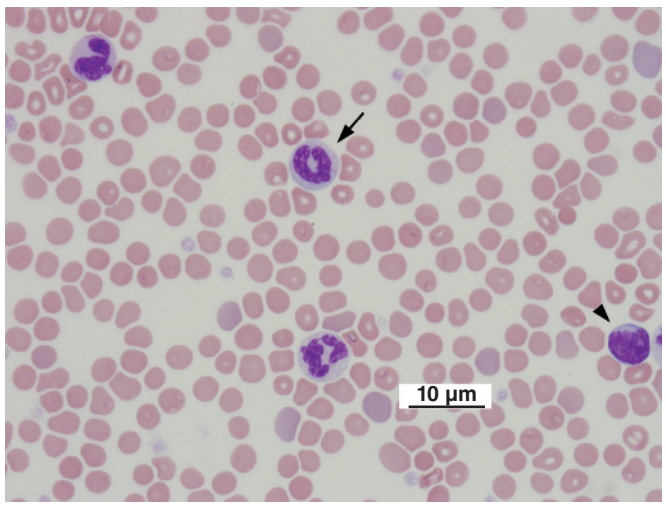


Fig. 1.31. Neutrophils, one band cell (arrow), and lymphocyte (arrowhead) in the blood film of an African hedgehog (*Atelerix albiventris*), Wright–Giemsa stain.

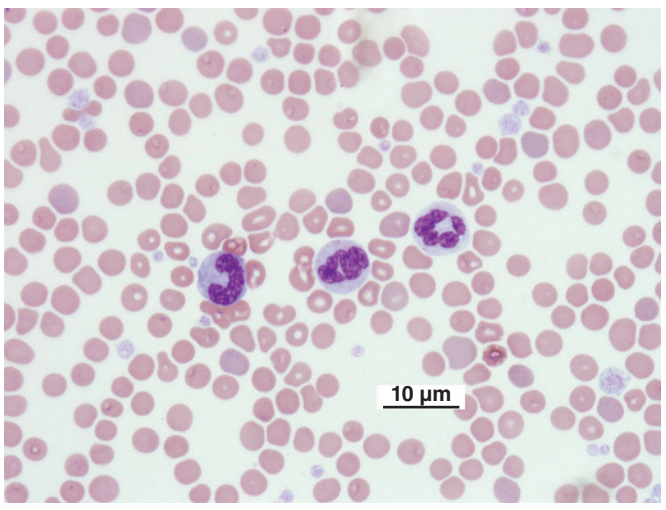


Fig. 1.30. Neutrophils in the blood film of an African hedgehog (*Atelerix albiventris*), Wright–Giemsa stain.

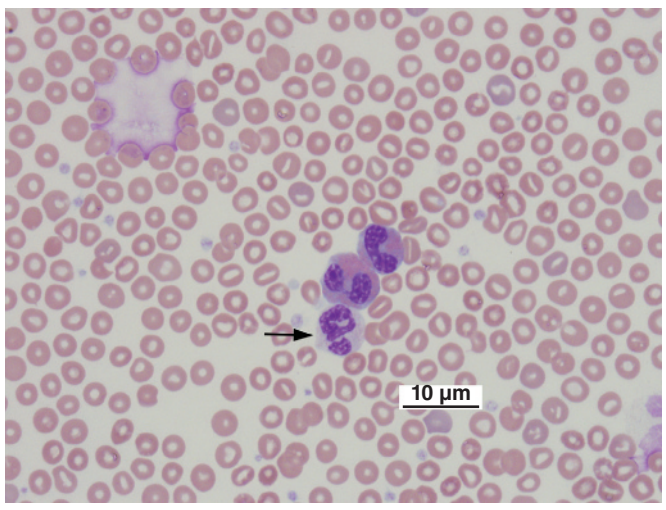


Fig. 1.32. Two eosinophils and a neutrophil (arrow) in the blood film of an African hedgehog (*Atelerix albiventris*), Wright–Giemsa stain.

Evaluation of Mammalian Erythrocytes

The hemoglobin contained within erythrocytes provides oxygen to the tissues. Mammalian erythrocytes are efficient in making this exchange primarily based upon their shape. Erythrocytes in mammalian blood films are anucleated, round (except those from camelids), and biconcave. Romanowsky-stained blood films from mammals often reveal erythrocytes with a distinct area of central pallor resulting from their biconcavity. This shape provides for the efficient exchange of oxygen by allowing the red blood cell to deform its shape to travel through blood vessels with a diameter smaller than its own. The morphology of the erythrocytes in the stained blood film is useful in providing clues as to the nature of red blood cell disorders, such an anemia.

Diagnostically, the important morphologic characteristics of mammalian erythrocytes include polychromatic, hypochromatic, microcytic, and macrocytic erythrocytes; poikilocytosis; and red blood cell inclusions. Appendix B: Tables B.1 and B.2 provide guidelines for evaluation of mammalian erythrocytes. Important erythrocyte structures include Heinz bodies, basophilic stippling, nucleated erythrocytes, and Howell-Jolly bodies. Other abnormalities such as Rouleaux formation and red blood cell agglutination should also be reported.

Polychromasia and hypochromasia are staining characteristics of erythrocytes that should be noted when

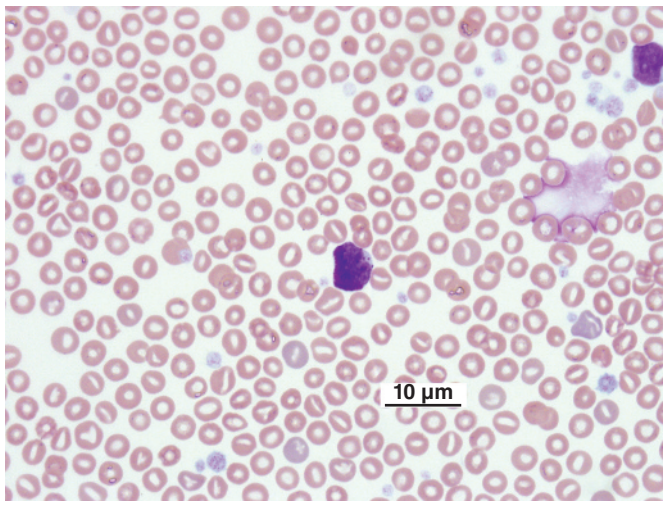


Fig. 1.33. Basophil in the blood film of an African hedgehog (*Atelerix albiventris*), Wright–Giemsa stain.

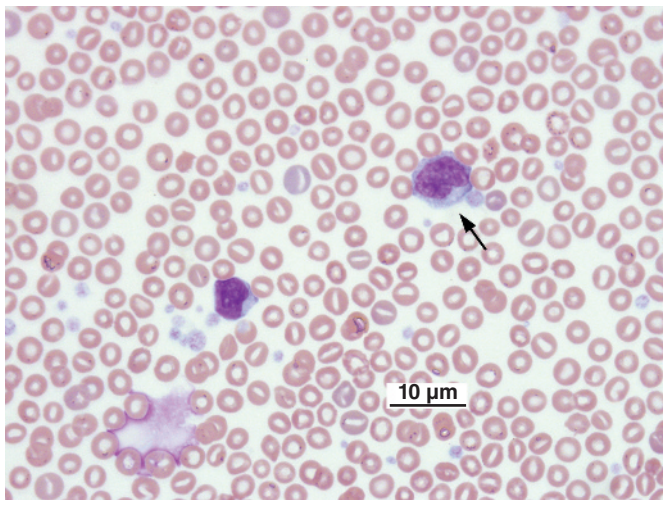


Fig. 1.34. Lymphocyte and monocyte (arrow) in the blood film of an African hedgehog (*Atelerix albiventris*), Wright–Giemsa stain.

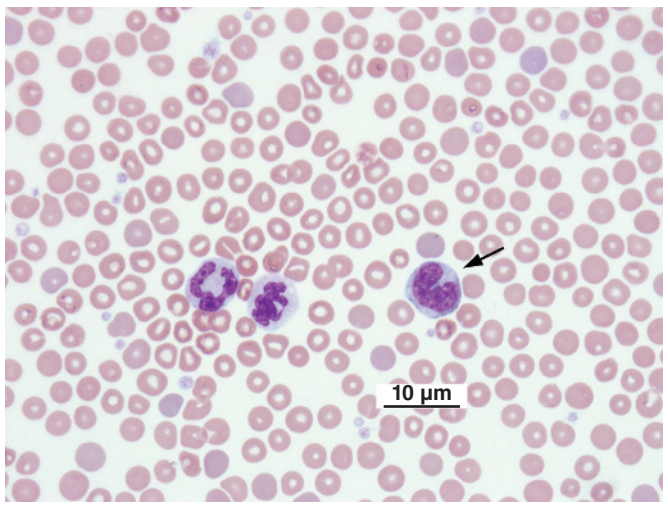
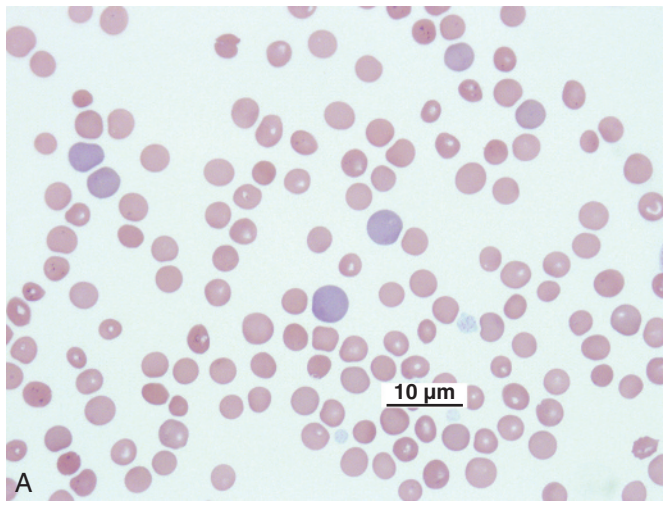


Fig. 1.35. Neutrophils and monocyte (arrow) in the blood film of an African hedgehog, Wright–Giemsa stain.



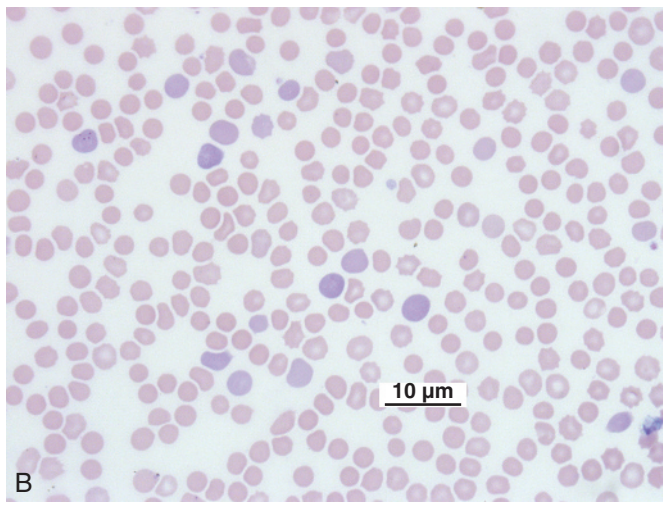


Fig. 1.36. (a) Polychromasia in the blood film of an African hedgehog (*Atelerix albiventris*), Wright–Giemsa stain; (b) polychromasia in the blood film of a ferret (*Mustela putorius furo*), Wright–Giemsa stain.

evaluating erythrocyte morphology. Polychromatic erythrocytes (reticulocytes) are young erythrocytes that have been released into circulation early, and are larger and more basophilic in color compared to mature erythrocytes (Figures 1.36a–1.36b). The degree of polychromasia (total number of polychromatic erythrocytes) may function as an aid in the determination of the cause of an anemia. Polychromasia tends to occur in association with blood loss and blood destruction anemias. Polychromasia is not present in anemias caused by erythroid hypoplasia or in an aplastic anemia.

Polychromatic erythrocytes as seen in Romanowsky-stained blood films are considered the same as the reticulocytes seen on blood films stained with vital stains, such as new methylene blue (Figure 1.37). Both are used as a measure of the regenerative response of the erythrocytes; however, the reticulocytes are easier to

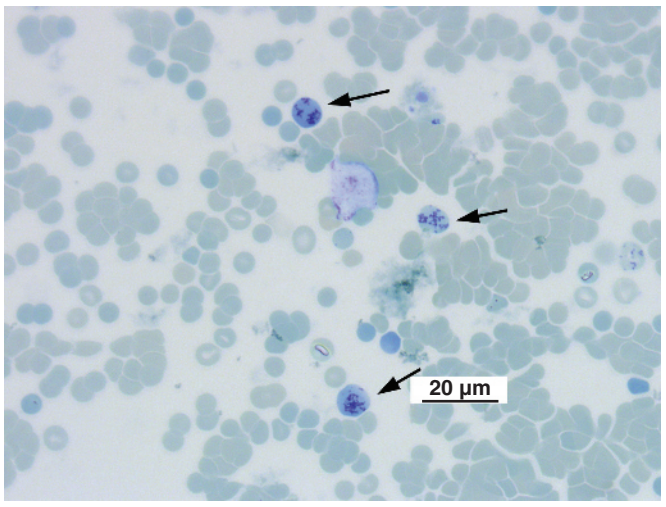


Fig. 1.37. Reticulocytes (arrows) in the blood film of a mammal, new methylene blue stain.

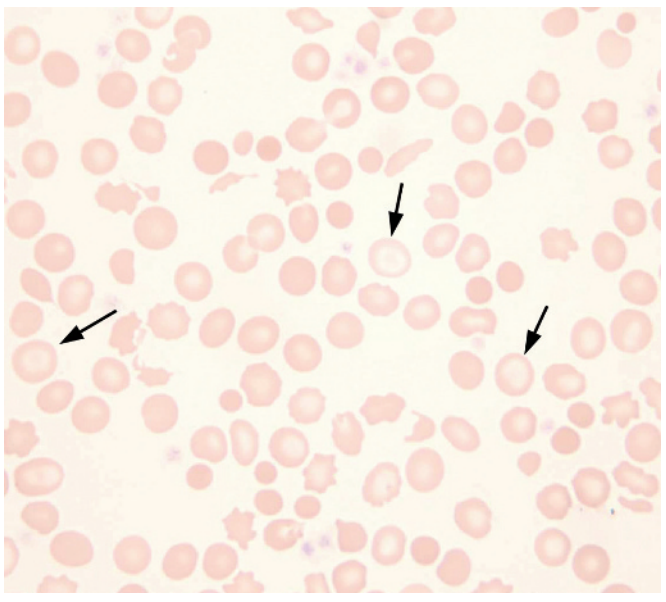


Fig. 1.38. Hypochromasia (arrows show hypochromic erythrocytes) in the blood film of an otter (*Lontra canadensis*), Wright–Giemsa stain (500×).

differentiate from mature erythrocytes. An absolute reticulocyte count, therefore, is easier to obtain than an absolute polychromatic cell count.

Hypochromasia is indicated by pale-staining erythrocytes with an increased area of central pallor (Figure 1.38). Hypochromatic erythrocytes indicate a state of iron deficiency. Iron deficiency in adult mammals is generally the result of chronic blood loss caused by blood-sucking parasites, gastrointestinal ulcers, inflammatory bowel disease, or neoplasms. Iron deficiency anemia in very young mammals is due to inadequate dietary iron.

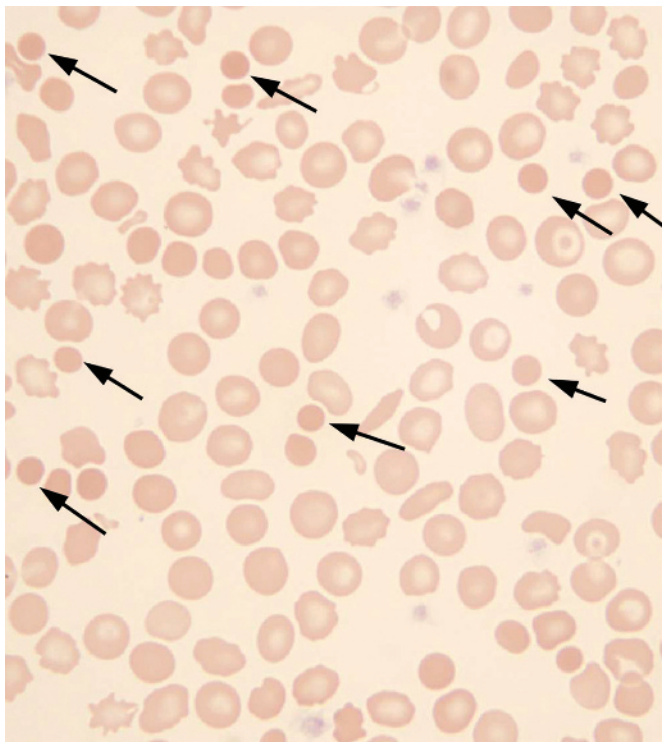


Fig. 1.39. Microcytic erythrocytes (arrows) in the blood film of an otter (*Lontra canadensis*), Wright–Giemsa stain (500×).

Evaluation of erythrocyte size provides important hematologic clues to the process of some diseases. The mean cell volume (MCV) is a measure of the average size of the erythrocytes present in a blood sample. A low MCV is associated with microcytic erythrocytes, which are red blood cells that are smaller than normal cells (Figure 1.39). Microcytic erythrocytes are usually associated with iron-deficiency anemia. A high MCV is associated with macrocytic erythrocytes (red blood cells that are larger than normal cells). Macrocytic erythrocytes are made up of polychromatic erythrocytes associated with erythroid regeneration (Figures 1.36a–1.36b).

Poikilocytosis is a general term used when erythrocytes with abnormal shapes are present in the blood film. Erythrocyte shape is an important feature of the hemogram, and important shape abnormalities include acanthocytes, echinocytes, keratocytes, and schistocytes. Acanthocytes are spiculated erythrocytes with irregularly spaced, spike-like surface projections that vary in length and thickness (Figure 1.40). The abnormal shape of the acanthocyte is considered to be associated with abnormal lipid content in the red cell membrane. Any disease causing alterations in lipid metabolism may result in appearance of acanthocytes in the blood film. Acanthocytes may also be present in some mammals with hemangiosarcoma.

Echinocytes are another type of spiculated red blood cell that may be noted in the blood films of

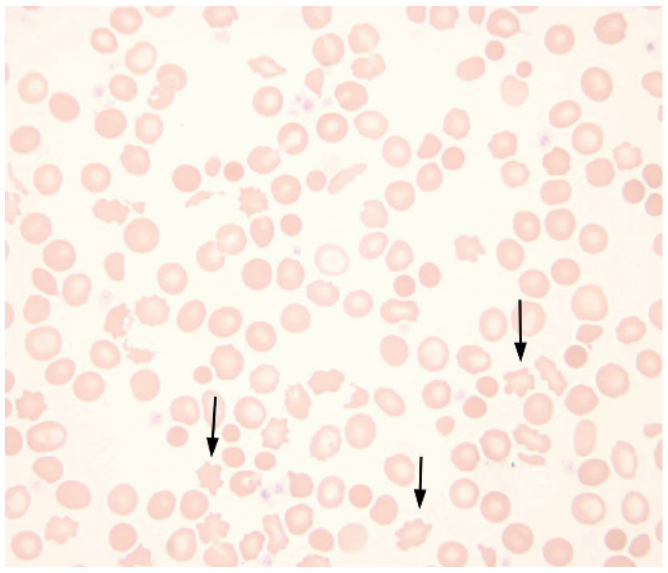


Fig. 1.40. Acanthocytes (arrows) in the blood film of an otter (*Lontra canadensis*), Wright–Giemsa stain (500×).

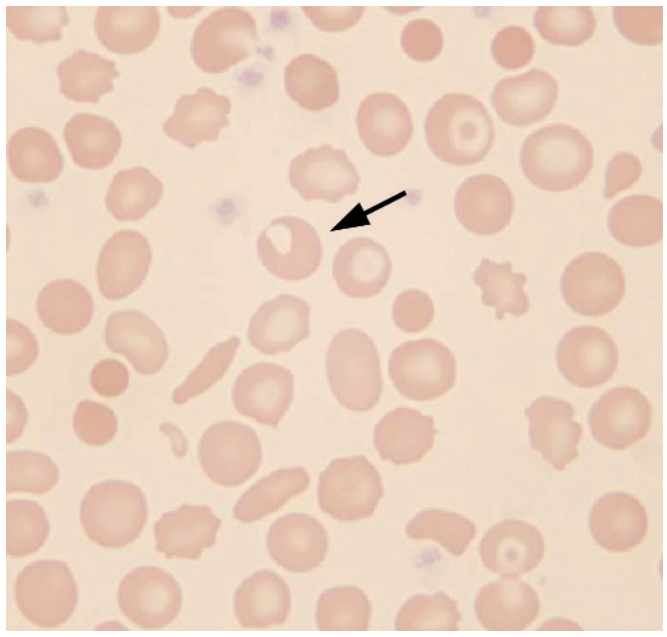


Fig. 1.42. Keratocyte (arrow) in the blood film of an otter (*Lontra canadensis*), Wright–Giemsa stain (500×).

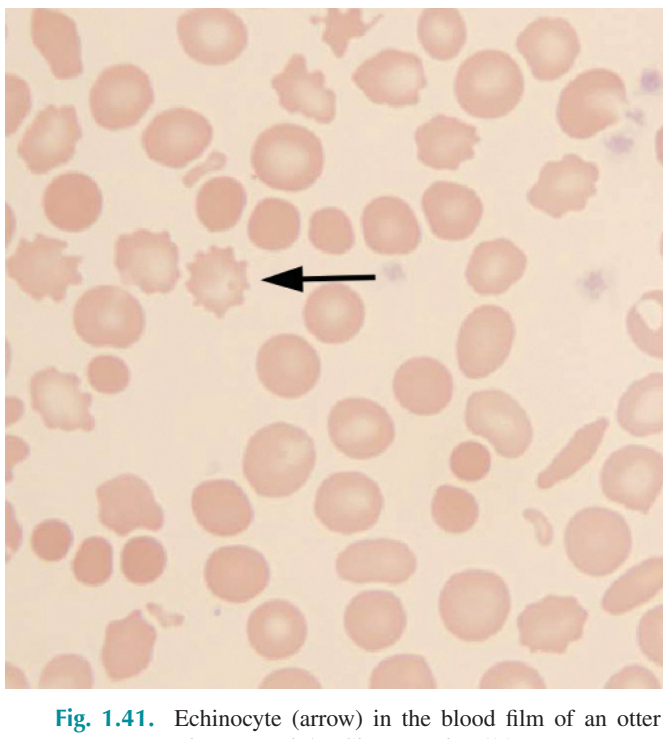


Fig. 1.41. Echinocyte (arrow) in the blood film of an otter (*Lontra canadensis*), Wright–Giemsa stain (500×).

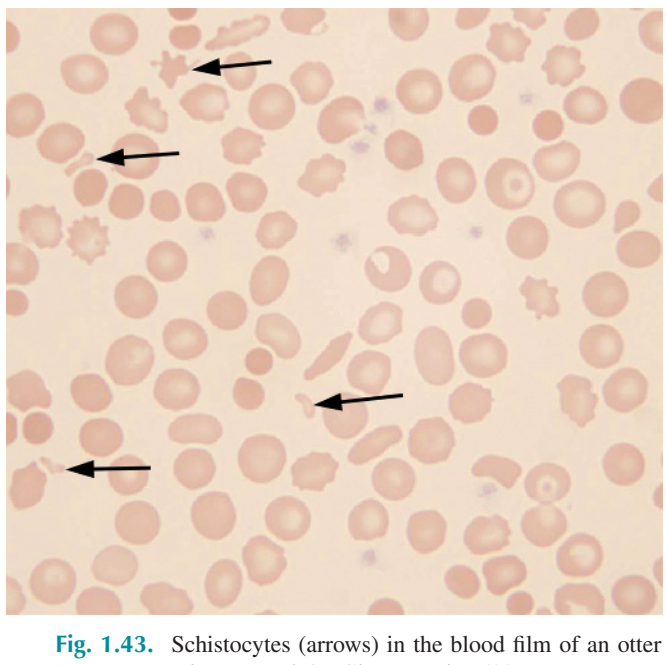


Fig. 1.43. Schistocytes (arrows) in the blood film of an otter (*Lontra canadensis*), Wright–Giemsa stain (500×).

some mammals. These cells differ in appearance from acanthocytes in that they have many short, uniformly sized and shaped, evenly spaced surface projections (Figure 1.41). Echinocytes usually occur as artifacts associated with blood films that are allowed to dry too slowly; however, certain pathologic conditions such as renal disease, lymphoma, exposure to certain drugs, and rattlesnake envenomation may result in echinocyte formation in the blood of some mammal species.

Keratocytes are red blood cells with two or more spicules that result from the lysis of a vacuole formed in association with oxidative damage to the red cell membrane secondary to iron deficiency (Figure 1.42). Schistocytes are red blood cell fragments present in the blood film (Figure 1.43). Formation of schistocytes may be associated with disseminated intravascular coagulopathy (DIC), hemangiosarcomas, and iron deficiency.

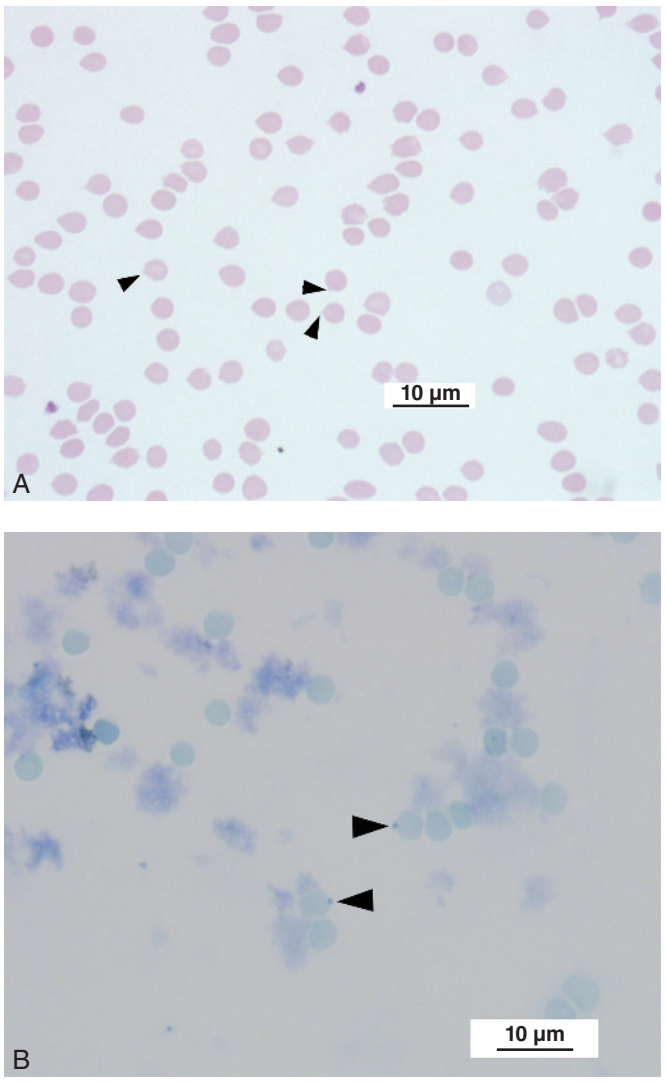


Fig. 1.44. (a) Heinz bodies (arrowheads) in the red blood cells of a ferret (*Mustela putorius furo*), Wright–Giemsa stain; (b) Heinz bodies (arrowheads) in the red blood cells of a ferret (*Mustela putorius furo*), new methylene blue stain.

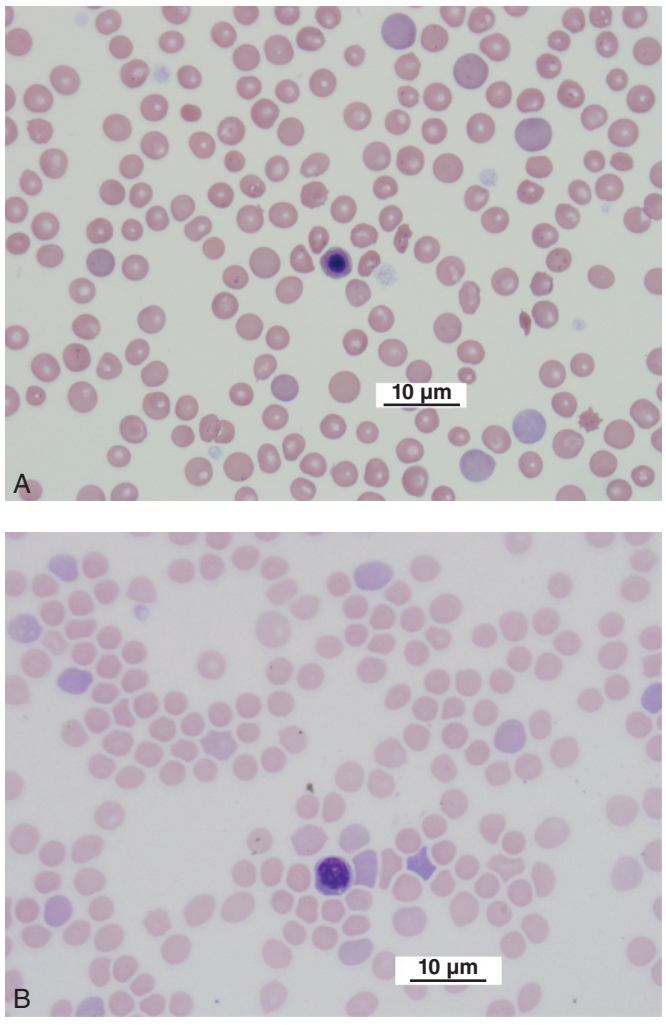


Fig. 1.45. (a) Nucleated erythrocyte in the blood film of an African hedgehog (*Atelerix albiventris*), Wright–Giemsa stain; (b) nucleated erythrocyte in the blood film of a ferret (*Mustela putorius furo*), Wright–Giemsa stain.

A variety of cellular structures or red blood cell inclusions can be associated with erythrocytes and should be noted. Heinz bodies are small, eccentric, single to multiple pale structures that often protrude slightly from the red cell margins (Figures 1.44a and 1.44b). Heinz bodies are caused by oxidative denaturation of hemoglobin and can be associated with certain plant chemicals (onions and garlic), drugs (acetaminophen and propofol), and diseases such as lymphoma and hyperthyroidism. Basophilic stippling appears in the erythrocyte as small basophilic granules present within the cytoplasm of the cell. Basophilic stippling is commonly associated with erythrocyte regeneration and commonly found in blood films from healthy animals, such as gerbils. Basophilic stippling may also be associated with nonanemic animals with lead poisoning, but this is generally a rare finding.

Nucleated erythrocytes are immature red blood cells that are released in an early stage of maturation from the bone marrow usually as part of a regenerative response to anemia or hypoxia (Figures 1.45a and 1.45b). An inappropriate release of nucleated erythrocytes may be seen with lead poisoning or a myelodysplastic condition as well. Howell–Jolly bodies are small, variably sized, round, dark-blue inclusions present in the cytoplasm of the erythrocyte (Figure 1.46). These inclusions represent nuclear remnants that occur as part of a regenerative response or may indicate suppressed splenic function.

Rouleaux formation and red blood cell agglutination occur when red blood cells group together and may be noted in the blood films of some animals. Rouleaux formation appears as linear stacking of erythrocytes and is often associated with increased plasma proteins, such as immunoglobulins, in domestic mammals. Erythrocyte agglutination may be identified by irregular to

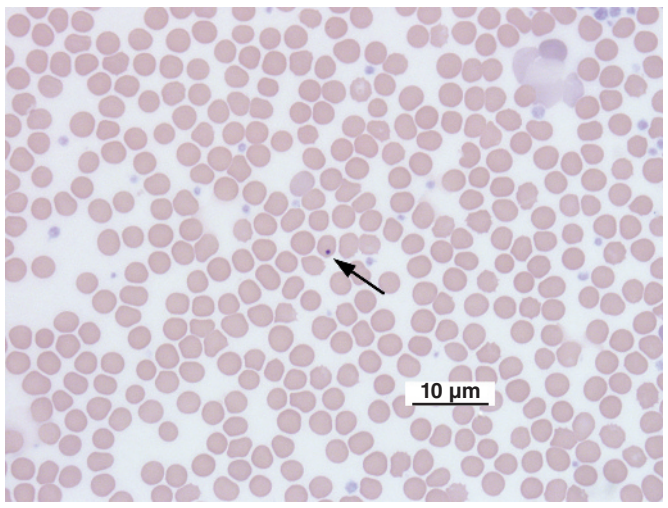


Fig. 1.46. Howell–Jolly body (arrow) in the cytoplasm of an erythrocyte in the blood film of a mouse (*Mus musculus*), Wright–Giemsa stain.

circular clumping of erythrocytes and is associated with immune-mediated hemolytic anemia. Rouleaux formation may be differentiated from erythrocyte agglutination by mixing a drop or two of blood with a drop of isotonic saline. Rouleaux formation will dissipate, whereas red cell agglutination persists after addition of the saline when the slide is viewed through the microscope.

Anemia

Anemia is a considered a reduction in the total mass of hemoglobin-bearing erythrocytes, which in turn results in a deficiency in oxygen transport. Anemias may be caused by many etiologies, but in general are caused by abnormal red blood cell loss that cannot be compensated by normal erythropoiesis, or decreased blood cell production, which cannot provide sufficient replacement of red cells that are lost normally.

Anemias are often classified according to their pathophysiology as hemolytic, hemorrhagic, or hypoplastic. Hemolytic anemia results from increased erythrocyte destruction. Hemorrhagic anemia results from erythrocyte loss via hemorrhage. Hypoplastic anemia results from decreased production of erythrocytes.

Nonregenerative Anemia in Mammals

Anemia associated with a lack of a regenerative response in the form of circulating immature erythrocytes (polychromatophilic erythrocytes or reticulocytes) is indicative of a nonregenerative anemia. This is a result of the erythropoietic tissue in the bone marrow and other hematopoietic tissues not meeting the demand of replacing lost erythrocytes. Most are classified as a normocytic, nonregenerative anemia.

A nonregenerative anemia in conjunction with normal neutrophil (or heterophil depending upon the species) and platelet concentrations may have either an extrinsic or intrinsic bone marrow defect. An extrinsic defect results in defective red blood cell production or decreased erythropoiesis resulting from conditions outside of the bone marrow, such as renal failure, endocrine disorders, inflammatory disorders, and nutritional deficiencies. Intrinsic bone marrow defects are caused by red blood cell hypoplasia, aplasia, or maturation defect within the bone marrow.

A nonregenerative anemia in conjunction with a neutropenia (or heteropenia depending upon the species) and thrombocytopenia is referred to as a pancytopenia. Pancytopenia can indicate stem cell injury or myelophthisic disorder. Myelophthisic disorders occur when nonhematopoietic neoplastic cells (such as lymphomas) crowd out the hematopoietic tissue. Stem cell injury can be reversible or irreversible depending upon the etiology. Irreversible stem cell injuries result from an intrinsic defect in the proliferative behavior and/or regulation of stem cell ability to differentiate into the various cell types during hematopoiesis. Causes of irreversible stem cell injury include exposure to certain drugs, chemicals, viruses, and radiation, as well as immune-mediated stem cell injury. Injury to the stem cells can result in a general lack of cell production resulting in aplastic anemia or an uncontrolled proliferation of neoplastic cells. Reversible stem cell injury, a transient condition, can also be caused by drugs, chemicals, viruses, radiation, and immune-mediated destruction of stem cells. Although reversible stem cell injury does not progress to neoplasia, it can lead to myelofibrosis.

Estrogen toxicosis is perhaps the most common cause of aplastic anemia (aplastic pancytopenia) in exotic mammals, especially intact female ferrets. Ferrets are induced ovulators and when mating does not occur, the female will stay in estrus for an extended period of time commonly resulting in marrow suppression from endogenous estrogen. This is a potentially fatal disorder. The exact mechanism of estrogen toxicosis is not known. One possible explanation is the secretion (possibly by thymic stromal cells) of an estrogen-induced substance that inhibits stem cells that causes an initial thrombocytosis and neutrophilia before the marrow suppression and aplastic anemia occurs (Bernard et al., 1983). Myelosuppression may result from the administration of excessive amounts of estrogen or from an idiosyncratic sensitivity to estrogen. Bone marrow suppression resulting from excessive concentrations of endogenous estrogen can also occur in male animals with Sertoli cell tumors or in females with cystic ovaries or granulosa cell tumors (Thrall, 2012a).

A chemical agent, such as a drug used to create immunosuppression or to treat neoplasia, that commonly cause reversible stem cell damage in domestic mammals

has a strong potential for creating the same disorders in nondomestic mammals. A list of these drugs can be found in clinical pathology references for domestic mammals (Thrall, 2012a). Likewise, stem cell destruction caused by drug-induced immune-mediated injury seen in domestic mammals may also occur in nondomestic mammals resulting in aplastic anemia.

A nonregenerative anemia associated with normal neutrophil (or heterophil) and platelet concentrations in the peripheral blood and a marked decrease in the population of bone marrow erythroid precursors without a decrease in granulocyte precursors and megakaryocytes is a pure erythrocyte aplasia. In domestic mammals, especially the dog, this condition is nearly always the result of immune-mediated destruction of erythroid precursors (Thrall, 2012a). Affected animals often reveal an arrest at some stage of erythroid precursor maturation, but a complete absence of erythroid precursors may also be seen. The presence of phagocytized rubricytes or metarubricytes aids in the diagnosis.

Anemia of chronic disease (disorders) resulting in red cell hypoplasia is a common cause of nonregenerative anemia in exotic animals. This results from conditions that are extrinsic to the marrow, such as inflammatory disease, chronic renal failure, endocrine disease, and nutritional deficiencies (Campbell and Grant, 2010). Inflammatory diseases normally result in a mild-to-moderate, nonregenerative, normocytic anemia. Anemia can result from inflammation caused by infectious agents, trauma, or neoplasia. Chronic inflammation leads to a chain of events that limits the availability of iron for erythropoiesis and decreases erythrocyte production and red blood cell life span resulting in anemia (Thrall, 2012a). The presence of an inflammatory leukogram, decreased serum iron concentration with a normal or increased serum ferritin concentration or normal, or decreased total iron-binding capacity will aid the diagnosis. Chronic renal failure leads to a moderate-to-severe (depending upon the severity of the renal failure), nonregenerative, normocytic anemia. The failing kidneys quit producing erythropoietin which in turn stops erythropoiesis. Domestic mammals, especially dogs, will demonstrate a mild, nonregenerative, normocytic anemia with various endocrine disorders, such as hypothyroidism and hypoadrenocorticism. These conditions have the potential for causing similar anemias in nondomestic mammals.

Common causes of nonregenerative anemia in nondomestic mammals include malignant neoplasia such as lymphoma, systemic infections, and hyperestrogenism (especially in the intact female ferret).

Regenerative Anemia in Mammals

Regenerative anemia is indicated by the bone marrow's attempt to compensate for the loss of red blood cells by increased erythrocyte production and early release of immature red cells. This is indicated by an increase in the number of polychromatic red blood cells on the Wright's stained blood film and increased reticulocyte concentration. The MCV may also be increased.

Reticulocytes are the stage of red blood cell maturation between metarubricytes and mature erythrocytes. They are evaluated by staining blood with a supravital dye, such as new methylene blue (1% in saline plus 1.6% potassium oxalate) or brilliant cresyl blue (1% in saline) to demonstrate ribosomal material in reticulocytes. Reticulocyte numbers in blood from exotic animals are generally counted manually on stained blood films and reported as a percentage of reticulocytes per 1000 red blood cells. Multiplying this percentage times the absolute erythrocyte count obtained by either the manual hemacytometer method or an automated method provides the absolute reticulocyte count.

Hemorrhagic Anemia

Blood loss anemia can occur from the acute or chronic loss of blood either externally or internally. Causes of acute hemorrhagic anemia include traumatic injury, blood loss during a surgical procedure, hemorrhagic ulcers (i.e., gastrointestinal ulcers or neoplasms), and bleeding disorders associated with coagulation defects. Such hemostatic disorders include thrombocytopenia, inherited coagulopathies, and acquired coagulopathies, such as warfarin toxicosis or disseminated vascular coagulopathy. Common causes of chronic blood loss include bleeding lesions, especially those within the gastrointestinal tract, and gastrointestinal or external parasites. Chronic blood loss from gastrointestinal ulcers is common in domestic ferrets and generally associated with *Helicobacter mustelae*.

During external blood loss, blood components such as iron and plasma protein are lost. As a result, the laboratory findings generally include either a low-normal plasma protein concentration or hypoproteinemia coupled with a regenerative anemia. Hypoproteinemia, when present, implies a recent or severe blood loss because protein is replaced much faster than the red blood cells. However, hypoproteinemia caused by other disorders than blood loss should also be considered. These include decreased protein intake, such as malabsorption, maldigestion, and starvation; decreased production of protein as seen in liver failure; or other types of protein loss, such as glomerulonephropathy and protein-losing enteropathy. Chronic external blood loss leads to iron-deficiency anemia.

Internal blood loss mimics hemolytic anemia because the red blood cells are broken down within the body and the protein and iron are not lost. Blood loss within the body is more difficult to detect than the loss of blood outside the body.

Acute Blood Loss

Initially, during acute blood loss, the PCV remains normal because both cells and plasma proteins are lost. Within a few hours, the PCV and plasma protein decrease as a result of dilution, as interstitial fluid is added to blood to restore normal vascular volume. This is followed by a pre-regenerative period because it generally takes 1–2 days following the acute blood loss for a regenerative response in the form of increased numbers of polychromatophilic erythrocytes (reticulocytes) to appear in the blood. Dehydration can delay the decrease in the hematocrit and hemodilution by excessive fluid therapy can reduce the hematocrit clouding the assessment of the true severity of the anemia. The presence of macrocytic, hypochromic erythrocytes indicates prior red blood cell regeneration.

In some animals, such as the dog, the presence of acanthocytes and schistocytes are associated with acute blood loss associated with a hemangiosarcoma. Laboratory findings associated with hemangiosarcomas include increased polychromasia (reticulocytosis), a transient hypoproteinemia, and a mild-to-moderate thrombocytopenia.

Chronic Blood Loss and Iron-Deficiency Anemia

Iron-deficiency anemia in adult animals is usually associated with chronic blood loss when iron stores are quickly depleted. Gastrointestinal bleeding, such as occurs with ulcers, neoplasms, or internal parasites, or heavy infestations of blood-sucking ectoparasites lead to iron-deficiency anemia. Because iron is required for hemoglobin synthesis, iron deficiency results in a deficiency in hemoglobin synthesis and iron-deficient erythrocytes undergo one or two additional cell divisions resulting in the formation of microcytes (Weiss, 2010). Therefore, laboratory findings associated with iron-deficiency anemia include a decreased MCV or the presence of microcytosis. The MCHC tends to decrease following the decrease in the MCV; however, it is commonly within the reference interval. Examination of the blood film reveals hypochromatic (pale) erythrocytes with increased central pallor (often only a narrow rim of lightly stained hemoglobin is present) and poikilocytosis. Keratocytes and schistocytes are common because of increased membrane susceptibility to oxidative damage. Initially the erythrocyte develops a blister or vacuole where inner membrane surfaces are cross-linked across the cell which eventually expands and breaks open to form the “apple-stem cells” and keratocytes (spiculated erythrocytes with two or more pointed projections) (Campbell and Grant, 2010; Thrall, 2012a). Projections from the keratocytes fragment from the cell to form schistocytes (cytoplasmic fragments). Iron-deficiency anemia usually demonstrates a regenerative response.

A thrombocytosis is present in some patients with iron-deficiency anemia. The mechanism for this is not known (it may be associated with increased erythropoietin or other cytokines) (Thrall, 2012a).

Iron deficiency is associated with a decreased serum iron concentration and low iron storage. Transferrin (a plasma glycoprotein that transports iron) saturation as measured by the total iron-binding capacity is usually increased in most species with iron deficiency, but may decrease in others. Assessment of iron storage can be determined by measuring serum ferritin (an acute phase protein); however, measuring serum ferritin can be difficult as it is a species-specific test and may be increased with iron deficiency in some species. Examination of a bone marrow aspirate for its iron content can also be used to assess iron storage. Bone marrow aspirates can be stained with Prussian blue to detect hemosiderin stored in macrophages. The absence of hemosiderin in macrophages when examining a bone marrow aspirate indicates low iron storage; however, there is species variability with this method as some noniron-deficient animals may lack stainable iron in their bone marrow. Thus, the determination of low serum iron and a decreased MCV in an anemic patient is usually adequate to diagnose iron-deficiency anemia.

Hemolytic Anemia in Mammals

Blood destruction (hemolysis) may be either intravascular or extravascular. This may occur as a result of an intrinsic (primary) defect, such as hereditary membrane defects or enzyme deficiencies, or of extrinsic (secondary) causes, such as erythrocyte parasites or immune-mediated destruction. Intravascular hemolysis occurs when there is actual lysis of erythrocytes within the vascular system. Extravascular hemolysis occurs when abnormal erythrocytes are phagocytized by macrophages, usually within the spleen or liver. Common causes of hemolytic anemia include immune-mediated mechanisms, erythrocyte parasites, and Heinz body formation as a result of oxidative damage caused by exposure to certain drugs and chemicals. Hemolytic anemia is typically regenerative type of anemia characterized by increased anisocytosis, macrocytosis, polychromasia, nucleated erythrocytes, and Howell–Jolly bodies in the blood film.

Heinz Body Anemia

Heinz bodies form in red blood cell following oxidative damage that denatures and precipitates hemoglobin. Heinz bodies are detected in the blood stained with the same supravital dyes, such as new methylene blue and brilliant cresyl blue, used to demonstrate ribosomal material in reticulocytes (Campbell and Grant, 2010). Romanowsky-stained blood films may reveal Heinz bodies as bulges from the surface of red blood cells,

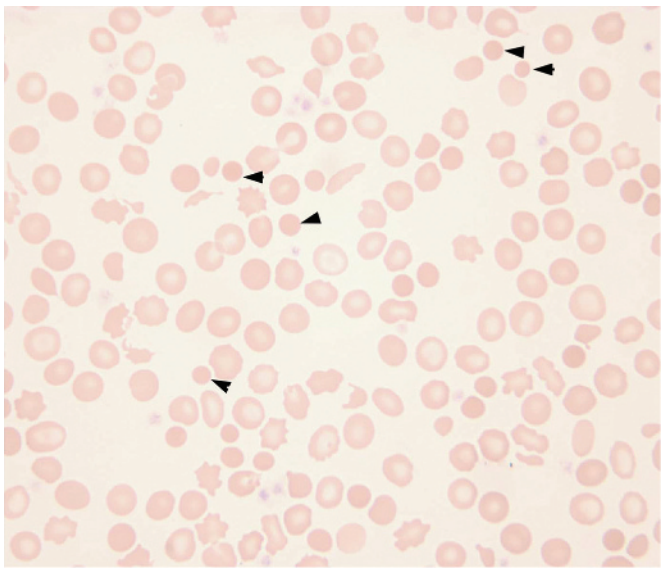


Fig. 1.47. Spherocytes (arrowheads) in the blood film of an otter (*Lontra canadensis*), Wright–Giemsa stain (500×).

lighter and refractile bodies compared to the rest of the hemoglobin of the red blood cell, or round bodies the color of hemoglobin in ghost cells (pale erythrocytes). Heinz bodies stained with supravital stains are lighter blue compared to the ribosomal material of reticulocytes and appear as large round bodies (e.g., 1–2 μm in diameter in guinea pigs and rabbits) or multiple, small granular bodies depending upon the species.

Immune-Mediated Anemia

Immune-mediated hemolytic anemia (IMHA) is caused by red cell destruction due to antibodies directed against erythrocytes or immune complexes attached to erythrocytes. This typically results in a regenerative anemia unless antibodies are formed against red blood cell precursors causing destruction of polychromatophilic erythrocytes or earlier erythrocytes. In domestic animals, this type of anemia can be related to disorders, such as infections, vaccinations, neoplasia, and administration of certain drugs (Thrall, 2012b).

The presence of spherocytes in the blood film as a result of partial erythrophagocytosis by macrophages is indicative of IMHA. Spherocytes are small erythrocytes that appear dense because they lack an area of lack central pallor (Figure 1.47). Spherocytes are difficult to detect in species in which the red cells normally lack central pallor. Red blood cell agglutination, another indication of IMHA, occurs when the cells are coated with autoantibody.

Hemoparasites

Microorganisms, such as blood parasites, that infect erythrocytes may result in intravascular hemolysis or extravascular hemolysis resulting in a hemolytic anemia. The majority of these microorganisms that cause anemia do so by immune-mediated extravascular hemolysis. This occurs when antibodies against the organism, immune complexes, or complement bind to infected erythrocytes which are removed from the blood by macrophages (erythrophagocytosis).

Erythrocytosis (Polycythemia)

Polycythemia and erythrocytosis are often used interchangeably by clinicians; however, erythrocytosis refers to an increase in the PCV (or hematocrit), red blood cell count, and hemoglobin concentration, whereas the term polycythemia implies that all blood cells, including leukocytes, are increased in concentration. A relative erythrocytosis refers to an increase in the PCV due to a decrease in plasma volume and hemoconcentration as seen with dehydration. In some domestic species, this can also occur with erythrocyte redistribution and body fluid shifts as seen with splenic contraction commonly associated with excitable animals. Absolute erythrocytosis refers to a true increase in the red cell mass. Primary absolute erythrocytosis (i.e., polycythemia vera) is a rare well-differentiated chronic myeloproliferative disorder (of humans, dogs, and cats) resulting in an autonomous proliferation of erythroid precursors independent of the erythropoietin concentration. Diagnosis of primary erythrocytosis is typically made by excluding causes of secondary erythrocytosis. Secondary absolute erythrocytosis results from excessive production of erythropoietin. This can be an appropriate overproduction of erythropoietin in response to systemic hypoxia as seen with certain congenital heart anomalies, chronic severe pulmonary disease, and hereditary hemoglobinopathies. An inappropriate secondary absolute erythrocytosis occurs with increased overproduction of erythropoietin in the absence of systemic hypoxia. This is associated with erythropoietin-secreting tumors or kidney disorders.

Relative Erythrocytosis

Patients with a relative erythrocytosis usually have clinical evidence of dehydration and a concurrent increase in plasma protein providing other conditions, such as a decreased protein intake, decreased protein production by the liver, or increased protein loss via the kidney or gastrointestinal tract are not involved. Rarely does the PCV exceed 60% with relative erythrocytosis (Hasler, 2000; Watson, 2000). Relative erythrocytosis resolves with treatment using replacement of fluids and electrolytes.

Effects of splenic contraction are typically seen only in those species of domestic mammals with normal high PCVs in association with exertion or excitement (epinephrine release). Splenic contraction results in a mild increase in the PCV with no increase in the plasma

protein concentration. Presence of an excitement leukogram (a mature neutrophilia and lymphocytosis) is supportive of this type of relative erythrocytosis.

Primary Absolute Erythrocytosis

Unlike most other types of hematopoietic neoplasia, this myeloproliferative disorder exhibits normal appearing erythroid cells with a normal maturation sequence. The human form of polycythemia vera is also associated with an abnormal proliferation of neutrophils (causing a leukocytosis) and platelets (thrombocytosis) and therefore is referred to as a primary absolute polycythemia. Abnormal proliferation of cells other than red blood cells is a rare occurrence in domestic animals with this disorder. Primary erythrocytosis has been reported in dogs, cats, horses, cattle, and llamas (Thrall, 2012c). It is likely that this disease occurs in exotic species as well but may be under reported.

Secondary Absolute Erythrocytosis

Physiologically appropriate erythrocytosis occurs with generalized hypoxia. When available, arterial blood gas analysis showing reduced arterial partial pressure of oxygen (PaO_2) or determination of hemoglobin oxygen saturation (SaO_2) is helpful in the detection of hypoxemia. Inadequate tissue oxygenation as detected by StO_2 less than 60 mmHg or SaO_2 less than 95% at sea level triggers an increase in erythropoietin production, which in turn stimulates erythrocyte production and release so that more oxygen can be carried to the tissues (Thrall, 2012c).

Physiologically inappropriate erythrocytosis occurs in the absence of generalized tissue hypoxia as determined by a normal or slightly decreased StO_2 and SaO_2. Tumors of the kidney are the most common cause of increased erythropoietin production in domestic mammals; however, production of erythropoietin-like substances from nonrenal tumors may also occur. Determination of serum erythropoietin concentration would be helpful in the diagnosis; however, there are no commercially available validated erythropoietin assays currently available for exotic animals.

Inflammatory Leukogram in Mammals

Mediators of Inflammation

Mammalian neutrophils and heterophils are the first line of defense against any inciting inflammatory signal, such as occurs with the invasion of microorganisms or tissue trauma (Appleberg, 2006). Cytochemical and ultrastructural features of these cells differ among species (Parmley, 1988). These cells contain a variety of granules that contain antimicrobial proteins, proteases, components of the respiratory burst (the release of chemically active oxygen molecules designed to kill invading organisms), and mediators of inflammation (Faurschou and Borregaard, 2003). The granule types have been best described in humans; however, although poorly studied, a large degree of species variability exists among other mammals (Nabity and Ramaian, 2010). In general, the neutrophils or heterophils of most mammalian species studied contain both primary and secondary granules that contain the same most frequently encountered enzymes within those granules. Many species of mammals, such as the rabbit, rat, and guinea pig, also have neutrophils/heterophils that contain a tertiary granule, but it may not necessarily correlate with that granule in human neutrophils. Studies involving the enzyme content of the entire neutrophil/ heterophil have also shown marked variation among mammalian species (Rausch and Moore, 1975). In these studies, myeloperoxidase and beta-glucuronidase were present in varying degrees of activity (generally lower when compared to humans) in all species of mammals. Small amounts of lysozyme activity were detected in these cells in rats and guinea pigs, but not in hamsters. Rabbit heterophils contain large amounts of elastase activity when compared to other mammalian species. Alpha-defensins have been detected in the neutrophils/heterophils of rabbits, guinea pigs, and hamsters, but not in the neutrophils of mice. Cathelicidins are found in the neutrophils/heterophils of rabbits, guinea pigs, rats, and mice (Linde et al., 2008).

Inflammatory Response

Inflammation is the most common blood leukocyte response in mammals. The inflammatory process involves the consumption, production, and release of neutrophils or heterophils. During this process, these cells are released from the bone marrow and delivered via the blood to the inflammatory lesion until it resolves. Peripheral blood concentrations of neutrophils or heterophils vary from severely decreased to markedly increased depending upon the balance between consumption at the site of the lesion and production and release by the bone marrow. In small mammals, most inflammatory processes result in some degree of neutrophilia or heterophilia, indicating that marrow releases more cells into the blood than are consumed at the site of inflammation. The inflammatory leukogram may be classified as mild to severe depending upon the number of neutrophils or heterophils and their morphology, such as the presence of a left shift and toxic changes. Severe (usually acute) inflammation is indicated by a neutropenia or heteropenia with a left shift as a result of greater consumption of these cells than can be delivered to the blood. The balance between neutrophil/heterophil consumption and delivery by bone marrow is affected by species differences as to the amount of these cells in reserve and the proliferative capacity of the marrow (Weiser, 2012b). These differences translate into

magnitudes of neutrophilia/heterophilia that can occur with inflammatory disease in each species. Species with large reserves would be expected to deliver large numbers of neutrophils/heterophils into peripheral circulation and therefore would be expected to exhibit a neutrophilia/heterophilia during acute inflammation. Species with small reserves may exhibit an initial neutropenia/heteropenia until bone marrow production has had time to catch up. For example, healthy rabbits are primarily lymphocytic meaning that small and large lymphocytes are the predominate leukocytes in the normal blood film. As a result, infections rarely cause a significant leukocytosis (>15 000 cells/μL) and acute infections may cause a leukopenia (<5000 cells/μL) with a normal differential or a heterophilia, thrombocytopenia, and increase in nucleated erythrocytes in the peripheral blood (Vennen and Mitchell, 2009).

In general, changes in the leukocyte morphology of most mammals are a reliable indication of disease. Given the amount of information known about the different small exotic mammal species, strains, and breeds, the most reliable criteria for infectious disease appear to be the presence of immature leukocytes, toxic neutrophils, and Döhle bodies, and not necessarily the total leukocyte and differential counts.

Mammalian neutrophils/heterophils found in the peripheral blood are present in either the circulating pool or the marginating pool. The circulating pool occurs in large blood vessels and consists of neutrophils/heterophils that do not interact with the endothelial wall of the vessel. Blood collected by venipuncture consists of samples taken from the circulating pool. The marginating pool consists of neutrophils/heterophils that are interacting with the endothelium of small blood vessels and capillaries.

Left Shift

The term "left shift" refers to an increased concentration of immature neutrophils or heterophils in blood. The immature cells usually consist of bands, but also could include increased numbers of metamyelocytes and myelocytes (Figure 1.48). A left shift with a neutrophilia/heterophilia indicates the presence of marked inflammation. A left shift with a neutropenia/heteropenia indicates severe consumption of neutrophils. This is seen with overwhelming infections where peripheral utilization of these cells is greater than their replacement.

Toxic Change

"Toxic change in neutrophils/heterophils" is a term referring to morphologic changes associated with inflammatory diseases that alter bone marrow production of these types of cells. In response to the inflammatory disease, an acceleration of neutrophil/heterophil production occurs, resulting in the production and release of early

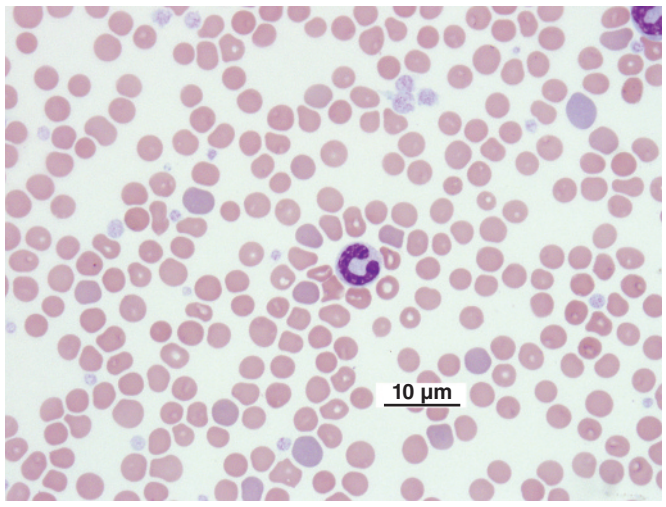


Fig. 1.48. Band cell in the blood film of an African hedgehog (*Atelerix albiventris*), Wright–Giemsa stain.

stage neutrophils/heterophils with retained organelles such as ribosomes. Retention of these organelles results in cytoplasmic basophilia and the presence of cytoplasmic vacuolation (Figures 1.49a–1.49c). Döhle bodies may also be present. Döhle bodies are composed of aggregates of endoplasmic reticulum and appear as gray-blue cytoplasmic inclusions.

Neutrophil/Heterophil Degeneration

The term "neutrophil/heterophil degeneration" is used to describe the cellular changes in cytologic samples that have been exposed to an unhealthy microenvironment, such as those produced by bacterial toxins or exposed epithelial surfaces. Degenerate neutrophils/heterophils found in peripheral blood films, therefore, are created by artifacts of sample preparation, such as prolonged storage of blood prior to preparing a blood film. Degenerate neutrophils/heterophils exhibit cytoplasmic vacuolation and nuclear swelling, which may be described cytologically as a pale-staining nucleus that has lost chromatin definition. Cell lysis can also occur with extreme degeneration. Pyknosis or karyorrhexis of leukocytes can occur with natural disease and toxicosis (e.g., xenobiotic administration) (Rovira and Kreklau, 2012) (Figure 1.50).

Type of Inflammation

Besides species differences in the amount of neutrophils/heterophils held in reserve and the proliferative capacity of the marrow, the type of inflammatory lesion will also influence the balance between consumption and marrow release and the leukogram. Closed cavity inflammatory lesions versus diffuse inflammation are examples of this. The increase in local blood flow and swelling in acute inflammatory lesions (such as an acute

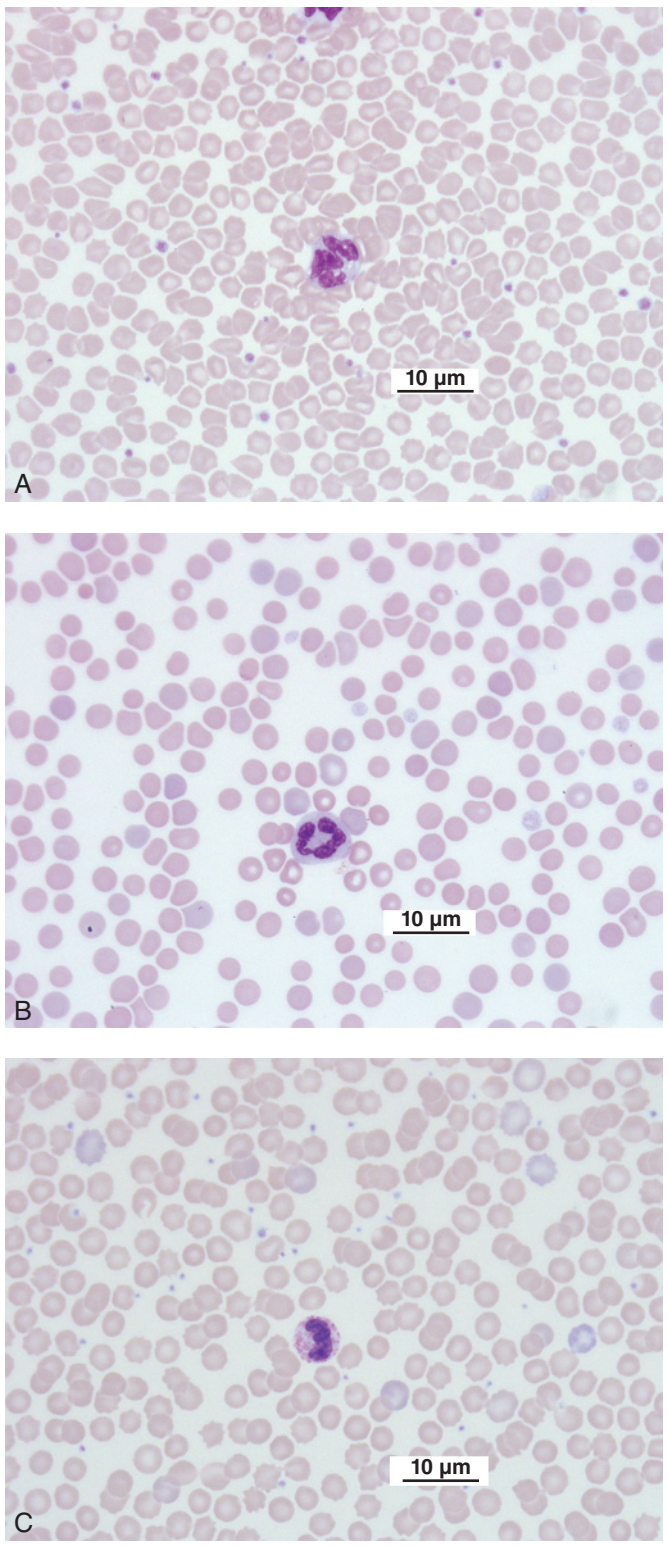


Fig. 1.49. (a) Toxic neutrophil in the blood film of a ferret (*Mustela putorius furo*), Wright–Giemsa stain; (b) toxic neutrophil in the blood film of an African hedgehog (*Atelerix albiventris*), Wright–Giemsa stain; (c) toxic heterophil in the blood film of a rabbit (*Oryctolagus cuniculus*), Wright–Giemsa stain.

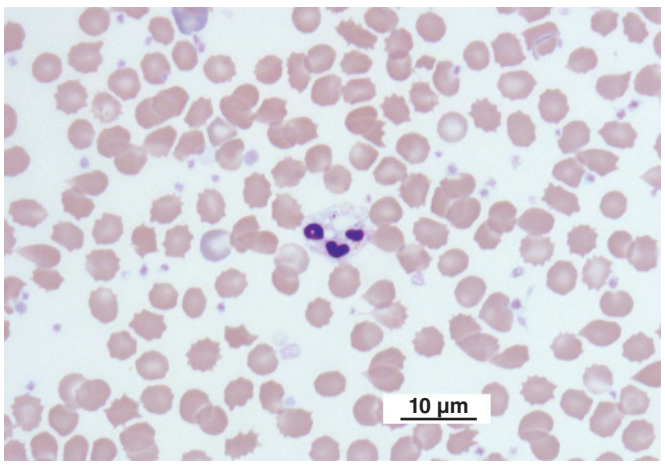


Fig. 1.50. Pyknosis of a heterophil in the blood film of a guinea pig (*Cavia porcellus*), Wright–Giemsa stain.

coelomitis associated with gastrointestinal rupture) leads to chemotactic factors and vascular changes that promote consumption of neutrophils/heterophils leading to a reduction of these cells in the peripheral blood, thus neutropenia or heteropenia. However, chronic, walled-off inflammatory lesions (such as a chronic walled-off abscess) often lead to very high neutrophil/heterophil concentrations in the peripheral blood because the rate of consumption of these cells is less than their production.

Assessment of the number of immature neutrophils/heterophils and lymphocytes present against the total population of neutrophils/heterophils can serve as a valuable means of evaluating the health status of a mammalian patient. For example, a neutrophilia/heterophilia with a left shift is indicative of an inflammatory disease, while a lymphopenia accompanied by a neutrophilia/heterophilia without a left shift is indicative of a corticosteroid response. A neutrophilia/heterophilia with a high normal lymphocyte count or a lymphocytosis is indicative of an excitement response. When a combined inflammatory and corticosteroid response is present, a lymphopenia with a neutrophilia/heterophilia with no left shift may be present.

A leukocytosis with an accompanying neutrophilia/heterophilia indicates a mild to marked response to a managed inflammatory condition. Severe inflammatory responses are indicated by the presence of a left shift and toxic neutrophils/heterophils. Some animals may not exhibit a leukocytosis, but will still demonstrate changes in the neutrophil/heterophil population and morphology.

Neutropenia/Heteropenia

Neutropenia/heteropenia occurs when neutrophils/heterophils are consumed at a rate faster than they are being produced and released into the peripheral circulation, and is often associated with acute inflammatory

disease or disease of marked severity. A neutropenia/heteropenia with a left shift and toxic changes that occurs in the absence of anemia and a normal platelet count is indicative of an acute inflammatory response associated with excessive peripheral utilization of neutrophils/heterophils. A neutropenia/heteropenia with no left shift, no anemia, and an adequate platelet count is indicative of an acute viral infection or an acute bone marrow injury. A neutropenia/heteropenia associated with a nonregenerative anemia and possibly a thrombocytopenia is indicative of a chronic bone marrow injury.

Other miscellaneous causes of neutropenia/heteropenia may occur in small exotic mammals. Immune-mediated neutropenia/heteropenia is a rare condition that results in a profound neutropenia/heteropenia in the peripheral blood and depletion of the maturation pool in the bone marrow. Injury to the stem cells in the bone marrow can occur with chemicals or drugs that affect rapidly dividing cells and may lead to a neutropenia/heteropenia. Injury to stem cells first appears as a profound neutropenia/heteropenia followed by a thrombocytopenia and eventually by a nonregenerative anemia. Infectious diseases, such as viral agents, may also cause stem cell injury.

Lymphopenia is generally indicative of corticosteroid excess, but may occur as a relative change in the neutrophil/heterophil to lymphocyte ratio in association with some inflammatory disease in some animals. For example, rabbits do not develop an overall leukocytosis with bacterial infections, but will develop an increase in the absolute heterophil concentration and decrease in the absolute lymphocyte concentration. Therefore, in the rabbit, evaluation of the H:L ratio and absolute heterophil and lymphocyte concentrations appear to be more reliable indicators of inflammatory disorders than the total leukocyte concentration. The neutrophil morphology may aid in distinguishing an infectious etiology from that of corticosteroid excess in mammals exhibiting a reversal of the N:L ratio. Evidence of toxic changes supports the presence of an infectious etiology.

Rabbits

As mentioned earlier, in the normal healthy rabbit, lymphocyte concentrations are higher than heterophil concentrations and rabbits do not typically develop a leukocytosis when bacterial infections are present, but will have an increase in the absolute heterophil concentration and possibly a decrease in the absolute lymphocyte concentration (Stieve-Caldwell et al., 2009; Campbell and Grant, 2010). This reversal of the H:L ratio is also associated with increases in serum cortisol concentrations where there is a decrease in the lymphocyte concentration. The presence of toxic changes in the heterophils is an important clue to an inflammatory response in the rabbit. Therefore, evaluation of the H:L ratio, the absolute heterophil and lymphocyte concentrations, and heterophil morphology appear to be more reliable indicators of inflammatory disorders in the rabbit than are total leukocyte concentrations.

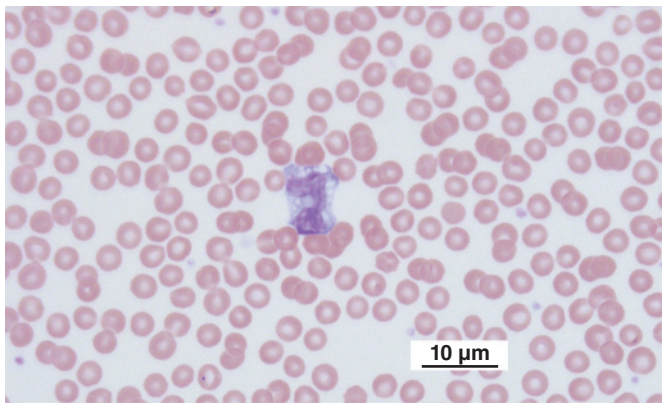


Fig. 1.51. A monocyte with numerous cytoplasmic vacuoles (reactive monocyte) in the blood film of a ferret (*Mustela putorius furo*), Wright–Giemsa stain.

Ferrets

In the normal healthy ferret, neutrophil concentrations are higher than lymphocyte concentrations and they demonstrate an increase in the neutrophil concentration and decrease in the lymphocyte concentration with increasing age (Kawasaki, 1994; Moore, 2000g). The total leukocyte count of healthy ferrets can be as low as 3000/µL (Lee et al., 1982; Kawasaki, 1994). Ferrets are unable to develop a marked leukocytosis with inflammatory disease; therefore, a concentration greater than 20 000/µL is unusual and a left shift is rare (Kawasaki, 1994).

Monocytosis

Mammalian monocytes engulf and degrade microorganisms, abnormal cells, and cell debris, and regulate immune responses and myelopoiesis (Figure 1.51). Monocytes in the peripheral blood are immature cells that migrate to inflammatory lesions in body tissues to become macrophages. A monocytosis may be seen with acute and chronic inflammatory conditions and occurs when there is an increased demand for monocytes. A corticosteroid response may also cause a monocytosis.

Neutrophilia of Excitement and Stress in Mammals

Neutrophilia of Excitement

In general, a leukocytosis and neutrophilia/heterophilia indicate that bone marrow production and

release of neutrophils/heterophils are exceeding peripheral demands. However, during excitement (known as the "fight-or-flight" response), epinephrine is immediately released causing neutrophils (heterophils) to leave the marginating pool and enter the circulating pool as a response to increased heart rate and blood flow through microcirculation. This increased blood flow through microcirculation is especially prominent in skeletal muscle; therefore, strenuous exercise just before bleeding often has the same effect. This typically results in a neutrophilia/heterophilia without a left shift, but may also result in a lymphocytosis. Depending upon the species, the numbers of these leukocytes nearly double as mature cells in microcirculation are swept from the marginating pool into the circulating pool. This excitement response is a common finding in many small exotic mammals, especially those not accustomed to handling.

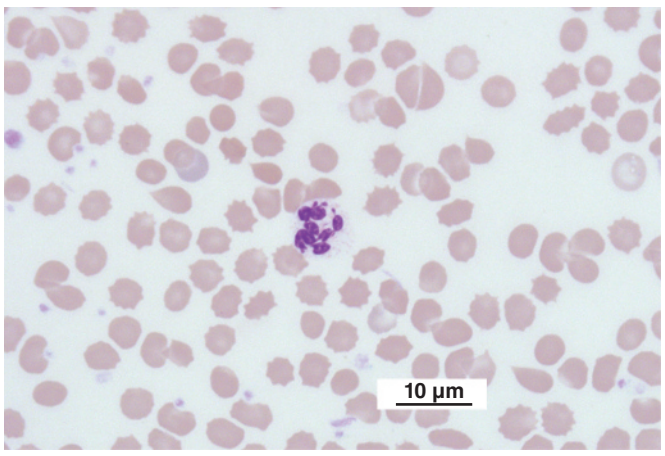


Fig. 1.52. Nuclear hypersegmentation in a heterophil in the blood film of a guinea pig (*Cavia porcellus*), Wright–Giemsa stain.

Neutrophilia of Corticosteroid Excess

Corticosteroid excess resulting from endogenous corticosteroid release in association with the physiologic stress that accompanies systemic disease or from an exogenous source, such as a therapeutic administration, causes predictable changes in the leukogram of most mammals. In response to major systemic illnesses (such as renal failure, dehydration, and inflammatory disease), metabolic disturbances (such as diabetic ketoacidosis), and pain (such as pain associated with trauma), cortisol is released from the adrenal gland following the release of adrenocorticotropic hormone from the pituitary gland. Other endogenous sources for an increase in plasma corticosteroids in small mammals include physical restraint, transport, a change in the cage population density, extremes in temperature, and loud sounds (Drozdowicz et al., 1990).

Corticosteroids, caused by endogenous corticosteroid excess or therapeutic administration of exogenous corticosteroids, typically result in predictable changes in the leukogram of small exotic mammals. Lymphopenia is the most consistent change observed because corticosteroids induce lymphocyte apoptosis (cell self-destruction) and may affect the recirculation of lymphocytes (Campbell and Grant, 2010). A mature neutrophilia/heterophilia (often doubling of these cells) is also commonly observed in the leukogram because corticosteroids cause a decrease in the stickiness of these leukocytes resulting in a decrease in the marginating pool. These leukocytes also stay in circulation longer than they normally would, which results in hypersegmentation.

Nuclear hypersegmentation (five or more segments or lobes) is a normal state in the progression of nuclear maturation in the neutrophil/heterophil and commonly occurs after the neutrophil/heterophil has left circulation (Figure 1.52). The presence of cells exhibiting hypersegmentation in the peripheral blood is an indication that the neutrophils/heterophils are staying in circulation longer than normal. This morphologic condition typically associated with prolonged corticosteroid effects on the neutrophils or heterophils.

A left shift is not associated with a corticosteroid affect unless there is coexisting inflammation. Less commonly seen changes to the leukogram may also include eosinopenia and monocytosis, but some species variability does occur among the small exotic mammals. For example, in the rabbit, a mature heterophilia and lymphopenia characterize glucocorticoid-mediated changes in the leukogram, which normally has more circulating lymphocytes than heterophils.

An inflammatory disorder is often accompanied by a steroid response. Assessment of the number of immature neutrophils/heterophils and lymphocytes present against the total population of neutrophils/heterophils can serve as a valuable means of evaluating the health status of a mammalian patient. For example, a neutrophilia/heterophilia with a left shift is indicative of an inflammatory disease, while a lymphopenia accompanied by a neutrophilia/heterophilia without a left shift is indicative of a corticosteroid response. A neutrophilia/heterophilia with a high normal lymphocyte count or a lymphocytosis is indicative of an excitement response. When a combined inflammatory and corticosteroid response is present, a lymphopenia with a neutrophilia/heterophilia with or without a left shift may be present.

Neutropenia in Mammals

Neutropenia/heteropenia occurs when neutrophils/heterophils are overwhelmingly consumed at a rate faster than they are being produced and released into the peripheral circulation, and is often associated with an acute inflammatory disease or a disease of marked

severity. This is especially notable with infections by pyogenic bacteria that often cause a degenerative left shift where the number of immature neutrophils/heterophils is greater than that of mature cells. A neutropenia/heteropenia with a left shift and toxic changes that occurs in the absence of anemia and a normal platelet count is indicative of an acute inflammatory response associated with excessive peripheral utilization of neutrophils/heterophils. Toxic changes may occur within a few days of the onset of the process (Weiser, 2012b)

A neutropenia/heteropenia with no left shift, no anemia, and an adequate platelet count is indicative of an acute viral infection or injury to rapidly dividing stem cells in the bone marrow. For example, ferrets with canine distemper typically exhibit a leukopenia and neutropenia (Wolf, 2009). Because neutrophils/heterophils have a higher turnover in circulating blood when compared to other hemic cells, a neutropenia/heteropenia develops before thrombocytopenia and a nonregenerative anemia. A neutropenia/heteropenia associated with a nonregenerative anemia and possibly a thrombocytopenia are indicative of a chronic bone marrow injury.

Reversible stem cell injury associated with an acute neutropenia/heteropenia that occurs without a left shift will frequently be brief (e.g., lasting only for 24–48 hours) as the marrow repopulates. This is followed by a left shift with progressively increasing mature neutrophil/heterophil concentrations leading to an inflammatory pattern that consists of a neutrophilia/heterophilia and left shift.

Depending upon the cause, a neutropenia/heteropenia associated with a reversible stem cell injury with a longer duration (e.g., several days or longer) will also result in thrombocytopenia and a nonregenerative anemia. Certain chemotherapeutic agents, total body ionizing radiation therapy, or estrogen toxicity can damage hematopoietic stem and progenitor cells or inhibit cell replication resulting in this type of hemogram.

Therefore, a neutropenia/heteropenia associated with a left shift and toxic change is likely associated with inflammatory disease (Campbell and Grant, 2010). However, if the neutropenia/heteropenia is not associated with a left shift but there is a combination of thrombocytopenia and nonregenerative anemia, then stem cell injury in the bone marrow should be considered.

Eosinophilia and Eosinopenia in Mammals

Eosinophilia

Eosinophilia is a nonspecific response that could be associated with parasite infestation, hypersensitivity reactions, or other agents chemotactic for eosinophils (Campbell and Grant, 2010; Weiser, 2012b). A peripheral eosinophilia does not always occur in the presence of tissue eosinophilic inflammation. Although the exact mechanism for eosinophilic inflammation in not well understood, it appears to be associated with eosinophil chemoattractants, such as interleukin-5 released from T cells sensitized by parasite antigens (both endoparasites and ectoparasites) or allergens and mast cell degranulation. (Herndon and Kayes, 1992). An eosinophilia is more likely to occur when parasites are present in tissues rather than in the lumen of the gastrointestinal tract (Young and Meadows, 2010).

Chronic eosinophilia mediated by mast cell degranulation is often associated with inflammation involving tissues that normally contain a high concentration of mast cells. Such tissues are species-specific, but generally include the skin, lung, gastrointestinal tract, and female reproductive tract. For example, eosinophilic inflammatory bowel disease, a poorly characterized gastroenteritis that may have an allergic component, of ferrets will occasionally produce a peripheral eosinophilia.

Eosinopenia

Eosinopenia has limited significance as healthy animals often have no eosinophils identified in their 100-cell leukocyte differential counts. However, it is known that corticosteroids cause eosinopenia. This is likely due to inhibition of mast cell degranulation, neutralization of circulating histamine, sequestration of eosinophils, and delayed release of eosinophils from the bone marrow (Young and Meadows, 2010). Catecholamines may also induce an eosinopenia by a β-adrenergic effect. Thus, during acute inflammation, an eosinopenia may occur due to increased corticosteroids and catecholamine release.

Basophilia in Mammals

The number of circulating basophils in any given species is, as a rule, inversely proportional to tissue mast cell concentration (Fortman et al., 2002). Basophilia has limited significance as healthy animals often have no basophils identified in their 100-cell leukocyte differential counts. Mammalian basophils participate in allergic and delayed hypersensitivity reactions; therefore, a basophilia is usually associated with an eosinophilia. The exact interpretation of the basophilia is usually unknown (Weiser, 2012b). Rabbits typically have more basophils than other exotic animal species. Often 5% of the leukocytes in the total leukocyte count are basophils, but they can be as high as 30% in rabbits with no apparent abnormalities (Campbell and Ellis, 2007).

Lymphopenia in Mammals

Corticosteroids, caused by endogenous corticosteroid excess or therapeutic administration of exogenous corticosteroids, create a transient and predictable

lymphopenia. Lymphopenia occurs because corticosteroids induce lymphocyte apoptosis (cell self-destruction) and may affect the recirculation of lymphocytes. Lymphopenia is often seen in acute infections because of stress-induced corticosteroid release and redistribution of lymphocytes where lymphocytes become trapped in lymphoid tissue or circulating lymphocytes migrate to antigenically stimulated lymph nodes (reactive lymph nodes) (Jain, 1993; Latimer, 1995). Lymphopenia may also be caused by viral diseases (Müller et al., 2009).

Reactive lymphocytes, also called immunocytes and transformed lymphocytes, are antigenically stimulated lymphocytes in the peripheral blood. They vary in size, nuclear chromatin pattern, number of visible nucleoli, and amount of cytoplasmic vacuolation; however, they are identified by their deeply basophilic cytoplasm (Figures 1.53a–1.53c). Some resemble lymphoblasts and those with a prominent perinuclear halo or Golgi resemble plasma cells.

Lymphadenitis may also cause lymphopenia as the efferent lymph flow from the inflamed lymph node prevents lymphocyte recirculation (Hopkins and McConnell, 1984). Loss of lymphocyte-rich fluid within the lumen of the intestine associated with a protein-losing enteropathy or ulcerative enteritis may also explain a lymphopenia (Kull et al., 2001). Likewise, loss of lymphocyte-rich fluid into a body cavity, such as chylothorax or chyloperitoneum, can result in sequestration of lymphocytes causing lymphopenia (Schultze, 2010).

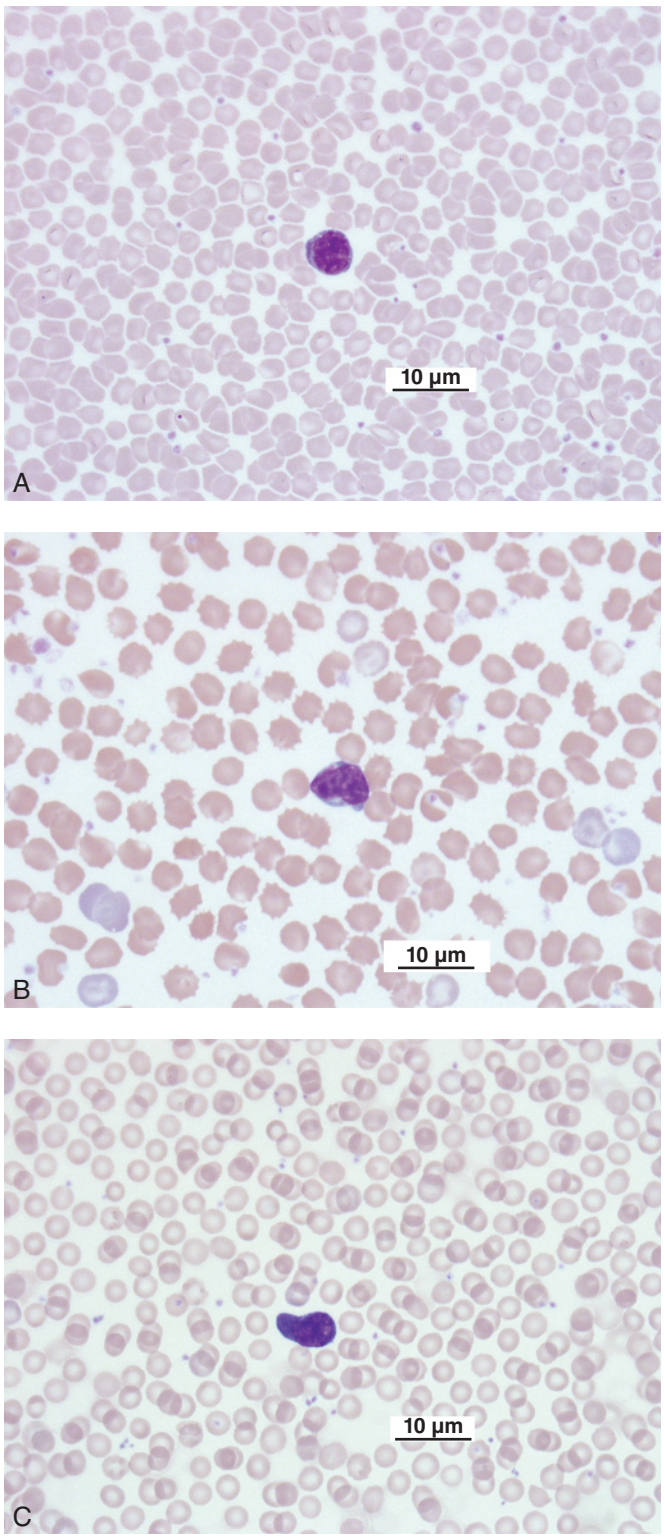


Fig. 1.53. (a) Reactive lymphocyte in the blood film of a ferret (*Mustela putorius furo*), Wright–Giemsa stain; (b) reactive lymphocyte in the blood film of a guinea pig (*Cavia porcellus*), Wright–Giemsa stain; (c) reactive lymphocyte in the blood film of a cottontail rabbit (*Sylvilagus floridanus*), Wright–Giemsa stain.

Lymphocytosis in Mammals

Lymphocytosis in the mammalian patient is usually associated with an excitement response or lymphocytic leukemia. An excitement response results in a moderate increase in the number of circulating normal-appearing small, mature lymphocytes. A marked increase in the lymphocyte count should be viewed with suspicion for a lymphoproliferative disorder such as lymphocytic leukemia. In general, a lymphoproliferative disorder is a lymphoid cell neoplastic disorder in the blood or bone marrow.

Lymphoma is a term used to describe lymphoid neoplasia involving solid tissue. Lymphocytic leukemia is the term used to describe lymphoid neoplasia involving blood or bone marrow. Lymphocytic leukemia is indicated by a marked increase in lymphocytes and is often accompanied by the presence of abnormal lymphocyte morphology. Lymphocytic leukemia is not commonly reported in small exotic mammals, but has been reported in the rabbit and ferret (Finnie et al., 1980; Toth et al., 1990; Boone et al., 1995).

Important lymphocyte morphology changes associated with lymphoid neoplasia include the presence of large lymphocytes (larger in size than neutrophils/

heterophils) with a pale-staining nucleus containing fine nuclear chromatin. The presence of a prominent nucleolus and increased cytoplasmic volume are additional supportive features of malignant lymphocytes. Occasionally, lymphocytes exhibit cytoplasmic vacuolation. Lymphocyte vacuolation can also be associated with toxins or storage disorders (Weiser, 2012b).

Myeloma is a term referring to a specific form of lymphoid neoplasia that involves plasma cell differentiation (see Chapter 20). These plasma cells are seen as discrete round to oval cells characterized by a moderate to large amount of moderately to deeply basophilic cytoplasm, a perinuclear clear area (Golgi), medium-sized round to oval eccentrically located nucleus with moderately granular to coarse chromatin, and mild-to-moderate anisocytosis and anisokaryosis. Often an associated monoclonal gammopathy is present as well.

A rare cause for a lymphocytosis is chronic inflammatory disease. The reason for this is that the immune system responds to the inflammation with lymphoid hyperplasia in lymphoid tissues but it rarely manifests as lymphocytosis in blood (Weiser, 2010). Persistent antigenic stimulation in chronic inflammatory diseases, especially those associated with certain infectious agents, may be more likely to cause a lymphocytosis (Schultze, 2010). In such cases the blood film often reveals a high proportion of large granular lymphocytes (chronic ehrlichiosis in the dog is an example of this). Such findings may also be seen occasionally in small exotic mammals.

Ferrets

The total leukocyte count and the lymphocyte count are often used as a means of monitoring ferrets for lymphoma. The total leukocyte count of ferrets with lymphoma may be normal or may demonstrate an absolute or relative lymphocytosis. Marked leukocytosis (leukocyte count greater than 20 000/μL) is often associated with lymphocytic leukemia or lymphoma (Erdman et al., 1992; Brown, 1997). Anemia, leukopenia, and thrombocytopenia may also be noted in some cases. Abnormal lymphocytes occasionally appear in the differential. As a general rule, a persistent absolute lymphocyte count greater than 3500/μL or a relative lymphocytosis (>60%) is considered suspicious, even if the total leukocyte count is normal.

Monocytosis

Mammalian monocytes engulf and degrade microorganisms, abnormal cells, and cell debris, and regulate immune responses and myelopoiesis. Monocytes in the peripheral blood are immature cells that migrate to inflammatory lesions in body tissues to become macrophages. Therefore, a monocytosis may be interpreted as a response to increased tissue demand for macrophages during acute and chronic inflammatory conditions and is typically associated with a neutrophilia/heterophilia. A monocytosis can be associated with necrosis, suppuration, malignant neoplasia, pyogranulomatous lesions, internal hemorrhage, hemolysis, immune-mediated disease, and trauma (Latimer, 1995). A corticosteroid response may also cause a monocytosis in some species. A monocytosis can also be associated with certain viral infections (Müller et al., 2009).

Thrombocytopenia and Thrombocytosis in Mammals

Thrombocytopenia occurs as a result of decreased platelet production, increased platelet destruction, or increased platelet utilization (consumption). Thrombocyte concentrations below 25 000/mL of blood may result in bleeding.

Decreases in platelet production are indicated by a decrease in the number of megakaryocytes in the bone marrow. Toxicities, whole-body irradiation, infectious agents, neoplastic conditions, and immune-mediated disorders can cause decreased platelet production thrombocytopenias in domestic mammals and are likely factors involved with such conditions in the small exotic mammals, as well (Baker, 2010). Chemicals that attack dividing cells will have a detrimental effect on all of the dividing cells in the bone marrow, including a loss of megakaryocytes. For example, ferret bone marrow is highly susceptible to estrogen-induced suppression. This is commonly seen in the panhypoplasia created by estrogen toxicity of intact female ferrets suffering from prolonged estrus. The mechanism for this is complex and poorly understood, but may involve serum inhibitors of hematopoiesis derived from T cells (Farris and Benjamin, 1993).

A thrombocytopenia associated with increased platelet destruction and consumption is generally associated with increased numbers of megakaryocytes in the bone marrow. Increased platelet destruction is a rare condition involving immune-mediated removal of platelets from peripheral circulation. Increased utilization of platelets is more common and is often associated with vascular injury, such as DIC, hemangiosarcoma or vasculitis, where excessive consumption of platelets is occurring.

Evaluation of the number of megakaryocytes in the bone marrow may be necessary to determine if the thrombocytopenia is due to a defect in platelet production or caused by platelet destruction or consumption. A lack of or decrease in the number of megakaryocytes would indicate a platelet bone marrow production defect, whereas a normal number or increased number of megakaryocytes would support the cause of thrombocytopenia being the result of platelet destruction or consumption.

It should be pointed out that blood loss alone does not result in a significant thrombocytopenia since the platelet concentration rarely is less than 100 000/μL following hemorrhage (Baker, 2010).

A pseudothrombocytopenia is an in vitro condition resulting from platelet clumping as identified by platelet clumps found on the feathered edge of the blood film. Excessive platelet clumping typically results from poor to difficult venipuncture (a common occurrence when dealing with small exotic mammals) that subsequently initiates platelet activation (Russell, 2010).

A general guideline to estimate the platelet count from a blood film is to count the number of platelets in ten 100× (oil-immersion) fields and multiply that number by 15 000 to obtain an estimated platelet count/μL (Russell, 2010). Another method to assess whether or not there are an adequate number of platelets on a blood film is to obtain the average number of platelets per oil-immersion field (magnification, 1000×). With this approach, the number of platelets is adequate if there are at least five platelets per oil-immersion field (Baker, 2010).

Thrombocytosis is a rare condition where there is an excessive amount of platelets present in the peripheral blood. The cause for increased platelet concentrations in the peripheral blood is a nonspecific condition that is generally not associated with clinical signs of disease. Examples of conditions that are associated with a thrombocytosis in domestic mammals include iron-deficiency anemia, inflammatory diseases, and epinephrine effects.

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