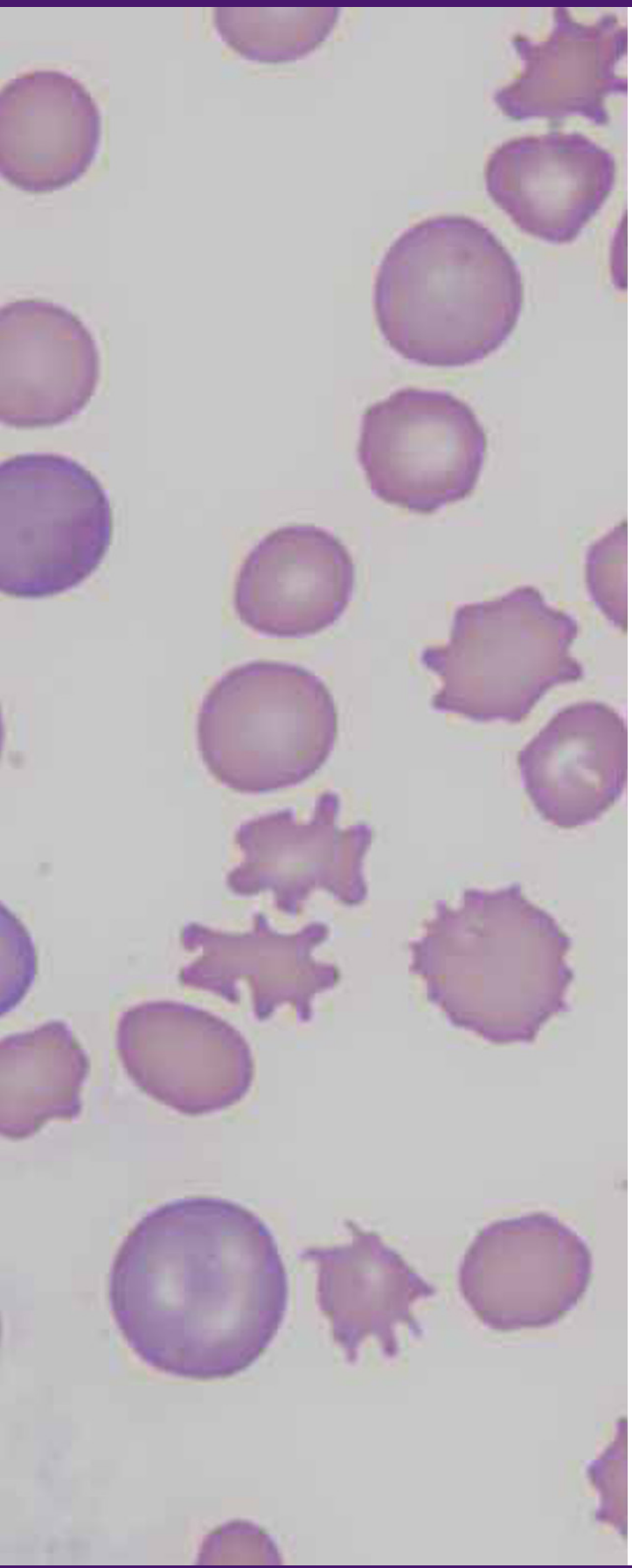

MARION L. JACKSON

VETERINARY CLINICAL PATHOLOGY

AN INTRODUCTION

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Veterinary Clinical Pathology

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Marion L. Jackson

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Preface

At the Western College of Veterinary Medicine, students are introduced to Clinical Pathology in year three of the undergraduate program. In year four, small groups of students rotate through the laboratory, which provides a dynamic setting to test and expand their knowledge. Several years ago, Dr. Gene Searcy, the most enthusiastic clinical pathologist I have known, made a dramatic change in the third-year course, by moving from didactic lectures with a few case examples, to case studies interjected with a few minilectures. Dr. Beverly Kidney, my colleague, and I have continued with this format, which is very well received by our students. Assigned readings provide the tools needed to understand and interpret laboratory data, and during classes, students are randomly chosen to discuss laboratory results from a book of case studies.

This textbook has evolved from the reading assignments for the undergraduate course and is intended to give the student a sound knowledge base with which to work. The book is not heavily referenced other than with standard physiology, clinical pathology, and medicine textbooks, and is not intended to be a review of the current literature. I have aimed for a clear, concise presentation of basic mechanisms without overwhelming the student. Cases at the end of each chapter (except Chapters 11 and 12) emphasize basic principles discussed in the text. The cases are real and, therefore, are not perfect. Not all results can be satisfactorily explained, which is true to life. Students are encouraged to interpret the case data on their own before reading my version. Complete interpretations are provided even though the student may not be familiar with all laboratory data until completion of the book. It is expected that instructors using this book will provide additional cases from their own diagnostic service for class discussion and to challenge the students. Laboratory periods complement our course by affording the opportunity to learn practical aspects of performing a CBC, blood and cytology smear evaluation, and doing a urinalysis.

If we as instructors can help students to become proficient in applying clinical pathology as a powerful diagnostic tool, then we have been successful. We are overjoyed when students grasp this subject and run with it; let us make it fun too.

Acknowledgments

I thank my mentor, Dr. Gene Searcy, and colleague, Dr. Beverly Kidney, for their encouragement and support with this project. Undergraduate students, graduate students, clinicians, technologists, staff, and colleagues within the Western College of Veterinary Medicine and Prairie Diagnostic Services have helped me to improve my teaching and writing, with their invaluable input, feedback and challenging questions. No doubt, I have made errors of various types in this book, and I welcome criticism and comments for improvements (please send me an e-mail: marion.jackson@usask.ca).

The whim to write this book only took root as a project for a sabbatical leave—a vital part of academic life. Dr. Nicole Fernandez, a former graduate student in our department, was of great assistance with the photographs, figures, tables, proofreading, and organization of the book. Dr. Juliane Deubner, our college medical illustrator and treasured resource, generated most of the figures, and Ms. Maeve Johnston, graphic designer and illustrator, drew the cells for the figures and the tables of erythrocyte morphology. I thank Ms. Priscilla Neufeld for her usual competence and reliability, and for working long and odd hours on the final product. The data for the case studies were generated by Prairie Diagnostic Services, which employs a team of veterinary professionals and technologists second to none. Mrs. Gloria Patry, Prairie Diagnostic Services, kindly provided the photographs of urine sediment findings. Ms. Kim Christiansen, Prairie Diagnostic Services, helped immensely with loose ends when deadlines loomed. Production staff of Blackwell Publishing are to be thanked for their guidance and creation of this textbook.

I am grateful to my husband, Dr. Vladimir Sopuck, and sons, Adam and Bennett, for supporting me through another adventure.

Veterinary Clinical Pathology

An Introduction

CHAPTER 1

Erythrocytes

Erythropoiesis

The production of erythrocytes from stem cell to mature circulating red blood cell (RBC) is known as erythropoiesis. Hemopoiesis refers to the production of all blood cells, including white blood cells (WBCs) and platelets. Erythropoiesis is most effective in the bone marrow, although other tissues may provide additional sites of RBC production. Erythrocytes deliver oxygen to tissues, remove carbon dioxide from tissues, and buffer acid–base changes in the circulation. Tissue oxygenation is the main regulator of RBC production, and conditions associated with tissue hypoxia stimulate the bone marrow to increase RBC production. When tissue oxygenation is adequate, total erythroid mass fluctuates very little, as a steady state exists between RBC production and RBC loss.

Hemopoietic stem cells have the ability to develop into common myeloid progenitors and common lymphoid progenitors (Fig. 1.1). Erythrocytes, megakaryocytes, and all leukocytes (except lymphocytes) are generated from common myeloid progenitors. The first level of committed differentiation to erythrocytes is within the precursor cell compartment. The bone marrow microenvironment provides the structural and biochemical support for normal hemopoiesis. Growth factors, transcription factors, adhesins, interleukins, and other mediators comprise a complex system that responds to increased demands when required and maintains a finely tuned balance under normal circumstances. The expression and availability of these factors influence the balance among the various committed lineages. Erythropoietin (EPO) is the most important growth factor for maintaining erythroid proliferation. If peripheral blood evaluation reveals an inadequate or unexplained bone marrow response, examination of the marrow is usually indicated.

The committed erythroid precursor undergoes up to five mitotic divisions over 5 days. The earliest recognizable erythroid precursor is the rubriblast, followed by differentiation sequentially to the prorubricyte, rubricyte, metarubricyte, polychromatophilic erythrocyte, and the mature erythrocyte (Fig. 1.2). Erythroid maturation correlates with a decrease in EPO receptors and an increase in transferrin receptors on the surface of red cell precursors. Transferrin receptors allow for incorporation of iron into erythrocytes, for hemoglobin synthesis. Hemoglobin comprises four globin chains, each bound to a heme molecule containing iron. Hemoglobinization of the red cell cytoplasm is most active during the rubricyte stage. Also, cell division stops during the rubricyte stage as hemoglobinization nears completion and the nucleus condenses. At the end of the metarubricyte stage, the pyknotic nucleus is extruded and phagocytized by local macrophages.

Although nucleated erythrocytes are not usually found in the peripheral blood, a low percentage of circulating polychromatophilic cells are present under normal circumstances in most species (e.g. about 1% in the dog). The horse is an exception in that immature erythrocytes are rarely released into the peripheral blood in this species, even when intense erythroid hyperplasia is occurring in the bone marrow in response to anemia. Residual ribosomes and RNA, reflecting the end of protein synthesis (mainly hemoglobin), are responsible for the purplish-blue coloration of polychromatophilic erythrocytes with Romanowsky-type stains,

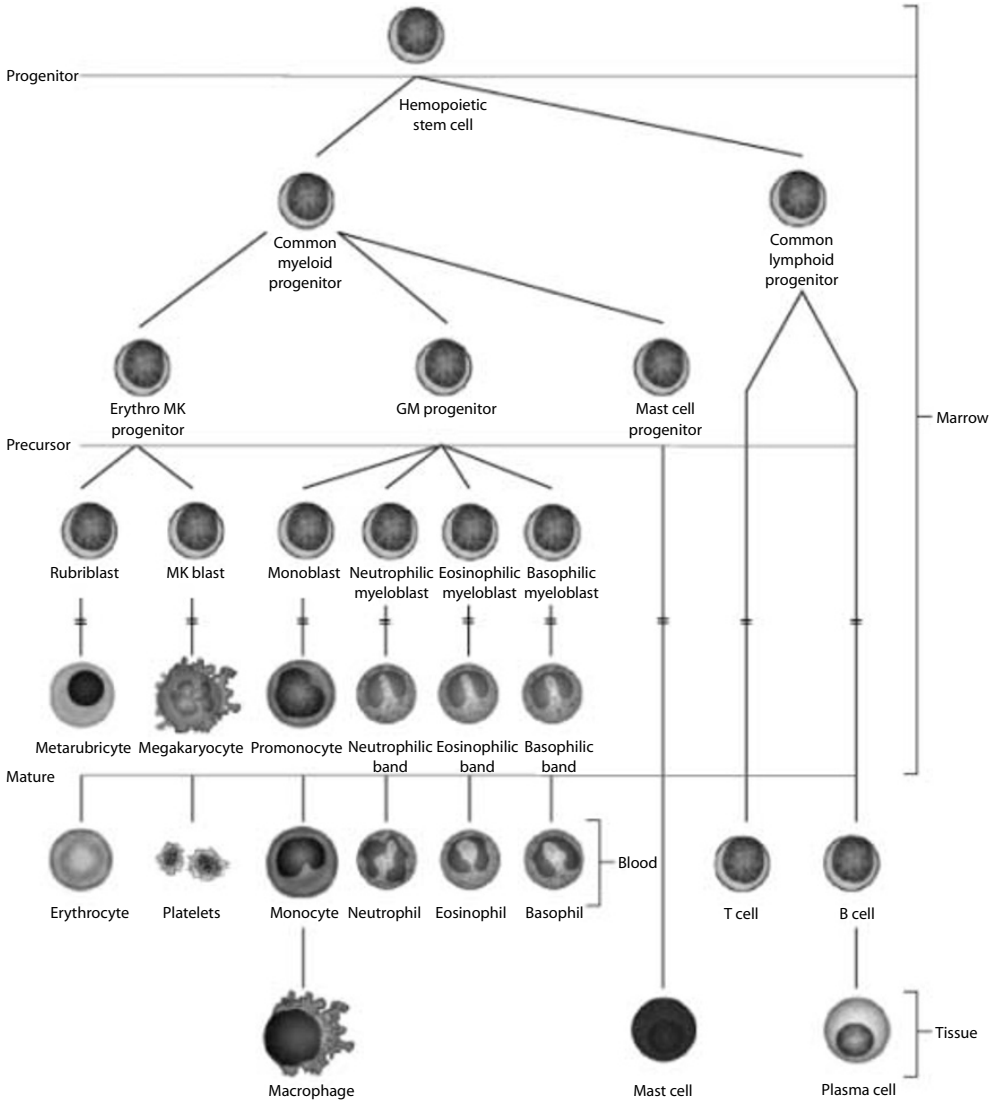


Figure 1.1 The cells of the blood and lymphoid organs and their precursors in the bone marrow.

such as Wright–Giemsa. Polychromatophilic erythrocytes lose their residual RNA within 24–48 hours of release from the bone marrow. When blood smears are stained with new methylene blue rather than Romanowsky stains, the residual RNA in immature RBCs is precipitated in clumps and the cells are known as reticulocytes.

Erythrocyte morphology

In the common domestic animals, mature erythrocytes are biconcave disks that are highly deformable, allowing them to travel through small capillaries and deliver oxygen to tissues

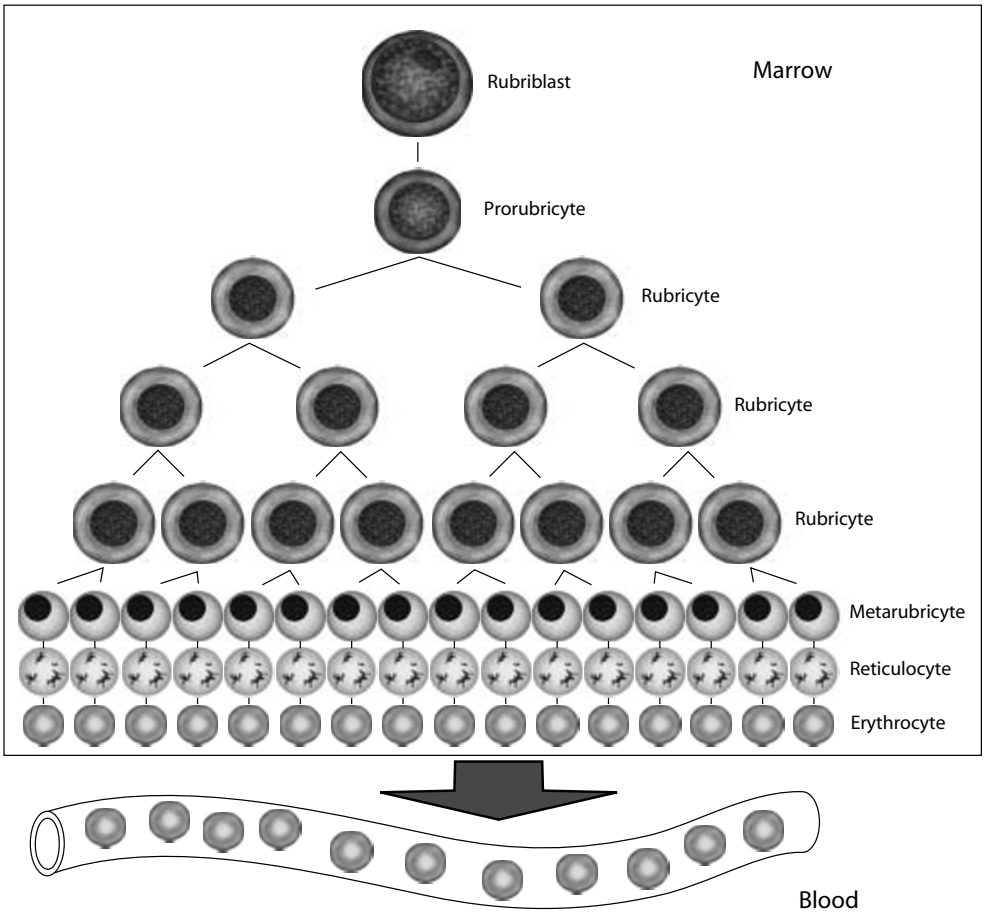


Figure 1.2 Erythrocyte kinetics. After stimulation by erythropoietin (EPO), cells in the committed erythroid compartment differentiate into rubriblasts, followed by mitotic division and maturation to mature erythrocytes.

(see Table 1.1). Erythrocyte aging and certain pathological conditions can cause RBCs to assume unusual shapes, which may result in increased rigidity. Rigid RBCs are susceptible to mechanical injury and are less effective in delivering oxygen. Exposure to stagnant environments (pooling of blood in a cavernous, hypoxic space), certain serum biochemical abnormalities, antibody-mediated membrane injury, and mechanical injury can alter the normal biconcave shape. Sometimes, RBC morphologic changes are associated with specific diseases or conditions, but the mechanism of the shape change is not clear (see Table 1.1 for diagrams of various types of RBC morphology).

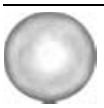
See Figs 1.3–1.24 for pictures of various types of RBC morphology.

The red cell wall is a typical lipid bilayer, comprising mainly phospholipids and cholesterol (Fig. 1.25). Membrane proteins and glycoproteins are inserted into the lipid bilayer, some in one leaflet only and others spanning the entire membrane. These integral proteins include hormone receptors and enzymes, which usually only partially penetrate into the bilayer, and transmembrane proteins, which include the

Table 1.1 Erythrocyte morphology

	**Acanthocytes** have projections of variable length that are unevenly spaced on the surface of the red cell. Acanthocytes may be seen as an incidental finding, as a consequence of a high-fat diet, with disorders of lipid metabolism, and with hemangiosarcoma. In the latter case, acanthocytes may form when red cells stagnate in cavernous spaces within the tumor, resulting in shifts in lipids in the RBC membrane.
	**Agglutination** is identified when red cells clump or cluster together in groups (not in rows) like a bunch of grapes. Agglutination must be differentiated from rouleaux. Polychromatophils do not participate in rouleaux formation but may agglutinate.
	**Anisocytosis** indicates variable red cell size.
	**Blister cells** appear as though they have a hole(s) punched through the periphery of the red cell. They are observed most often in feline blood films. Blister cells may result from oxidative injury.
	**Codocytes** (target cells) have a dark central area of hemoglobin, surrounded by a pale zone that in turn is surrounded by a peripheral rim of hemoglobin. Up to 50% of canine red cells may be codocytes; they are rarely observed in other species. Increased numbers of codocytes may be present with hepatic disease.
	**Dacryocytes** are red cells shaped like tear drops. They are considered artifactual if all points are oriented in the same direction. This artifact may be due to poor blood film preparation or lipemia. Increased numbers of non-artifactual dacryocytes may be seen with myelofibrosis.
	**Eccentrocytes** have eccentric hemoglobin distribution due to annealing of a crescent of red cell membrane that excludes hemoglobin. The hemoglobinated portion of the eccentrocyte stains darkly due to a higher concentration of hemoglobin in that portion of the cell. They indicate oxidative damage to the RBC membrane and may be accompanied by RBCs with Heinz bodies.
	**Echinocytes** are thought to be formed either as a result of erythrocyte dehydration or by expansion of the outer leaflet of the red cell membrane. **Echinocytes I** are red cells with an angular shape or short, blunt projections. They are often due to artifact, such as occurs with sample aging prior to smear preparation or excessive EDTA exposure.
	**Echinocytes III** are spherical red cells with sharp projections of equal length that are evenly spaced on the surface of the red cell. They may be increased in animals with renal disease and/or electrolyte disturbances. They can also occur artifactually for similar reasons described for echinocytes I.
	**Echinoelliptocytes** are oval to cigar-shaped red cells with projections of equal length that are evenly spaced on the surface of the red cell. They may be seen in cats with hepatobiliary disease and are rare in other species.
	**Elliptocytes** are oval to cigar-shaped cells. Red cells from Camelidae are normally elliptical.

Table 1.1 (*Continued*)

	Ghost cells are red cells that have been leached of hemoglobin. They are evidence of intravascular hemolysis.
	**Hypochromasia** refers to red cell pallor due to inadequate synthesis of hemoglobin. Hypochromic red cells have a large area of central pallor that gradually darkens towards the periphery of the red cell. Immature RBCs may appear hypochromic due to their large size. Small (microcytic), hypochromic RBCs can be seen with iron deficiency and disorders of iron utilization.
	**Keratocytes** are crescent-shaped cells. They are formed from mechanical shearing (usually due to fibrin strand deposition) of the red cell. Keratocytes are often accompanied by schizocytes (fragments).
	**Leptocytes** are thin, macrocytic red cells with a membrane surface area that exceeds hemoglobin content. The membrane tends to wrinkle or fold, forming twisted (like figure 8) cells. They are sometimes seen with hepatic disease.
	**Macrocytes** (left) are larger than normal red cells.
	**Microcytes** (right) are smaller than normal red cells.
	**Polychromasia** refers to red cells that appear blue–gray with Romanowsky dyes. They correspond to reticulocytes on blood films stained with supravital dyes (e.g., new methylene blue, NMB). Polychromatophils are young cells with a high RNA content and, as such, are larger than mature red cells and have a different staining character. Increased numbers indicate red cell regeneration.
	**Reticulocytes** can be identified on blood films stained with supravital dyes. NMB precipitates nucleic acids (like RNA) as dark blue deposits. Increased numbers indicate red cell regeneration. They correspond to polychromatophils on Romanowsky-stained smears.
	**Rouleaux** are stacks of red cells. Equine and feline erythrocytes readily form rouleaux. Excessive rouleaux formation in any species may be associated with hyperproteinemia.
	**Schizocytes** are red cell fragments attributed to mechanical red cell injury/shearing (see keratocytes).
	**Spherocytes** (left) are small, dark, round RBCs that are formed by the removal of altered red cell membrane without concurrent loss of hemoglobin. Spherocytes have no central pallor. They may be seen with immune-mediated hemolytic anemia.

(*Continued*)

Table 1.1 (*Continued*)

	**Unclassified poikilocytosis** is used when red cell shape defies description. This term may be used to describe the peculiar (and often abundant) poikilocytosis seen in normal calves, deer, goats, and pigs, which may actually be an *in vitro* artifact.
	**Basophilic stippling** refers to diffuse blue speckling (with Romanowsky stains) within red cells. This basophilia is due to the presence of cytoplasmic RNA and reflects red cell immaturity. Increased numbers of red cells with basophilic stippling often accompany other features of red cell regeneration (especially in ruminants) such as polychromasia and reticulocytosis. Lead poisoning interferes with metabolic pathways in developing erythrocytes and may result in the presence of RBCs with basophilic stippling and metarubricytes in the peripheral blood when there is no anemia or only mild anemia.
	**Heinz bodies** are difficult to visualize with Romanowsky stains where they may be visible as eccentrically located refractile bodies or blebs on the periphery of the red cell. They are better visualized and quantified on blood films stained with NMB, where they stain greenish blue. They indicate oxidative damage to red cells and may be seen along with eccentrocytes. Small Heinz bodies may be seen in high numbers on blood films from normal, non-anemic cats.
	**Nuclear remnants** are small, round, dark purple, erythrocyte inclusions representing a portion of the otherwise extruded nucleus. They are usually single and located close to the periphery of the red cell. Excessive numbers may be seen post-splenectomy or with hypofunctioning of the spleen.
	**Nucleated red blood cells** (NRBCs) are enumerated per 100 leukocytes. Greater than 5 NRBCs/100 WBCs is significant and may indicate bone marrow damage or hypoxia. NRBCs may accompany a regenerative response when anemia is present, but should not be used as the only criterion of RBC regeneration. The total leukocyte count should be corrected if there are $\geq$5NRBCs/100 WBCs.

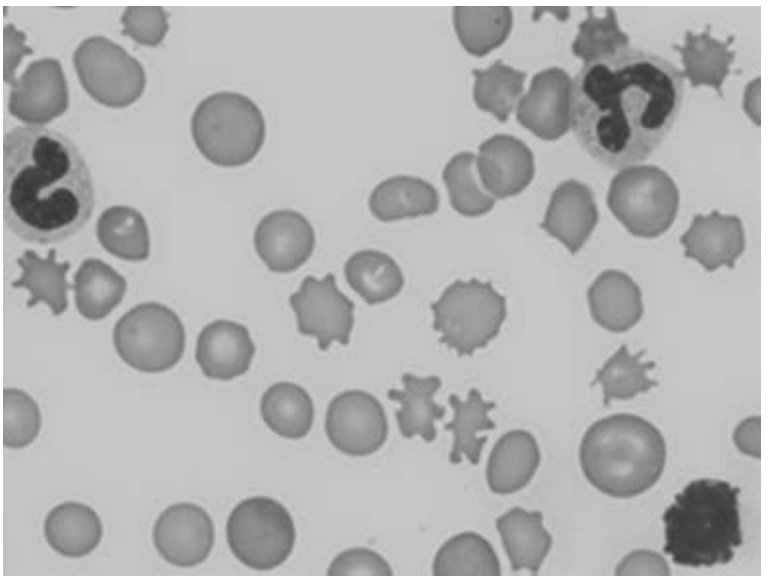


Figure 1.3 Canine blood film showing acanthocytes (also see color section).

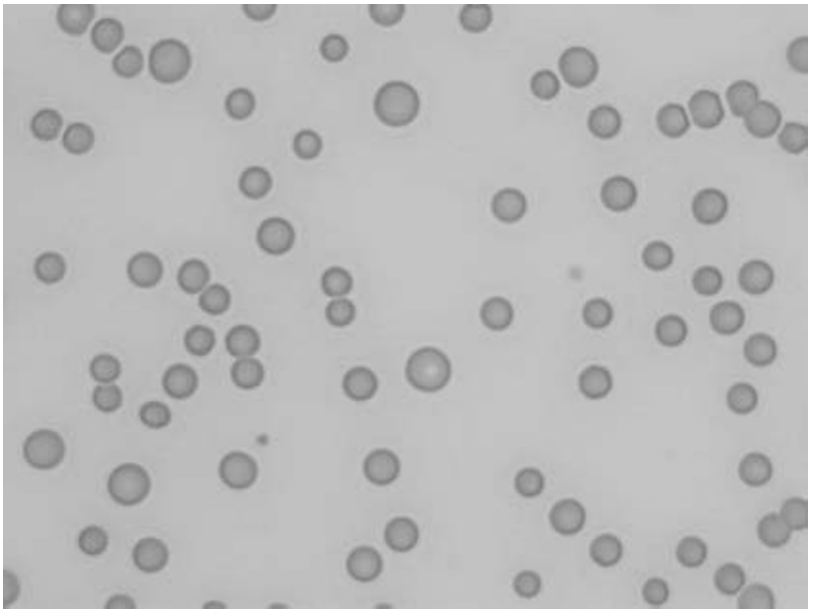


Figure 1.4 Canine blood film showing anisocytosis (also see color section).

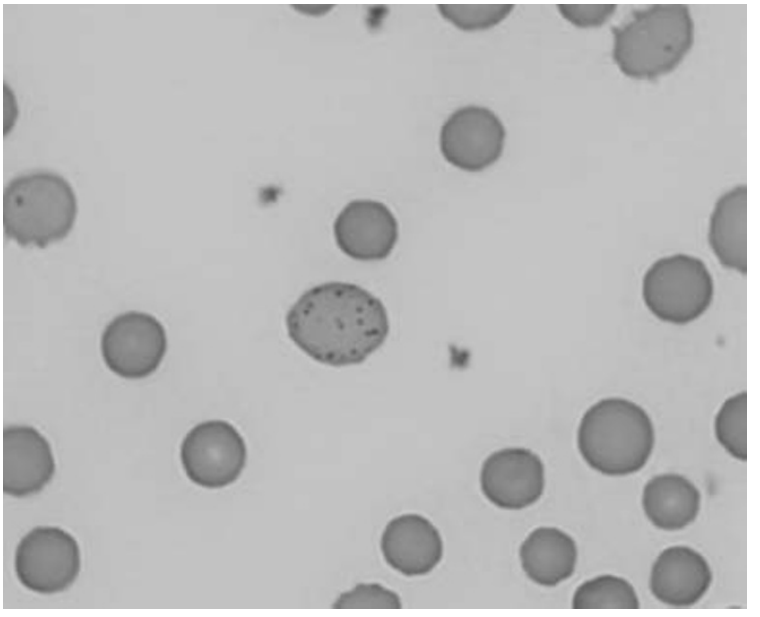


Figure 1.5 Bovine blood film showing basophilic stippling within a macrocyte as part of the regenerative response (also see color section).

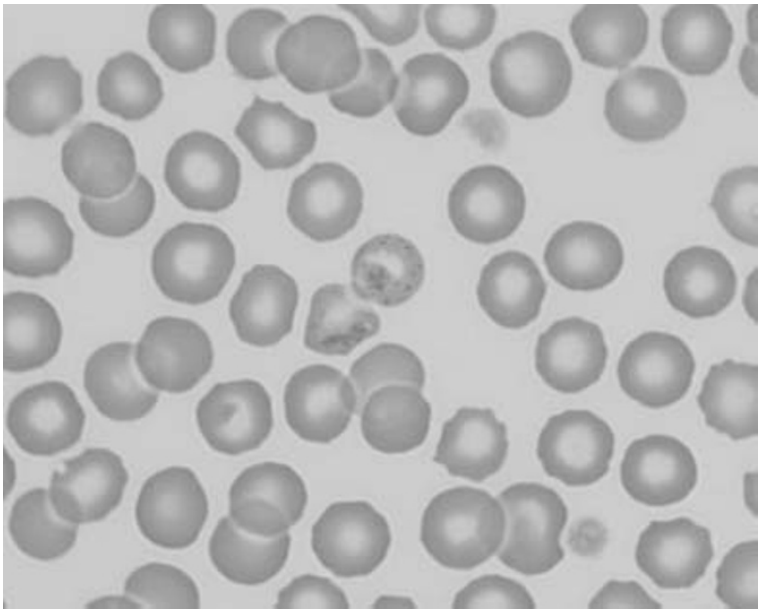


Figure 1.6 Canine blood film showing basophilic stippling due to lead toxicosis (also see color section).

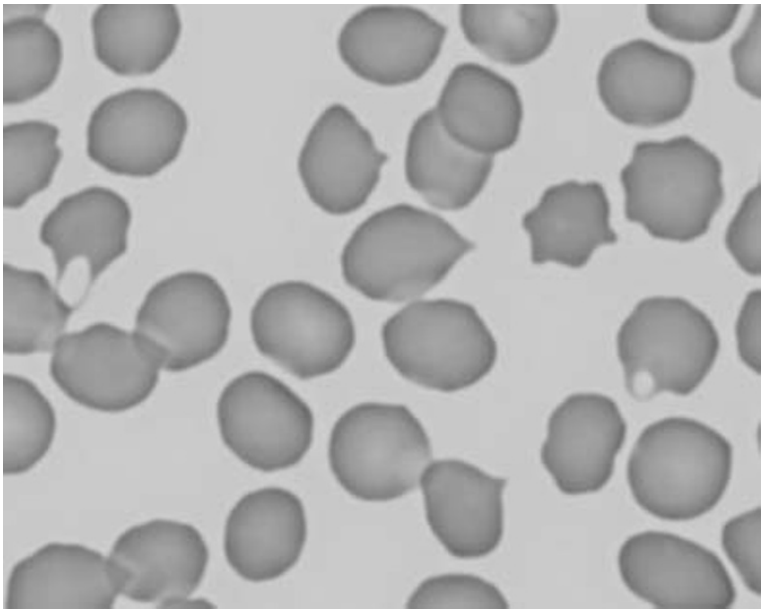


Figure 1.7 Canine blood film showing blister cells due to oxidative damage (also see color section).

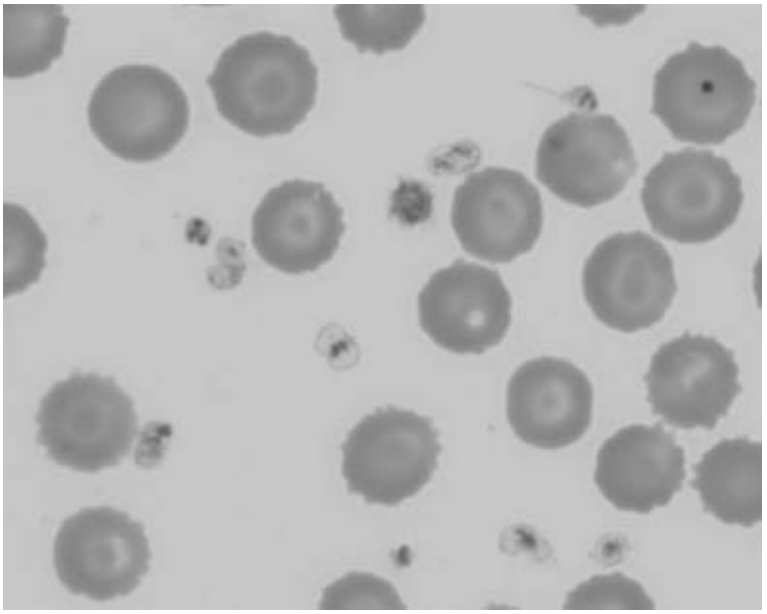


Figure 1.8 Canine blood film showing codocytes. Up to 50% codocytes may be normal in a dog (also see color section).

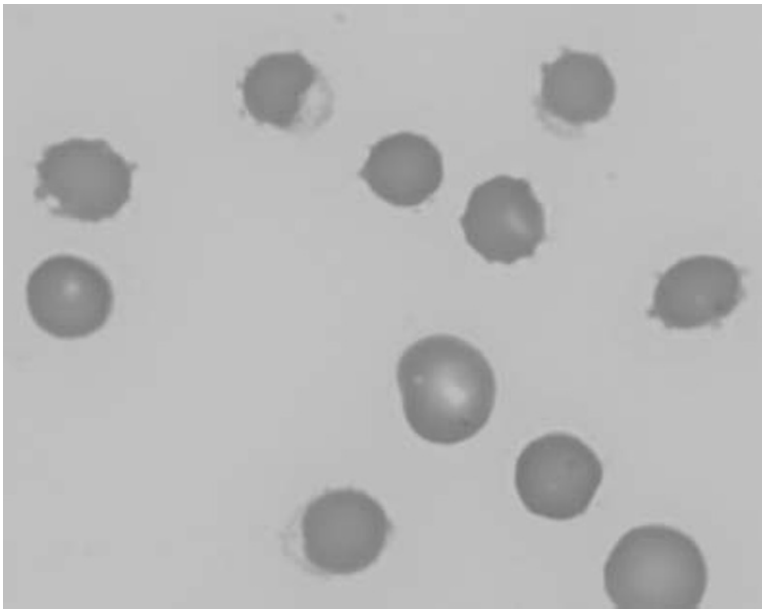


Figure 1.9 Canine blood film showing eccentrocytes due to oxidative damage (also see color section).

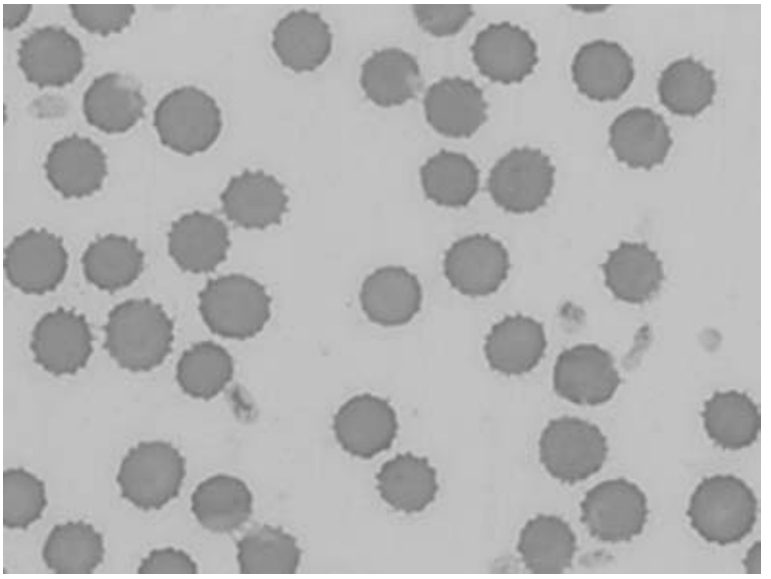


Figure 1.10 Canine blood film showing echinocytes I (also see color section).

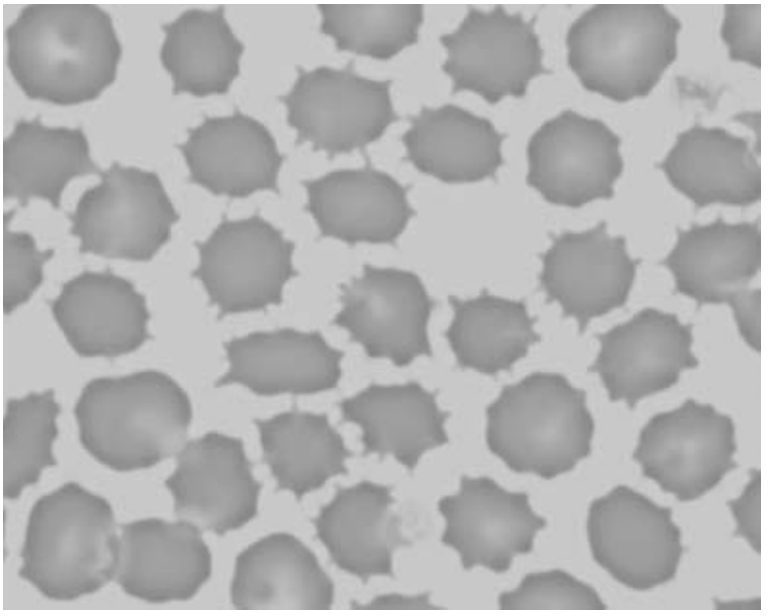


Figure 1.11 Canine blood film showing echinocytes III (also see color section).

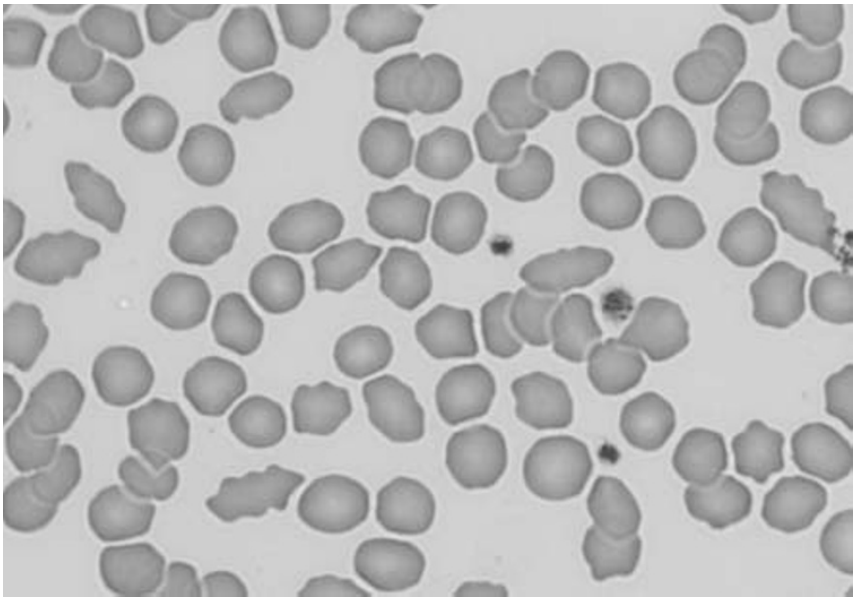


Figure 1.12 Feline blood film showing echinoelliptocytes (also see color section).

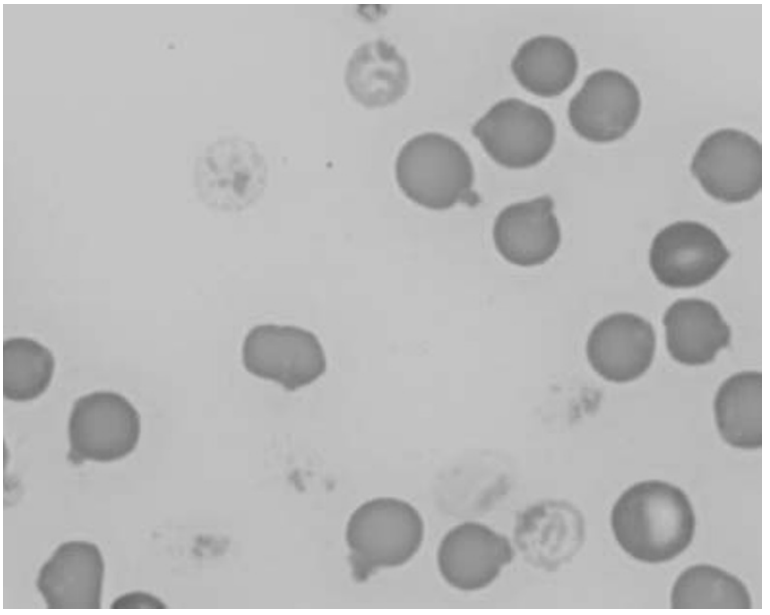


Figure 1.13 Canine blood film showing Heinz bodies and ghost cells due to oxidative damage. There are also several polychromatophils (also see color section).

Figure 1.14 Canine blood film stained with new methylene blue, to demonstrate Heinz bodies (also see color section).

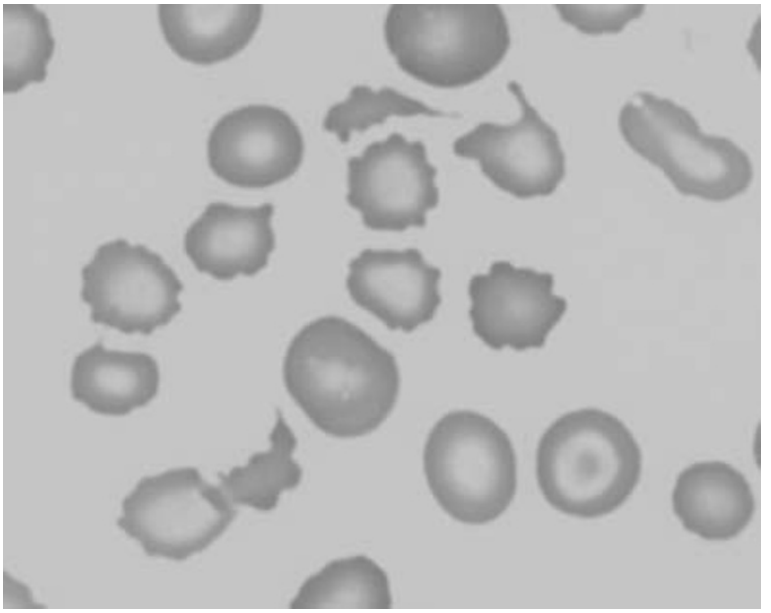


Figure 1.15 Canine blood film showing keratocytes and schizocytes (erythrocyte fragments) due to fibrin strand injury. A codocyte and a polychromatophil also appear in the field (also see color section).

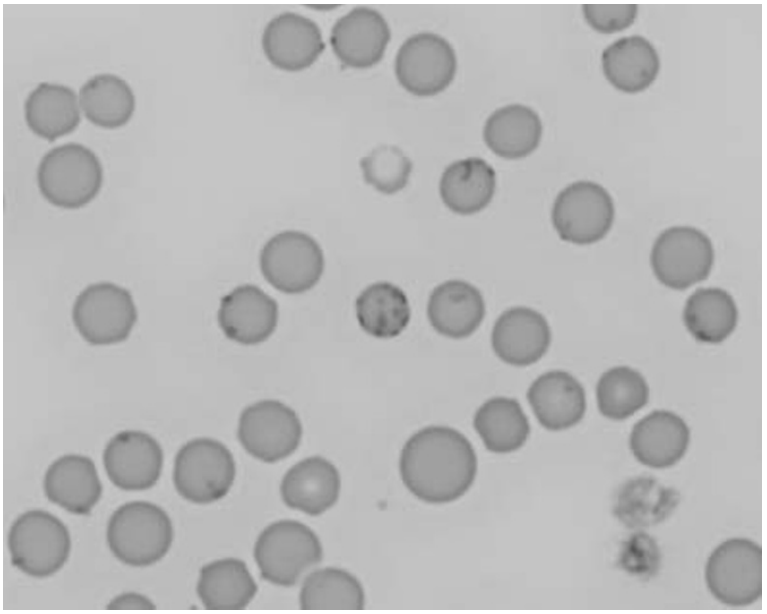


Figure 1.16 Feline blood film showing *Mycoplasma hemofelis* organisms (hemobartonellosis). Organisms are not always visible in blood smears from infected cats (also see color section).

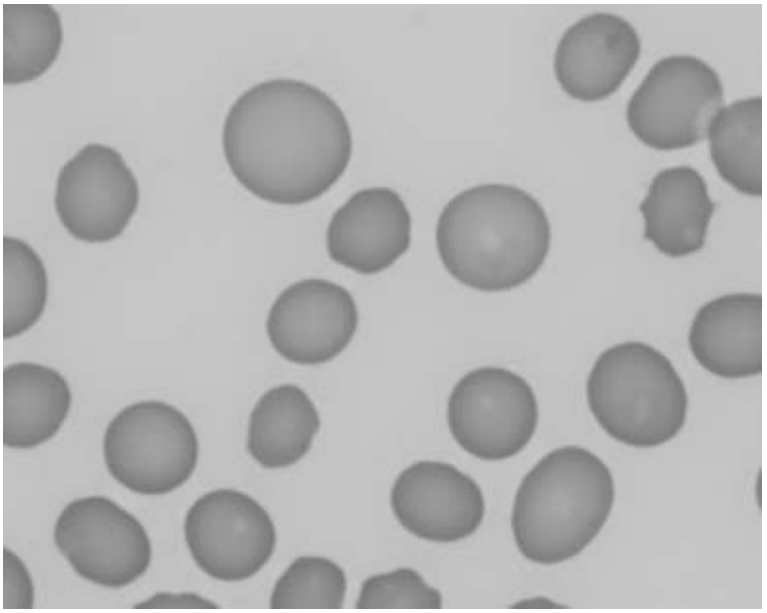


Figure 1.17 Canine blood film showing several macrocytes (also see color section).

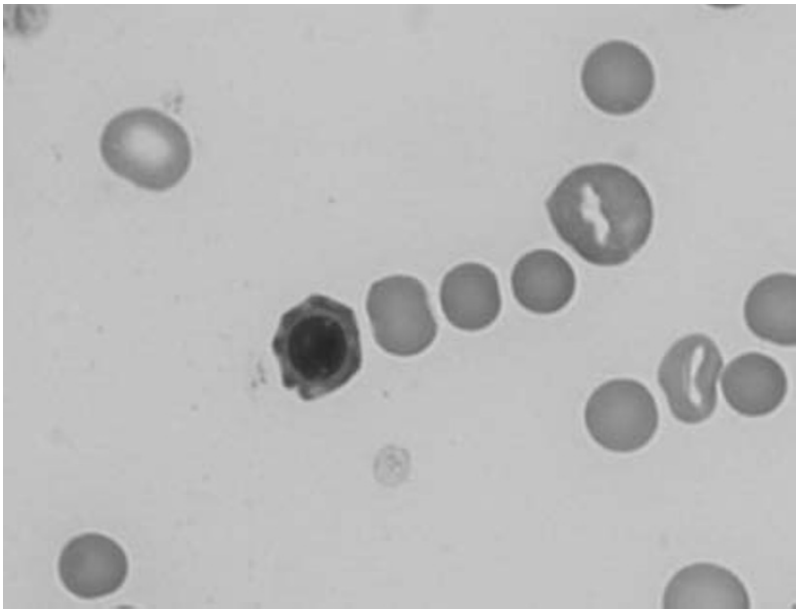


Figure 1.18 Canine blood film showing a metarubricyte There is also a polychromatophilic macrocyte in the field (also see color section).

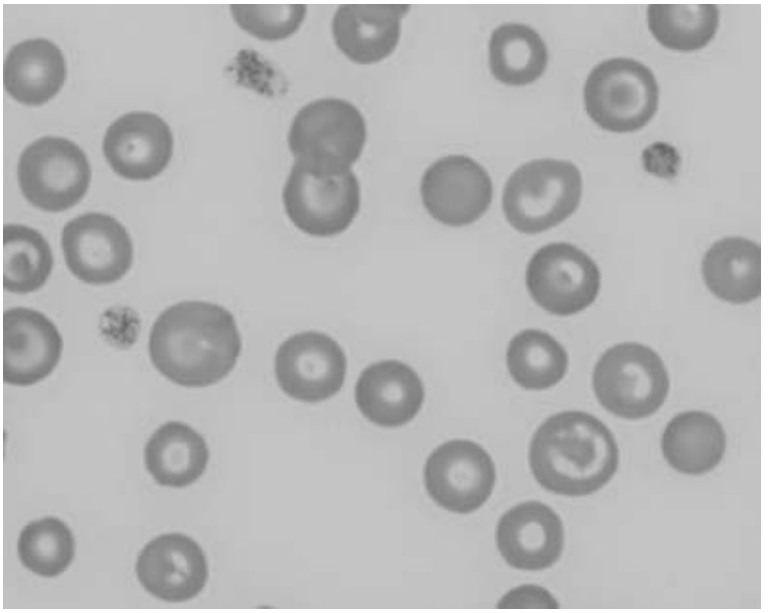


Figure 1.19 Canine blood film showing microcytic, hypochromic erythrocytes consistent with iron deficiency anemia. There are two polychromatophils in the field, indicating that the anemia is regenerative (also see color section).

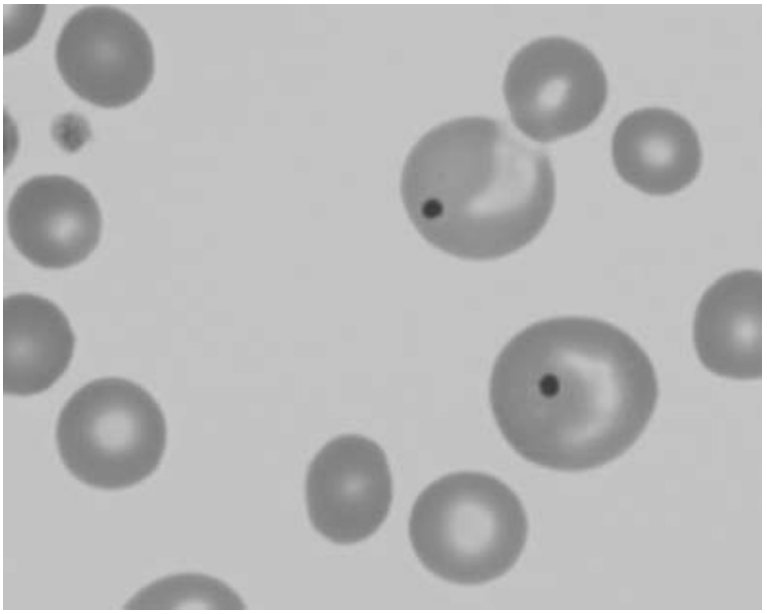


Figure 1.20 Canine blood film showing two macrocytes with nuclear remnants (also see color section).

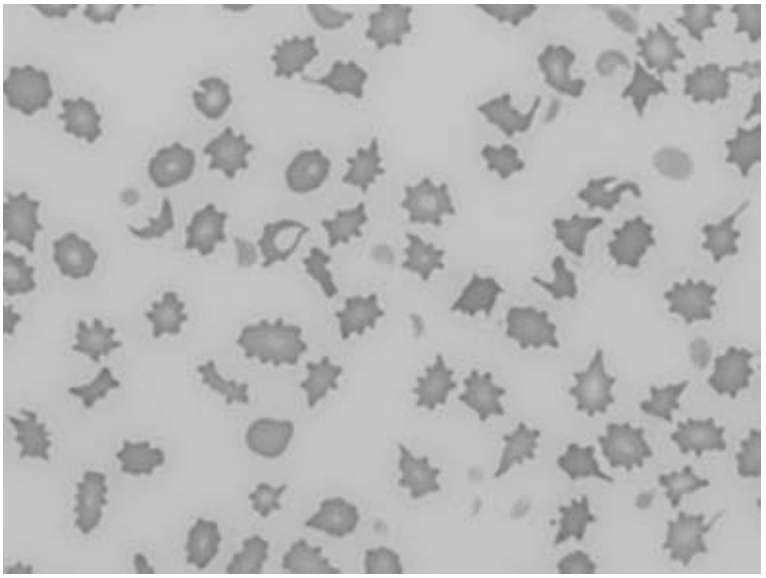


Figure 1.21 Bovine (calf) blood film showing marked poikilocytosis (normal?) (also see color section).

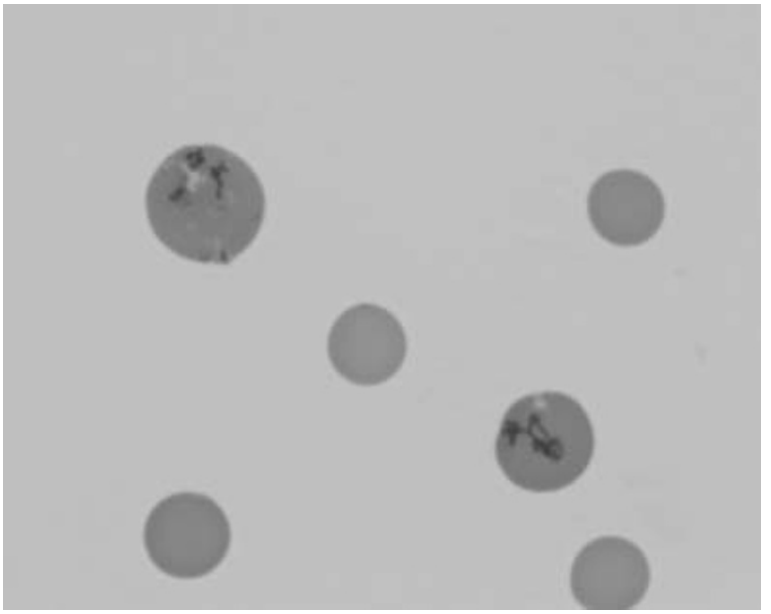


Figure 1.22 New methylene-blue-stained Canine blood film showing reticulocytes (also see color section).

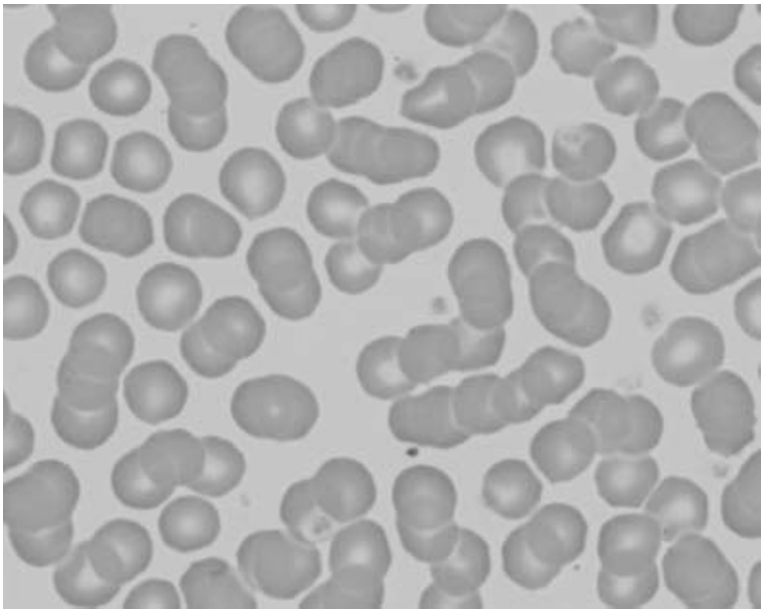


Figure 1.23 Canine blood film showing rouleaux formation (also see color section).