CHAPTER

9

HEMATOLOGY

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Evaluation of the avian hemogram involves counting the various blood cells per microliter of blood as well as cytologic evaluation of the cells. The techniques involved in the evaluation of the avian hemogram are easily performed by in-house veterinary laboratory personnel. Because avian blood does not store well (eg, during transport), hematologic results obtained soon after collection are preferred over those performed several hours later.[6,18,34]

Blood volume in birds depends on the species and varies from 5 ml/100g in the Ring-necked Pheasant to 16.3 to 20.3 ml/100g in the racing pigeon. In general, birds are better able to tolerate severe blood loss than mammals, which is due to their greater capacity for extravascular fluid mobilization. However, there is a marked variation among avian species in response to blood loss, which may be a reflection of differences in blood volume or extravascular fluid depots. In healthy Mallard Ducks and racing pigeons, a blood volume equivalent of up to three percent of the body weight can be collected. In Passeriformes, pheasants and Psittaciformes, up to one percent of the body weight can be collected with few ill effects (0.9 ml from a 90 g cockatiel).

Blood can be collected from a variety of sites in avian patients. The choice of a blood collection site is influenced by the species of bird, preference of the collector, physical condition of the patient and volume of blood needed. For best results, venous blood should be collected for hematologic studies. Blood collected from capillaries (eg, blood from clipped nails) often results in abnormal cell distributions and contains cellular artifacts such as macrophages and material not normally found in peripheral blood (Figure 9.1). Blood to be used for hematology should be collected into a collection tube containing EDTA (ethylenediaminetetraacetic acid) as the anticoagulant. Other anticoagulants, such as heparin, interfere with cell staining and create excessive cell clumping, resulting in erroneous cell counts and evaluations (Color 9.3)[6,18,34]

Processing the Avian Hematologic Sample

Blood Collection

Jugular venipuncture is a procedure that can be used for collecting blood from most avian species.[6,18,34,38,71] It is the method of choice for small birds that do not have other blood vessels large enough for venipuncture. The right jugular vein is usually chosen over the left for blood collection because in many birds it is the larger of the two. To collect blood from the jugular vein, the bird is properly restrained with the head and neck extended (Figure 9.2). Extending the neck encourages the highly movable jugular vein to fall into the jugular furrow. In many species, there is a featherless tract of skin (apterium) overlying the jugular vein; therefore, lightly wetting the feathers with alcohol in this area will aid in the visualization of the vein. Blood is collected into a syringe, and the size of needle is governed by the size of the vein. Complications of jugular venipuncture include difficulty in proper restraint of the bird or stabilization of the vein and hematoma formation. Improper attention to technique and hemostasis can cause a large hematoma to form during or following jugular venipuncture. However, jugular venipuncture becomes a skill perfected with practice, and complications are infrequent in skilled hands.

Venipuncture of the ulnar or wing vein is a common method for obtaining blood from medium to large birds. A needle is inserted into the vein, which is found crossing the ventral surface of the humero-radioulnar joint (elbow) (Figure 9.3). Blood is either aspirated into a syringe or allowed to drip from the needle hub into a microcollection device. Collecting blood in this manner reduces but does not eliminate hematoma formation. A variety of these devices is available.[a-c] These collecting tubes contain EDTA for hematology studies, are plain (with or without a serum separator) or contain heparin (lithium heparin is the preferred form) for blood chemistry studies. Hematoma formation, which can be severe, is common when the ulnar vein is used for blood collection. A needle with an extension tube, such as a butterfly catheter,[d] aids in stabilization during sample collection to minimize tearing of the vein.

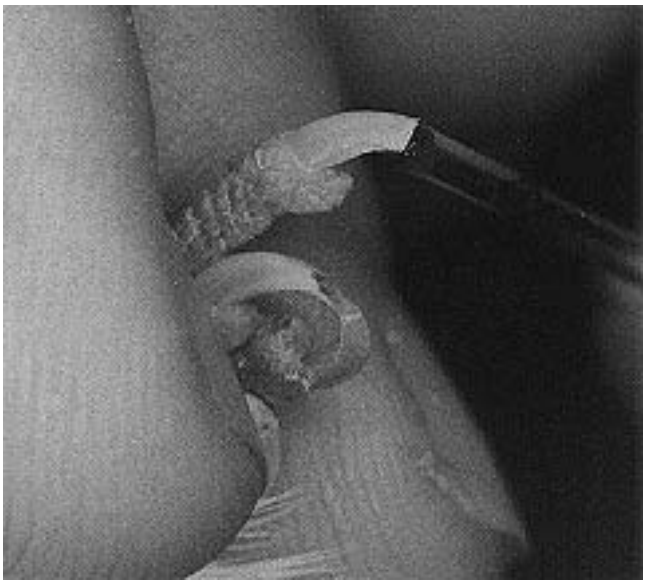


FIG 9.1 Blood for hematologic evaluation should be collected from a free-flowing venous source. Blood collected from a toenail clip may yield abnormal cell distributions and cellular artifacts.

Venipuncture of the medial metatarsal (caudal tibial) vein, which lies on the medial side of the tibiotarsus at the tibiotarsal-tarsometatarsal joint, is another common method for blood collection in medium to large birds (Figure 9.4).[6,18] The primary advantages of this method over other methods of blood collection is that the surrounding leg muscles protect the medial metatarsal vein from hematoma formation and, in some species, the leg is more easily restrained than the wing.

Blood can be collected from the occipital venous sinus of birds. This technique should be reserved for birds used in research or for blood collection prior to euthanasia,[6,78] because of the potential for injuring the brainstem. When properly executed, however, this method can be safely used for collecting repeated blood samples from birds. The bird must be completely restrained. The head is held firmly in a flexed position in a straight line with the cervical vertebrae. The occipital venous sinus is just below the skin in the space between the base of the skull and the first cervical vertebra. To collect blood from this sinus, an evacuated tube with needle and holder is required. The needle is passed through the skin at a 30 to 40° angle to the cervical vertebrae on the dorsal midline just above the sinus. Following penetration through the skin, the evacuated tube is advanced in the holder, allowing penetration of the tube stopper by the needle within the holder. The needle is advanced slightly downward to penetrate the venous sinus.

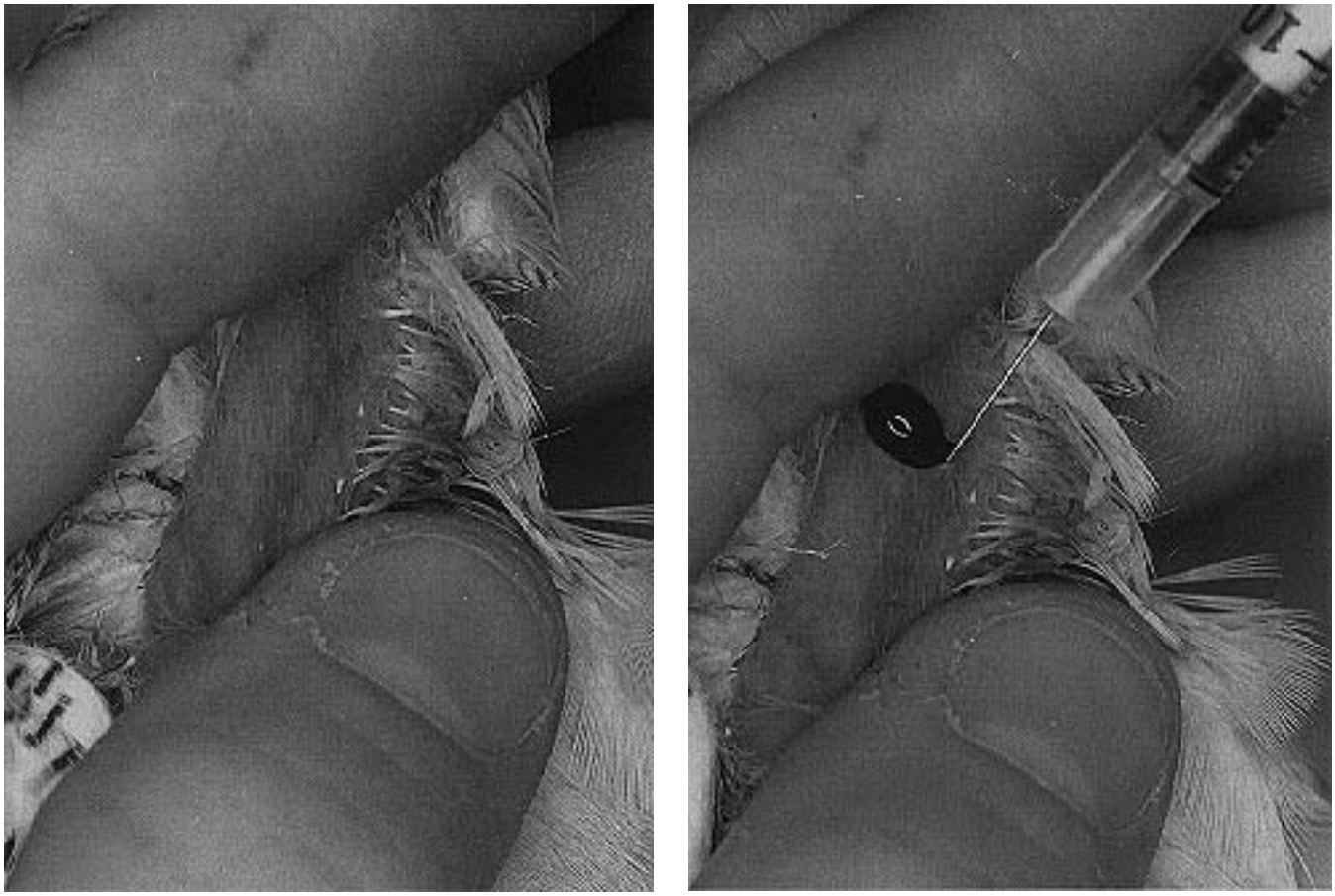


FIG 9.2 The right jugular vein (left) is the preferred site for blood collection in most species of birds. The vessel is easy to visualize and is larger than the left jugular vein. The neck and head are held in extension, and the mid-cervical area is lifted slightly to improve the angle for venipuncture. The vessel is occluded at the thoracic inlet (right) to facilitate distention and blood collection. Note the featherless tract (apterium) overlying the right lateral neck and jugular furrow. The vessel can be entered from either a cranial or caudal direction.

When this occurs, blood will rapidly fill the evacuated tube. Cardiac punctures should be used only for blood collection prior to euthanasia.[1,45,77]

Laboratory Techniques

After the blood is collected, a blood film is made. The film can be made either from blood containing no anticoagulant (especially if blood parasites are suspected) or blood containing EDTA. EDTA will cause hemolysis of erythrocytes in some birds including Corvidae, currasows, Crowned Cranes, hornbills and Brush Turkeys. Prolonged exposure to EDTA may result in increased disruption of cells in the blood film in some species (Color 9.3). Therefore, when using an anticoagulant, a blood film should be made immediately following blood collection. Heparin should be avoided whenever possible for hematologic studies. Heparinized blood contains artifacts such as clumping of cells (especially leukocytes in counting chambers) and frequently results in staining artifacts (Color 9.3).[6,34] When preparing a blood film, the standard two-slide wedge technique used in mammalian hematology usually works well with avian blood.[6,12,67] It is advisable to use precleaned, beveledged microscope slides to minimize cell damage during preparation of blood films. Peripheral blood films can also be made using a two-coverglass technique. A drop of blood is placed in the center of one coverglass;[16,17] a second coverglass is placed on top of the first, and the two are pulled apart as the blood begins to spread between the two surfaces. A similar technique using a microscope slide and a rectangular coverglass (24 mm x 50 mm) can be used to prepare a film on a microscope slide rather than on coverglasses, making the sample easier to stain.[6,18] Using the two-coverglass or microscope slide-coverglass methods should be considered if the standard two-slide wedge technique creates excessive smudging of the cells. Most veterinarians and technicians accus-

FIG 9.3 The cutaneous ulnar vein should be considered an inferior site to the right jugular vein for blood collection. The vessel (top) is easy to access on the ventral surface of the wing, but hematoma formation is common. Note that the bevel of the needle is up and the brachial vein is being occluded with the thumb. A small gauge needle (bottom) is used to minimize hematoma formation and is threaded into the vessel to decrease "wobble" and endothelial damage from the needle tip.

tomed to performing the two-slide wedge technique with mammalian blood have little difficulty in using the same technique to prepare proper avian blood films.

A variety of hematologic stains can be used to evaluate the air-dried blood film. Romanowsky stains, such as Wright's, Giemsa, Wright-Giemsa, Wright-Leishman or May-Grunwald and their combination, are preferred[6,18,34] (see Chapter 10). Wright's stain has been the standard in veterinary hematology, and all cell descriptions and illustrations used in this text are based upon that stain. These descriptions also apply to a great extent to the other commonly used quick stains, which essentially are modifications of the classic Wright's stain procedure.[e,f] The use of an automatic slide stainer[g] simplifies the staining procedure and provides a means for consistency and high quality staining by removing variations that occur with hand-staining procedures.

After making a blood film, the remainder of the blood sample is used to obtain a packed cell volume (PCV), hemoglobin concentration and cell count. The PCV is obtained by centrifuging a microhematocrit tube full of blood at 12,000 G for five minutes. The hemoglobin concentration is measured spectrophotometrically by using the manual or automated cyanmethemoglobin method after centrifugation removal of free red cell nuclei and membrane debris.

The red blood cell (RBC) count is obtained either by automated or manual methods. The automated cell counters[h] provide a quick, reliable method for obtaining a RBC count in birds. The two manual methods that can be used are the erythrocyte Unopette system[i] (standard method in mammalian hematology) or Natt and Herrick's method. The latter method requires the preparation of a methyl violet 2B diluent.[55] A 1:200 dilution of the blood is made using this solution and a diluting pipette. After mixing, the diluted blood is discharged into a Neubauer-ruled hemacytometer and the cells are allowed to settle to the surface for five minutes before enumeration. The red blood cells are counted using the four corner squares and one central square of the central large primary square of the hemacytometer. The number of red cells counted is multiplied by 10,000 to obtain the RBC count per microliter of blood. Appropriate secondary squares are counted on each grid and the counts are averaged.

FIG 9.4 The medial metatarsal vein can be used to collect smaller quantities of blood from medium- to large-sized birds. This vessel is supported by the soft tissues of the leg and in comparison to other blood collection sites, hematoma formation is rare (courtesy of Kathy Quesenberry).

The mean corpuscular values can be calculated using the PCV, hemoglobin and RBC count values. The mean corpuscular volume (MCV) and mean corpuscular hemoglobin concentration (MCHC) are useful in the characterization of the erythrocytes, especially in the evaluation of anemia.

$$\text{MCV} = \frac{\text{PCV} \times 10}{\text{RBC count}}$$

$$\text{MCH (pg)} = \frac{\text{Hemoglobin} \times 10}{\text{RBC}}$$

$$\text{MCHC} = \frac{\text{Hemoglobin} \times 100}{\text{PCV}}$$

A reticulocyte count can be useful in the evaluation of the red cell regenerative response. The erythrocytes are stained with a vital stain, such as new methylene blue stain, and the reticulocytes are identified as red blood cells that contain distinct rings of aggregated reticulum encircling the cell nucleus (Color 9.1).[6,34] Other erythrocytes may contain varying amounts of reticulum, but those with the distinct ring of aggregated reticulum surrounding the cell nucleus appear to be cells that have recently entered the peripheral circulation, and thus reflect the current regenerative response.

The white blood cell (WBC, leukocyte) count of birds is obtained using manual techniques because the presence of nucleated erythrocytes and thrombocytes interfere with the counting of white blood cells using electronic cell counters. The current methods of choice for obtaining a total leukocyte count in birds are the indirect method using the eosinophil Unopette brand 5877 system or the direct leukocyte count using Natt and Herrick's method.[6,13,18,55] Estimation of leukocyte numbers from a blood film should be reserved for those occasions when a quantitative count is unavailable or when there is suspicion of error in a value obtained from the other methods.

The indirect eosinophil Unopette brand method involves the filling of the 25 µl pipette with blood, mixing the blood with the phloxine B diluent in the vial provided in the system and charging the hemacytometer chamber for cell counting. The blood-phloxine mixture should not be allowed to stand in the Unopette vial for longer than five minutes or erythrocytes may also be stained. The charged hemacytometer should stand undisturbed for at least five minutes to allow the cells to settle to the surface of the counting grid. It is advisable to keep the charged hemacytometer in a humid chamber to prevent dehydration of the sample if the chamber is going to sit for longer than five minutes. The granulocytes that stain distinctly red (heterophils and eosinophils) are counted in both sides of the hemacytometer (representing 18 large squares). Therefore, a total absolute heterophil and eosinophil count is obtained. A WBC count is obtained by determining a leukocyte differential from the peripheral blood film and using the formulas in Table 9.1.

A total thrombocyte count can be obtained using the Natt and Herrick's method; however, thrombocytes tend to clump, making an accurate count difficult to achieve. A subjective opinion as to the number of thrombocytes present can be made from the peripheral blood film. An average of one to two thrombocytes are present in monolayer oil immersion (100 x) fields in blood films of normal birds. Numbers less than this suggest a thrombocytopenia and those greater suggest a thrombocytosis. An estimate of the thrombocyte count can be made from the peripheral blood film by obtaining the average number of thrombocytes in five monolayer oil immersion fields. This

represents the average number of thrombocytes per 1000 erythrocytes in most species of birds.

A more accurate method would be to count the number of thrombocytes per 1000 erythrocytes in the blood film. The number of thrombocytes per 1000 erythrocytes is multiplied by the erythrocyte count and divided by 1000 to obtain an estimated thrombocyte count per µl of blood. If the actual erythrocyte count is not known, then 3,500,000 can be used to represent the average number of erythrocytes per µl of blood in most species of birds having an average PCV of 45%. If the PCV is below 40 or above 50, the estimated thrombocyte count (est T) can be corrected using the following formula:

$$\text{Corrected est T} = \frac{\text{est T X observed PCV}}{\text{normal PCV (averages 45\%)}}$$

Cell Identification

Erythrocyte Morphology

The normal mature avian erythrocyte is oval with a centrally positioned oval nucleus. The cytoplasm is abundant and stains a uniform orange-pink, resembling the cytoplasm of mammalian erythrocytes (Color 9.1). The nucleus of the mature erythrocyte is condensed and stains dark purple. The nuclear chromatin is uniformly clumped. The red cell nuclei vary with age, becoming more condensed and darker staining as the cells age.

Variations from the typical mature erythrocyte are occasionally seen in the peripheral blood of birds. Avian erythrocytes frequently demonstrate diffuse polychromasia. Polychromatic erythrocytes demonstrate cytoplasmic basophilia and have nuclei that are less condensed compared to mature erythrocytes (Color 9.1). Immature round erythrocytes (eg, rubricytes) may also be found in the peripheral blood of birds. These developmental stages have been described in this chapter with the discussion of the evaluation of hematopoietic tissue. Occasionally, round erythrocytes with oval nuclei may be found, especially in anemic birds. This is suggestive of an asynchronous maturation of the cell nucleus and the cytoplasm, probably owing to accelerated erythropoiesis. Anucleated, oval erythrocytes (erythroplastids) are rare findings in peripheral blood films of birds (Color 9.2). The shape of the red blood cell may appear irregular, or smudging may occur as a result of artifacts created by the preparation of the film. The most common artifact found in peripheral blood films of birds is the rupturing of cells during preparation of the film (Color 9.3). The majority of these cells appear to be erythrocytes. The free red cell nuclei appear as amorphous, pink-to-purple material on the film. Other abnormal findings include variations in the location of the cell nucleus within the cell and nuclei having indentations, constrictions or protrusions (Color 9.3). Perinuclear rings are usually artifacts of staining (eg, a form of cellular crenation). Cytoplasmic basophilic stippling is also indicative of abnormal erythrocyte morphology. Hypochromasia is indicated by pale-staining cytoplasm, cytoplasmic vacuoles and round, pyknotic nuclei (Color 9.2). Agglutination of erythrocytes in the blood film is a rare, abnormal finding.

TABLE 9.1 Formulas for Determining WBC counts

The total heterophil and eosinophil count T(h+e) is obtained by using the formula given for the eosinophil Unopette brand system:

$$T(h+e)/mm^3 = \text{cells counted} \times 10 \times 32 / 18$$

The total leukocyte count (TWBC) is obtained using the leukocyte differential and the following formula:

$$TWBC/mm^3 = (T(h+e) / \%\ \text{heterophils + eosinophils}) \times 100$$

The TWBC can be calculated using the formula:

$$TWBC/mm^3 = \frac{\text{number of cells counted} \times 10 \times 32 \times 100}{(\%\ \text{heterophils + eosinophils}) \times 18}$$

This formula can be simplified by using the formula:

$$TWBC/mm^3 = \frac{\text{number of cells counted} \times 1.111 \times 16 \times 100}{\%\ \text{heterophils + eosinophils}}$$

or

$$TWBC/mm^3 = \frac{\text{number of cells counted} \times 1778}{\%\ \text{heterophils + eosinophils}}$$

The Natt and Herrick's method is a direct method for obtaining a TWBC and utilizes the same dilution and charged hemacytometer used to obtain a RBC count. The dark-staining leukocytes are counted in the nine large squares of the hemacytometer chamber. The TWBC is obtained using the following formula:

$$\text{TWBC count}/mm^3 = \frac{(\text{total leukocytes in 9 squares}) \times 10 \times 200}{9}$$

or simplified to:

$$TWBC/mm^3 = (\text{total leukocytes in 9 squares} + 10\%) \times 200$$

Hematology

Illustrations for Colors 9.1 to 9.10 are computer-generated reproductions of blood cells originally printed in Lucas AJ, Jamroz C: Atlas of Avian Hematology, USDA Monograph 25, Washington DC, 1961.

Color 9.1
Normal erythropoiesis (Wright's-Leishman stain unless otherwise noted).

a) Rubriblast with prominent nucleolus, finely granular chromatin pattern and dark-blue cytoplasm.

b) Prorubricyte with moderately granular chromatin pattern and dark-blue cytoplasm. A nucleolus is not present.

c-g) Various stages of developing polychromatophilic erythrocytes. As maturation progresses, the nuclear chromatin pattern condenses, the cytoplasm becomes less basophilic and the nuclear and cell shapes transform from round to elliptical. The presence of these cells in the blood indicates polychromasia or erythrocyte regeneration.

h,i) Appearance of polychromatophilic erythrocytes as reticulocytes following new methylene blue staining. Ribosomes are stained and aggregate as particulate material around the nucleus.

j,k) Mature erythrocytes contain abundant hemoglobin, which imparts an orange color to the cytoplasm. As erythrocytes continue to mature or age, the cell and nuclear shapes become more elongate, and the chromatin pattern is extremely condensed.

l) Early polychromatophilic erythrocyte in mitosis. These cells are observed most commonly in bone marrow smears but are rare in peripheral blood.

Color 9.2
Common erythrocyte abnormalities in the stained blood smear.

a-c) Poikilocytes are misshapen erythrocytes. These cells may assume a variety of shapes including a unipolar-to-bipolar, spindle appearance. Cytoplasmic constrictions also may be present.

d) A macrocyte is an enlarged erythrocyte with voluminous cytoplasm and a condensed, displaced nucleus. These cells may be observed with certain forms of anemia.

e,f) Microcytes are small erythrocytes with a minimum of cytoplasm. These cells are associated with lack of iron or iron deficiency anemia.

g) Anucleated erythrocytes are observed infrequently. These cells also are called erythroplastids.

Color 9.3
Common erythrocyte artifacts resulting from improper collection or preparation of blood smears.

a) Smudge cell resulting from traumatic disruption of a blood cell, usually an erythrocyte.

b) Diffuse, non-refractile, cytoplasmic vacuolation suggesting cytoplasmic edema from loss of membrane integrity. These changes often result from cellular damage during blood smear preparation.

c) Refractile artifact caused by water or air trapped between the cell membrane and mounting medium or immersion oil. This artifact is commonly mistaken for a hemoparasite.

d) Staining artifact seen most commonly in smears from blood collected in heparin and subjected to Romanowsky staining (Wright's or Giemsa staining).

e) Intact erythrocyte nucleus following cellular disruption.

Color 9.4
Normal thrombocytopoiesis.

a) Thromboblast containing an indistinct nucleolus, finely granular chromatin pattern and basophilic cytoplasm.

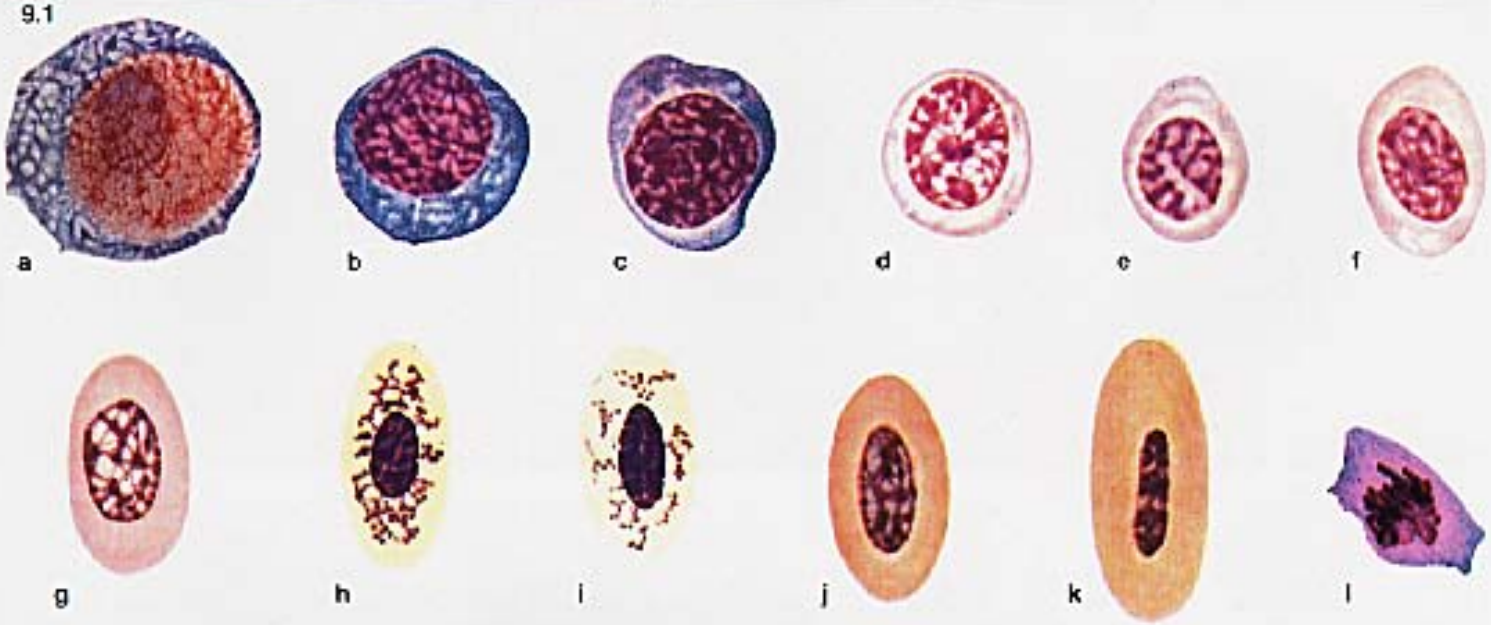
b,c) Immature thrombocytes containing round nuclei, a moderately granular chromatin pattern and moderately blue cytoplasm.

d) Late immature thrombocyte. As maturation progresses, the cellular and nuclear profiles become more elliptical. Cytoplasmic vacuolation and granules may appear.

e-g) Mature thrombocytes are elliptical with a round-to-oval nucleus, condensed chromatin pattern and light-blue vacuolated cytoplasm. One to three cytoplasmic granules may be present, but granules may vary from absent to abundant.

h,i) Shrunken, degenerating thrombocytes with pointed cytoplasmic projections or a condensed nucleus. These cells are observed more frequently in old blood specimens.

9.1



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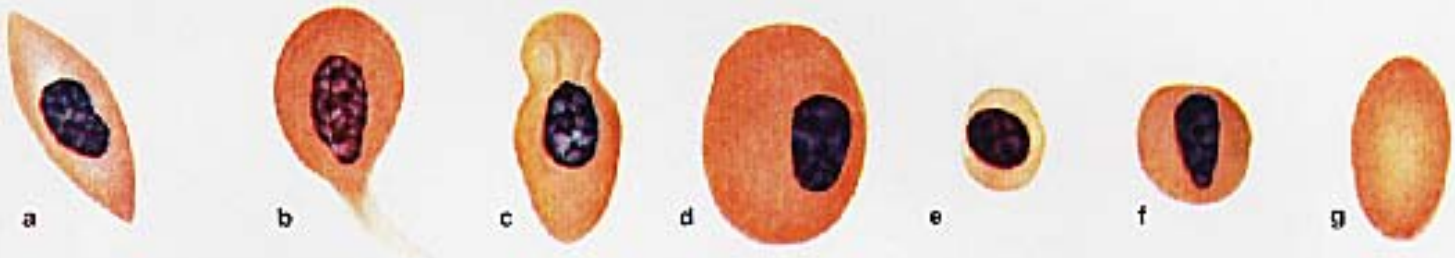

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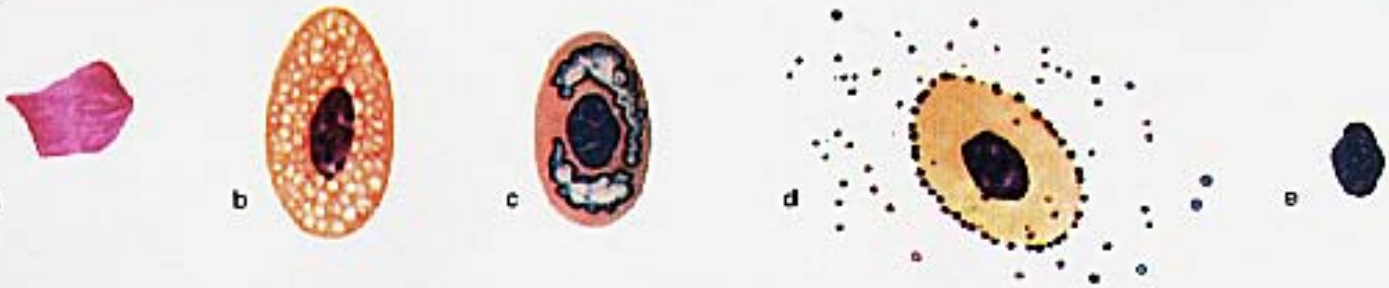

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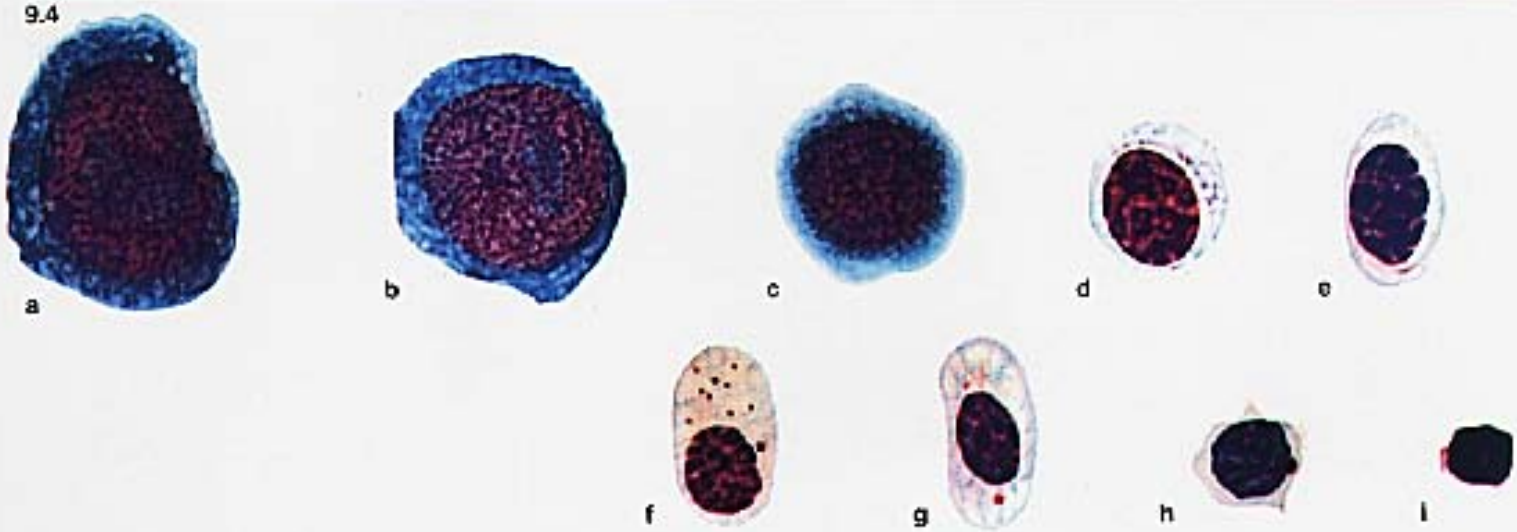

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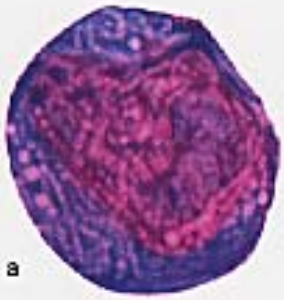
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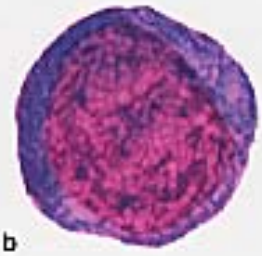
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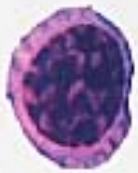
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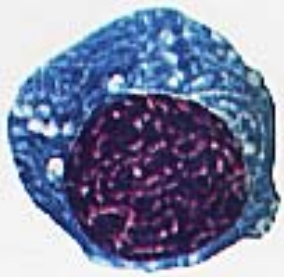
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9.6


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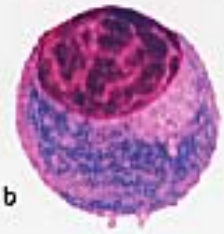
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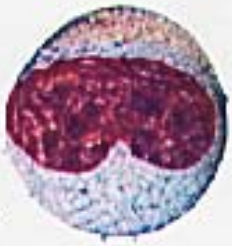
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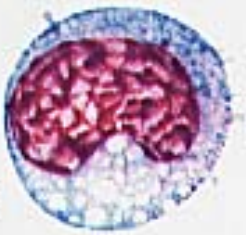
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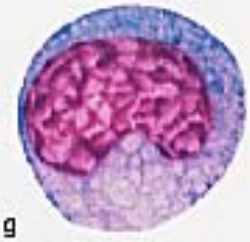
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i

Hematology

Color 9.5
Normal lymphopoiesis.

a,b) Lymphoblasts with a round to slightly irregular nucleus, fine chromatin pattern, multiple nucleoli and basophilic cytoplasm. These cells may be found within the bone marrow in health, but suggest neoplasia when observed within the peripheral blood.

c) Small lymphocyte with a scant rim of basophilic cytoplasm. These cells are often confused with thrombocytes by an inexperienced microscopist.

d) Typical small lymphocyte with an eccentric nucleus and scant basophilic cytoplasm.

e) Small lymphocyte with cytoplasmic azurophilic granules. In mammals, these cells are "killer" lymphocytes.

f) Large lymphocytes are observed infrequently in the blood. Usually, monocytes are misidentified as large lymphocytes.

g,h) Degenerating lymphocytes with cytoplasmic blebs or broad pseudopodia. These cells are seen most frequently in old blood specimens.

i) Plasma cells are the ultimate expression of a B-lymphocyte. These cells are round-to-rectangular with an eccentric nucleus, condensed chromatin pattern, abundant royal-blue cytoplasm and a pale golgi zone. Plasma cells are observed frequently in cytologic preparations, but are rare in blood smears.

j) An immunocyte is an antigenically stimulated lymphocyte. These large cells contain a round-to-scalloped nucleus, condensed chromatin pattern and dark-blue cytoplasm. Scattered immunocytes may be observed in the blood smear following antigenic stimulation from immunization or disease.

Color 9.6
Monocyte development.

a-c) Monocytes with oval to slightly indented nuclei, a variable nuclear-to-cytoplasmic ratio and gray cytoplasm. These cells often are misidentified as lymphocytes.

d,e) Mature monocytes with an indented to bilobed nucleus, abundant gray cytoplasm and occasional pseudopodia.

f,g) Monocytes with cytoplasmic vacuolation or increased dust-like eosinophilic cytoplasmic granules. Cytoplasmic vacuolation usually occurs if the blood specimen is allowed to stand before making blood smears. The eosinophilic granules are lysosomes.

h,i) Two degenerating monocytes are present. The first monocyte has nuclear edema and cytoplasmic vacuolation. The second monocyte has broad pseudopodia or cytoplasmic blebs. These cells are observed most frequently within old blood specimens.

Leukocyte Morphology

The granulocytic leukocytes of birds are heterophils, eosinophils and basophils.[6,18,34,36,44] The heterophil is a round cell with distinct eosinophilic cytoplasmic granules (Color 9.9). These granules are oval to spindle-shaped and often contain a distinct refractile body in the center of the granule. The mature heterophil nucleus is lobed, usually containing fewer lobes than mammalian neutrophils (Color 9.8). The nuclear chromatin contains heavy chromatin clumping. The cytoplasm of normal mature heterophils is colorless and nonvacuolated (Color 9.8).

Avian eosinophils are round granulocytes and contain distinct round-to-oval cytoplasmic granules that lack the central refractile body seen in heterophil granules (Color 9.9). The cytoplasmic granules of eosinophils typically stain brighter or differently from heterophil granules in the same blood film. The intense eosinophilic appearance of eosinophil granules is most likely related to the high concentration of arginine.[19] The cytoplasm of avian eosinophils stains clear blue. The eosinophil nucleus is lobed and generally stains darker than the nuclei of heterophils (Color 9.9). There is variation in the morphologic appearance of the eosinophils of several avian species.[34,41]

The normal basophil is slightly smaller than the heterophil and has a colorless cytoplasm that contains strongly basophilic granules (Color 9.10). These granules often dissolve or coalesce in alcohol-based stains, such as the Romanowsky stains. Avian basophils have round-to-oval, non-lobed nuclei that are often hidden by cytoplasmic granules (Color 9.10).

The mononuclear leukocytes found in the peripheral blood of birds are lymphocytes and monocytes (Color 9.5, 9.6). The mature avian lymphocytes are round cells that frequently "mold" around adjacent cells in the blood film. These cells have high nucleus to cytoplasm (N:C) ratios. The nucleus is usually centrally positioned and round with a scant amount of homogeneous blue cytoplasm appearing as a small band surrounding the nucleus (Color 9.5). Avian lymphocytes often vary in size, and the larger lymphocytes that have pale-staining nuclei may be confused with monocytes. The nuclear chromatin of mature lymphocytes is densely clumped. Occasionally, the cytoplasm of small mature lymphocytes may contain irregular projections.

Monocytes are the largest leukocytes found in the peripheral blood films (Color 9.6). They vary in shape from round to ameboid. Monocytes have an abundant amount of cytoplasm compared to lymphocytes. The cytoplasm generally stains darker than the cytoplasm of normal lymphocytes. The cytoplasm of monocytes has a finely granular, blue-gray appearance and often contains vacuoles. Often two distinct cytoplasmic zones can be seen in monocytes: a light-staining area adjacent to the nucleus and a darker staining area on the periphery. The cytoplasm of monocytes may occasionally contain fine, dust-like eosinophilic granules. The nucleus of monocytes generally contains less nuclear chromatin clumping as compared to mature lymphocytes. The shape of the monocyte nucleus is variable, ranging from round to bilobed.

TABLE 9.2 Characteristics of Blood Cells Using Wright's Stain

Erythrocytes	Dark purple nucleus; orange-pink cytoplasm
Heterophils	Violet, lobed nucleus; colorless cytoplasm, orange-red, rod-shaped granules in most species
Eosinophils	Violet, lobed nucleus; blue cytoplasm; red-orange, round granules in most species
Basophils	Purple, non-lobed nucleus; dark purple cytoplasm granules
Monocytes	Purple nucleus; abundant, finely granular, blue-grey cytoplasm
Lymphocytes	Dark purple, non-lobed nucleus; pale blue, homogeneous cytoplasm
Thrombocytes	Dark purple nucleus; colorless cytoplasm; red granules

On occasion, abnormal-appearing leukocytes are found in the peripheral blood films of birds (Color 9.5). Immature heterophils are abnormal findings in avian blood films, and their appearance has been described in the evaluation of avian hematopoietic tissue at the end of this chapter. The immature stages most commonly found are heterophil myelocytes and metamyelocytes. In general, immature heterophils have increased cytoplasmic basophilia, nonsegmented nuclei and immature cytoplasmic granules compared to mature heterophils (Color 9.5). Usually when immature heterophils are found on a blood film, mature heterophils can also be found.

Mature heterophils appear to show toxic changes in a manner similar to the toxic changes identified in mammalian neutrophils.[6,12,67] Signs of toxicity include increased cytoplasmic basophilia, vacuolation, abnormal granules, degranulation, and degeneration of the nucleus (Color 9.18). The degree of toxicity is reported subjectively on a scale of +1 to +4, where the lower rating reflects slight change and the higher indicates severe change. A +1 toxic heterophil shows increased cytoplasmic basophilia (Color 9.8). An

overall assessment of the staining of the blood film must be made to assure the hematologist that the film is not overly stained blue, giving the impression that the heterophils have increased basophilia. A +2 toxic heterophil has increased cytoplasmic basophilia, vacuolation and partial degranulation (Color 9.11). A +3 toxicity shows a deeper cytoplasmic basophilia, vacuolation and abnormal granulation (Color 9.8).

Abnormal granulation is indicated by the presence of granules that vary in appearance from the typical rod-shaped eosinophilic granules (eg, large, pale, round eosinophilic granules and small, deeply basophilic granules). A +4 toxic heterophil resembles a +3 toxic heterophil except the cell nucleus has undergone karyorrhexis or karyolysis. The number of toxic heterophils present is an indication of severity and suggestive of duration of an inflammatory response. A slight number (25% or less) of toxic heterophils may be present in the early stages of disorders responsible for their occurrence. As the disorder becomes increasingly severe, the number of toxic heterophils will increase. A marked number (greater than 25%) of toxic heterophils is common in birds showing this heterophil abnormality. It is common for birds with toxic heterophil changes to have all of their heterophils affected on the blood film. Clinically, these birds will be severely compromised.

Cytologic indications for reactivity in lymphocytes include increased cell size, increased cytoplasmic basophilia, the presence of azurophilic cytoplasmic granules and smooth nuclear chromatin (Color 9.12). Blast-transformed lymphocytes have a deeply basophilic cytoplasm and smooth nuclear chromatin (Color 9.5). Blast-transformed lymphocytes may also have nucleoli and distinct Golgi. Occasionally, plasma cells can be found in the peripheral blood of birds. These are relatively large lymphocytes with eccentric, mature-appearing nuclei; abundant, deeply basophilic cytoplasm; and prominent Golgi adjacent to the nucleus. Lymphocytes containing azurophilic granules (large purple cytoplasmic granules) are considered abnormal in birds. Lymphocytes having scalloped cytoplasmic margins are found occasionally in avian blood films; however, large numbers of these cells are considered abnormal.[5,62] Immature lymphocytes in the peripheral blood films are also considered to be abnormal (Color 9.5).

An occasional monocyte having a few cytoplasmic vacuoles is normal, but the presence of large numbers of highly vacuolated monocytes is abnormal.

Cells that contain large granules that fill the cytoplasm are frequently found in blood films of birds. Often these granules fail to stain or may stain blue.[6,34,41] These cells are common in blood films of some species of birds (eg, cockatoos) and suggest either staining artifact or represent variation owing to different cytochemical properties of these cells compared to other avian species. The differential for the type of cell involved includes eosinophils, basophils and rarely, Mott cell variant of plasma cells.

Careful examination of the blood film most often reveals normal staining basophils and no evidence of lymphoid reactivity (which may support the possibility of Mott cells being present), but there are no eosinophils present that stain normally. Based on these characteristics, the majority of these cells have been identified as eosinophils.[6, 34, 41]

Thrombocyte Morphology

Birds have nucleated cells (thrombocytes) rather than cytoplasmic fragments as platelets that participate in blood coagulation. Thrombocytes are derived from a distinct line of cells found in hematopoietic tissue. Mature thrombocytes are small oval cells that appear more rounded than the erythrocytes (Color 9.4). The nucleus is pyknotic and the cytoplasm is colorless in mature cells. The cytoplasm may contain one or more red granules and small vacuoles or clear spaces (Color 9.4). Thrombocytes, like mammalian platelets, tend to clump in blood films. Thrombocytes are differentiated from small, mature lymphocytes by having a colorless, nonhomogeneous cytoplasm; small, round, red cytoplasmic granules; and a smaller N:C ratio. Small mature lymphocytes have high N:C ratios with a scant amount of blue, homogenous cytoplasm (Color 9.5).

Abnormal thrombocyte cytology includes the presence of reactive and immature thrombocytes. Reactive thrombocytes are usually found in aggregates, have a diffusely eosinophilic cytoplasm (suggesting release of chemicals from the granules) and irregular cytoplasmic margins. Reactive thrombocytes tend to be more spindle-shaped than nonreactive thrombocytes (Color 9.4).

Immature stages of thrombocytes are occasionally found in the blood film of birds (Color 9.4). The mid-immature and late-immature thrombocytes are most often seen when immature cells are present.

Interpretation of the Avian Hemogram

Interpretation of the Erythron

The normal PCV of birds ranges between 35 and 55 percent. A PCV less than 35 percent is indicative of anemia, and a PCV greater than 55 percent is suggestive of dehydration or polycythemia. An increase in red cell polychromasia is indicative of red blood cell regeneration (Color 9.17). In normal birds, the number of polychromatic erythrocytes (or reticulocytes) found in the peripheral blood film ranges between one and five percent of the erythrocytes. An anemic bird with a five percent or less degree of polychromasia (or reticulocytosis) is responding poorly to the anemia or there has not been enough time for the bird to demonstrate a significant response. An anemic bird showing a ten percent or greater degree of polychromasia is exhibiting a significant regenerative response. The presence of immature erythrocytes (eg, rubricytes) in the peripheral blood along with an increase in polychromasia is indicative of a marked regenerative response.

Some common causes of anemia in birds are discussed in Table 9.3.

Hypochromasia can be associated with certain nutritional deficiencies in birds, especially iron deficiency. Hypochromasia has also been seen in lead toxicosis.[34,42] Lead toxicosis may also create a dichotomous population of erythrocytes in the blood film of a nonanemic bird. In such cases, small senescent, mature erythrocytes with pyknotic nuclei and young erythrocytes (eg, rubricytes) are present in the blood film without the appearance of normal, mature erythrocytes. This condition resembles the inappropriate release of nucleated erythrocytes in the blood of nonanemic dogs suffering from lead poisoning. Basophilic stippling in the cytoplasm of erythrocytes is a rare finding with lead poisoning in birds.[42] Basophilic stippling may be associated with erythrocyte regeneration and hypochromic anemia.

Polycythemia is rarely reported in birds.[74] Increases in the PCV (relative polycythemia) are usually associated with dehydration in birds; however, absolute polycythemia can also occur. The conditions often associated with absolute polycythemia in mammals are expected to be the causes of this condition in birds as well.

TABLE 9.3 Causes of Anemia in Birds[2,10,11,20,24,32,33,39,48,49,61,63,84]

Blood-loss Anemia
(Appears regenerative except in the peracute stage)

1. Traumatic injury
2. Parasitism (ticks, *Dermanyssus* mites, coccidia)
3. Primary coagulopathy (rarely reported in birds)
4. Toxicity resulting in a coagulopathy (aflatoxicosis and coumarin poisoning)
5. Organic disease (ulcerated neoplasm, gastrointestinal ulcers, organ rupture)

Hemolytic Anemia (Regenerative)

1. Red blood cell parasites (*Plasmodium, Aegyptianella* and, rarely, *Haemoproteus* and *Leucocytozoon*)
2. Bacterial septicemia (salmonellosis and spirochetosis)
3. Toxicity (mustards and petroleum products)
4. Immune-mediated (rarely reported in birds)

Depression Anemia (Nonregenerative)

1. Chronic diseases (tuberculosis, chlamydiosis, aspergillosis, neoplasia)
2. Hypothyroidism
3. Toxicity (lead poisoning and aflatoxicosis)
4. Nutritional deficiencies (iron and folic acid deficiencies)
5. Leukemia (lymphoid leukemia and erythroblastosis)

Interpretation of the Leukogram

There is wide variation in the normal leukograms among birds of the same species. Therefore, values of diagnostic importance must differ greatly from normal reference intervals, which are generally much broader than those obtained from domestic mammals. Preparing normal reference values on healthy individual birds is the best method for evaluating blood parameters of a bird during illness. When these specific values have not been determined, the avian clinician must rely on reference intervals obtained from several birds that are presumed to be healthy. It is best to use values obtained in the laboratory that routinely performs the clinician's avian profiles. Published values obtained from other laboratories can be used as a guide, but may differ from the avian clinician's routine laboratory.

In general, total leukocyte counts greater than 10,000/µl are considered suggestive of leukocytosis in tame, adult psittacine birds. The total leukocyte

count in the blood of normal psittacine birds not accustomed to handling may be high (greater than 10,000/µl) owing to a physiologic leukocytosis. The general causes of a leukocytosis include infection (general or localized), trauma, toxicities, hemorrhage into a body cavity, rapidly growing neoplasms and leukemias. The leukocyte differential aids in the assessment of the leukocytosis. Because a leukocytosis is often caused by inflammation, a heterophilia is usually present.

Although avian heterophils lack the myeloperoxidase and alkaline phosphatase of mammalian neutrophils, studies of their ultrastructure, cytochemistry and function suggest they perform a similar function in the inflammatory response.[14,35,46,52,60] The magnitude of the heterophilia usually indicates the magnitude or severity of the initiating inflammatory process. Although avian heterophils do not produce hydrogen peroxide during phagocytosis, they do contain lysosomal enzymes and have a bactericidal function.[23,50,59,60,75,76] A leukocytosis and heterophilia can be associated with infectious agents (eg, bacteria, fungi, chlamydia and parasites) and noninfectious etiologies (eg, traumatic injury and toxicities). A slight to moderate leukocytosis, heterophilia and lymphopenia can result from either an exogenous or endogenous excess of glucocorticosteroids (stress response).[3,15,31,56,69,83] Species that normally have high numbers of circulating lymphocytes may develop a leukopenia and lymphopenia in the initial stress response, but up to 12 hours later show a leukocytosis and heterophilia.[15] Birds that normally have higher numbers of circulating heterophils than lymphocytes often show a less dramatic change in the leukogram initially. A marked leukocytosis and heterophilia are often associated with chlamydiosis, avian tuberculosis and aspergillosis.

Immature heterophils occur rarely in the peripheral blood of most species of birds. When present, they generally represent an overwhelming peripheral demand for heterophils and a depletion of the mature storage pool in the hematopoietic tissues.[73] The presence of immature heterophils in the peripheral blood usually indicates a severe inflammatory response, especially in association with a leukopenia (degenerative left shift) (Color 9.18). A marked number of immature heterophils may be associated with a granulocytic leukemia, a rare condition in birds.

The presence of toxic heterophils is also uncommon in the peripheral blood of birds. When present, they suggest the presence of a septicemia or toxemia (especially associated with bacterial toxins affecting the microenvironment of the hematopoietic tissue). The greater the degree of toxicity, the more severe the condition. The presence of a marked number of +4 toxic heterophils indicates a poor prognosis for survival in birds (Color 9.18).

The general causes of leukopenias in birds are depletion of peripheral leukocytes and depression or degeneration of leukopoiesis. Leukopenias associated with heteropenias can be associated with certain viral diseases (eg, Pacheco's disease virus) and overwhelming bacterial infections.[58,64] A leukopenia and heteropenia associated with immature heterophils is suggestive of exhaustion of the mature heterophil storage pool owing to excessive peripheral demand for heterophils. A depression of the hematopoietic tissue is indicated by a leukopenia, heteropenia and few, if any, immature heterophils. A degenerative response is indicated by the presence of a leukopenia, heteropenia, immature heterophils and toxic heterophils. This degenerative response can be differentiated from depletion only by the presence of toxic heterophils or by following the decreasing leukocyte count with serial leukograms. Leukopenias associated with lymphopenias have been reported in early response to corticosteroids in some species of birds.[5,15] A lymphopenia also may be expected with certain viral diseases; however, viral causes have not been well documented in birds.

A lymphocytosis may be expected with antigenic stimulation associated with certain infections. The presence of many reactive lymphocytes is also suggestive of antigenic stimulation. An occasional reactive lymphocyte may be found in the blood film of normal birds. A marked lymphocytosis with or without the presence of immature lymphocytes can occur with lymphocytic leukemia. A marked lymphocytosis, with the majority of cells appearing as small mature lymphocytes with scalloped cytoplasmic margins, is suggestive of lymphoid neoplasia.[5,6,62]

A monocytosis can be found with certain diseases that produce chemotactic agents for monocytes. These conditions include avian chlamydiosis, mycotic and bacterial granulomas and massive tissue necrosis.[32] It should be emphasized that although these disorders can create a peripheral monocytosis, it may not always occur. A monocytosis can also occur in birds on a zinc-deficient diet.[82]

The function of the avian eosinophil is unclear.[54] Although this avian granulocyte was given the name

eosinophil, there is evidence that its function may differ from the mammalian eosinophil. Thus, conditions responsible for inducing avian eosinophilias most likely differ from those causing mammalian eosinophilias. Eosinophilias associated with gastrointestinal nematode infections have occasionally been reported; however, it has been difficult to induce this condition experimentally using parasite antigens.[47] Studies suggest that avian eosinophils may participate in delayed (Type IV) hypersensitivity reactions.[51] Idiopathic eosinophilias occur sporadically in birds, and more research is needed to clarify the meaning of this condition.

As with avian eosinophils, the exact function of basophils in birds is unknown. Avian basophils are similar to mammalian basophils in their ability to produce, store and release histamine.[9] Basophils appear to participate in the initial phase of the acute inflammatory response in birds, but this is not always reflected as a basophilia in the leukogram.[8,54] Because basophils appear to play a role in early inflammation and possibly hypersensitivity reactions in birds, a peripheral blood basophilia may suggest the presence of these conditions.

Interpretation of Thrombocyte Changes

Avian thrombocytes play a primary role in hemostasis in a manner similar to mammalian platelets. They may also have a phagocytic function and participate in removing foreign material from the blood.[19,30] A normal thrombocyte count ranging between 20,000 and 30,000/µl of blood or 10 to 15/ 1000 erythrocytes can be used as a general reference for most birds.[6,19] Thrombocytopenias are usually indicative of excessive peripheral demand for thrombocytes, although a depression in thrombopoiesis should be considered. Thrombocytopenias are often seen with severe septicemias, where a combination of excessive peripheral demand for thrombocytes and depression of thrombocyte production may occur. A thrombocytosis may reflect a rebound response following hemorrhage or recovery from other conditions associated with excessive utilization of thrombocytes. Often a regenerative response can be detected by the presence of immature thrombocytes in the peripheral blood film.

Identification of Common Blood Parasites

For a complete review of avian parasites see Chapter 36.

The diagnosis of *Haemoproteus* is made by the detecting characteristic intraerythrocytic gametocytes in peripheral blood films. Only the gametocyte stage of this organism appears in the peripheral blood, whereas schizogony occurs in the tissues (eg, lung, spleen and liver).[70] The mature gametocyte contains yellow-to-brown, refractile pigment granules (Color 9.21). The typical mature gametocyte occupies greater than 50 percent of the red cell cytoplasm, partially encircles the host cell nucleus forming the classic "halter-shape" and causes little displacement of the red cell nucleus. It is rare for more than one mature gametocyte to occur in a cell. Macrogametocytes stain blue with Romanowsky stains and have pigment granules dispersed throughout the cytoplasm of the parasite. The smaller microgametocytes stain pale blue to pink with pigment granules appearing in spherical aggregates. If blood containing *Haemoproteus* organisms is allowed to stand at room temperature for a few hours prior to preparing a blood film, gametes may be released from the cells and found in the extracellular spaces of the blood film. The macrogametes appear as spheres that resemble the macrogametocytes within the red cell cytoplasm. The microgametes appear as small spindle-shaped structures. When gametes are found, it should be considered as an artifact of blood film preparation because these structures normally leave the host red cell following ingestion by the intermediate insect host (hippoboscid flies).

Leucocytozoon is easily identified from blood films because it grossly distorts the host cell (usually immature erythrocytes) that it parasitizes. Like *Haemoproteus*, only the gametocyte stage of *Leucocytozoon* occurs in the peripheral blood of birds (Color 9.22).[70] The large, round-to-elongated gametocytes cause the host cell to enlarge and appear to have two nuclei: the host cell nucleus pushed to the margin of the cell and the parasite nucleus, a pale-pink nucleus within the parasite. The parasitized cell usually has tapered ends with the remnants of the cell membrane trailing away from the cell. The macrogametocyte stains dark blue with a condensed nucleus and occasional cytoplasmic vacuoles. The microgametocyte stains light blue with a diffuse, pale-pink nucleus. Gametocytes of *Leucocytozoon* lack the refractile pigment granules found in *Haemoproteus*.

The intraerythrocytic gametocytes of *Plasmodium* spp. are often confused with those of *Haemoproteus* spp. because they also contain refractile pigment granules. However, *Plasmodium* gametocytes usually occupy less than 50 percent of the host cell

cytoplasm, and those of some species alter the position of the red cell nucleus (Color 9.26). Two key features that aid in the detection of *Plasmodium* are the presence of schizogony in the peripheral blood and gametocytes or schizonts in blood cells other than erythrocytes.[70] Schizonts appear as round-to-oval intracytoplasmic inclusions that contain dark-staining merozoites. The number of merozoites produced depends upon the species of *Plasmodium*. As with *Haemoproteus*, *Plasmodium* macrogametocytes stain darker than the microgametocytes. Both *Plasmodium* and *Haemoproteus* infections may reveal small, ring-like forms (trophozoites) in the cytoplasm of infected erythrocytes. In rare cases, only these forms may be seen, and it is impossible to identify the parasite involved. In such situations, resampling a week or more later will often reveal the developed forms having the characteristics described for either *Plasmodium* or *Haemoproteus*. Mosquitos (*Culex* and *Aedes* spp.) are the intermediate hosts for *Plasmodium*.

Microfilaria are frequently found in the peripheral blood of a variety of birds.

Atoxoplasma sp. is identified by its characteristic sporozoite within the cytoplasm of mononuclear leukocytes, especially lymphocytes (Color 9.27).[40] The sporozoites appear as pale-staining, round-to-oval intracytoplasmic inclusions that compress the host cell nucleus and create a characteristic crescent shape to the nucleus. This organism can be found in the per-ipheral blood films or imprints of tissues such as the lung, liver and spleen.

Aegyptianella can occur within the cytoplasm in one of three forms: 1) anaplasma-like initial bodies appearing as small (less than one micrometer in diameter), round, basophilic inclusions; 2) intermediate stages resembling *Babesia* and measuring between one and two micrometers in diameter; and 3) large, round-to-elliptical forms measuring between two and four micrometers in length.[70] *Aegyptianella* spp. are considered to be pathogenic to many species of birds (primarily Passeriformes) but may be difficult to detect because the parasitemia stage of the disease is often very short and easily missed.

Evaluation of the Hematopoietic Tissue

Hematopoiesis occurs primarily in the bone marrow of post-hatch birds; however, hematopoietic activity may also be found in various internal organs (eg, liver and possibly spleen).[4,19] A bone marrow sample should be obtained for cytologic evaluation in avian patients with persistent nonregenerative anemia, thrombocytopenia, panleukopenia and heteropenia. Bone marrow evaluation is also indicated for suspected cases of leukemia or if unexplained abnormal cells are found in the peripheral blood. An evaluation of the hemogram should accompany any bone marrow evaluation to properly assess hematopoiesis.

Bone Marrow Collection

In general, the proximal tibiotarsus just below the femoral-tibiotarsal joint (knee) is the preferred site for bone marrow collection in most birds.[6,79] After surgical preparation of the skin either on the cranial or medial aspect of the proximal tibiotarsus, a small stab incision through the skin is made using a scalpel blade. A bone marrow aspiration biopsy needle is pushed through the thin cortex and into the marrow space using clockwise-counterclockwise rotational movements. Once the needle has entered the marrow space, the stylet is removed from the needle and a syringe is attached to gently aspirate a small amount of marrow into the needle lumen. Excessive pressure during aspiration should be avoided to prevent peripheral blood contamination of the sample. Following aspiration, the needle is removed from the bone and the syringe is detached from the needle. The syringe is filled with air and reattached to the needle hub. Using the air in the syringe, the marrow within the needle lumen is forced onto a microscope slide. A second slide is placed across the first on top of the marrow sample. As the two slides are pulled horizontally apart, two marrow films are made for cytologic examination.

Marrow can also be obtained from the sternum (keel) of some birds with the biopsy needle inserted into the widest part of the sternal ridge.

Bone marrow biopsy needles commonly used include pediatric Jamshidi bone marrow biopsy-aspiration

Hematology

Color 9.7
Early myeloblast with a fine chromatin pattern, nucleolar ring and dark-blue cytoplasm. The myeloblast is the progenitor cell for the heterophil (Color 9.8), eosinophil (Color 9.9) and basophil (Color 9.10).

Color 9.8
Heterophil development.

a) Myeloblast with fine chromatin pattern, nucleolus and light-blue cytoplasm.

b) Promyelocyte with oval nucleus, pale-blue cytoplasm and round to slightly irregular metachromatic cytoplasmic granules.

c) Heterophil myelocyte with an oval nucleus and a mixture of metachromatic granules and scattered round-to-rod-shaped eosinophilic granules.

d) Heterophilic metamyelocyte with a slightly indented nucleus.

e) Heterophilic band with U-shaped nucleus.

f,g) Mature, segmented heterophils with numerous needle-shaped granules. The granules may obscure nuclear detail, making assessment of lobulation difficult.

h) Heterophil with mild toxic changes including cellular swelling, partial degranulation and a basophilic cast to the cytoplasm.

i,j) Toxic heterophils with progressive granule dissolution leaving the round granule core intact. The cell is slightly swollen and has basophilic cytoplasm. This cell may occasionally be confused with an eosinophil, except for retention of a few needle-shaped granules. Stain-induced heterophil degranulation is not associated with cytoplasmic basophilia.

k) Heterophil with non-staining cytoplasmic granules. This may be an artifact resulting from exposure of the blood smear to formalin vapor during transport or mailing.

l) Disrupted heterophil showing typical needle-shaped granules. These smudge cells are ignored during the leukocyte differential count.

m) Smudged heterophil with dissolution of the outer granule matrix leaving intact round central cores. Granule dissolution may cause an eosinophil-like appearance; however, smudge cells are ignored during the leukocyte differential count.

Color 9.9
Eosinophil development.

a) Late myeloblast with condensing chromatin pattern and light-blue cytoplasm.

b) Eosinophilic myelocyte with an oval nucleus and scattered, variably sized, round, secondary granules.

c) Eosinophilic myelocyte with a slightly indented nucleus and round, red-orange granules.

d) Eosinophilic band with U-shaped nucleus and round, red-orange granules.

e) Segmented eosinophil with a lobulated nucleus and abundant secondary (specific) granules.

f) Disrupted or smudged eosinophil. These cells are ignored during the leukocyte differential count.

Color 9.10
Basophil development.

a,b) Basophilic myelocytes with round nuclei and round, variably sized, metachromatic granules.

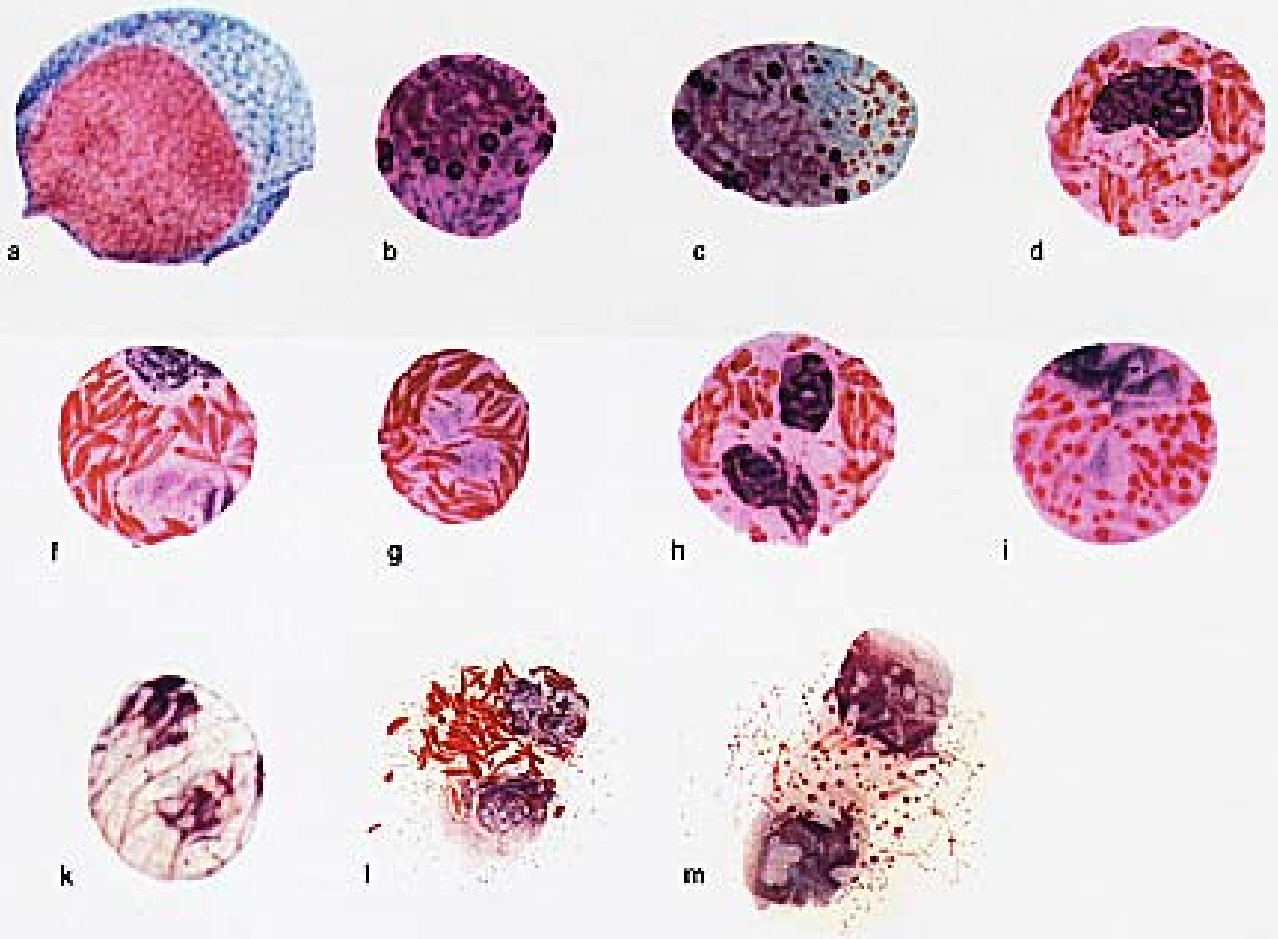
c,d) Basophils with round, intensely stained, metachromatic granules. Basophil granules have high affinity for Romanowsky stain, often resulting in poor staining of the cell nucleus. In addition, cytoplasmic granules may obscure nuclear detail.

e) Basophil degranulation may occur with disease or as an artifact of blood smear staining.

f) Disrupted basophil (smudge cell). These cells are ignored during the leukocyte differential count.

9.7

9.8



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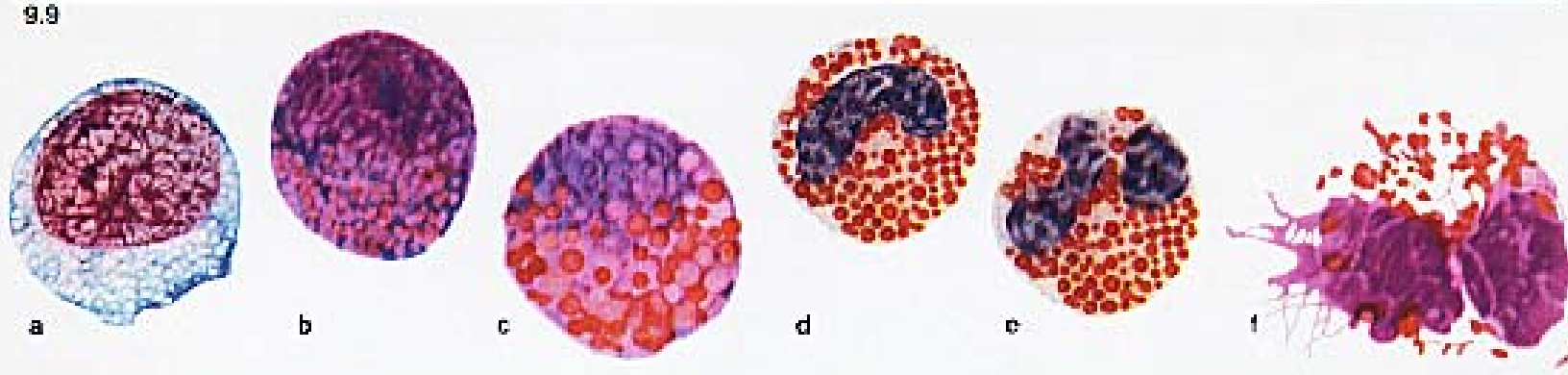

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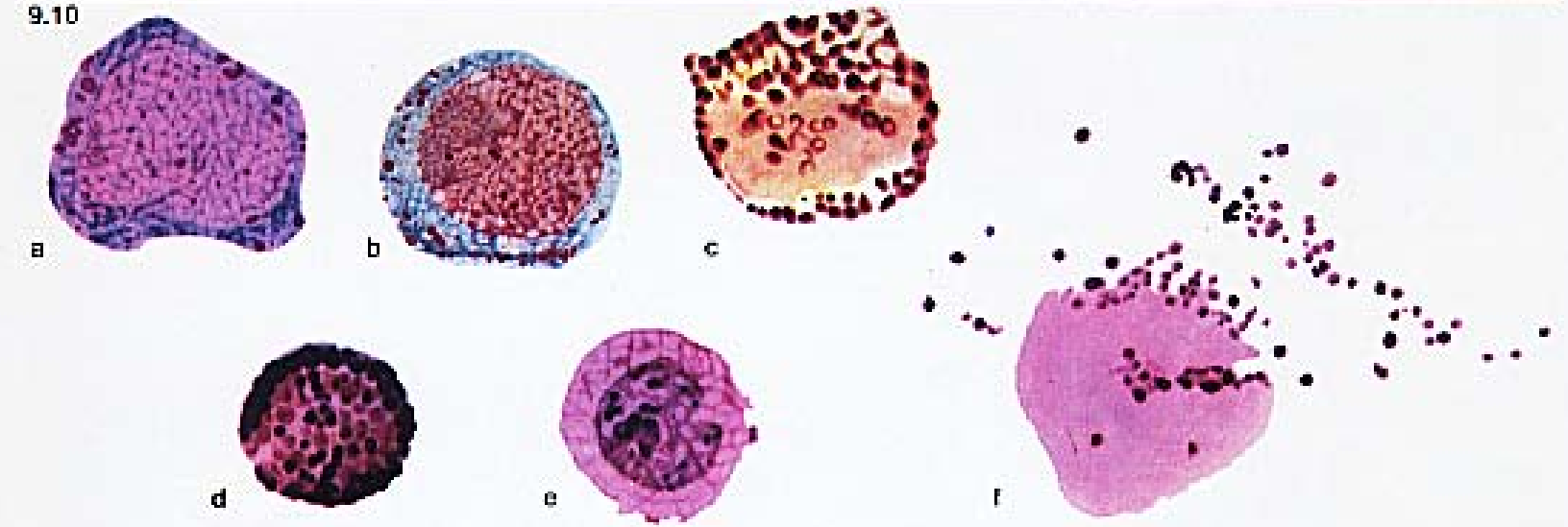

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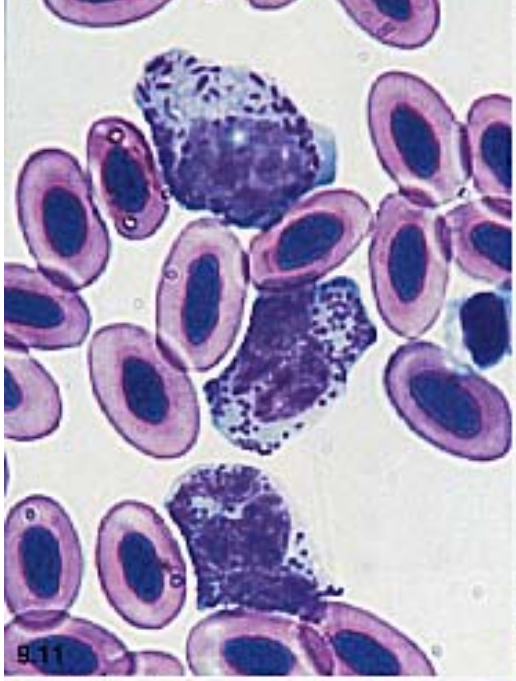
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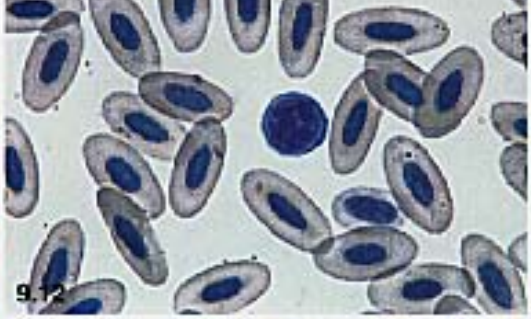
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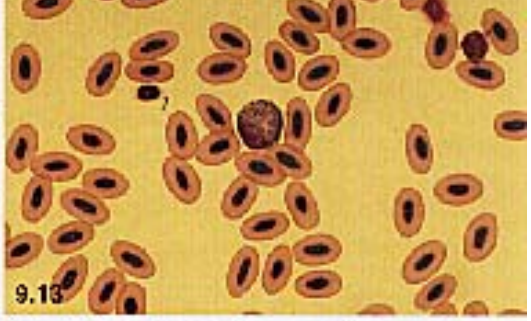
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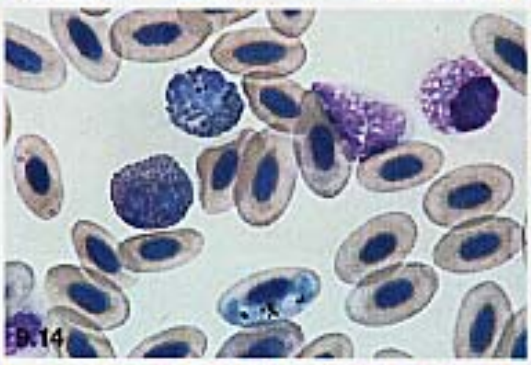
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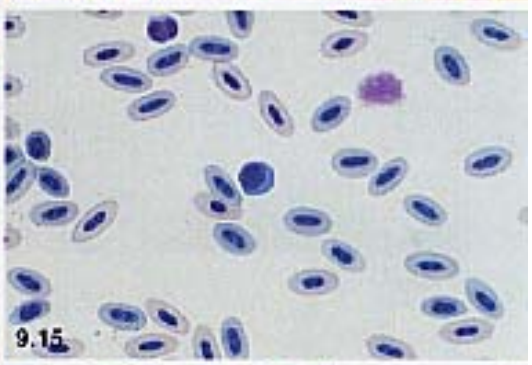
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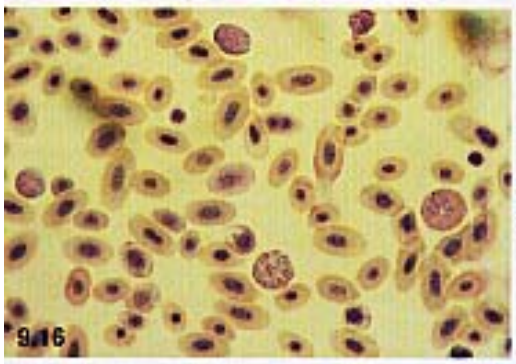
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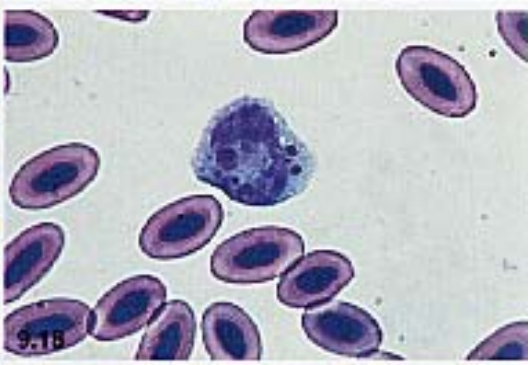
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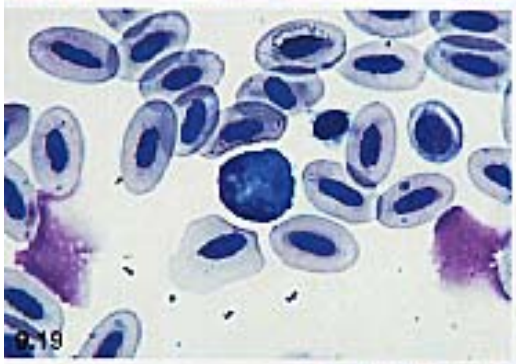
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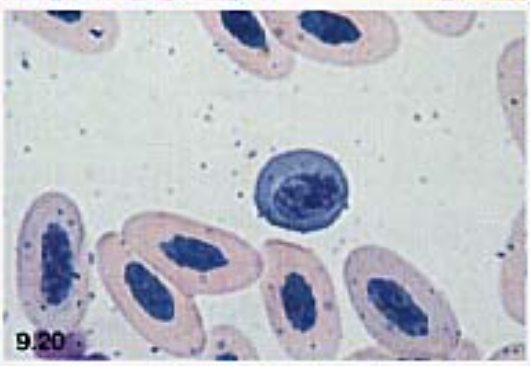
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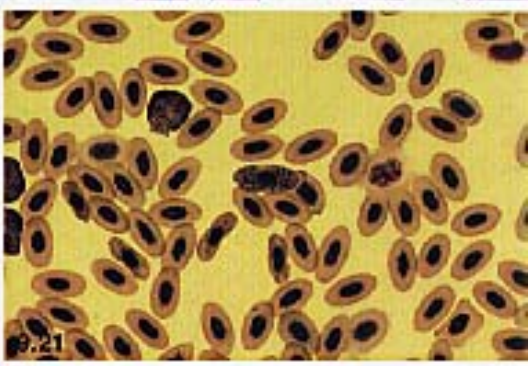
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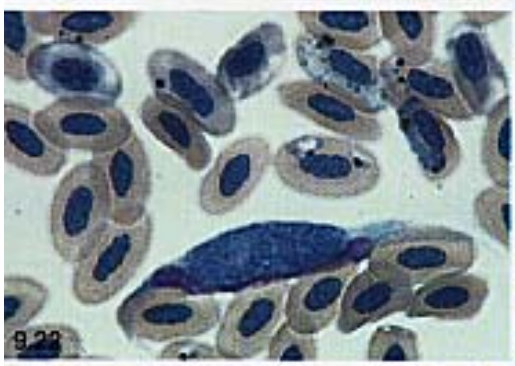
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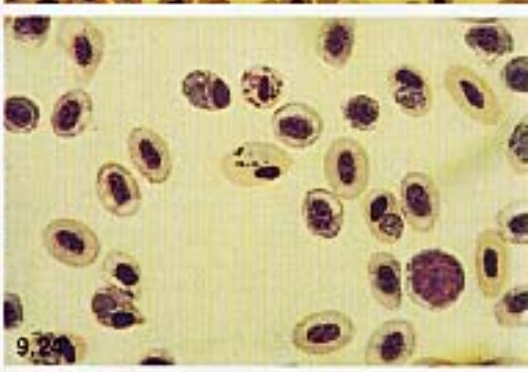
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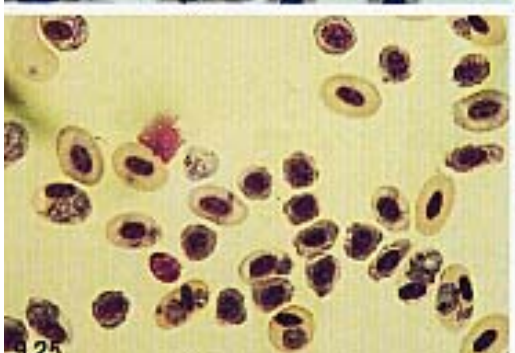
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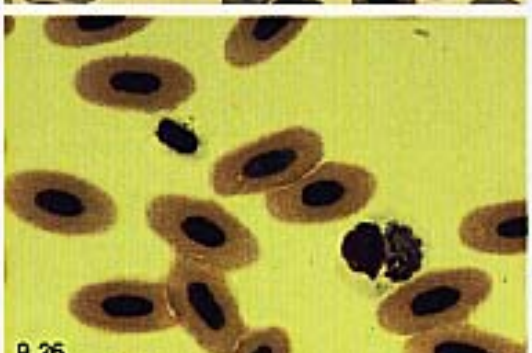
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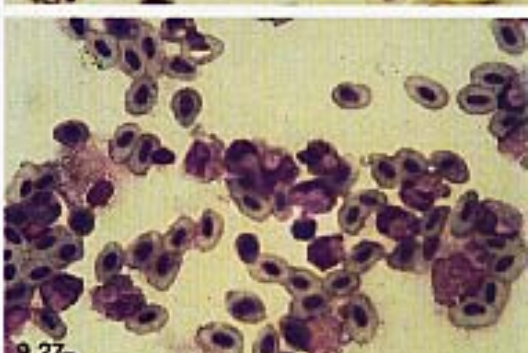
9.27

Hematology

All photographs on this page were provided courtesy of Terry W. Campbell.

Color 9.11
An adult female Red-tailed Hawk was presented with clinical signs of lethargy, a marked reduction of the pectoral muscle mass and diarrhea. A diagnosis of salmonellosis was made based upon necropsy and bacterial culture two days later. The hemogram revealed: PCV = 28%, RBC = 1,950,000/ mm^3, WBC = 31,876/ mm^3, heterophils = 28,050/ mm^3, lymphocytes = 1,594, monocytes = 638/ mm^3 and eosinophils = 1,594/ mm^3. A toxic heterophil (2+ toxicity) and two eosinophils are shown. Wright's stain.

Color 9.12
A Blue-fronted Amazon Parrot was presented with multifocal, depigmented, nonraised lesions involving the skin on the feet. Serum chemistries and erythrocyte parameters were within normal limits. The leukogram revealed the following: WBC = 7,543/ mm^3, heterophils = 1,886/ mm^3, lymphocytes = -4,601/ mm^3, monocytes = 830/ mm^3 and eosinophils = 226/ mm^3. Typical reactivity of lymphocytes demonstrated by Wright's stain.

Color 9.13
A critically ill Green-cheeked Amazon Parrot was presented for evaluation. The bird was extremely weak and unable to perch. Severe uveitis was present in both eyes. The bird was housed in a room where varnish was being applied to furniture. Important hematologic findings included: PCV = 40%, total protein = 5.0 gm/dl, WBC = 16,140/ mm^3, heterophils = 13,880/ mm^3 (10% of the heterophils were myelocytes and 20% were metamyelocytes), lymphocytes = 807/ mm^3, monocytes = 1,453/ mm^3 and thrombocytopenia. A heterophilic myelocyte is demonstrated by Wright's stain.

Color 9.14
A juvenile Red-tailed Hawk was presented with marked reduction of the pectoral muscle mass. Radiographic evaluation revealed a fracture of the left coracoid bone. The hematologic findings included: PCV = 37%, Hb = 11.5 g/dl, RBC = 2,250,000/ mm^3, 4% polychromasia, total protein = 2.6 g/dl, WBC = 32,932/ mm^3, heterophils = 25,687/ mm^3, lymphocytes = 1,976/ mm^3, monocytes = 988/ mm^3, eosinophils = 3,952/ mm^3 and basophils = 392/ mm^3. A heterophil, eosinophil, basophil and *Haemoproteus* gametocyte (0.7% of the erythrocytes contained these gametocytes) are demonstrated by Wright's stain.

Color 9.15
A Blue and Gold Macaw was examined 93 hours following severe blood loss that resulted from a traumatic injury to the foot. The hemogram revealed: PCV = 21%, RBC = 1,660,000/ mm^3, Hb = 5.8 g/dl, WBC = 19,457/ mm^3, heterophils = 12,255/ mm^3, lymphocytes = 6,615/ mm^3, monocytes = 97/ mm^3 and basophils = 389/ mm^3. Twenty-two percent of the erythrocytes exhibited polychromasia, and an occasional immature erythrocyte was noted. Polychromasia and a round, immature erythrocyte (early polychromatic rubricyte) are demonstrated by Wright's stain.

Color 9.16
The hemogram from a Harris Hawk that died three days later from an acute pneumonia revealed a moderate anemia, leukocytosis, heterophilia and left shift. Marked anisocytosis and polychromasia are illustrated as well as a binucleate erythrocyte (which were frequently seen in the blood film). A heterophil, eosinophil, two lymphocytes, a thrombocyte and an immature erythrocyte (basophilic rubricyte) can also be seen by Wright's stain.

Color 9.17
A juvenile Bar-headed Goose was presented for weakness and weight loss. Six other geese in the group appeared normal. The hemogram revealed: PCV = 28%, Hb = 9.0 g/dl, RBC = 1,950,000/ mm^3, 11% polychromasia, WBC = 39,556/ mm^3, heterophils = 31,645/ mm^3, lymphocytes = 5,645/ mm^3, monocytes = 1,187/ mm^3 and basophils = 791/ mm^3. Blood lead levels were normal. The erythrocyte morphology revealed polychromasia, hypochromasia and stippled basophilia. Wright's stain.

Color 9.18
A juvenile Red-tailed Hawk was presented with open fractures of the left radius and ulna and poxvirus lesions along the margins of the beak and on the feet. The bird was extremely depressed and died within 24 hours of presentation. The hemogram showed: PCV = 35%, Hb = 11 g/dl, RBC = 3,020,000/ mm^3, WBC = 42,240/ mm^3, monocytes = 3,802/ mm^3, eosinophils = 1,267/ mm^3 and basophils = 422/ mm^3. The majority of the heterophils appeared extremely toxic (4+). Wright's stain.

Color 9.19
An adult African Grey Parrot was presented with a history of an intermittent seizure disorder and was successfully treated for hypocalcemia. The hemogram revealed: PCV = 49%, RBC = 2,940,000/ mm^3, WBC = 15,740/ mm^3, heterophils = 6,453/ mm^3, lymphocytes = 8,814/ mm^3, monocytes = 315/ mm^3 and eosinophils = 157/ mm^3. Eosinophils with large, blue cytoplasmic granules are demonstrated by Wright's stain.

Color 9.20
An adult Red-tailed Hawk was presented with multiple gunshot wounds and open fractures involving the right radius and ulna. The hemogram revealed: PCV = 16%, Hb = 5.3 g/dl, RBC = 1,300,000/ mm^3, WBC = 15,740/ mm^3, heterophils = 6,453/ mm^3, lymphocytes = 8,814/ mm^3 and monocytes = 472/ mm^3. The reticulocyte count was 20% and there were many immature erythrocytes present. A mid-polychromatic rubricyte is demonstrated by Wright's stain.

Color 9.21.
Haemoproteus sp. gametocytes in a blood film stained with Wright's stain from a Great Horned Owl.

Color 9.22
Haemoproteus and *Leukocytozoon* spp. gametocytes in a blood film stained with Wright's stain from a Great Horned Owl.

Color 9.23
Haemoproteus sp. microgametes in a blood film stained with Wright's stain from a Screech Owl.

Color 9.24
Plasmodium sp. gametocytes and schizonts in the cytoplasm of erythrocytes from a Skua stained with Wright's stain.

Color 9.25
Plasmodium sp. gametocytes in the cytoplasm of erythrocytes and extracellular space in a Wright's-stained blood film from a Skua.

Color 9.26
Plasmodium sp. gametocyte in the cytoplasm of a thrombocyte in a Wright's-stained blood film from a Mississippi Kite.

Color 9.27
Numerous intracytoplasmic *Atoxoplasma* sp. inclusions within lymphocytes from a lung imprint of a Siskin.

needles[j] and disposable Jamshidi Illinois-Sternal/Iliac aspiration needles. Disposable spinal needles can be used to sample small birds because they contain a stylet to facilitate passage of the needle through the cortex without occlusion of the needle lumen with bone.

Erythropoiesis

The terminology describing the different stages of erythrocytic development varies in the literature.[6,19,34,44] In general, there are six recognizable stages involved in red cell development. The earliest recognizable stage is the rubriblast (proerythroblast) (Color 9.1). This cell has large, prominent nucleoli or nucleolar rings. The round nucleus is centrally positioned within the cell. The coarsely granular chromatin is atypical for most blast-type cells. The abundant cytoplasm stains deeply basophilic and contains fine, clear spaces (mitochondrial spaces). Rubriblasts have high N:C ratios, typical of immature cells.

The second stage in erythrocyte development is the prorubricyte (basophilic erythroblast). This cell resembles the rubriblast, but the nucleoli are either absent or indistinct, and the cytoplasm lacks the mitochondrial spaces of the rubriblast (Color 9.1).

The next three stages are the rubricyte stages. These are round-to-slightly oval cells that are smaller than rubriblasts and prorubricytes. Rubricytes are divided into three groups based upon their appearance in the cytologic sample. In order of increasing maturation they are the basophilic rubricyte (early polychromatic erythroblast), early polychromatic rubricyte (late polychromatic erythroblast) and late polychromatic rubricyte (orthochromic erythroblast). The basophilic rubricyte has a high N:C ratio, homogeneous basophilic cytoplasm and round nucleus with distinct chromatin clumping.

The early polychromatic rubricyte appears smaller than the basophilic rubricyte and is the first stage of red cell development in which hemoglobinization of the cytoplasm can be detected with Wright's stain. The hemoglobin gives the cytoplasm a gray, slightly eosinophilic appearance. The nucleus appears smaller with increased density, and the cytoplasm is more abundant when compared to the previous stage of development. The late polychromatic rubricyte is a round-to-slightly oval cell with an eosinophilic gray-to-weakly eosinophilic cytoplasm (Color 9.1). This cell appears to have increased cytoplasmic volume when compared to the previous stage, and the nucleus may appear oval with irregularly clumped chromatin. The penultimate stage of erythrocyte development is the polychromatic erythrocyte, which resembles the oval, mature erythrocyte except for the cytoplasmic basophilia and nuclear chromatin that appear less condensed than the pyknotic nucleus of the mature cell (Color 9.1).

Granulopoiesis

Avian granulopoiesis appears to follow developmental stages similar to those described for mammalian granulocytes.[6,34,44] These stages are the myeloblast (granuloblast), progranulocyte (promyelocyte), myelocyte, metamyelocyte and mature granulocyte.

Myeloblasts are large, round cells with a narrow rim of cytoplasm that appears less basophilic than that of rubriblasts.[44] In general, the nucleus is round with a delicate reticular chromatin pattern and distinct nucleoli. No cytoplasmic granules are present. The myeloblast stage is common to all the granulocytes (Color 9.7).

The next stage toward maturation is the progranulocyte. These are large cells with cytoplasmic granules and light blue cytoplasm. The granules are variable in appearance. An attempt has been made to differentiate progranulocytes into their respective granulocytic cell lines based upon the appearance of the cytoplasmic granules.[44] Heterophil progranulocytes contain orange spheres (primary granules) or rings and dark magenta granules or rings. The ring forms are thought to be characteristic of the heterophil cell line. Eosinophil progranulocytes lack the dark magenta granules and rings and contain only brightly staining orange spheres (primary granules). Basophil progranulocytes have magenta granules that appear smaller than those of heterophil progranulocytes and have fewer ring forms. The nucleus of progranulocytes is typically eccentric in its cellular position, has a delicate reticular chromatin pattern and often has indistinct margins.

The myelocytes are smaller than the progranulocytes and contain the specific granules (secondary granules) for each cell line. Heterophil myelocytes are round cells with light blue cytoplasm containing primary granules, magenta granules and rings and the definitive rod-shaped heterophil granules. The definitive granules occupy less than 50 percent of the cytoplasmic volume. Eosinophil myelocytes contain primary and secondary granules. The specific or secondary granules occupy less than 50 percent of the

cytoplasmic volume. The basophil myelocyte has magenta granules and mature basophil granules (secondary granules) that occupy less than 50 percent of the cytoplasmic volume. The nucleus of myelocytes is round and has coarsely granular chromatin.

Metamyelocytes resemble myelocytes, except the cell nucleus is slightly indented and may have distinct chromatin clumping. Heterophil metamyelocytes have definitive, rod-shaped granules that occupy greater than 50 percent of the cytoplasmic volume. The primary granules and magenta spheres and rings may be present, but fewer in number than the previous stage. The definitive granules of the eosinophil and basophil series also occupy greater than 50 percent of the cytoplasmic volume in their respective metamyelocyte stages. The basophil myelocyte nucleus remains round.

The granulocytic cell series will occasionally reveal a band cell stage similar to that described in mammalian granulocytes. However, the cell nucleus is often hidden by the cytoplasmic granules (especially in heterophils), making it difficult to differentiate the band cell from mature cells. Mature avian basophil nuclei do not segment.

Thrombocytopoiesis

The developmental stages involved in thrombopoiesis are the thromboblast, early-immature thrombocyte, mid-immature thrombocyte, late-immature thrombocyte and mature thrombocyte (Color 9.4).[6,44] As the cell develops toward maturity, the cell size decreases, the N:C ratio decreases, the nucleus becomes increasingly pyknotic and cytoplasm becomes less basophilic.

Thromboblasts are large, round-to-ameboid-shaped cells with a narrow rim of deeply basophilic cytoplasm surrounding the round nucleus. The nuclear chromatin often appears punctate, making nucleoli difficult to detect. The cytoplasm may contain small clear spaces.

The early-immature thrombocyte is smaller than the thromboblast. It has a round-to-oval nucleus and smaller N:C ratio than the previous cell. The cytoplasm is basophilic with small, clear spaces or vacuoles. The nuclear chromatin is irregularly clumped and nucleoli are absent. The mid-immature thrombocyte appears slightly elongated or irregular with a pale blue, vacuolated cytoplasm. Specific red cytoplasmic granules may be seen at this stage. The nucleus usually has marked chromatin clumping. The late-immature thrombocyte is an oval cell that has the appearance of the elongate, mature thrombocyte, except the cytoplasm is a pale blue and the nuclear chromatin is less condensed.

Lymphopoiesis

Lymphocyte development may be seen occasionally when evaluating hematopoietic tissue (Color 9.5). Three distinctive stages can be identified for lymphocyte development: lymphoblasts, prolymphocytes and mature lymphocytes. Lymphoblasts are large, round lymphocytes with high N:C ratios. The nucleus has smooth chromatin, in comparison to the mature cell, and contains distinct nucleoli. The cytoplasm of lymphoblasts stains deeply basophilic.

Prolymphocytes resemble lymphoblasts but are slightly smaller, lack nucleoli and have a less basophilic cytoplasm. In normal lymphoid tissue, lymphoblasts and prolymphocytes represent less than ten percent of the lymphoid cells. Thus, the majority of the cells should be mature lymphocytes with the heavy nuclear chromatin clumping, high N:C ratio and scant amount of blue, homogeneous cytoplasm.

Other Bone Marrow Cells

Other cells frequently encountered in bone marrow samples include osteoclasts, osteoblasts, monocytes, plasma cells and mitotic figures. Osteoclasts are large, multinucleated cells that are ameboid in shape. The abundant cytoplasm is weakly basophilic and often contains vacuoles and small red granules of various shapes. The nuclei are round-to-oval and usually contain distinct nucleoli. Osteoblasts are large cells that vary in shape. The oval-to-round nucleus is eccentrically positioned in the cell. The abundant, foamy, basophilic cytoplasm contains a prominent clear space (Golgi) that is located a distance from the nucleus.

Products Mentioned in the Text

a. Microtainer - Becton Dickinson, Rutherford, NJ
b. Samplette, Monoject, Sherwood Medical, St. Louis, MO
c. Capiject, Terumo, Elkton, MD
d. Abbott Hospitals Inc, North Chicago, IL
e. Diff Quik, American Scientific Products, McGraw Park, IL
f. Hemacolor, Miles Laboratories Inc, Elkhart, IN
g. Hema-Tek, Ames Division of Miles Laboratories Inc, Elkhart, IN
h. Coulter Counter, Coulter Electronics, Inc
i. Unopette System, Becton-Dickinson, Rutherford, NJ
j. Kormed Corp., Minneapolis, MN

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