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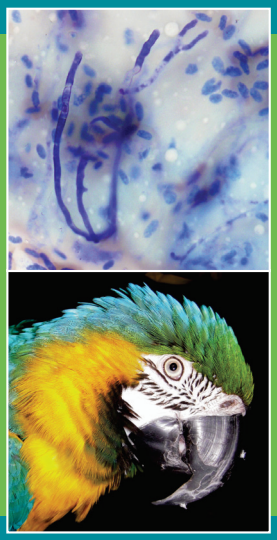

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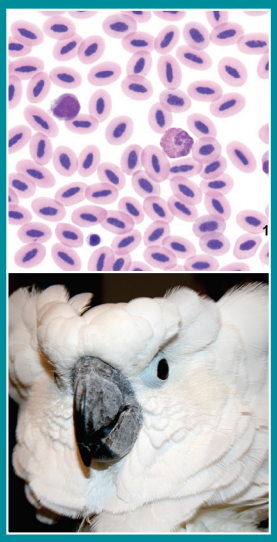

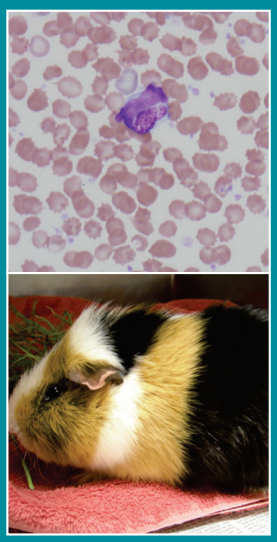

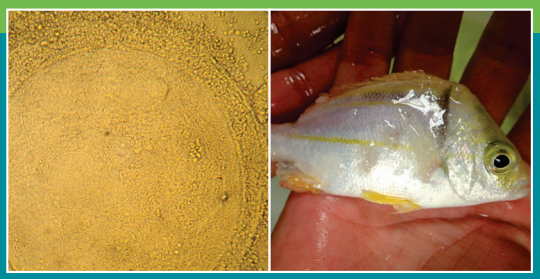

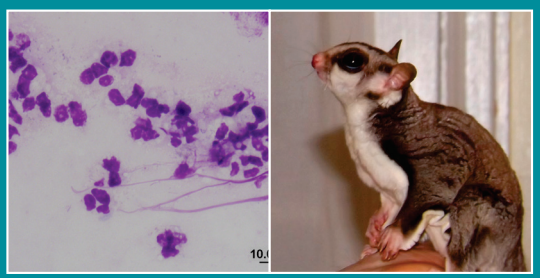

Terry W. Campbell

WILEY Blackwell

Exotic Animal Hematology and Cytology

■ FOURTH EDITION

Exotic Animal Hematology and Cytology

FOURTH EDITION

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CONTENTS

SECTION VI WET MOUNT MICROSCOPY OF FISH

PREFACE

This book serves as a comprehensive reference on exotic animal hematology and cytology of all major species by providing practical hematologic and cytologic information involving small exotic mammals, birds, reptiles, amphibians, and fish. It is designed to act as both an atlas and a text. Veterinarians and veterinary technicians in clinical practice, clinical pathologists, laboratory technicians, veterinary students, veterinary pathologists, and those engaged in avian and exotic animal research are the target audience for this book. This edition has been reorganized from previous editions to provide a more user-friendly disease-based chapter structure. This new structure is designed to better match how most users of previous editions search for information in the book.

Chapters 1 through 5 provide information on hemic cytology and hematology with each major exotic animal group separated into its own individual chapter. Chapter 6 discusses the common blood parasites of exotic animals. Chapter 7 provides information on bone marrow interpretation when dealing with exotic animal patients. Chapters 8–12 provide guidance for blood collection in the various animal groups and Chapter 13 guides the readers in bone marrow sample collection. Chapter 14 covers hematologic techniques used in the clinical or research laboratory. Chapters 15–17 provide information on the normal cytology with each major exotic animal group separated into its own individual chapter. Chapter 18 discusses the cytology of inflammation. Chapter 19 provides information on the cytology of tissue hyperplasia or benign neoplasia, whereas Chapter 20 discusses malignant neoplasia. Chapter 21 covers interpretation of effusions. Chapter 22 provides information related to the identification of important infectious disease agents. Chapter 23 guides the readers in cytologic sample collection and evaluation. Chapters 24 and 25 provide information on wet-mount microscopy, which is especially useful in the evaluation of aquatic patients.

A good quality microscope is one of the most useful diagnostic tools available for veterinarians in clinical practice and necessary for evaluating hemic cytology and cytology specimens. Many of the disorders affecting the hematology and cytology of exotic animal patients can easily be diagnosed in-house without the delay of using an outside commercial laboratory, thus providing the opportunity of treating the patient more quickly with disease-specific therapy. Therefore, this book serves as a resource for in-house hematology and cytology diagnosis for the exotic animal hospital.

The reader will find that for the most part, the basic principles of hematology and cytology, such as sample collection, preparation, and interpretation, of exotic animals are the same as those for domestic mammals. Therefore, knowledge of the hematology and cytology of domestic mammals will greatly enhance the understanding of the information provided in this book.

The majority of the photomicrographs in this book were taken of Wright–Giemsa stained blood films or cytology slides using 1000× magnification (oil immersion or 100× objective). Sizing bars have been added to the newer images. Other photomicrographs were taken from slides using lower magnifications, such as 400× or 500×, or stained with other stains, such as Diff-Quik, acid-fast, Natt and Herrick's, phloxine B, Macchiavello's, or Giménez. Wet-mount images used in the diagnosis of fish and amphibian diseases were taken from videomicroscopy images using primarily 400× magnifications with the specimens under a glass coverslip.

ACKNOWLEDGMENTS

As I sit at my desk, on July 10, 2014, I have before me the final pages of the manuscript to the fourth edition of this book with the original title, *Avian Hematology and Cytology*, addressing notes from the editors. I soon begin to mull over the events that got me to this place and how things have changed over time. In 1987, I was working as a clinical instructor in the Veterinary Teaching Hospital at Kansas State University. I had just completed a residency and PhD program in veterinary clinical pathology and was hired to start an exotic animal medicine service in the hospital. One day (the exact one, I cannot remember), I was approached by a representative (whose name also I do not remember) from Iowa State University Press who asked the question: "I understand that you have material that might be published in book form." Prior to that moment, I had not spoken to anyone about my publishing goals. I had never considered writing a book; instead, I had planned on publishing a series of articles in a veterinary journal for practicing veterinarians on the subjects of avian hematology and cytology. I agreed to submit a prospectus, which consisted of a representative chapter of a proposed book. The proposal was accepted and the rest is history. To this day, I do not know who had sent the publishing representative my way. That person, whoever he or she may be, should know that I still have not decided if that action on his or her part was a blessing or a curse. But I wish to thank you for acknowledging my work as being something worthy of sharing with others.

Dr. Robert Quick from Crete, Nebraska (who happens to be my father-in-law), was noted among other character traits for saying, "One should never outlive his projects." This book just might be one of those projects for me. The time and energy it took to write this fourth edition, considering the format changes and addition of new references and images, was like writing a new book.

I offer my profoundest thanks to my dear wife, Susie, for her love and never-failing support for all of my projects. I also wish to thank my lads, Brian, Aiden, Ian, Bryce, and Taylor, for bringing joy and balance into my life.

SCIENTIFIC NAMES USED IN TEXT

Mammals

African hedgehog (*Atelerix albiventris*)
African lion (*Panthera leo*)
Bengal tiger (Panthera tigris tigris)
Chinchilla (*Chinchilla lanigera*)
Cottontail rabbit (*Sylvilagus floridanus*)
Domestic ferret (*Mustela putorius furo*)
Domestic mouse (*Mus musculus*)
Domestic rabbit (*Oryctolagus cuniculus*)
Domestic rat (*Rattus norvegicus*)
Gerbil (*Meriones unguiculatus*)
Guinea pig (*Cavia porcellus*)
Hamster (*Mesocricetus auratus*)
North American river otter (*Lontra canadensis*)
Sugar glider (*Petaurus breviceps*)

Birds

African Gray parrot (*Psittacus erithacus*)
Atlantic puffin (*Fratercula arctica*)
Bald eagle (*Haliaeetus leucocephalus*)
Barn owl (*Tyto alba*)
Barred owl (*Strix varia*)
Black-neck stilt (*Himantopus mexicanus*)
Black-throated laughing thrush (*Garrulax chinensis*)
Blue and gold macaw (*Ara ararauna*)
Blue-fronted amazon parrot (*Amazona aestiva*)
Buderigar (*Melopsittacus undulatus*)
Caribbean flamingo (*Phoenicopterus ruber*)
Chukar (*Alectoris chukar*)
Cockatiel (*Nymphicus hollandicus*)
Domestic chicken (*Gallus gallus domesticus*)
Domestic duck (*Anas platyrhynchos domestica*)
Domestic turkey (*Meleagris gallopova*)
Eclectus parrot (*Eclectus roratus*)
Emu (*Dromaius novaehollandiae*)
Ferruginous hawk (*Buteo regalis*)
Gannet (*Morus* spp.)
Goffin cockatoo (*Cacatua goffiniana*)
Golden eagle (*Aguila chrysaetos*)
Great horned owl (*Bubo virginianus*)
Greater Indian hill mynah (*Gracula religiosa intermedia*)
Green-cheeked conure (*Pyrrhura molinae*)
Green-wing macaw (*Ara chloropterus*)
Grey-cheeked parakeet (*Brotogeris pyrrhoptera*)
Gyrfalcon (*Falco rusticolus*)
Hyacinth macaw (*Anodorhynchus hyacinthinus*)
Java rice bird (*Lonchura oryzivora*)
Kestrel (*Falco sparverius*)
Lesser sulfur crested cockatoo (*Cacatua sulphurea*)
Magpie (*Pica pica*)
Mallard duck (*Anas platyrhynchos*)
Military macaw (*Ara militaris*)
Moluccan (or Salmon-crested) cockatoo (*Cacatua moluccensis*)
Orange-winged amazon parrot (*Amazona amazonica*)
Peach-faced lovebird (*Agapornis roseicollis*)
Peregrine falcon (*Falco peregrinus*)
Pine siskin (*Carduelis pinus*)
Quail (*Colinus* spp.)
Red tailed hawk (*Buteo jamaicensis*)
Rock dove, pigeon (*Columbia livia*)
Rose-ringed parakeet (*Psittacula krameri*)
Skua (*Stercorarius* sp.)
Spectacled amazon parrot (*Amazona albifrons*)
Sun conure (*Aratinga solstitialis*)
Timneh African Gray parrot (*Psittacus erithacus timneh*)
Turkey vulture (*Cathartes aura*)
Umbrella cockatoo (*Cacatua alba*)
Western screech owl (*Megascops kennicottii*)
White-winged wood duck (*Cairina scutulata*)
Yellow-headed amazon parrot (*Amazona oratrix*)
Yellow-naped amazon parrot (*Amazona auropalliata*)

Reptiles

American alligator (*Alligator mississippiensis*)
Ball python (*Python regius*)
Bearded dragon (*Pogona vitticeps*)
Burmese mountain tortoise (*Manouria emys*)
Burmese python snake (*Python bivittatus*)
Common boa constrictor (*Boa constrictor*)
Emerald tree boa (*Corallus caninus*)

Green iguana (*Iguana iguana*)
Green sea turtle (*Chelonia mydas*)
Green tree python (*Morelia viridis*)
Jackson's chameleon lizard (*Trioceros jacksonii*)
Leopard gecko (*Eublepharis macularius*)
Leopard tortoise (*Stigmochelys pardalis*)
Loggerhead sea turtle (*Caretta caretta*)
Malayan box turtle (*Cuora amboinensis*)
Ramsay's (or Woma) python (*Aspidites ramsayi*)
Red-eared slider (*Trachemys scripta elegans*)
Reeve's (or Chinese pond) turtle (*Chinemys reevesii*)
Spectacled caiman (*Caiman crocodiles*)
Sulcata tortoise (*Geochelone sulcata*)
Three-toed box turtle (*Terrapene carolina trunguis*)
Veiled chameleon (*Chamaeleo calyptratus*)
Water dragon (*Intellagama lesueurii*)
Wood turtle (*Glyptemys insculpta*)

Amphibians

Boreal toad (*Bufo boreas boreas*)
Poison dart frog (family Dendrobatidae)
Rough skinned newt (*Taricha granulosa*)
Tiger salamander (*Ambystoma tigrinum*)
White's tree frog (*Litoria caerulea*)
Woodhouse toad (*Bufo woodhousii*)

Fish

Atlantic spadefish (*Chaetodipterus faber*)
Black tip reef shark (*Carcharhinus melanopterus*)
Carp, Koi (*Cyprinus carpio*)
Clown knifefish (*Chitala chitala*)
Dwarf Gourami (*Trichogaster lalius*)
Freshwater Angelfish (*Pterophyllum scalare*)
Goldfish (*Carassius auratus*)
Green sunfish (*Lepomis cyanellus*)
Guppy (*Poecilia reticulate*)
Iridescent shark catfish (*Pangasianodon hypophthalmus*)
Orbicular batfish (*Platax orbicularis*)
Plecostomus (*Hypostomus plecostomus*)
Rainbow trout (*Oncorhynchus mykiss*)
Red Oscar (*Astronotus ocellatus*)
Red-tailed catfish (*Phractocephalus hemioliopterus*)
Regal tang (*Paracanthurus hepatus*)
Roundtailed chub (*Gila robusta*)
Sandbar shark (*Carcharhinus plumbeus*)
Sharpnose puffer (*Canthigaster* spp.)
Southern ray (*Dasyatis americana*)
Tinfoil barb (*Barbonymus schwanenefeldii*)
White spotted bamboo shark (*Chiloscyllium plagiosum*)
White sturgeon (*Acipenser transmontanus*)
White tip reef shark (*Triaenodon obesus*)
Wolf eel (*Anarrhichthys ocellatus*)
Yellow tang (*Zebrasoma flavescens*)

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 retroorbital 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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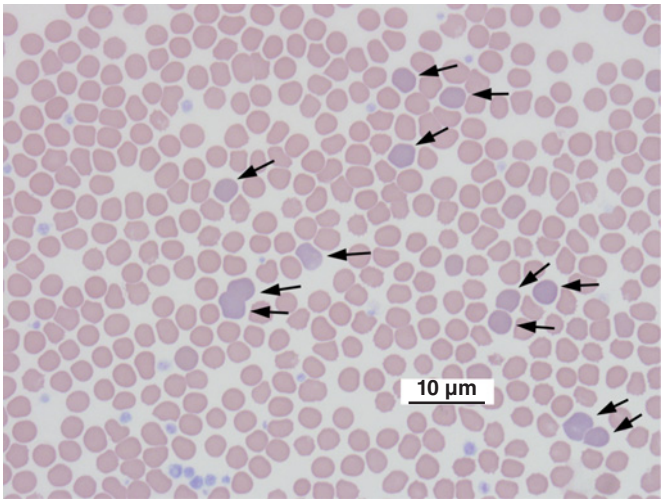


Fig. 1.1. Polychromatic erythrocytes (arrows) in the blood film of a mouse (*Mus musculus*), Wright–Giemsa stain.

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.

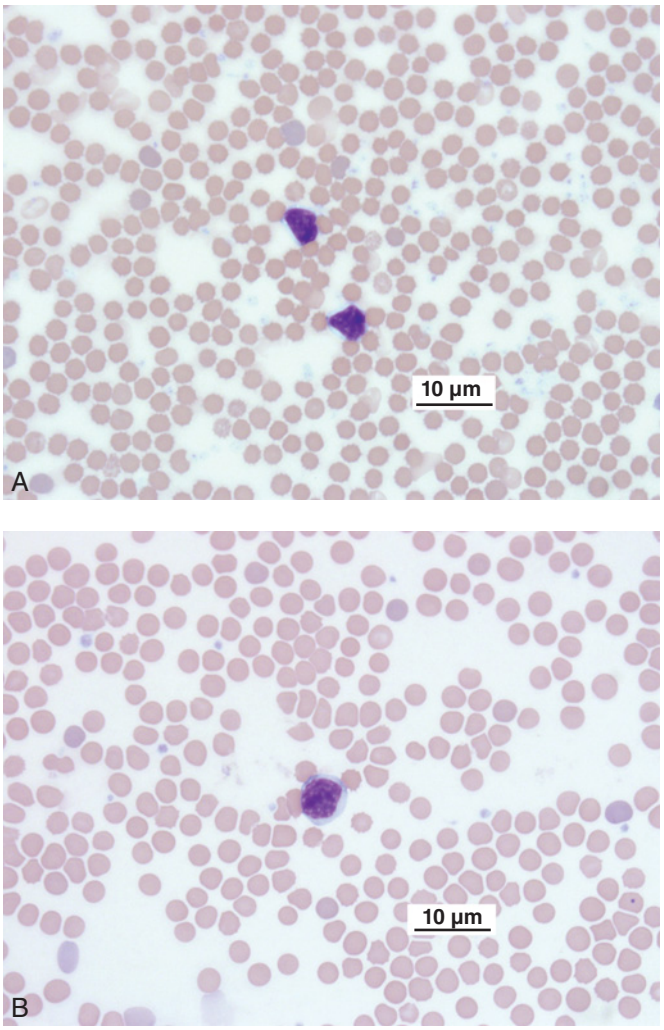
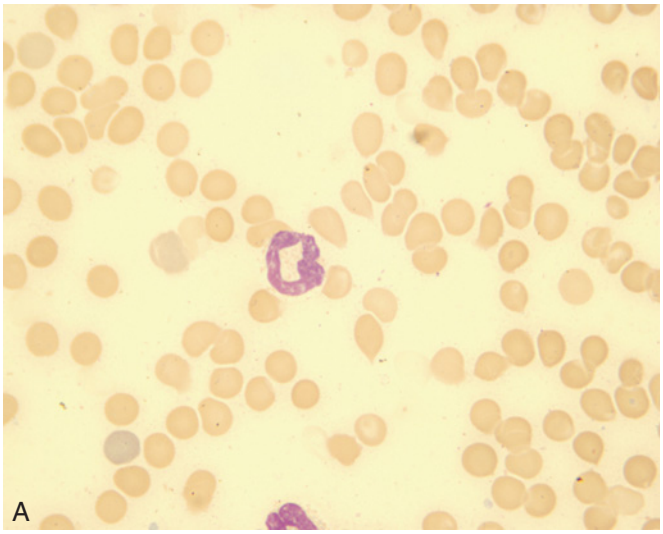


Fig. 1.2. (a) Small lymphocytes in the blood film of a mouse (*Mus musculus*), Wright–Giemsa stain; (b) large lymphocyte in the blood film of a mouse (*Mus musculus*), Wright–Giemsa stain.

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



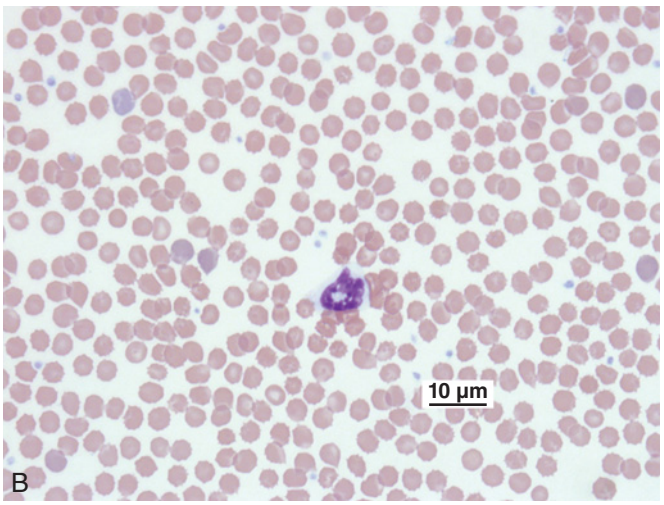
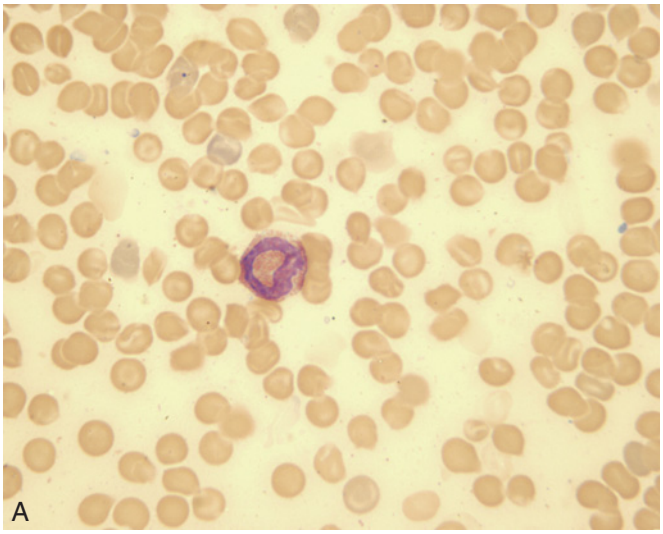


Fig. 1.3. (a) Neutrophil in the blood film of a domestic rat (*Rattus norvegicus*), Wright–Giemsa stain (1000×); (b) neutrophil in the blood film of a mouse (*Mus musculus*), Wright–Giemsa stain.



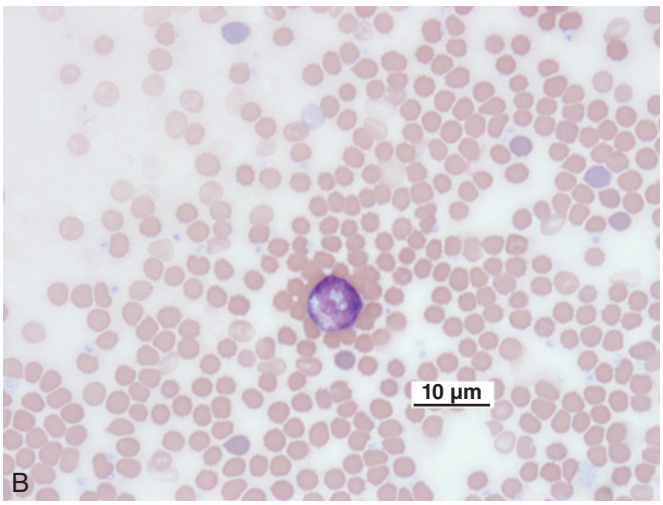


Fig. 1.4. (a) Eosinophil in the blood film of a domestic rat (*Rattus norvegicus*), Wright–Giemsa stain (1000×); (b) eosinophil in the blood film of a mouse (*Mus musculus*), Wright–Giemsa stain.

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

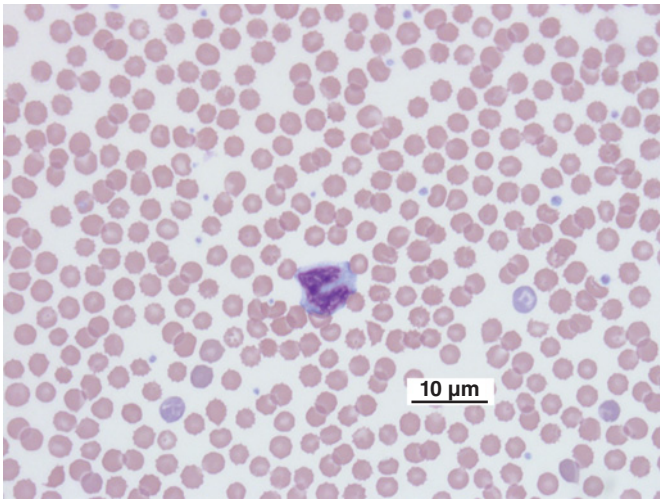


Fig. 1.5. Monocyte in the blood film of a mouse (*Mus musculus*), Wright–Giemsa stain.

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

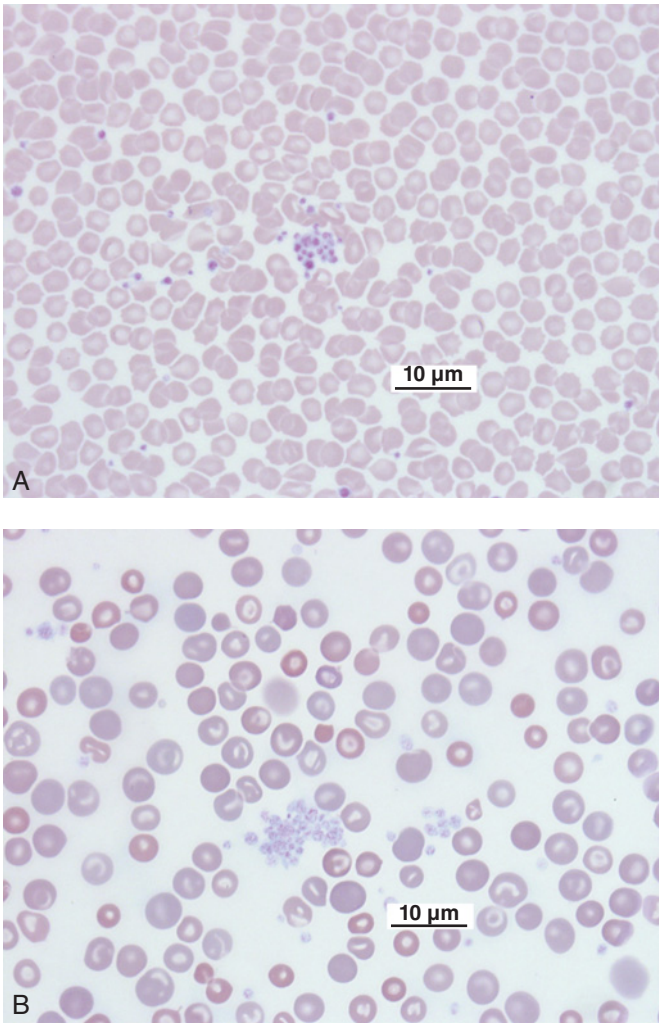


Fig. 1.6. (a) Platelets in the blood film of a ferret (*Mustela putorius furo*), Wright–Giemsa stain; (b) platelets in the blood film of an African hedgehog (*Atelerix albiventris*), Wright–Giemsa stain.

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

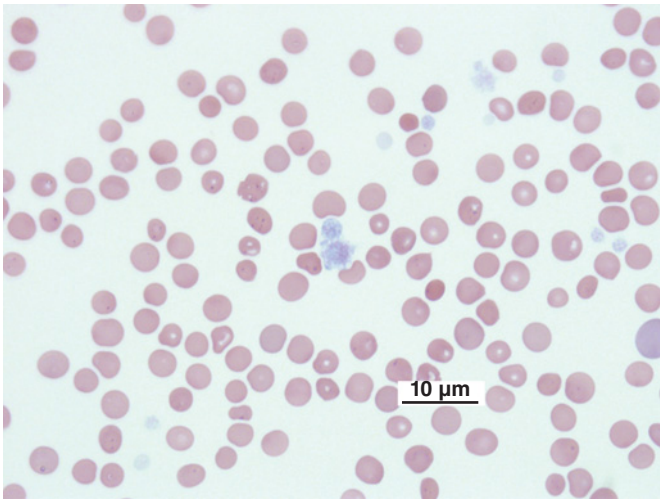


Fig. 1.7. Large platelets in the blood film of an African hedgehog (*Atelerix albiventris*), Wright–Giemsa stain.

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,

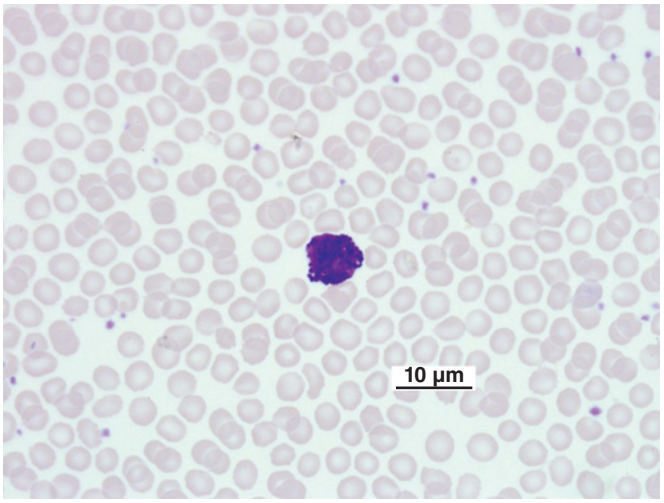


Fig. 1.8. Normal erythrocytes and a basophil in the blood film of a guinea pig (*Cavia porcellus*), Wright–Giemsa stain.

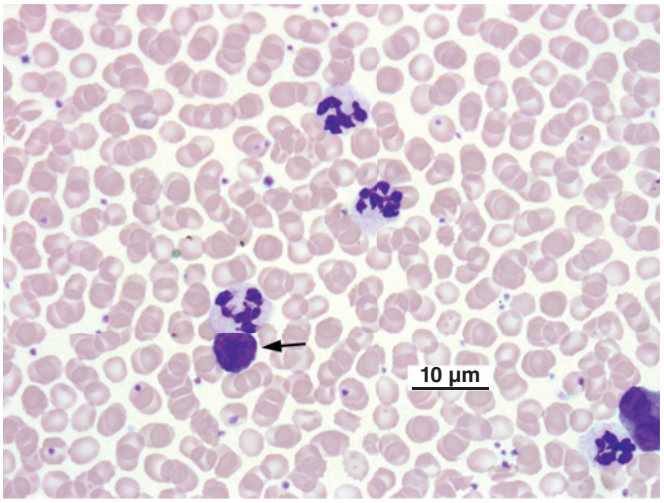


Fig. 1.9. Heterophils and lymphocyte (arrow) in the blood film of a guinea pig (*Cavia porcellus*), Wright–Giemsa stain.

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

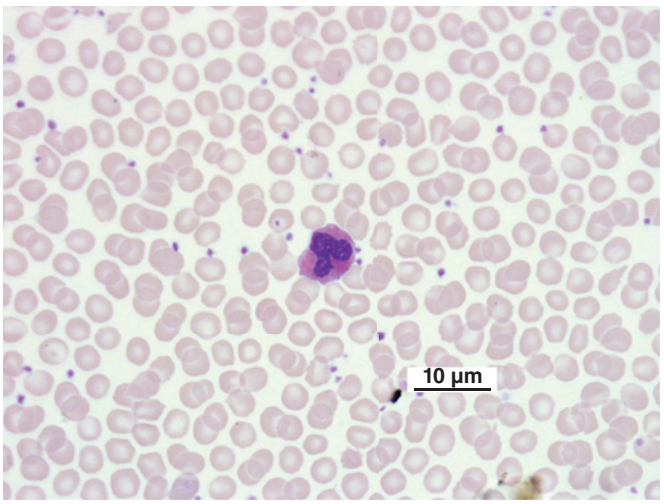


Fig. 1.10. Eosinophil in the blood film of a guinea pig (*Cavia porcellus*), Wright–Giemsa stain.

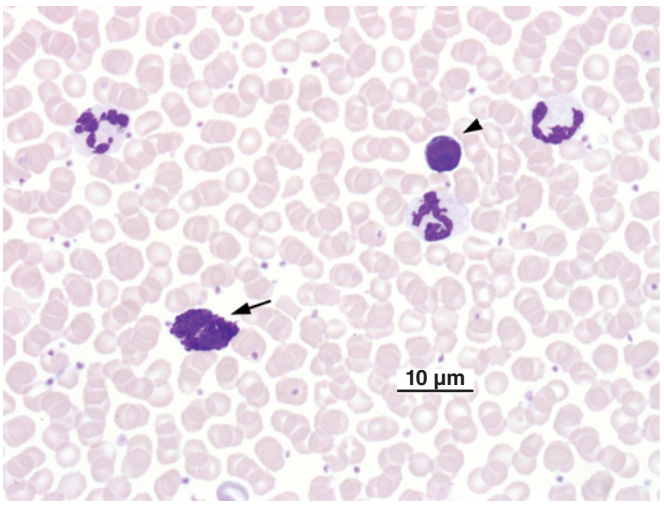


Fig. 1.11. Basophil (arrow), heterophils, and lymphocyte (arrowhead) in the blood film of a guinea pig (*Cavia porcellus*), Wright–Giemsa stain.

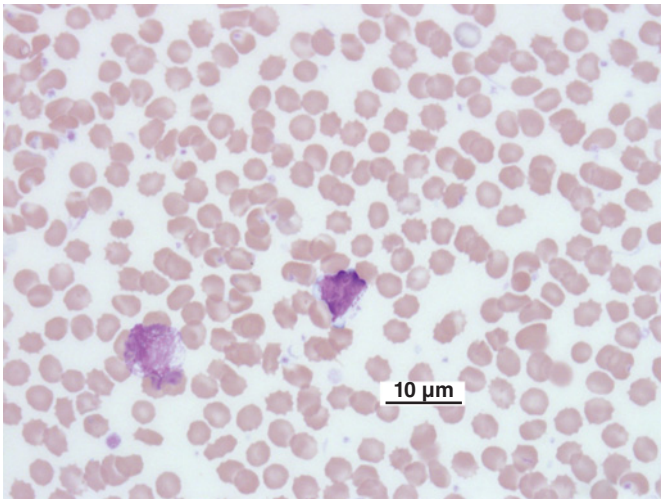


Fig. 1.12. Large lymphocyte with azurophilic granules in the blood film of a guinea pig (*Cavia porcellus*), Wright–Giemsa stain.

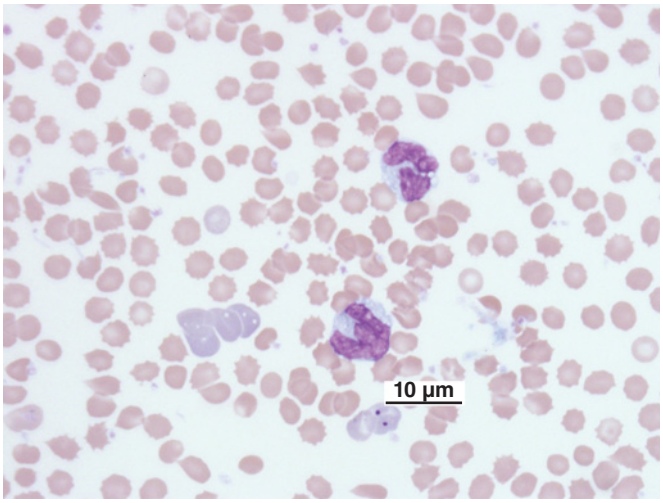


Fig. 1.13. Monocytes in the blood film of a guinea pig (*Cavia porcellus*), Wright–Giemsa stain.

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

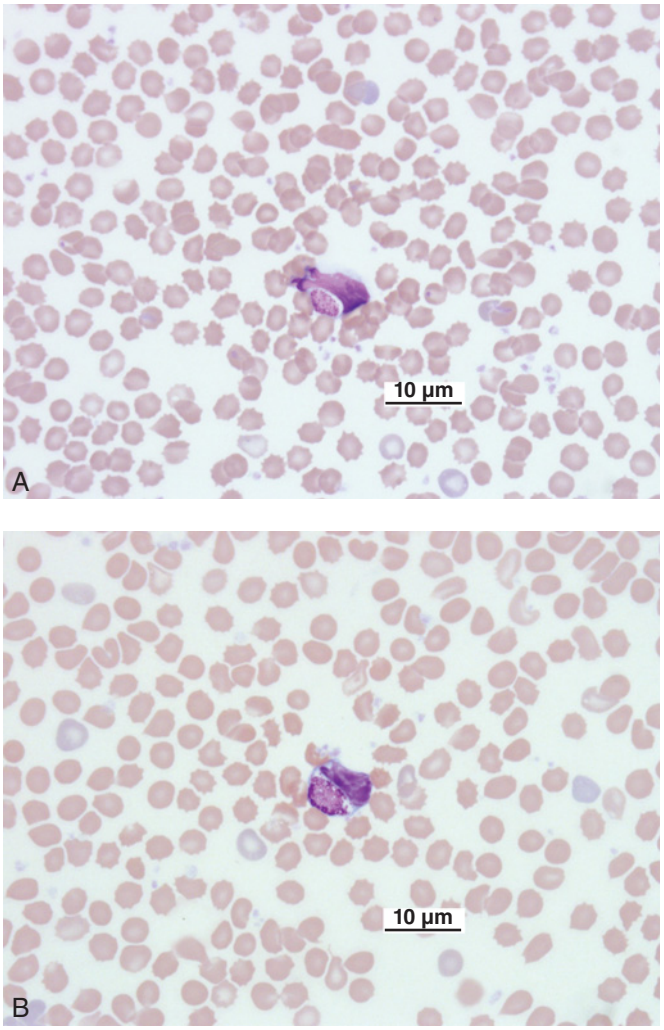


Fig. 1.14. (a) Lymphocyte with Kurloff body in the blood film of a guinea pig (*Cavia porcellus*), Wright–Giemsa stain. (b) Lymphocyte with Kurloff body in the blood film of a guinea pig (*Cavia porcellus*), Wright–Giemsa stain.

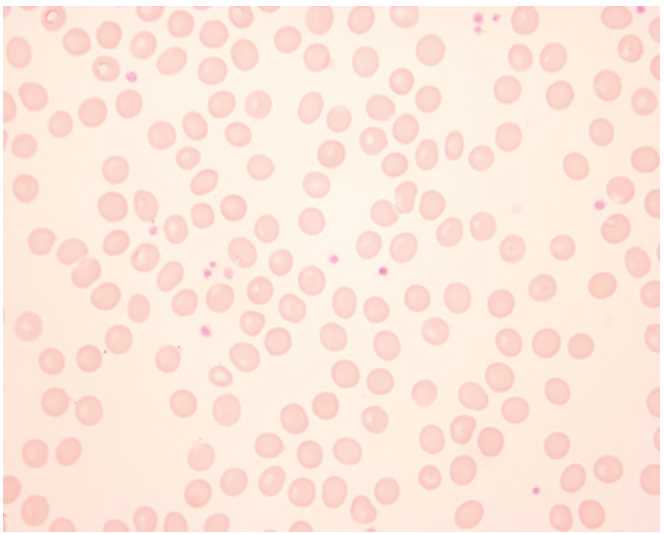


Fig. 1.15. Normal erythrocytes in the blood film of a chinchilla (*Chinchilla lanigera*), Wright–Giemsa stain (1000×).

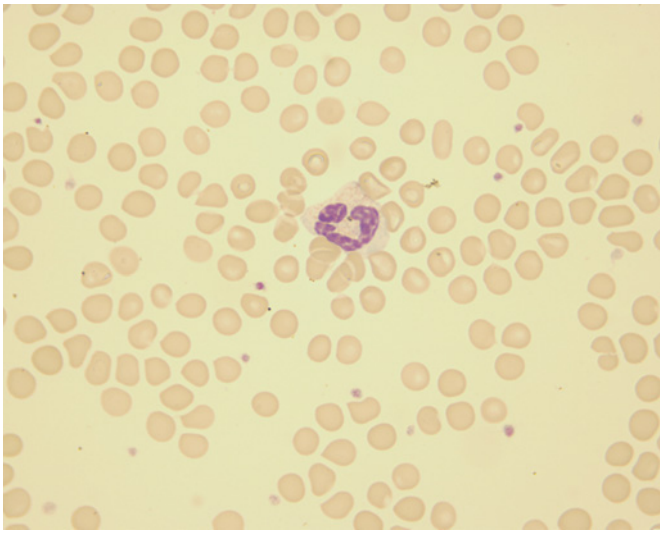


Fig. 1.16. Heterophil in the blood film of a chinchilla (*Chinchilla lanigera*), Wright–Giemsa stain (1000×).

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

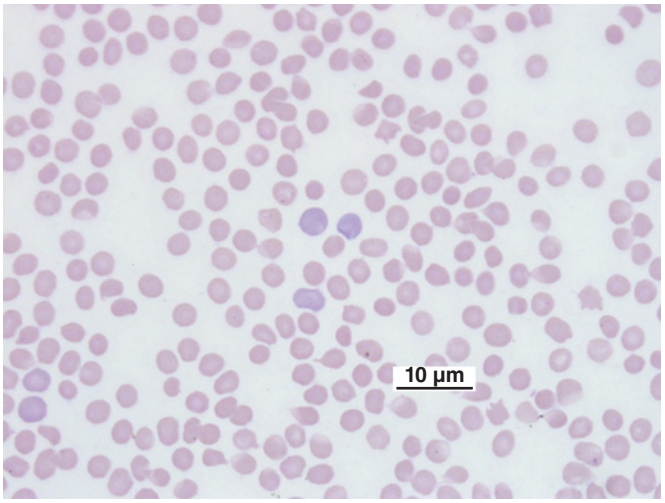


Fig. 1.17. Normal erythrocytes in the blood film of a rabbit (*Oryctolagus cuniculus*), Wright–Giemsa stain.

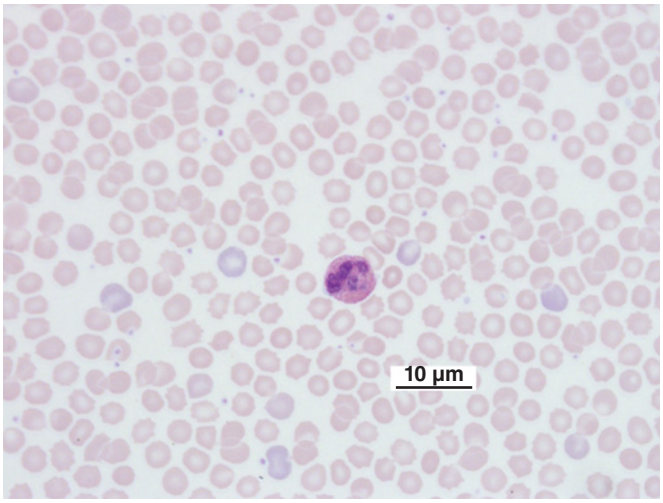


Fig. 1.18. Heterophil in the blood film of a rabbit (*Oryctolagus cuniculus*), Wright–Giemsa stain.

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

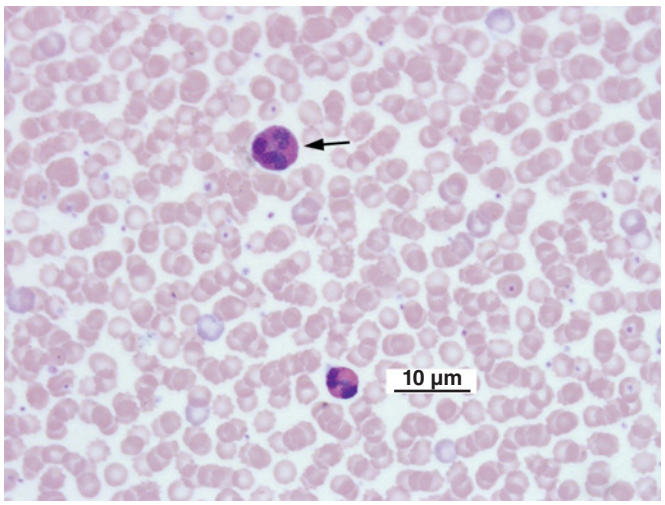


Fig. 1.19. Eosinophil (arrow) and heterophil in the blood film of a rabbit (*Oryctolagus cuniculus*), Wright–Giemsa stain.

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,