John E. Whalan

A Toxicologist's Guide to Clinical Pathology in Animals

Hematology, Clinical Chemistry, Urinalysis

Springer

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- Hematology
- Clinical Chemistry
- Urinalysis

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Much of this Work was first published in Chinese language in 2014 by Shanghai Popular Science Press under the title: 实验动物临床检验指南 (A Guide to Clinical Pathology in Animals).

ISBN 978-3-319-15852-5 ISBN 978-3-319-15853-2 (eBook)
DOI 10.1007/978-3-319-15853-2

Library of Congress Control Number: 2015936603

Springer Cham Heidelberg New York Dordrecht London
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Preface

For many toxicologists, the evaluation of hematology, clinical chemistry, and urinalysis data can be the most daunting part of animal toxicity studies. When dozens of parameters are measured for each animal at regular intervals throughout a study, there may be hundreds or even thousands of data points to consider.

What does it mean when a parameter value increases for an individual or for a group? What does it mean when it decreases? When a parameter change is statistically significant does that mean it is biologically significant? What other parameters can be used to strengthen a diagnosis? What is causing these changes? The answers to these questions can be found in veterinary clinical pathology textbooks, of course, and every toxicologist should own at least one or two; but searching for diagnostic information in textbooks can be difficult and time consuming.

Many years ago, I began keeping a notebook of key information and diagnoses for the clinical pathology parameters used in toxicology studies. As my notebook grew over the years into a handbook, I shared more than 150 copies with my fellow toxicologists. It is because of their favorable reviews and encouragement that my handbook has now been published.

The intent of this handbook is to provide a user-friendly resource that puts the most relevant information at your fingertips. It is written as one toxicologist to another. I sincerely hope you find this handbook to be useful.

I wish to thank my charming wife, Chipper, for her patience and support and for her pen drawing of a mouse. I also wish to thank my lovely daughters, Bridget and Lorena, for their encouragement; and my grandchildren, Kathleen, Alex, and Brooklyn, for their boundless curiosity. Finally, many thanks to Manika Power and her colleagues at Springer Publishing who brought this book to fruition.

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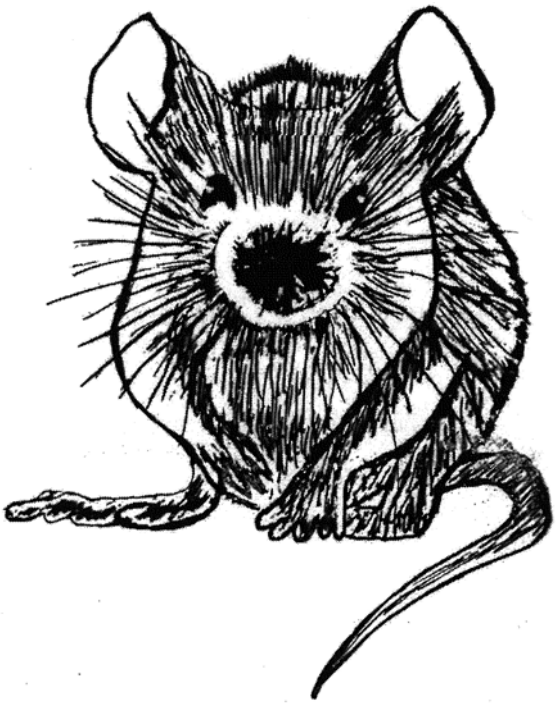

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Abbreviations

ACD	Acid citrate dextrose
ACTH	Adrenocorticotropic hormone
ADP	Adenosine 5′-diphosphate
A:G, A/G	Albumin/globulin ratio
Alb	Albumin
ALKP	Alkaline phosphatase
Alk Phos	Alkaline phosphatase
ALP	Akaline phosphatase
ALT, ALAT	Alanine aminotransferase
AP	Alkaline phosphatase
APTT, aPTT	Activated partial thromboplastin time
ASAT	Aspartate aminotransferase
AST	Aspartate aminotransferase
Bands	Band neutrophils
Bas, Basos	Basophils
Bicarb	Bicarbonate
Bili	Bilirubin, total
BT	Bleeding time
BUN	Blood urea nitrogen
Ca, Ca^{++}, Ca^{2+}, Calc	Calcium
CB	Conjugated bilirubin
CBC	Complete blood count
C-Bili	Conjugated bilirubin
ChE, CHE	Cholinesterase
Chol	Cholesterol, total
CK	Creatine kinase
Cl, Cl^{-}	Chloride
CMIR	Cell mediated immune response
CPK	Creatine phosphokinase
Cr, Cre, Creat	Creatinine

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CT	Clotting time
D-Bili	Bilirubin, direct
DIC	Disseminated intravascular coagulation
Diffs	Differential leukocyte count
dL	Deciliter
ECF	Extracellular fluid
EDTA	Ethylenediaminetetraacetic acid
Eos, Eosins	Eosinophils
EPO	Erythropoietin
ESR	Erythrocyte sedimentation rate
F	Fibrinogen
FBG	Fibrinogen
FCM	Flow cytometry
FDP	Fibrin degradation products
Fe^{3+}	Ferric iron
Fe^{2+}	Ferrous iron
g/mol	Grams per mole (molecular weight)
GDH	Gutamate dehydrogenase
GFR	Glomerular filtration rate
GGT	Gamma-glutamyl transferase
GGTP	Gamma-glutamyl transpeptidase
G.I.	Gastrointestinal
GLD, GLDH	Glutamate dehydrogenase
GLOB	Globulins
GLP	Good laboratory practices
GLU, Gluc	Glucose
GOT	Glutamic oxaloacetic transaminase
GPT	Glutamic pyruvic transaminase
GTP	Gamma glutamyl transpeptidase
Hb, HB	Hemoglobin
Hgb, HGB	Hemoglobin
HCO_3^-	Bicarbonate
HCT, Hct	Hematocrit
HSC	Hematopoietic stem cell
Ht	Hematocrit
I-Bili	Bilirubin, indirect
ICF	Intracellular fluid
I.M.	Intramuscular
I. Neut	Immature (band) neutrophils
I.P.	Intraperitoneal
IU	International units
I.V.	Intravenous
K, K^+	Potassium
LCFA	Long-chain fatty acid
LD, LDH	Lactate dehydrogenase

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LPF	Low-power field
Lym, Lymphs	Lymphocytes
M	Molarity
MAF	Macrophage activating factor
MCH	Mean corpuscular hemoglobin
MCHC	Mean corpuscular hemoglobin concentration
MCV	Mean corpuscular volume
M:E ratio	Myeloid:erythroid ratio
MetHb	Methemoglobin
Mg, Mg^{++}, Mg^{2+}	Magnesium
MIF	Migration inhibiting factor
mL	Milliliter
Mon, Monos	Monocytes
MPD	Myeloproliferative disorder
MPS	Mononuclear phagocyte system
N	Normality; nucleus
Na, Na^{+}	Sodium
N-Band	Band neutrophil
Neuts	Neutrophils
N:L	Neutrophil:lymphocyte ratio
5′-NT	5′-nucleotidase
NPN	Nonprotein nitrogen
nRBC, NRBC	Nucleated erythrocytes
N. Seg	Segmented neutrophils
NucRBC	Nucleated erythrocytes
ODC	Ornithine decarboxylase
OECD	Organisation for Economic Co-operation and Development
Osmol	Osmolality
P, P_i, Phos	Phosphorus, inorganic
pCO_2, $P(a)CO_2$	Partial pressure carbon dioxide
PCV	Packed cell volume (hematocrit)
PF	Plasma fibrinogen
PGOT	Plasma glutamic oxaloacetate transaminase
PGPT	Plasma glutamic pyruvic transaminase
pH	pH (acidity, alkalinity)
Plate	Platelets (thrombocytes)
PMN	Polymorphonuclear
pO_2, $P(a)O_2$	Partial pressure oxygen
PO_4^{3-}	Phosphate
PP:F	Plasma protein:fibrinogen ratio
Pro Time	Prothrombin time
PT	Prothrombin time
PTT	Partial thromboplastin time
RBC	Red blood cell (erythrocyte)
RES	Reticuloendothelial system

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Ret, Retics	Reticulocytes
SAP	Serum alkaline phosphatase
SDH	Sorbitol dehydrogenase
Sed. Rate	Erythroid sedimentation rate
Segs	Segmented neutrophils
SG	Specific gravity
SGOT	Serum glutamic oxaloacetic transaminase
SGPT	Serum glutamic pyruvic transaminase
SHBG	Sex hormone-binding globulin
SOP	Standard operating procedures
$t_{½}$	Half life
T-Bili	Bilirubin, total
Thromb	Thrombocytes (platelets)
TBA	Bile acids, total
TG	Triglycerides
TP	Total protein
T. PROT	Total protein
TRIG	Triglycerides
UA	Uric acid
UB	Unconjugated bilirubin
UCB	Unconjugated bilirubin
U-Bili	Unconjugated bilirubin
UN	Urea nitrogen
Urobl	Urobilinogen
WBC	White blood cell (leukocyte)

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Chapter 1
The Fundamentals

Abstract The Fundamentals chapter covers basic information that every toxicologist needs to know when evaluating animal hematology, clinical pathology, and urinalysis data. It begins with a description of the clinical pathology tests routinely required by animal toxicity test guidelines to assess the health status of animals and discover evidence of toxicity. The value of using clinical pathology panels to further investigate specific diseases and toxicity is explained. This is followed by a description of reference ranges ('normal' ranges) and how they can be used to recognize anomalous values that may indicate the presence of disease or toxicity. The importance of identifying 'outlier' values is stressed along with the reasons why they should be eliminated from consideration. There is a discussion of the pitfalls of statistical analysis and how to distinguish between statistical and biological significance. Finally, the importance of considering multiple pieces of data is stressed to establish a weight-of-the-evidence diagnosis of animal health and toxicity.

1.1 Introduction

Clinical pathology provides a quantifiable way to assess animal health and to diagnose disease and toxicity. Organic damage can be detected within a few seconds to a few days, and often before clinical signs and microscopic lesions appear. Dozens of tests can be performed using small volumes of blood or urine. These tests are more revealing than merely observing an animal and far less traumatic than biopsy or surgery. Clinical pathology measurements are routinely used to:

- screen animals to uncover illness, toxicity, and genetic disease,
- diagnose disease,
- monitor the progression or reversal of disease and toxicity, and
- monitor therapeutic drug regimens.

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J.E. Whalan, *A Toxicologist's Guide to Clinical Pathology in Animals*,
DOI 10.1007/978-3-319-15853-2_1

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Clinical pathology encompasses the following:

Hematology—The study of the cellular components of the blood including erythrocytes (red blood cells), leukocytes (white blood cells), thrombocytes (platelets), and the blood forming tissues (e.g. bone marrow).
Clinical Chemistry—The study of the chemical composition of the liquid portion of blood.
Urinalysis—The study of the chemical and cellular composition of urine.
Cytology—The study of cells, including their origin, structure, function, and pathology.
Parasitology—The study of parasites and parasitism (not covered in this guide).

This handbook provides diagnostic hematology, clinical chemistry, and urinalysis information and briefly describes the formation of the various blood cells. Readers seeking information on myeloproliferative disorders and parasitology are advised to search textbooks that specialize in these topics.

1.2 Routine Clinical Pathology Testing

In 1996, the Joint Scientific Committee for International Harmonization of Clinical Pathology Testing (IHCPT), which represents ten scientific organizations, provided minimum clinical pathology test recommendations for use in routine animal toxicity and safety studies (Weingand et al. 1996). These recommendations have been widely accepted and continue to be followed to this day.

The Organisation for Economic Co-operation and Development (OECD) has developed guidelines for an array of toxicity studies by multiple routes of exposure (OECD 2009). Each test guideline lists recommended clinical pathology tests. These guidelines are internationally agreed testing methods used by government, industry and independent laboratories to identify and characterize potential hazards of new and existing chemical substances. Toxicology test guidelines can be found at this OECD website: http://www.oecd-ilibrary.org/environment/oecd-guidelines-for-the-testing-of-chemicals-section-4-health-effects_20745788.

The U.S. Environmental Protection Agency has also recommended clinical pathology parameters for its various test guidelines (U.S. EPA 2007). Toxicology test guidelines can be found at this EPA website: http://www.gpo.gov/fdsys/granule/CFR-2007-title40-vol31/CFR-2007-title40-vol31-part798/content-detail.html.

Clinical pathology recommendations can vary depending on which test guideline is being used. Table 1.1 summarizes the IHCPT, OECD, and U.S. EPA recommendations for hematology, clinical chemistry, and urinalysis testing for a chronic toxicity study. Additional tests may be performed as needed for diagnostic purposes.

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Table 1.1 IHCPT, OECD, and U.S. EPA recommended clinical pathology tests for a chronic toxicology study

Hematology test	**IHCPT**[a]	**OECD**[b]	**U.S. EPA**[c]
Erythrocyte (RBC) count	√	√	√
Red blood cell morphology	√		
Reticulocytes	O		
Hemoglobin concentration	√	√	√
Hematocrit (packed cell volume)	√	√	√
Mean corpuscular volume (MCV)	√	√	
Mean corpuscular hemoglobin (MCH)	√	√	
Mean corpuscular hemoglobin concentration (MCHC)	√	√	
Total leukocyte (WBC) count	√	√	√
Absolute differential leukocyte count	√	√	√
Platelet (thrombocyte) count	√	√	√
Prothrombin time	√	√	
Activated partial thromboplastin time	√	√	
Bone marrow cytology slides	O		

Clinical pathology test	**IHCPT**[a]	**OECD**[b]	**U.S. EPA**[c]
Alanine aminotransferase (ALT)	√	√	√
Albumin	√	√	√
Alkaline phosphatase (ALP)	√	√	
Aspartate aminotransferase (AST)	√	√	√
Bilirubin, total	√	√	√
Blood urea nitrogen (BUN)	√	√	√
Calcium	√	√	√
Cholesterol, total	√	√	√
Chloride			√
Creatine phosphokinase			√
Creatinine	√	√	√
Gamma glutamyltransferase (GGT)	√	√	√
Globulin	√		
Glucose	√	√	√
Glutamate dehydrogenase	√	√	
5′-Nucleotidase (5′-NT)	√	√	
Ornithine decarboxylase	NR		√
Phosphorus			√
Potassium	√	√	√
Sodium	√	√	√
Sorbitol dehydrogenase (SDH)	√		
Total bile acids (TBA)	√	√	
Total protein	√	√	√

(continued)

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Table 1.1 (continued)

Urinalysis test	IHCPT[a]	OECD[b]	U.S. EPA[c]
Appearance (color and turbidity)	√	√	√
Bilirubin		√	√
Glucose	√	√	√
Ketone		√	√
Microscopy of sediment	NR		√
Occult blood		√	√
pH	√	√	
Total protein	√	√	√
Specific gravity (osmolality)	√	√	√
Urobilinogen		√	
Volume	√	√	√

NR not recommended, *O* optional when indicated
[a]Weingand et al. (1996)
[b]OECD (2009)
[c]U.S. EPA (2007)

1.3 Clinical Pathology Panels

A clinical pathology *panel* (also known as a *battery* or *profile*) is a collection of tests routinely performed to confirm good health, or to diagnose a disease or toxic effect. If, for example, one wishes to determine whether an animal has liver disease, a hepatic panel such as this might be performed:

Hepatic panel	
ALT (small animals)	Bile acids
AST	5′-NT
SDH (large animals)	Urine bilirubin
AP	Urine urobilinogen
GGT	Total protein (TP)
GLDH	Albumin
SDH	Protein electrophoresis
Total bilirubin	Prothrombin time (PT)
Direct bilirubin	Partial thromboplastin time (PTT)

Because each parameter in this panel has its own advantages, limitations, and specificity for diagnosing liver damage, the measurement of multiple parameters increases the likelihood of a correct diagnosis through a weight-of-evidence approach. A panel may be modified as prudence and costs allow. A collection of panels can be found in Chap. 9.

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1.4 How to Evaluate Clinical Pathology Data

When evaluating clinical pathology data, one looks for test values that suggest something may be adverse in an animal. An *anomaly* is any value judged to be significantly outside of a 'normal' range or *reference range* for healthy animals. Anomalies provide clues to the presence and cause of disease or toxicity. The following sections discuss how to evaluate clinical pathology data and stress the importance of using multiple tests in conjunction with histopathology data to diagnose disease and toxicity. Also discussed are confounding factors and complications, the use of reference ranges, and how to determine whether a statistically significant anomaly is biologically (clinically) significant.

1.4.1 Weight of the Evidence

When evaluating clinical pathology data, a toxicologist must think like a detective. All evidence must be considered together. One clue is seldom enough. Information that is limited or erroneous can lead to misdiagnosis. Would you undergo surgery based on a single clinical pathology value?

Ideally, one anomalous parameter should be substantiated with other parameters and, if possible, with histopathology and organ weight data. As shown in the example below, if an animal in a toxicity study has elevated aspartate aminotransferase (AST), it may have damage to the liver, cardiac muscle, or skeletal muscle. It is also possible that the AST measurement is erroneous. If, however, the animal also has elevated levels of alkaline phosphatase (ALP), alanine aminotransferase (ALT), and gamma-glutamyl transferase (GGT), it is clear that the liver is affected because the only organ common to all four parameters is the liver:

Parameter	Target organs
AST	*Liver*, cardiac muscle, skeletal muscle
ALP	*Liver*, bone, thyroid
ALT	*Liver*, cardiac and skeletal muscle
GGT	*Liver*, cardiac muscle, pancreas, kidney

The degree of deviation of each parameter from its reference range indicates the extent of liver damage. Histopathology can confirm and further characterize the nature of liver disease. It is worth noting that hepatocellular hypertrophy and increased liver weight, common findings in toxicity studies, are generally not adverse findings but rather evidence of adaptation in a healthy liver due to increased endoplasmic reticulum in response to a xenobiotic (Hall et al. 2012).

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1.4.2 What Can Go Wrong?

Well, actually a lot can go wrong when collecting and analyzing biological samples. Mistakes and confounding factors are minimized when a laboratory has good training, complies with Good Laboratory Practices (GLPs) and their own Standard Operating Procedures (SOPs), and when analytical equipment is regularly maintained and calibrated. Still, errors happen; which is why it is so important to understand how biological factors, analytical glitches, and technician mistakes can impact clinical pathology data and one's evaluation of these data:

Biological Factors

- *Sex*—Increased muscle mass in reproductive males results in increased creatinine, creatine kinase, cholesterol, triglycerides, urate, and triiodothyronine. Reproductive females have increases in alanine and aspartate aminotransferases, alkaline phosphatase, total protein, albumin, calcium, magnesium, aldolase, prolactin, and growth hormone; and decreases in hemoglobin, bilirubin, iron, and ferritin. Most of these effects are caused by estrogen. [See also *Reproductive Effects* below.]
- *Diet*—Serum sodium, potassium, phosphate, chloride, magnesium, alkaline phosphatase, bilirubin, urea, glucose, cholesterol, triglycerides, total proteins, albumin, and occult fecal blood can increase or decrease depending on the diet.
- *Time of day*—Daily cyclic changes occur in serum cortisol, aldosterone, growth hormone, prolactin, thyroid stimulating hormone, renin, and testosterone.
- *Stress*—Plasma cortisol, growth hormone, prolactin, serum iron, ferritin cholesterol, and urinary catecholamines are altered by stress. Stress can be caused by animal shipment and handling.
- *Posture*—Going from a lying to standing posture shifts body water from the vascular compartment to the interstitial compartment causing <10 % increases in serum or plasma protein, albumin, cholesterol, triglycerides, bilirubin, alkaline phosphatase, alanine and aspartate aminotransferases, other enzymes, protein—bound hormones, iron, and the 50 % of calcium that is bound to albumin.
- *Aging of animals*—Serum phosphate and alkaline phosphatase decrease after puberty, and cholesterol and creatinine levels increase in adults. Many hormones decrease with age (e.g. testosterone, angiotensin, and renin).
- *Exercise*—Serum creatine kinase, lactate dehydrogenase, aspartate aminotransferase, urea, creatinine, urate, calcium, magnesium, growth hormone, cortisol, prolactin, and plasma renin and catecholamines are increased by exercise or exertion.
- *Health status*—In any group of animals, there are likely to be several individuals that appear healthy but are actually ill.
- *Hydration of the animal*—The lack of water (e.g., defective automatic watering devices) or overhydration (e.g. IV administration of fluids) can concentrate or dilute many analytes.

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- *Reproductive effects*—During pregnancy, plasma volume increases approximately 30 % and erythrocyte volume increases. There is a decrease in serum urea, creatinine, proteins, albumin, iron, sodium, potassium, calcium, and magnesium; and an increase in ceruloplasmin, transferrin, some proteins, lipoproteins, cholesterol, triglycerides, and placenta—derived enzymes (e.g. alkaline phosphatase). Menstrual and estrous cycle fluctuations are seen in luteinizing hormone, estrogens, and progesterone. Rats have leukopenia during estrus.
- *Biological variation*—Intra-individual and inter-individual variations.
- *Genetic factors*—High-normal, and low-normal values for individuals are usually an expression of genetic diversity. In the case of inbred laboratory strains, whole populations may have high or low normal values since they share nearly identical genetic information, and they are susceptible to genetic drift.
- *Genetic drift*—A shift in clinical pathology values for an animal strain over several generations.
- *Environmental influences*—Seasonal changes are due to such factors as changes in activity level, length of day, temperature, and diet. Environmental influences tend to be minimal in a toxicology laboratory. Cedar chip bedding or excessive ammonia can induce liver enzymes.
- *Differences between animal studies*—Whenever comparing data from two or more studies, consider differences in age, sex, diet, strain, health, environment, stress, husbandry, and technicians.

Analytical Glitches

- *Inaccuracy*—Deviation from the true value due to procedural and reagent deviations, and differences in analyzers, technicians, and sampling techniques.
- *Imprecision*—A lack of repeatability in a series of measurements due to mechanical, electronic, and chemical variations. Imprecision is expressed in standard deviations.
- *Drug interactions*—Anomalies may be due to physiologic effects or chemical or physical interference with the analytical method.
- *Iatrogenic effects*—Surgery and intramuscular injections can cause increases in creatine kinase, lactate dehydrogenase, aspartate aminotransferase, and aldolase. Squeezing an animal can cause huge increases in liver enzymes.

Technician Errors

- *Errors in labeling*—Mixing up samples is a persistent human error that is difficult to detect.
- *Use of the wrong anticoagulant*—Using the wrong anticoagulant, failure to use one when needed, or using one when one is not called for can make a sample useless. Also, having too much anticoagulant for the volume of blood collected can result in a faulty value due to dilution.
- *Improper or lengthy storage of samples*—A failure to speedily analyze or refrigerate samples can have a significant effect on the measurement of some parameters such as blood gases, glucose, inorganic phosphorus, electrolytes, nitrogenous compounds, and enzymes.

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- *Improper centrifugation*—When blood is improperly centrifuged, cellular components may be released into the plasma, which may affect analysis.
- *Contamination of the sample*—Urine is often contaminated with fecal matter. Blood samples may be diluted with interstitial fluid. Dirty sampling equipment can also be a source of contamination.

1.4.3 Reference Ranges

Every toxicology laboratory should periodically develop its own *reference ranges* for the animals they use based on species and strain, age, gender, animal supplier, sample collection methods, and analytical methods. A reference range is a 'statistically normal' range for a measured parameter with upper and lower limits encompassing 95 % of a group's values (±2 standard deviations). In theory, the remaining 5 % may be thought of as being slightly outside the normal range. Reference ranges should be periodically updated to account for new analyzers and *genetic drift* across several generations of animals.

Reference ranges are based on values from healthy, untreated animals, and they should not include outliers that might skew the ranges. An *outlier* is a test value for a single animal that is markedly different from others in its group. An outlier may result from illness or an error in sampling, storage, or measurement. It may also be iatrogenic, such as elevated liver enzymes when a rodent is squeezed during bleeding.

Because inbred animals have little biological variation between individuals, their reference ranges tend to be narrow; whereas outbred animals, such as dogs and primates, can have considerable intra-individual variation and thus broader reference ranges. When evaluating data from outbred animals, it is a good idea to consider baseline (pre-treatment) data for individual animals because what seems to be an abnormally high or low value may, in fact, be normal for a given animal. Reference ranges can come from three sources, ranging from most relevant to least relevant:

Concurrent Controls Reference ranges are based on control animals from the same study as the treated animals.

Advantages

- The reference ranges are always applicable because they are based on the same source and population of animals.
- The control animals experience the same husbandry, handling, and environmental conditions as the treated animals.
- The reference ranges reflect all changes due to aging, sampling, testing equipment, etc.

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Disadvantage

- The reference ranges may be based on a small population.

Baseline Values Reference ranges are based on pretreatment values for the animals on study.

Advantage

- The reference ranges are based on the actual animals in a study, so each animal can be compared against its own baseline (pretreatment) values and also group means.

Disadvantages

- The reference ranges are constant throughout a study. They do not change as animals age, and they do not reflect changes in sampling, testing equipment, etc.
- The reference ranges may be based on a small population.

Historical Values Reference ranges are based on a large number of untreated (negative control) animals from recent studies of the same species and strain.

Advantage

- The reference ranges represent a large population of animals.

Disadvantages

- Historical conditions may differ from current conditions with regards to age, sex, strain, supplier, diet, fasting, husbandry, anesthesia, sampling site (e.g. tail vein, jugular vein, orbital plexus), sampling technique, storage, technicians, reagents, testing equipment, etc.
- Historical reference ranges from an animal supplier (e.g. Charles River®) may not reflect the conditions of the laboratory where the animals are housed and tested.
- Historical reference ranges are susceptible to *genetic drift*, which can lead to misinterpretation.

Although concurrent control data are the ideal source for reference ranges, there may be too few animals to derive robust reference ranges. One way around this problem is to pool a laboratory's control values from two or more recent studies performed in the same animal species and strain. Reference ranges are not provided in this handbook because: (1) ranges can vary considerably from species-to-species and from laboratory-to-laboratory and (2) ranges can become obsolete after several years due to genetic drift. Published reference ranges should be used with caution.

Reference ranges are routinely reported in laboratory study reports but they are often omitted from journal articles. A risk assessor is at a distinct disadvantage when reference ranges are not reported.

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1.4.4 Statistical and Biological Significance

> *He uses statistics as a drunken man uses lamp posts—for support rather than illumination.*
> (Andrew Lang (1844–1912, Scottish writer and critic))

Andrew Lang's 'lamp post' analogy is highly relevant to clinical pathology data. Although statistical analyses are routinely used to identify clinical pathology anomalies, they can frequently lead to misdiagnosis. Indeed, just because a computer says a value is statistically significant does not mean it is biologically or clinically significant. When interpreting clinical pathology data, it is *biological (clinical) significance* that matters, not *statistical significance*. The Joint Scientific Committee for International Harmonization of Clinical Pathology Testing offered the following counsel regarding statistics (Weingand et al. 1996):

> Appropriate statistical methods should be used to analyze clinical pathology data (Gad and Weil 1989). Regardless of the outcome of statistical analysis, scientific interpretation is necessary for the ultimate determination of test material treatment effects. Statistical significance alone should not be used to infer toxicological or biological relevance of clinical pathology findings. Additionally, the absence of statistical significance should not preclude the possibility that test material treatment effects exist. The concurrent control data are more appropriate than historical reference ranges for comparison with test material treatment groups.[1]

The following examples demonstrate why a statistical analysis is a poor substitute for experience and judgment:

- A group of rats has statistically significant decreases in erythrocytes, hematocrit, and hemoglobin, which suggests the rats might be anemic. Considering that the decreases are only 6 % for all three parameters, these decreases are neither biologically significant nor evidence of anemia.
- A sizeable <u>increase</u> in alkaline phosphatase is often an indication of liver damage, especially when supported by histopathological evidence. Conversely, a <u>decrease</u> in alkaline phosphatase, which can occur when animals are fasted prior to bleeding, is not clinically significant. It is surprising how often journal articles report a statistically significant decrease in alkaline phosphatase and erroneously attribute it to liver toxicity.
- Normal AST values in rats are about 100 IU/L. Occasionally, one rat in a group may have an extremely high value (e.g., 5,000 IU/L). This can happen if a technician squeezes the rat while collecting blood, causing a release of AST into the bloodstream. A single value this large can raise the mean for an entire group to a statistically significant level. The squeezed rat's AST value is an *outlier* and must be eliminated from group consideration, but a statistical program cannot make this distinction. Just as it would be a mistake to diagnose this single rat as having liver damage, it would be worse to misdiagnose the whole group as having liver damage.

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These examples demonstrate the need to carefully consider individual animal data, especially when the data seem illogical. Or as William W. Watt, an English sports journalist, said,

> *Do not put your faith in what statistics say until you have carefully considered what they do not say.*

Confidence in a diagnosis increases when a group of animals is evaluated together. In a toxicology or clinical study, for example, periodic clinical pathology measurements are made and group data for each parameter are presented as means and standard deviations. The values for dosed animals are compared against those of the controls. This makes it possible to diagnose and monitor illness and to identify dose-related toxic effects, adaptations, and reversals over time. Generally, the more a value exceeds a reference range, the more severe the effect.

No one-size-fits-all threshold exists for biologically significance in a given test. There are many contextual factors that may increase or decrease concern for certain changes. Just because a value is outside of a reference range does not necessarily mean it is biologically significant. For parameters that are regulated within narrow limits, such as the electrolytes Na, Cl, and K, a small change of perhaps 10 % beyond the upper or lower range may be biologically significant. Conversely, parameters with more variability require larger changes before they may be considered biologically significant. For liver enzymes (ALP, ALT, AST, GGT, and SDH), a threefold elevation beyond an upper range is generally considered to be biologically significant and evidence of hepatotoxicity; but this is only a rule-of-thumb.

Clinical pathology interpretation is subjective and can vary from one toxicologist to another. For every parameter, the threshold for biological significance should be based on the context of sound study design and analysis including sample size and statistical power. An evaluation should take into account attributes of individual species because what is abnormal in most species may be normal in the species under consideration (see Chap. 4, Species Specifics).

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Chapter 2
Hematology Highlights

Abstract The Hematology Highlights chapter begins with a description of hematopoiesis—the process by which all blood cell species are formed—that takes place primarily in the bone marrow but also in the liver, and spleen. A section on erythrocytes (RBCs) examines the causes of anemia, the morphologic classifications of anemias, the process whereby healthy bone marrow responds to anemia, the causes of erythrocytosis, and 24 major causes of hemolysis. The section on leukocytes (WBCs) describes the formation of neutrophils, lymphocytes, eosinophils, monocytes, and basophils and the role each plays in the immune system. A discussion of differential leukocyte counts describes how each of the leukocyte species can be presented as either percentages of WBCs or as absolute cell counts. An example illustrates why evaluating differential cell counts only as percentages can often lead to a misdiagnosis. Finally, the formation of thrombocytes (platelets) and the key role they play in hemostasis are described.

2.1 Hematopoiesis

In a process called *hematopoiesis*, all blood cell types are produced through pathways that begin with multipotent stem cells called *hemocytoblasts* and *hemoblasts*. Figure 2.1 illustrates the hematopoietic pathways for each blood cell type. Each cell lineage undergoes a series of proliferative phases (mitotic division) and maturation phases, after which they enter a storage pool of mature cells for release into general circulation and, in the case of some leukocytes, distribution to the body. All blood cell types except thrombocytes undergo a series of mitotic divisions followed by several maturation stages during which they shrink in size. Thrombocyte production is unique in that the marrow precursor cells undergo polyploidy without cell division. This results in very large cells with multilobed nuclei that then break up into hundreds of platelets. Hematopoiesis primarily occurs in the bone marrow but it can also occur in the spleen and liver, especially in laboratory mice and rats. The rodent spleen produces mostly erythrocytes but it can also produce granulocytes and megakaryocytes.

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J.E. Whalan, *A Toxicologist's Guide to Clinical Pathology in Animals*,
DOI 10.1007/978-3-319-15853-2_2

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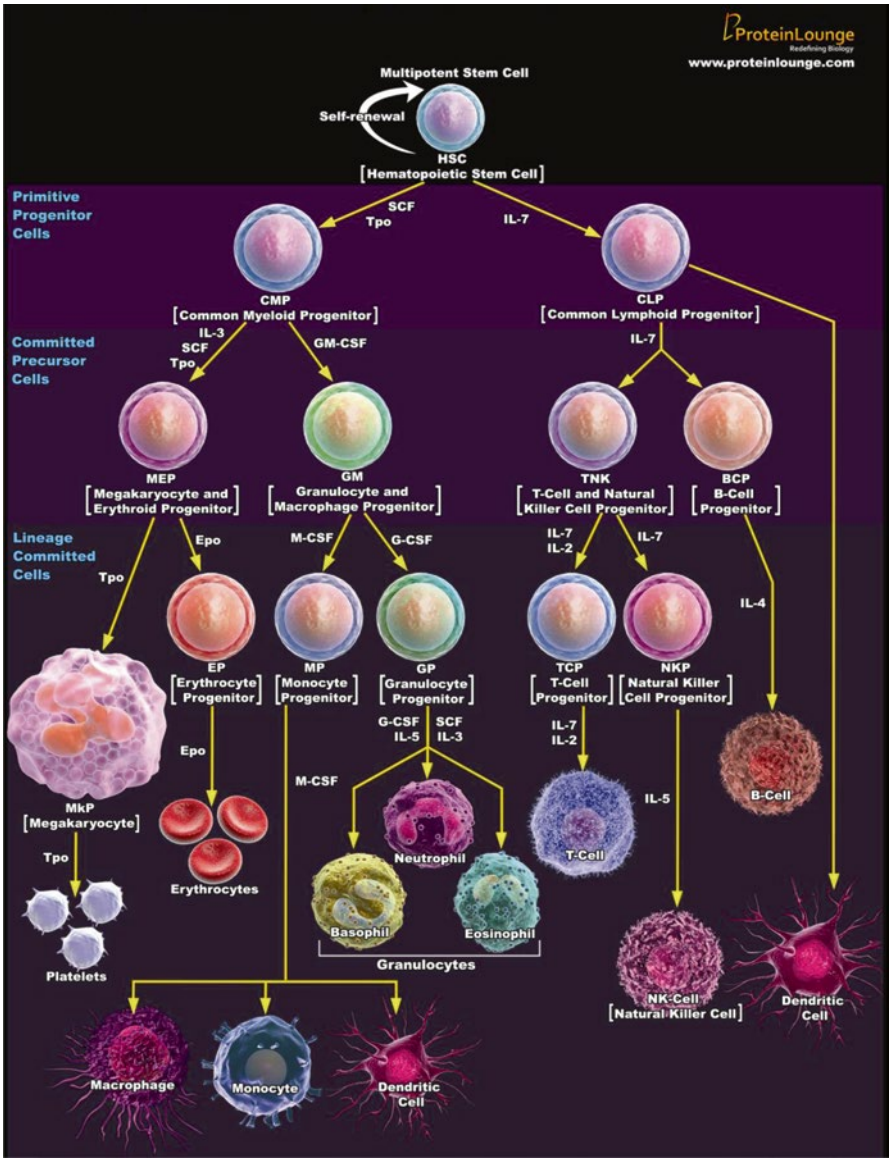

Fig. 2.1 Hematopoiesis from multipotent stem cell

Flow cytometry (FCM) is the preferred method for counting RBCs, WBCs, reticulocytes, and platelets. Reticulocyte counts can also be made by counting ≥1,000 erythrocytes in a smear stained with new methylene blue. Hemoglobin is measured by the cyanmethemoglobin method. Wright-stained blood smears are used to microscopically evaluate erythrocyte, leukocyte, and platelet abnormalities,

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leukocyte counts, differential leukocyte counts, parasites, platelet counts, and bacteria. Wet mounts are preferred for morphology studies since erythrocyte shape is unaltered, and artifacts are minimized. Erythrocyte morphology is further described in the Hematology Diagnosis chapter and the Hematology Glossary. An increase in a cell count has the suffix—*philia* or—*osis* (e.g. neutrophilia, monocytosis). A decrease in a cell count has the suffix—*penia* (e.g. lymphopenia).

Figure 2.1 illustrates the hematopoietic pathways by which various blood cells are produced from multipotent stem cells. Each step of this process is regulated by the cytokines and growth factors adjacent to the yellow arrows. [This illustration was created by proteinlounge.com and is used with their kind permission.]

2.2 Erythrocytes

Approximately one million senescent erythrocytes are replaced every second in the dog. In response to anemia, such as from blood loss or disease, a healthy dog's bone marrow can produce erythrocytes at 6–8 times the normal rate. The factors needed for erythrocyte production are erythropoietin (EPO, the key cytokine for regulating RBC production), globulins, iron, cobalt, copper, hematopoietic factor, protoporphyrin, vitamins B_2, B_6, and B_{12}, niacin, folic acid, and thiamine. The lack of any of these can lead to anemia, polycythemia, and abnormal erythrocytes.

Anemia is a decrease in the erythrocyte count, hematocrit, and/or hemoglobin. Anemia is not a disease, but rather a sign of disease or trauma. There are four general causes of anemia:

- *Blood loss*—trauma, surgery, coagulopathies, blood-sucking parasites, intestinal or genitourinary bleeding, internal hemorrhage, ulceration, and poisoning (warfarin, bracken fern, sweet clover).
- *Hemolytic anemias*—the bursting of RBCs caused by bacterial and viral infections, chemicals (e.g. lead, saponin, copper, and certain drugs such as phenothiazine), poisonous plants, metabolic diseases, and hemolytic diseases.
- *Bone marrow depression*—physical agents (radiation), chemicals (bracken fern, estrogen, phenylbutazone, some antibiotics), parasites, infectious agents, chronic infections, nephritis, liver disease, endocrine deficiencies, and myeloproliferative disease.
- *Nutritional deficiencies*—vitamin, mineral, and protein deficiencies.

Anemias are either *regenerative* or *non-regenerative* (sometimes called *responsive* or *non-responsive* anemias). In regenerative anemias, the bone marrow increases erythrocyte production to replace lost or hemolyzed cells, and releases large (macrocytic) immature RBCs such as *nucleated erythrocytes* and *reticulocytes*. This response is not seen in non-regenerative anemias.

Table 2.1 shows how anemias can be morphologically classified using two erythrocyte indexes—mean corpuscular volume (MCV) and mean corpuscular hemoglobin concentration (MCHC). Typically, *macrocytic* anemias are regenerative

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Table 2.1 Morphologic classification of anemias

MCV	MCHC	Interpretations
Normocytic[a]	Normochromic	Normal Depression anemias (excluding certain nutritional deficiencies and some cases of myeloproliferative disorders in the cat)
Normocytic[a]	Hypochromic ↓	Early iron deficiency, depressed erythrogenesis, disease in domestic animals
Macrocytic[b] ↑	Normochromic	Pernicious anemia in primates Vitamin B_{12} and folate deficiencies Cobalt deficiency in ruminants Erythemic myelosis and erythroleukemia in cats Defective erythrogenesis as in Poodle macrocytosis Antimitotic drugs, liver disease, splenectomy
Macrocytic[b] ↑	Hypochromic ↓	Temporary anemia following blood loss or hemolysis, usually accompanied by reticulocytosis
Microcytic[c] ↓	Normochromic	Normal for Japanese Akita dogs. Iron deficiency in progression
Microcytic[c] ↓	Hypochromic ↓	Iron and copper deficiencies and chronic blood loss. Pyridoxine (vitamin B_6) deficiency

[a]Normocytic anemias are generally non-regenerative (non-responsive)
[b]Macrocytic anemias are generally regenerative (responsive)
[c]Microcytic anemias may be regenerative or non-regenerative

(responding), *normocytic* anemias are non-regenerative (non-responding), and *microcytic* anemias may be either.

The opposite of anemia—*erythrocytosis or polycythemia*—is an increase in erythrocyte mass in the blood. Both terms are used interchangeably. The most common causes for erythrocytosis in laboratory studies are stress-induced splenic contraction or *hemoconcentration* (*relative erythrocytosis*) when an animal is deprived of water. Erythrocytosis may have primary or secondary causes:

- In *primary erythrocytosis*, there is splenomegaly, a dramatic increase in erythrocyte production despite low serum EPO levels, and increased production of granulocytes and thrombocytes. Primary erythrocytosis is a myeloproliferative disease. It is also called *primary polycythemia*, *polycythemia vera*, and *polycythemia rubra vera.*
- In *secondary erythrocytosis*, there is an increase in the erythrocyte count in the circulating blood because of excessive EPO production, either due to systemic hypoxia (appropriate) or an EPO secreting tumor in the kidney (inappropriate). Unlike primary erythrocytosis, there is no increased production of granulocytes and thrombocytes. It is also called *Secondary Polycythemia*.

A common finding in toxicity studies is *hemolysis*, the lysing (bursting) of erythrocytes with the release of hemoglobin. Hemolysis occurs naturally in the spleen, liver, and lymph nodes, which each have abundant macrophages. Hemolysis in peripheral blood can be caused by many conditions, diseases, toxicities, pharmaceuticals, and sampling errors, as demonstrated in Table 2.2.

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Table 2.2 Major causes of hemolysis

• Inherited conditions (e.g., thalassemia, hematoglobinopathy, sickle cell anemia)	• Antiviral agents (e.g., Ribavirin®)
• Metabolic diseases	• Hemodialysis
• Hemolytic diseases	• Transfusion reactions
• Autoimmune hemolytic anemia	• Malaria
• Poikilocytes (abnormally shaped RBCs)	• Tick-borne diseases
• Improper specimen collection (improper venipuncture, use of a small or large-bore needle, rapid transfer of blood from a syringe to a tube)	• RBC parasites (e.g., babesiosis)
• Improper specimen processing (vigorous mixing, exposure to heat or cold)	• Arsenic
• Bacterial and viral infections (e.g., clostridial organisms)	• Poisonous plants
• Pharmaceuticals (e.g., sulfones, dapsone, quinine, nitrofurantoin, sulfonamides, phenazopyridine phenothiazine)	• Metals (chromium/chromates, platinum salts, cis-platinum, nickel compounds, copper, lead)
	• Nitrites
	• Rho immune globulin (WinRho®)
	• Toxins (e.g., snake bite venom)
	• Saponin
	• IV infusion of water (hypotonic solution)
	• Capillary constriction or trauma (as in the feet of long-distance runners)

2.3 Leukocytes

Leukocytes, also known as *white blood cells* or *WBCs*, play a critical role in the immune system. The five major populations of leukocytes include *neutrophils*, *eosinophils*, *basophils*, *monocytes*, and *lymphocytes*.

The *polymorphonuclear (PMN) granulocytes*, including neutrophils (also called *heterophils*), eosinophils, and basophils; and *mononuclear macrophages*, including *monocytes* and tissue *macrophages*, are involved in nonspecific mechanisms of host resistance. All these cells are phagocytic toward foreign material. The PMN granulocytes are the first line of defense because they do not require prior experience with the foreign material (antigens) and they are highly mobile. If PMNs cannot control an infection, antigen-sensitized *T-lymphocytes* excrete *lymphokines* that attract macrophages to the site of infection. Macrophages become very powerful killers.

Leukocyte counts (WBC counts), can vary widely due to circadian effects and the choice of bleeding sites. A physiologic leukocytosis due to the release of epinephrine can result from exercise, stress, and the apprehension associated with venipuncture. This can lead to misdiagnosis. In small animals such as rats, mice, and rabbits that have high lymphocyte counts, stress causes lymphopenia and neutrophilia such that a marked decrease in circulating lymphocytes can result in leukopenia. Species with high neutrophil counts have leukocytosis, neutrophilia,

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and lymphopenia when stressed. The release (or administration) of corticosteroids causes neutrophilia, lymphopenia, and eosinopenia. This results in a leukocytosis in species with N:L (neutrophil:lymphocyte) ratios >1.0, and a leukopenia in species with N:L ratios <1.0 (see Neutrophil:Lymphocyte Ratio in Chap. 5, Hematology Diagnosis).

2.3.1 Neutrophils

Neutrophils are the predominant leukocyte in most species. Neutrophils are a type of granulocyte (the others being eosinophils, and basophils). The term granulocyte is often used to mean neutrophil. Neutrophils are both phagocytic and bacteriocidal, and are the first line of defense against microbial infection. Their most notable feature is a segmented polymorphic nucleus. It is because of this structure that neutrophils are often called segmented granulocytes. The extent of segmentation varies between species. In females of some species, the nucleus may have a drumstick-shaped appendage of extra chromatin called a *Barr body*.

The cytoplasm has pale granules that contain enzymes and other chemicals needed by neutrophils to combat microbial infection. The contents of these granules varies among species. The neutrophils in some species, such as birds, are called *heterophils* because of the diverse ways that they stain. For example, the neutrophils (heterophils) in the rabbit, guinea pig, rat, and chicken contain red cytoplasmic granules that makes them resemble eosinophils. Lemur neutrophils stain azurophilic (blue).

Neutrophils undergo three phases: the intramedullary phase, the intravascular phase, and the tissue phase. During the *intramedullary phase*, neutrophils are formed in the bone marrow and released as mature neutrophils. *Band* or *stab* neutrophils (immature neutrophils) are in small numbers in the blood, but are released early from the marrow in response to infection. The marrow contains an abundant storage pool of mature neutrophils (a 5 day supply in dogs) that allows for rapid replacement.

Intravascular phase neutrophils are either in the *circulating pool* (in the blood), or in the *marginal pool* (adhering to the walls of capillaries). Both pools are equivalent in size and interchangeable depending on health status. Neutrophils in the marginal pool are older and are most abundant in lung capillaries. Neutrophils collected during venipuncture are from the circulating pool. The $t_{½}$ for circulating neutrophils is approximately 6 h. The circulating pool and the marginal pool make up the *total blood granulocyte pool*. The marginal pool in cats is twice as large as the circulating pool.

Any neutrophils not lost in the lungs, urine, saliva, and G.I. tract enter the *tissue phase*. They pass through intact blood vessels (*diapedesis*) and enter tissues in response to inflammation. In severe tissue trauma, there is a substantial migration into the lesion along with increased neutrophil production and release from the marrow. After an initial neutropenia, the total blood granulocyte pool increases.

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Blood neutrophil counts are a reflection of neutrophil production (granulopoiesis), release of neutrophils from the marrow, cell senescence, margination, and migration into the tissues. Fear, stress, extreme exertion, and the release of epinephrine and norepinephrine can cause physiological neutrophilia due to a shift of the marginated neutrophils back into the circulating pool. This shift can result in a sudden two to threefold increase in neutrophils. This can lead to misdiagnosis in animals not conditioned to venipuncture. Neutrophilia following administration of corticosteroids is due to the release of stored mature neutrophils from the marrow and from the marginal pool. It can be difficult to distinguish from a single complete blood count (CBC) whether neutrophilia is due to a stress response or inflammation.

In response to infection, circulating neutrophils marginate and enter tissues. This causes an initial *neutropenia*. The marrow then releases stored neutrophils that also marginate and enter tissues. The neutropenia persists despite an increased total blood granulocyte pool. As infection progresses, granulopoiesis increases and new (as opposed to stored) neutrophils are released, resulting in neutrophilia.

Band neutrophils are immature neutrophils. A small number of band neutrophils are always found in the blood. Whenever infection places a demand on the bone marrow to release more neutrophils, an increased number of immature neutrophils are released into the blood. This is called a *left shift*. As the released cells become more immature, the left shift is said to be more severe. The degree of left shift can be defined as follows:

Slight—release of band neutrophils
Moderate—release of many band neutrophils and some metamyelocyte neutrophils
Marked—release of many myelocytes and some promyelocytes
Severe—release of myeloblasts

The left shift may be either regenerative or degenerative:

Regenerative—The left shift is due to increased granulopoiesis in healthy bone marrow. There is an increase in immature neutrophils, but they are outnumbered by mature neutrophils.

Degenerative—The left shift is due to inhibited granulopoiesis and septicemia and characterized by delayed maturation, so immature neutrophils outnumber mature neutrophils. WBC counts are normal or decreased.

In a *right shift*, there is an increased proportion of aged neutrophils in the blood as characterized by hypersegmentation of the nucleus (five or more lobes). It is caused by corticosteroids, vitamin B_{12} and folate deficiency, and reduced cell mitosis in the marrow. It may also be an artifact in stored blood. Right shifts are seen in humans, but rarely in domestic animals.

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2.3.2 Eosinophils

Eosinophils mediate allergic and inflammatory reactions, and destroy parasites such as helminths. The eosinophil is a granular leukocyte and is distinguished by its bright pink or orange cytoplasmic granules and a smooth polymorphic nucleus. The granules are rod-shaped in cats. Although their appearance varies widely between species, all eosinophils are parasiticidal, bacteriocidal, and phagocytic.

Eosinophils are produced mostly in the marrow, but also in the thymus, spleen, and cervical lymph nodes. They spend only a few hours (one hour in dogs) in the peripheral circulation before migrating into tissues. They tend to congregate near mast cells, and are found mostly in the bone marrow and ports-of-entry such as the skin, gastrointestinal tract, and lung. Thus, a CBC may not be a reliable measure of eosinophils in affected tissues. Most species have their lowest eosinophil counts in the morning.

2.3.3 Basophils

Basophils are involved in hypersensitivity reactions and inflammation. The basophil is a granular leukocyte that has an irregular, pale-staining multilobed nucleus (2–3 lobes), and a small number of cytoplasmic granules whose size and staining characteristics vary between species. Basophils are morphologically similar to mast cells, and serve some of the same functions. They produce and store histamine, heparin, serotonin, hyaluronic acid, and other vasoactive substances, and release these chemicals upon stimulation. After leaving the bone marrow, basophils spend only a few hours in peripheral circulation before migrating into body tissues. Basophilia is rare, but may coincide with eosinophilia. Basophils are found in small numbers except in rabbits. As a rule, species with many basophils have few mast cells, and species with many mast cells, have few basophils.

2.3.4 Monocytes

Monocytes are formed in the bone marrow and are typically the largest of the leukocytes. They are found in small numbers in the blood. The nucleus can have a variety of shapes (generally ovoid or kidney shaped). The cytoplasm has a ground glass appearance and contains small purplish granules and large vacuoles. Monocyte nuclei degenerate quickly in old blood.

Monocytes are phagocytic and routinely attack the more difficult pathogens. After briefly circulating in the blood, monocytes enter body tissues and cavities where they become ‘fixed’ macrophages until they are stimulated by inflammation to become ‘free’ macrophages. The effectiveness of a monocyte increases when it

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becomes a macrophage. Unlike neutrophils, monocytes are capable of mitosis while residing in tissues. Corticosteroids inhibit the function of monocytes and macrophages. Since macrophages are rarely found in the blood, monocytes are counted instead.

Monocytes are components of the Mononuclear Phagocyte System (MPS) that includes monocytes and monocyte precursors in the blood and marrow, and macrophages of the lymph nodes, spleen, lung, bone marrow, connective tissue, Kupffer cells (liver), and other organs. In those species that have very low monocyte counts in health, it may be difficult to identify monocytopenia.

2.3.5 Lymphocytes

Lymphocytes vary widely in size. They are round and have a single round or oval nucleus. They are produced mostly in the bone marrow, but also in lymphoid organs (including the lymph nodes, thymus, and spleen), and in the gut-associated lymphoid tissues (including tonsils, Peyer's patches in the small intestines, and the appendix). Immature lymphocytes are large, have dispersed chromatin, basophilic cytoplasm, and smooth chromatin. Mature lymphocytes are smaller and have smaller nuclei with chromatin clumping. When stimulated, mature B-lymphocytes and T-lymphocytes can revert to immature blast forms (*blastogenesis*), and can then divide and mature.

There are four major lymphocyte categories. *B-lymphocytes* and *T-lymphocytes* comprise about 90 % of the lymphocytes, with the balance being *natural killer cells* and *null cells*. B-lymphocytes are short-lived but T-lymphocytes are long-lived.

B-lymphocytes, or *B-cells* (also called bursa-derived lymphocytes in birds, thymus independent lymphocytes, bursa equivalent lymphocytes, and bone marrow derived lymphocytes in mammals) originate in the bursa in birds, and in the bone marrow in mammals, and are not acted upon by the thymus. They are involved in humor immunity (HI), which involves antibody production. Antibody production generally requires interaction between antigens, macrophages, B-lymphocytes, and T-lymphocytes. Antigen recognition varies among species and strains.

When mature (small) B-lymphocytes become sensitized, they can revert to a blast form, divide, and differentiate into *plasma cells* in a process called *blastogenesis*. Plasma cells have a large basophilic cytoplasm. They form in the lymph nodes, spleen, and bone marrow. They disseminate to all parts of the body, but concentrate in the lymph nodes, spleen (white pulp and perivascular sheaths), and connective tissue. They are rarely found in the blood. Plasma cells are the most prolific producers of antibodies (immunoglobulins) with each plasma cell producing only one type of antibody.

T-lymphocytes, or *T-cells* (also called thymus-derived or thymus-dependent lymphocytes) can kill cells in cell mediated immune responses (CMIR) that include pathogen resistance to viruses, fungi, and protozoa, autoimmunity, graft and tumor rejection, response to malignancies, and delayed hypersensitivity reactions). They

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also regulate antibody response by stimulating and suppressing antibody production by B-lymphocytes. T-lymphocytes (and B-lymphocytes to a lesser extent) secrete lymphokines in response to antigenic stimulation. Lymphokines are soluble protein mediators that have a variety of significant effects on target cells, macrophages, and other leukocytes. T-lymphocytes are able to produce other T-lymphocytes. Disruption in the immunoregulatory function of T-lymphocytes can result in autoimmune disease. There are several types of T-lymphocytes:

- *Cytotoxic T cells*—T-lymphocytes with previous antigenic exposure that recognize and destroy foreign cells.
- *T Helper cells* (*Th cells*)—progeny of antigen-stimulated T cells that stimulate B-lymphocytes to produce antibodies.
- *T suppressor cells* (*Ts cells*)—progeny of antigen-stimulated T cells that modulate humor response by regulating T-helper cells and the antibody production of B-lymphocytes. They are involved in autoimmune diseases.
- *T memory cells*—very long-lived cells that allow rapid antigen response because of their ability to retain antigen experience over long periods of time.
- *Killer cells*—null cells, which resemble mature lymphocytes, and are capable of antibody-dependent cellular cytotoxicity (ADCC). They attack cells coated with specific IgG antibody. Also called K cells and killer lymphocytes.
- *Natural killer cells* (*NK cells*)—immature (large) null cells that have broad activity because they do not require antibodies to interact with foreign material such as bacteria, viruses, tumors, and transplanted tissue. NK cells provide the first response to tumors and viruses. They are activated in response to interferons and macrophage-derived cytokines.
- *Null cells* (also called *non-T*, *non-B lymphocytes*) have surface antigens that differ from those seen on B and T-lymphocytes. They may be T lymphocyte precursors, but their ontogeny and purpose is uncertain. Null cells are seen in systemic lupus erythematosus.

Lymphocytes are able to recirculate (mobilize); that is, they leave the blood, enter lymphatic tissues, and return to the blood. This allows immunocompetent lymphocytes to distribute widely and yet target antigens in large numbers when needed. Immune competence is adequate at birth, complete within a few months, and gradually tapers off with age.

Changes in leukocyte counts generally reflect the status of cell production in the marrow, but this is <u>not</u> the case with lymphocytes. Mature lymphocytes are stored in a pool of unknown location, and their numbers are approximately 10-times that found in circulation. Lymphocytosis and lymphopenia are terms used to describe relative increases and decreases in circulating lymphocyte counts, which in turn are reflections of production, destruction, maturation, and recirculation (mobilization). Corticosteroids inhibit lymphocyte production, especially in rats, mice, and rabbits.

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Table 2.3 Differential leukocyte count

	Control group		Dosed group	
	%	10^3 cells/mm^3	%	10^3 cells/mm^3
Total WBC	–	6.0	–	2.0
Neuts, Seg	20	1.2	60	1.2
Neuts, Band	0	0	0	0
Lymph	80	4.8	40	0.8
Eos	0	0	0	0
Bas	0	0	0	0
Mon	0	0	0	0

2.3.6 Differential Leukocyte Counts

Leukocyte data are typically presented as a total leukocyte (WBC) count and an absolute differential leukocyte count. An absolute differential leukocyte count presents cell counts for all leukocyte species in the blood. Neutrophils are reported as both band (immature) and segmented (mature) cells. Changes in the differential leukocyte count over time can tell us something about an animal's health. For example, an increase in lymphocytes relative to neutrophils suggests there is a chronic infection, and a marked increase in eosinophils suggests there may be a parasitic infection.

Differential leukocyte data are sometimes presented only as percentages (e.g., 20 % neutrophils, 70 % lymphocytes, etc.). Making a diagnosis based on percentages can be misleading when there is a significant change in total WBCs, as demonstrated in Table 2.3. In this example, each leukocyte species is presented as both a percent of WBCs and as an absolute cell count. For simplicity, the percentages for band neutrophils, eosinophils, basophils, and monocytes are zero.

If we only look only at the percentages in this table, we notice that the dosed group has a threefold increase in neutrophils (60 % v 20 %) and a twofold decrease in lymphocytes (40 % v 80 %) relative to the controls. This interpretation is flawed because it fails to account for the threefold decrease in the total WBC count in the dosed animals (2.0 v 6.0 × 10^3 cells/mm^3). A comparison of the absolute cell counts yields a very different and more accurate interpretation:

- The total WBC count decreased threefold (2.0 v 6.0 × 10^3 cells/mm^3).
- The neutrophils did not increase threefold as suggested by the percentages, but rather remained unchanged (1.2 × 10^3 cells/mm^3 for both groups).
- Lymphocytes severely decreased in the dosed group (0.8 × 10^3 cells/mm^3) compared to the controls (4.8 × 10^3 cells/mm^3), which accounts for the decrease in total WBCs.
- Thus, there was a severe lymphocytopenia that caused a leukopenia.

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This example demonstrates why it is better to evaluate differential data using absolute values instead of percentages. A statistical analysis of the differential percentages would have flagged the wrong values. When differential data are presented only in percentages, absolute counts can easily be calculated using the following equation:

$$Total WBC \times \% \, Leukocyte \, Species = Absolute \, Cell \, Count$$

e.g.: $6.0 \times 10^3 \, WBC / mm^3 \times 20\% \, Neutrophils = 1.2 \times 10^3 \, Neutrophils / mm^3$

2.4 Thrombocytes (Platelets)

Thrombocytes, also called *platelets*, play a major role in blood coagulation and are critically important for maintaining *hemostasis*. A failure to maintain hemostasis results in extended bleeding times and *purpura* (leakage of erythrocytes into tissues). Mammalian thrombocytes are derived from cytoplasmic fragmentation of megakaryocytes that form in the bone marrow. *Megakaryocytopoiesis* (the production of megakaryocytes) is unique in that the marrow precursor cells undergo polyploidy without cell division. This results in a multilobed nucleus (8–64 N) and a large cytoplasmic volume. The cytoplasm of the productive megakaryocytes breaks up to form mature blood platelets. A single megakaryocyte can produce 2,000–8,000 platelets with the largest ones producing the most platelets.

Megakaryocytopoiesis is stimulated by *thrombopoietin*, a glycoprotein hormone produced in the kidneys and liver. Thrombopoietin can increase platelet production as much as eightfold. Thrombocytopenia (usually due to decreased or destroyed platelets) stimulates an increase in the number, size, and nuclear ploidy of megakaryocytes. This results in an increased number of circulating platelets within 2–3 days. Large platelets are an indication of increased marrow production. Small platelets are seen in idiopathic thrombocytopenia. Megakaryocytopoiesis is inhibited by a factor produced in the spleen. Inhibition is seen following a transfusion.

Avian thrombocytes are produced in the bone marrow. They are mononucleated and similar to, but smaller than, avian erythrocytes. They are never called platelets. Mammalian platelets are slightly biconcave, may be round, oval, or elongated, and have clear cytoplasm. They are small in rats, guinea pigs, mice, sheep, horses, and oxen; medium in dogs, pigs, and humans; and large in cats.

Epinephrine, whether injected or released in response to stress, causes contraction of the spleen and the release of platelets into circulation. Approximately 30–40 % of the platelets are stored in the spleen in health, and as much as 90 % can be stored in splenomegaly. Senescent and damaged platelets are removed by the spleen, liver, and bone marrow.

Platelets do not adhere to the endothelial linings of vascular walls or to each other until they are exposed to subendothelial collagen following injury. Adenosine

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5′-diphosphate (ADP) is released from the damaged vessel wall that, in the presence of Ca^{++}, stimulates platelets to become spherical and acquire spines (*filopodia)*. The platelets then begin adhering to the site of injury, basement membranes, and elastic fibers. Platelets release the contents of their granules, including proteins, fibrinogen, Ca^{++} and more ADP, which causes more platelets to accumulate and to aggregate. Vasoconstriction (a neurologic reflex) and clotting work jointly to stop bleeding a few minutes after trauma.

Blood is fully capable of clotting in the *intrinsic pathway* (as occurs when blood is exposed to glass in a collection tube), but the addition of thromboplastin from the site of trauma facilitates clotting in what is known as the *extrinsic pathway*. After coagulation is promoted via the intrinsic or extrinsic pathway, the rest of the coagulation process occurs by the *common pathway*. Numerous *coagulation factors* (listed in the hematology glossary) are involved in these three pathways. The principle roles of platelets are adhesion, aggregation, and viscous metamorphosis (the conversion of plasma prothrombin to thrombin). The efficiency of coagulation and the magnitude of coagulopathies become obvious when a medium or large vessel is cut. Ineffective clotting will lead to prolonged hemorrhage, whereas inappropriate or extensive clotting can lead to vascular obstruction.

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Chapter 3
Blood and Urine Sampling

Abstract Every clinical pathology measurement can be undermined by poor sample collection, and there are many ways that samples can be compromised. A long list of tests can be ruined by red blood cell hemolysis and the presence of bilirubin because of improper blood collection technique. Similarly, a failure to properly fast animals can cause serum to be lipemic, which can interfere with many tests. Blood should be drawn from the same site, and at the same time of day throughout serial samplings. This is essential in rodents since their leukocyte counts fluctuate widely due to circadian effects and sampling site. Cell counts in all species differ depending on the collection site. When an animal is stressed during blood collection, there is a release of corticosteroids, splenic contraction, and the release of additional cells into circulation; and blood chemistries are also affected. Four methods for collecting urine are described along with their strengths and weaknesses. There is no ideal way to preserve urine so the means used are dictated by the evaluations needed.

3.1 Blood Sampling

Whenever drawing blood from animals, care should be taken to minimize subcutaneous hematomas, vascular damage, and thrombophlebitis. The animal should be at rest since activity and stress can significantly alter cell counts. It is generally prudent to draw duplicate samples, though this may not be possible with small animals.

Blood can be drawn with syringes, capillary tubes, and evacuated blood collection tubes (e.g. Vacutainers®). Collecting blood with a syringe is a two-step process. First the blood is drawn through a needle of appropriate size, then the needle is removed (to minimize hemolysis) and the blood is transferred to a tube. Capillary tubes gently remove blood from an exposed capillary bed or a lanced site. They run the risk of contamination with body fluids, dirt, hair, dander, and microbes unless the site is carefully cleaned.

Evacuated blood collection tubes are the most popular way to collect blood. They offer a one-step process that allows one or more tubes to be filled with a single venipuncture. They can cause small veins to collapse, however. Once blood is collected, tubes that contain a precise amount of anticoagulant should be inverted

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J.E. Whalan, *A Toxicologist's Guide to Clinical Pathology in Animals*,
DOI 10.1007/978-3-319-15853-2_3

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several times to allow the anticoagulant to mix with the blood. Capillary tubes are also available with premeasured anticoagulant. Syringes may be prewetted with anticoagulant before sampling. Excessive dilution of blood with anticoagulants can be avoided by always drawing a full volume of blood, or by using dry anticoagulants.

Pink tinted serum is a sign of hemolysis and the release of the pigment hemoglobin. These samples should be rejected because the release of cellular material will interfere with measurements of potassium, magnesium, calcium, phosphorus, iron, chloride, bromide, blood urea nitrogen, bilirubin, albumin, pyruvic acid, lactate dehydrogenase, alkaline phosphatase, bromsulphalein, ALT, AST, arginase, acid phosphatase, corticotrophin, icterus index, lactic acid, lipase, NPN (nonprotein nitrogen), sex hormone-binding globulin (SHBG), and pH. The major causes of hemolysis during sampling are excessive turbulence or vacuum when drawing blood into or emptying a syringe, vigorous shaking, chilled glassware, vigorous ringing of clots prior to centrifuging, high temperatures (e.g. during shipping), and wet or dirty needles, syringes, and glassware. The use of evacuated blood collection tubes minimizes these problems.

Capillary blood, such as that drawn from digits or heel pricks, can differ from venous blood. Serum or plasma calcium, potassium, and protein are lower, glucose is higher, and there tends to be more hemolysis. Lipemic serum often results from bleeding unfasted animals. This serum should be rejected since it may interfere with measurements of ALT, AST, total protein, albumin, cholesterol, bilirubin, icterus index, amylase, thymol, turbidity, platelet count, and uric acid. Prolonged contact with clots can affect measurements of glucose, alkaline phosphatase, alanine and aspartate aminotransferases, lactate dehydrogenase, and iron.

Serum is the cell-free portion of blood from which fibrinogen has been removed by clotting. It is obtained by allowing blood to sit at room temperature for 30–45 min to remove the fibrinogen by clotting. *Plasma* (serum that contains fibrinogen) is collected with a syringe or tube containing an anticoagulant to prevent clotting. Blood samples are centrifuged (>3,000 G for >15 min) and the plasma or serum is pipetted from the top of the tube into a clean container. Improper centrifugation will not remove all platelets so lactate dehydrogenase (LDH) may be released from lysed platelets. Alkaline phosphatase (ALP) and urate measurements may also be affected. The following anticoagulants are most frequently used for tests requiring whole blood or plasma:

- *EDTA* (disodium and tripotassium salts of ethylenediaminetetraacetic acid) is a chelating agent that inhibits clotting by binding with calcium to form insoluble calcium salts. It is the most commonly used anticoagulant. EDTA does not affect blood cell morphology or staining.
- *Heparin* (sodium, lithium, and ammonium heparin) has the least effect on clinical chemistry measurements and erythrocyte morphology because it is a natural product. It inhibits thrombin and thromboplastin, so it is not suitable for agglutination or prothrombin time tests. It must always be used when collecting blood for blood gas analyses. It is not recommended for blood smears because it interferes with the staining of leukocytes, and may cause leukocyte clumping.

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- *Oxalates* (ammonium, potassium, lithium, and sodium oxalates) form insoluble calcium oxalate that interferes with blood coagulation. The oxalates are not used for coagulation studies. Because they inhibit lactate dehydrogenase (LDH), they cannot be used for electrolyte and nitrogen measurements. Leukocytic degeneration begins after approximately 1 h.
- Potassium, sodium, and lithium oxalates cause cell shrinkage (6–8 %), and ammonium oxalate causes cell swelling. Heller's and Paul's double oxalate is a mixture of ammonium and potassium oxalates. This mixture can be used for most hematology studies including morphology because the shrinkage caused by one oxalate is offset by the other.
- *Citrates* (trisodium and lithium citrates) combine with calcium to form the insoluble salt calcium citrate. They are used for coagulation studies and to preserve blood for transfusion. Sodium citrate causes cell shrinkage. Lithium citrate is generally not used except for measurements of minerals in blood.
- *Sodium Fluoride/Thymol* (10:1) is generally not used except to inhibit glycolysis (glucose test).
- *ACD Solution B* (Acid Citrate Dextrose) is used to preserve blood for transfusions.

It is important to use the proper anticoagulant to prevent chemical interference. Blood collection tubes are color-coded according to the additives they contain. Every laboratory has its preferred tube choices. A Vacutainer tube guide wall chart may be downloaded at this website: http://www.bd.com/vacutainer/pdfs/plus_plastic_tubes_wallchart_tubeguide_vs5229.pdf.

3.1.1 Hematology Sampling

When drawing blood for hematology studies, hemolysis should be avoided and the sample must not be allowed to clot. The preferred anticoagulant is potassium EDTA since it has minimal effect on cell morphology and it preserves platelets. Blood smears for differential leukocyte counts should be made immediately with fresh blood, although blood that contains EDTA may also be used within 15 min. If the blood cannot be evaluated immediately, it should be refrigerated at 4 °C. Storing blood can result in reduced leukocyte and platelet counts and artifacts in erythrocyte and leukocyte morphology. The plasma hemoglobin sample must be centrifuged immediately, and the plasma removed before refrigeration. Hematocrit measurements made with a microhematocrit are collected in heparinized capillary tubes.

Since blood is often drawn at the same time for hematology and clinical chemistry measurements, the animals are usually fasted; but fasting is not necessary for hematology samples. Provided the animals have access to drinking water, there should be no significant impact on any hematologic parameters. Blood should be drawn from the same site, and at the same time of day throughout serial samplings. This is essential in rodents since their leukocyte counts fluctuate widely due to

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circadian effects and sampling site. Cell counts in all species differ depending on the sampling site. It is important to avoid animals becoming excited during bleeding since this will cause a release of corticosteroids, splenic contraction, and the release of additional cells into circulation.

Bone marrow can be aspirated from live animals with a needle (a drop will do). Bone marrow can also be collected within a few hours of death. Marrow is typically collected from the femur of rodents by opening opposite ends of the femur or sternum and flushing out the marrow with saline. Dog marrow is collected from the iliac crest, rib, sternum, or proximal humerus. It is not collected from the femur because the marrow is nearly all replaced by fat in adult dogs. Marrow can also be collected with a sable brush. Marrow is typically mixed with saline and EDTA. Bone marrow is smeared on a glass slide and allowed to dry for microscopic evaluation. Marrow can also be prepared for flow cytometry, histopathologic evaluation, or in vitro culturing.

3.1.2 Clinical Chemistry Sampling

Arterial blood is used for blood gas determinations (pCO_2 and pO_2) and must not contain air bubbles. Venous blood is generally used for all other clinical chemistry measurements since it is easier and safer to obtain. The chemistry of arterial and venous blood is nearly identical with the exception of blood gases. Serum is used for most chemistry measurements because it does not contain anticoagulants that may dilute or interact with the component being measured.

Lithium heparin is used as an anticoagulant when testing plasma components such as fibrinogen. Anticoagulants are not used for most tests, however. The blood is allowed to clot and then it is centrifuged and the supernatant serum separated for analysis. Serum should be protected from light, treated with preservatives, or refrigerated as needed. For example:

- Glucose undergoes enzymatic glycolysis unless it is refrigerated and treated with fluoride (an enzyme inhibitor).
- Enzymes (except for lactate dehydrogenase), blood urea nitrogen, creatinine, and uric acid are unstable, so they should be evaluated immediately or the samples preserved at refrigeration/freezing temperatures.
- Bilirubin is broken down by strong light, so blood should be kept in the dark.

Animals should be fasted for several hours. For small species, an overnight fast borders on starvation because of their rapid rates of metabolism. Water should be available during a fast. Fasting prevents lipemia, which may interfere with some tests. Animals tested at regular intervals during a long-term study should be bled at approximately the same time of day to prevent circadian fluctuations. Also sources of stress should be minimized since they can have significant effects on clinical chemistry.

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3.2 Blood Sampling Sites

The collection of blood requires a certain level of expertise in order to collect a clean sample. When drawing blood from small animals, small samples should be collected to avoid adverse effects to the animal (e.g., anemia) and the toxicology study. For example, there is a 20 % loss of blood when 0.5 mL of blood is drawn from a 20 g mouse. Table 3.1 lists preferred sites for drawing blood from a variety of species, as well as possible complicating factors:

Table 3.1 Preferred sites for drawing blood

Birds: Small volumes of blood can be collected by clipping a toenail (causes the release of tissue thromboplastin), puncturing the comb, or snipping off a comb tip. Large volumes can be collected from the brachial, cutaneous ulnar, right jugular and medial metatarsal veins. Hematomas are common
Cats: Blood can be drawn in large volumes from the jugular, cephalic, femoral, and lateral saphenous veins, or from the heart of an anesthetized cat. Arterial blood can be drawn from the femoral artery. Excitement causes splenic contraction resulting in increases in erythrocytes, hematocrit, hemoglobin, and thrombocytes. Fear and muscular activity cause increases in neutrophils and lymphocytes
Cattle: Small volumes of blood can be collected by nicking the ear or tail. Large volumes can be collected from the jugular vein
Dogs: Blood can be drawn in large volume from the jugular, cephalic, femoral, and lateral saphenous veins, or from the heart of an anesthetized dog. Arterial blood can be drawn from the femoral artery. Excitement or strenuous exercise causes increases in neutrophils. Fear and prolonged activity cause increases in lymphocytes and erythrocytes. Fear during sampling may cause splenic contraction resulting in increases in erythrocytes, hematocrit, and hemoglobin
Ferrets: Small volumes of blood can be collected from the tail vein or by clipping a toenail. Larger volumes can be drawn from the jugular or cephalic veins or the heart of an anesthetized animal
Gerbils: Small volumes of blood can be collected by clipping a toenail. Larger volumes can be drawn from the heart of an anesthetized animal, or the orbital venous plexus
Goats: Small volumes of blood can be collected by nicking the ear or tail. Large volumes can be collected from the jugular vein
Guinea pigs: Small volumes of blood can be collected from the orbital venous plexus, dorsal ear vein, jugular vein, or from a clipped toenail. Larger volumes can be drawn from the femoral artery, femoral vein, or the heart (under anesthesia)
Hamsters: Small volumes of blood can be collected from the orbital venous plexus, from the prewarmed amputated tail tip (caudal vein), or from a slit foot pad. When collecting blood from the caudal vein, massaging the tail will cause dilution with tissue fluids. Larger volumes can be drawn from the heart, jugular vein, or femoral vein of an anesthetized animal
Horses: Small volumes of blood can be collected by nicking the ear or tail. Large volumes can be collected from the jugular vein. Erythrocyte counts can be increased 10–15 % by the stress of venipuncture. Cells separate readily from the plasma because of rouleaux formation, so samples must be mixed before evaluation

(continued)

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Table 3.1 (continued)

Mice: Small volumes of blood (30–80 μL) can be collected from the orbital venous plexus, from the prewarmed amputated tail tip (caudal vein), or from a clipped toenail. When collecting blood from the caudal vein, massaging the tail will cause dilution with tissue fluids. Larger volumes can be drawn from the jugular vein, or the heart of an anesthetized animal. A maximum of 0.5–0.7 mL of blood may be drawn from healthy mice. Leukocyte counts are higher in tail blood. High reticulocyte counts are common
Primates: Because of their strength and dexterity, nonhuman primates must be either restrained or anesthetized for blood collection. Venous blood can be drawn from the femoral, cephalic, jugular, and saphenous veins, and arterial blood can be drawn from the femoral artery. Blood may be drawn from the heart of an anesthetized animal
Rabbits: The marginal ear vein is the site of choice for collecting moderate volumes of blood. Larger volumes can be drawn from the auricular artery or the heart of an anesthetized animal. Rabbit veins are thin and tend to tear
Rats: Small volumes of blood can be collected from the orbital venous plexus, from the prewarmed amputated tail tip (caudal vein), or from a clipped toenail. When collecting blood from the caudal vein, massaging the tail will cause dilution with tissue fluids. Larger volumes can be drawn from the jugular vein, or the heart of an anesthetized animal. Leukocyte counts are higher in tail blood. High reticulocyte counts are common
Sheep: Small volumes of blood can be collected by nicking the ear or tail. Large volumes can be collected from the jugular vein
Swine: Small volumes of blood can be collected by nicking the ear or tail. Large volumes can be collected from the jugular vein

3.3 Urine Sampling

Animal urine may be collected in four ways:

1. *Metabolism cage*—This is often the best method for collecting serial urine samples and for diagnosing polyuria.
2. *Free-flow sample*—A sample of free-flow urine, preferably mid-stream, may be collected from large animals. Fecal contamination can affect analysis.
3. *Catheterization*—This procedure is prone to contamination from blood, tissue, sterilants, and lubricants.
4. *Cystocentesis*—This involves aspirating urine into a syringe via a needle placed through the abdominal wall into the bladder. This procedure is prone to contamination from blood or tissue.

Standing urine will become cloudy (due to precipitation of salts), lose bilirubin (via light-activated oxidation), and become basic (loss of CO_2 and production of ammonia by bacteria). Because enzymes in urine are unstable, samples that cannot be evaluated immediately are typically refrigerated (4 °C) for a few hours. Preservatives can cause dilution effects and are generally not needed except during transport. Chemicals that control bacterial growth may interfere with analytical

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methods. Acidification may dissolve or form crystals. Since there is no ideal way to preserve urine, the means used are dictated by the evaluations needed:

- *Refrigeration*—Samples may be usable for several hours, particularly if the urine is acidic. Refrigerated urine must be warmed to room temperature prior to analysis.
- *Freezing*—Freezing slows bacterial growth and decomposition of metabolites and preserves urine for at least 10 weeks. The freezing and thawing of urine causes a decrease in enzymes and proteins. It impedes sediment examination because it damages cells and casts. Freezing is recommended for measurements of sodium, potassium, chloride, calcium, magnesium, and phosphate; but not recommended for measurements of epinephrine, norepinephrine, hydroxyproline, antidiuretic hormone, creatinine, and urea nitrogen. Urine must be warmed to room temperature for most analyses.
- *Acidification*—Urine can be acidified by adding 0.1 N HCl. Cells, casts, and some other components can be preserved for several hours in acidic urine. Crystals tend to form in acidic urine, but crystals found in alkaline urine may dissolve once they are acidified.
- *Ascorbic Acid*—Ascorbic acid prevents bilirubin oxidation. It is not suitable for glucose measurements that use glucose oxidase.
- *Formaldehyde*—Urine preserved with formaldehyde is suitable for evaluation of cells, casts, and microbial growth. It is not suitable for glucose measurements that use glucose oxidase.
- *Thymol*—Thymol is an excellent antimicrobial preservative, but can yield false positives for protein.
- Toluene—Toluene can prevent microbial growth. Since it is a solvent, care must be taken in the selection of collecting and storage containers.
- *Chloroform*—Chloroform can prevent microbial growth, and can preserve urine for about 24 h.
- *Boric Acid*—Boric Acid is a good antibacterial.
- *Metaphosphoric Acid (HPO_3)*—Metaphosphoric Acid can preserve vitamin C.

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Chapter 4
Species Specifics

Abstract As difficult as clinical pathology interpretation may be for humans, it is far more complex when numerous other species are considered. Clinical pathology diagnosis is generally similar across species but there are many species-specific differences that must be considered to avoid erroneous diagnosis. For example, birds have nucleated red blood cells but mammals do not. It is normal for red blood cells to resemble a stack of coins (rouleaux) in cats, dogs, guinea pigs, and rabbits, but this is a sign of disease in other species. Mouse leukocyte, lymphocyte, and eosinophil counts can be threefold higher in the morning than in the evening. Alkaline phosphatase is a good indicator of cholestasis except in cats. Manual restraint of rats during sampling can cause increases in plasma protein, calcium, and magnesium; and decreases in blood pH and glucose. The urine of healthy cats tends to be cloudy. Rabbit urine tends to be basic and turbid and it is high in volume because rabbits do not concentrate their urine as well as other animals. Only by understanding peculiarities for a given species can one avoid calling adverse what is actually a normal finding. This chapter provides specific information for the species most commonly encountered in toxicology studies.

4.1 Birds

Hematology Birds have lower RBCs and higher WBCs than mammals. Erythrocytes are nucleated, and typically elliptical and biconvex, with as many as 10 % of them being reticulocytes in healthy birds. Chicks have low RBCs, but they reach adult levels within a week. Lymphocytes are the most numerous leukocytes. Basophils are common, especially in pheasant. All hematopoiesis occurs in the bone marrow but B-lymphocytes differentiate in the bursa of Fabricius, which is located near the cloaca. Mammals do not have an analogous organ to the bursa. Thrombocyte counts are slightly lower in adults, particularly in males. The speed of clotting in birds and mammals is similar. Thrombocytes are nucleated and function similarly to mammalian cells, although they do have phagocytic abilities. Avian thrombocytes are never called platelets. Birds lack clotting factors XI and XII. Clotting relies primarily on the extrinsic pathways. Heterophils (the avian equivalent to neutrophils) and eosinophils look similar, and lymphocytes, monocytes, and thrombocytes look similar.

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J.E. Whalan, *A Toxicologist's Guide to Clinical Pathology in Animals*,
DOI 10.1007/978-3-319-15853-2_4

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Automatic counting of erythrocytes is complicated by the presence of nuclei. Cyanmethemoglobin measurements of hemoglobin will be falsely elevated because of light scattering by the RBC nuclei unless the reagent blood mixture is centrifuged. Hematopoietic neoplasia is common in birds. Bone marrow cannot be collected from live birds.

Clinical Chemistry Alkaline phosphatase (ALP) increases due to liver disease in carnivorous birds but not in herbivorous birds.

4.2 Cats

Hematology Male and female cats have similar values. RBC counts and Hb levels are lower, and reticulocytes counts are higher in kittens under 3 months old. Nucleated RBCs are found in kittens (≤12 weeks old), but rarely in cats (except those with myeloproliferative disorders). HCT and Hb decrease during the first months of life and there is microcythemia. RBCs, HCT, and Hb gradually increase to adult levels. Reticulocyte counts are very low except during recovery from blood loss anemia. RBCs, HCT, and Hb decrease during late pregnancy, then quickly rebound at postpartum. Erythrocytes tend to be anisocytotic (varying in size), and they become smaller and more numerous with age. Erythrocyte diameters range from 5.4 to 6.5 μm and rouleaux formation is common. Abnormal erythrocyte shapes (poikilocytosis) are common in disease states, such as blister cells caused by oxidative injury. Heinz bodies and Howell Jolly bodies may be seen in small numbers.

WBC counts vary widely since healthy cats can draw on a marginal pool containing three times the number of circulating neutrophils. Neutrophils are the most numerous leukocytes. Band neutrophils and basophils are rare in healthy cats, and metamyelocytes are never found. Eosinophils have rod-shaped granules. Leukocyte and monocyte counts are lower in kittens under 3 months old.

Excitement (such as during venipuncture) stimulates splenic contraction and the release of erythrocytes and platelets. Fear, apprehension, rage, and muscular activity cause an increase in neutrophils and lymphocytes. Because of difficulties in drawing blood, clotting may interfere with analysis, especially with electronic counters. Crenation is a common problem. The *H. Felis* parasite causes feline infectious anemia followed by macrocytic hemolytic anemia, and erythrocytes may lose their normal biconcave shape and become spherocytes or stomatospherocytes. ACTH and corticosteroid therapy cause neutrophil and monocyte counts to increase, and lymphocyte and eosinophil counts to decease.

Clinical Chemistry Excitement can significantly affect chemistry values for an individual cat over time. Age and sex have little effect. Total plasma protein increases in old cats, mostly due to increased gamma globulin. Alkaline phosphatase is a poor indicator of cholestasis in cats. The half-life of the hepatic alkaline phosphatase isoenzyme is approximately 10 % that of the dog.

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Urinalysis Feline urine tends to be acidic. Bilirubinuria is rare in cats. The urine of healthy cats tends to be cloudy due to lipid droplets.

4.3 Dogs

Hematology Male and female values are similar. Erythrocyte counts, HCT, and Hb decrease during the first months of life, but then gradually increase to adult levels. Erythrocytes tend to be anisocytotic (varying in size), and they become smaller and more numerous with age. Erythrocyte diameters range from 6.9 to 7.3 μm. Macrocytosis is common in poodles. Some rouleaux formation occurs in dogs. Abnormal erythrocyte shapes (poikilocytosis) are common in disease states. Reticulocytes are <3 % (except in puppies) and nucleated red blood cells are rare. Fear, such as during venipuncture, can cause splenic contraction yielding a significant increase in erythrocytes (erythrocytosis). Hemoglobin levels are increased by diets containing vitamin B-complex and other hematopoietic factors. Growing dogs have low fibrinogen levels, and thus will have negative corrected ESR values.

WBCs decrease with age (due to decreases in lymphocyte counts), and they fluctuate due to diurnal, seasonal, and physiologic effects. Neutrophil counts remain fairly constant and eosinophil counts increase somewhat throughout life. Leukocyte counts peak at pregnancy term. There is wide variation in WBC counts between breeds. Neutrophils are the most numerous leukocytes (three to fivefold higher than lymphocytes). Band neutrophils are in small numbers, and metamyelocytes are never found in the blood of healthy dogs. Monocytes can be easily mistaken for band neutrophils, but their cytoplasm stains bluish.

Bacterial infection can cause a shift to the left as seen by the release of band neutrophils, metamyelocytes, and even less mature cells. A shift to the right (neutrophil hypersegmentation) occurs in response to corticosteroid therapy. Lymphocyte counts are depressed during canine distemper. Basophils are rare in healthy dogs. Eosinophil counts are elevated during parasite infections (e.g., hookworm). ACTH and corticosteroid therapy cause neutrophil and monocyte counts to increase, and lymphocyte and eosinophil counts to decrease.

Clinical Chemistry There are no significant sex differences. Bilirubin measurements in dogs are unreliable, except in cases of bile duct obstruction with decreased renal function, and prolonged bile duct obstruction. Compared to adults, dogs 6 months to 2 years old have increased total protein, albumin, and globulins and decreased transaminase (ALT and AST) levels. Alkaline phosphatase is increased slightly in acute hepatocellular necrosis, while ALT and sorbitol dehydrogenase (SDH) are markedly increased. Alkaline phosphatase is markedly increased in biliary obstruction. Venous partial pressure CO_2 (pCO_2) is elevated between 1 and 3 years. Older dogs have increased glucose and bilirubin values, and decreased blood urea nitrogen values. Beagles and some other strains are prone to Factor VII deficiency, and may have prolonged prothrombin times. Increased prothrombin times in

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these dogs should not be used as a measure of liver function. Hypercalcemia is seen in young dogs with renal failure. Because Dalmatians are not able to metabolize uric acid in their livers, they can have hyperuricemia (elevated uric acid in the serum) and they are susceptible to gout and uric acid stones in the kidneys and bladder.

Urinalysis Canine urine tends to be acidic and high in volume. Mild bilirubinuria is normal.

4.4 Guinea Pigs

Hematology Guinea pigs have significant variations due to sex, strain, time of day, and diet. Males have higher erythrocyte counts than females. Guinea pigs have the largest erythrocyte diameter of common laboratory animals (7.5 μm), and there is some anisocytosis, microcytosis, polychromasia, and rouleaux formation. Erythrocyte counts, HCT, and Hb peak at 4 months, then decline to adult levels. Reticulocytes decrease after 2 months, but increase in anemia remission, along with anisocytosis, polychromasia, nucleated RBCs, and Howell Jolly bodies.

WBCs increase with age over the first year. Neutrophils are sometimes called heterophils or pseudoeosinophils. Basophils are rare and distinctive. Lymphocytes are the most numerous leukocytes and there are more small lymphocytes than large ones. As many as 4 % of lymphocytes are Kurloff cells, which contain one or more cytoplasmic inclusions called Kurloff bodies that are rich in neutral mucopolysaccharides. Kurloff cells, which are only found in guinea pigs and especially in pregnant females, may function as natural killer cells. Platelet counts are not affected by age or sex. M:E ratio is greater than 1.

Clinical Chemistry Guinea pigs must have dietary vitamin C to prevent hypovitaminosis C (scurvy), which can affect the metabolism of carbohydrates, cholesterol, and amino acids. Ketosis occurs due to pregnancy, toxemia, obesity, and stress, and is usually fatal. Total plasma proteins peak at 4–6 months, then decrease to adult levels.

Urinalysis Ketosis occurs due to pregnancy, toxemia, obesity, and stress, and is usually fatal.

4.5 Hamsters

Hematology Sex differences are not found in hamsters. Hemoglobin is elevated in young animals and starving adults. Reticulocytes are common, and increase significantly following blood loss. Reticulocytes and polychromatophilic erythrocytes are

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common. Nucleated RBCs are rare in adults, but common in newborns. WBC counts peak during the nocturnal period when hamsters are most active. Neutrophils are often called heterophils. Lymphocytes are the most numerous leukocytes and there are more small lymphocytes than large ones. Basophils and eosinophils are rare. M:E ratios are >1.

During hibernation, blood volume, RBCs, HCT, and Hb increase, leukocytes (mostly lymphocytes) and platelet counts decrease, there are equal numbers of neutrophils and lymphocytes, and hematopoiesis is suppressed.

Clinical Chemistry When collecting blood from the caudal vein, massaging the tail will cause dilution with tissue fluids that can significantly affect most clinical pathology parameters.

4.6 Mice

Hematology Values are similar for both sexes, but there are significant differences between strains and sampling sites. The RBCs, HCT, Hb, and WBCs increase during the first months of life, then decrease to adult levels. Erythrocyte anisocytosis (variation in size) and polychromasia are common. Reticulocytes are common, and some normoblasts are found. Hematopoiesis takes place in the bone marrow but also in the spleen and liver. The spleen produces mostly erythrocytes, but also granulocytes and megakaryocytes. The spleen is 50 % larger in males than in females. Hereditary anemias include W anemia, Hertwig's anemia, Steel anemia, siderocytic anemia, hemolytic anemia, and jaundice. Leukemia is common in mice, with some strains being more susceptible than others.

Lymphocytes are the most numerous leukocytes. During the first year of life, neutrophil (also called heterophil) counts increase, and lymphocyte counts decrease. Leukocyte, lymphocyte, and eosinophil counts peak in the morning and can be three-fold higher than in the evening. WBCs in tail blood can be 1.5 to 5-fold higher than in cardiac blood. WBCs in tail blood are lower during activity than during rest. Basophils are rare. Platelet counts are not affected by age or sex. High corticosteroid levels cause decreased lymphocyte counts. M:E ratios are >1.

Clinical Chemistry When collecting blood from the caudal vein, massaging the tail will cause dilution with tissue fluids that can significantly affect most clinical pathology parameters. ALT, AST, and LDH levels in orbital plexus blood increase on subsequent bleedings (between 1 min and 48 h). CPK is decreased and glucose is increased in cardiac blood. CO_2 anesthesia alters glucose and blood gas values. There are strain differences in AST, glucose, and chloride. Age, sex, and nutrition have minimal effects. Amyloidosis (accumulation of starch-like material) in kidneys and other organs is common in mice.

Urinalysis Calculi are rare.

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4.7 Primates

Hematology There is wide variation between primate species, particularly with regard to leukocytes. Adult leukocyte levels are reached by 12 weeks. HCT is lowest at 2 weeks but then gradually increases to adult levels at 2 years. Nucleated RBCs are rare. Reticulocyte counts are low except in young animals. Reticulocytes increase following blood loss.

The majority of leukocytes at birth are neutrophils, but thereafter lymphocytes are the most numerous. Excitement during venipuncture can cause leukocytes to nearly double, mostly because of an increase in neutrophils. Monocytes and basophils are rare. All hematopoiesis occurs in the bone marrow. M:E ratios are >1.

Clinical Chemistry Humans and lower primates vary widely with respect to age, sex, diet, race, environmental conditions, and biological variation. Compared to humans, lower primates have higher alanine and aspartate transaminases (ALT and AST), acid phosphatase, beta and gamma globulins, amylase, sodium, calcium, and inorganic phosphorus; and lower alkaline phosphatase, creatine kinase, serum bilirubin, cholesterol, uric acid, albumin, and A/G ratios. AST is higher in young monkeys, and alkaline phosphatase is higher in young and small monkeys. Primates must have dietary vitamin C to prevent hypovitaminosis C, which can affect the metabolism of carbohydrates, cholesterol and amino acids.

Urinalysis Monkey urine is more acidic and higher in volume that human urine. Urinary tract problems are rare.

4.8 Rabbits

Hematology Wild rabbits have higher RBCs, HCT, and Hb than domesticated rabbits. Reticulocytes are common (as much as 4 % of RBCs), especially in the young animals, and peak at 2 months before decreasing to adult levels. Rouleaux formation, polychromasia, anisocytosis (variation in RBC size) and microcytes are normal. Following anemia, anisocytosis, polychromasia, nucleated RBCs, and Howell Jolly bodies can be found.

WBCs are higher in males and increase with age, peaking twice—at approximately 5 months for lymphocytes, and at 12 months for neutrophils. Considerable leukocyte fluctuation can be caused by diet, circadian rhythms (maximum counts in the early afternoon) and individual variability. Lymphocytes are more plentiful than neutrophils in young rabbits, but in adults the neutrophil and lymphocyte counts are equivalent. Neutrophils, which resemble eosinophils, are sometimes called pseudoeosinophils, heterophils, and amphophils. Basophils, eosinophils, and monocytes are common. Ten to fifteen percent of the leukocytes are basophils.

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A high corticosteroid level causes increased HCT and neutrophil counts, and decreased lymphocyte counts. Erythrocyte and neutrophil counts, HCT, Hb, and sedimentation rate increase during an enteritis complex. Platelets peak at 1–2 months then decrease to adult levels. M:E ratios are generally <1.

Clinical Chemistry Age, sex, and strain have minimal effect on chemistry values. Values can vary widely for BUN, ALT and AST. Plasma fibrinogen increases with age. An enteritis complex is identified by increased albumin, globulin, BUN, and serum lipase glucose; and a decrease in sodium, potassium, and chloride.

Urinalysis Because rabbits are herbivores, their urine tends to be basic (pH 8–9) and turbid. Their urine is also high in volume because they do not concentrate their urine as well as other animals. Specific gravity can vary considerably (e.g., between 1.003 and 1.036) due to the presence of mineral deposits, and there may be traces of glucose and protein. An enteritis complex is characterized by acidic urine with increased albumin and decreased urobilinogen.

4.9 Rats

Hematology RBCs, HCT, and Hb values increase during the first 4 months of life. RBCs, HCT, and Hb are slightly lower in females. RBC anisocytosis (variation in size) and polychromasia are common. Reticulocytes and nucleated RBCs are common, with reticulocytes being highest in young rats (98 % of erythrocytes). Reticulocyte counts decrease with age.

WBCs increase with age, with peaks at 3 and 6 months (mostly due to lymphocytes). Neutrophil counts peak at 1 year. Neutrophils are also called heterophils. Lymphocytes are the most numerous leukocytes and there are more small lymphocytes than large ones. Monocytes can account for as much as 6 % of the WBC count. Basophils are rare. The spleen and the bone marrow are both hematopoietic organs. The spleen is capable of producing erythrocytes and granulocytes, and platelets bud off of resident megakaryocytes. Platelet counts are not affected by age or sex. M:E ratios are >1.

Compared to cardiac blood, tail blood is much higher in leukocytes. WBCs are lower during activity than during rest. Excitement resulting from blood sampling may cause leukocytosis. Stress caused by ether anesthesia causes elevated RBCs, HCT, and Hb due to splenic contraction, and increased WBCs due to redistribution from peripheral blood. RBCs increase with heat and decrease with cold. Fasting can cause increases in RBCs, HCT, and Hb. High corticosteroid levels cause decreased lymphocyte counts. Rats have leukopenia during estrus. Rats are prone to leukemia, but less so than mice. Germ-free rats have decreased WBCs and increased RBCs, HCT, and Hb compared to unprotected rats.

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Clinical Chemistry Values can vary between strains. When collecting blood from the caudal vein, massaging the tail will cause dilution with tissue fluids that can significantly affect most clinical pathology parameters. Older rats have higher glucose levels. Young male rats (<4 months) have high serum creatinine levels. Manual restraint during sampling can cause increases in plasma protein, calcium, and magnesium; and decreases in blood pH and glucose. With age, plasma fibrinogen, total protein, and gamma globulin increase while albumin tends to decrease. Although rats do not have gall bladders, their bile ducts can be obstructed with a resultant increase in liver enzymes. Fasting and reduced food consumption result in decreased ALP due to a decrease in the intestinal ALP isoenzyme.

Urinalysis Rat urine tends to be basic, yellow, and high in volume. Clear or watery urine is a sign of chronic renal disease (e.g. renal amyloidosis). While renal disease is common in rats, some proteinuria is normal. Urolithiasis (renal calculus formation) is common in germ-free rats.

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Chapter 5
Hematology Diagnosis

Abstract The three diagnosis chapters—Hematology, Clinical Chemistry, and Urinalysis—are intended to be the most informative and most used parts of this handbook. There is a lot of useful information packed into these chapters. Once animal clinical pathology data have been evaluated and anomalous values for several parameters have been identified, these chapters can help one ascertain what these anomalies signify. Next to the name of each parameter are its common abbreviations. For each parameter, there are listings for organs that may be affected, specimen handling information, and supportive tests that may be used to confirm a diagnosis. These are followed by a brief description of the parameter including its strengths and weaknesses and other need-to-know information. Next to up and down arrows are potential diagnoses for when a parameter's value is increased or decreased. When there is a name for an increase or decrease (e.g., polycythemia or anemia), that is also provided. The Hematology Diagnosis section is broken up into four sections—Erythrocytes, Leukocytes, Hemostasis, and Bone Marrow—for a total of 23 hematology parameters.

The general format used in this chapter (Hematology Diagnosis) and Chaps. 6 (Clinical Chemistry Diagnosis) and 7 (Urinalysis Diagnosis) is as follows:

Parameter Name [Abbreviations]

Affected Organs: Organs or tissues that may be affected, e.g., liver, kidneys, etc.
Specimen Handling: Information on the type of sample needed, preservation, and storage.
Supportive Tests: Other parameters that may be useful for supporting or clarifying a diagnosis.

A brief description is provided for each parameter, including its strengths and limitations.

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J.E. Whalan, *A Toxicologist's Guide to Clinical Pathology in Animals*,
DOI 10.1007/978-3-319-15853-2_5

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↑	**[Name of the condition when a value is elevated, e.g., Hyperglycemia]**
	Listing of potential causes for an elevated value including diseases and toxicities as well as drug and chemical interferences
↓	**[Name of the condition when a value is decreased, e.g., Hypoglycemia]**
	Listing of potential causes for a decreased value including diseases and toxicities as well as drug and chemical interferences. 'Not clinically significant' means a decrease does not occur or is irrelevant

Hematology diagnostic information is presented in the following order:

Erythrocytes

Erythrocytes (red blood cells, red blood cell count, red corpuscles, RBC)
Erythrocyte Morphology (red blood cell morphology)
Hematocrit (packed cell volume)
Hemoglobin
Mean Corpuscular Hemoglobin
Mean Corpuscular Hemoglobin Concentration
Mean Corpuscular Volume
Nucleated Erythrocytes (metarubricytes, normoblasts)
Reticulocytes (polychromatic erythrocytes)

Leukocytes

Leukocytes, Total (white blood cells, white blood cell count, white corpuscles, WBC)
Differential Leukocyte Count
Basophils
Eosinophils
Lymphocytes
Monocytes
Neutrophils (Mature or Segmented Neutrophils/Granulocytes)
Neutrophils, Band (Immature or Stab Neutrophils/Granulocytes)
Neutrophil: Lymphocyte Ratio

Hemostasis

Activated Partial Thromboplastin Time
Bleeding Time
Prothrombin Time
Thrombocytes (platelets)

Bone Marrow

Myeloid: Erythroid Ratio

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5.1 Erythrocytes

5.1.1 Erythrocytes [RBC]

Affected Organs: Marrow, G.I., kidneys.
Specimen Handling: Whole blood (preserved with EDTA) may be stored 24 h at room temperature, 48 h at 4 °C.
Supportive Tests: Complete blood count (CBC), M:E ratio, serum iron, total iron binding capacity, serum ferritin, iron-binding protein, marrow iron stores, fecal occult blood.

Erythrocytes, also known as red blood cells or red corpuscles, contain hemoglobin and transport oxygen to the body. Erythrocytes are nucleated in all vertebrates except mammals. A mammalian erythrocyte is a mature cell (i.e. not nucleated) unless specified otherwise.

The erythrocyte count is a measure of the number of circulating erythrocytes per unit volume of blood ($\times 10^{12}$ cells/L in SI units or $\times 10^{-6}$ cells/mm^3 in non SI units). Erythrocyte counts, hematocrit, and hemoglobin values generally rise and fall together. Excitement caused by venipuncture and exercise can cause these parameters to rise significantly due to splenic contraction. High altitude can also cause them to increase. These values fluctuate widely in young animals, and they are naturally high in ferrets (especially in males). The erythrocyte count is performed by electronic counter (the counter must be calibrated for the erythrocyte size of the species), or microscopically by the hemocytometer method.

Anemia is a significant decrease in the erythrocyte count, hematocrit, hemoglobin concentration, and/or quantity of oxygen carrying hemoglobin due to blood loss or impaired blood production (secondary to a disease state). Anemias are classified according to erythrocyte size (based on MCV values), and hemoglobin content (based on the MCHC values) as described in Chap. 2.

The opposite of anemia—*erythrocytosis or polycythemia*—is an increase in erythrocyte mass in the blood. Both terms are used interchangeably. The most common causes for erythrocytosis in laboratory studies are stress-induced splenic contraction, as can occur during bleeding, or *hemoconcentration* (*relative erythrocytosis*) when an animal is deprived of water. Erythrocytosis may have primary or secondary causes as described in Chap. 2.

↑	**[Polycythemia, Erythrocytosis]**
	Overproduction of RBCs is usually caused by erythropoietin (EPO) stimulation following tissue hypoxia, or overcompensation following marrow suppression
	Dehydration, anoxemia (e.g. high altitudes), methemoglobinemia, splenic contraction in response to stress or excitement (e.g., handling)
	Polycythemia vera, congenital heart disease, bone marrow hyperplasia, renal carcinoma with increased EPO production, enlargement of liver and spleen

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↓	**[Anemia, Erythropenia, Erythrocytopenia]**
	Usually caused by aplastic anemia, marrow suppression, or loss of blood such as bleeding into the gastrointestinal tract (ulcer, colon cancer) or genitourinary tract
	Deficiency in iron, copper, vitamin B, or protein needed for erythrogenesis
	Coagulopathy (such as vitamin K or clotting factor deficiency), thrombocytopenia, or von Willebrand disease (in dogs)
	Pregnancy, prolonged menstruation, abortion
	Malaria, arboviruses in primates, gut parasitism (e.g. coccidia and hookworm)
	Feline infectious anemia (*E. felis* and *H. felis*)
	Malignant lymphoma (primates)
	Hydremia (e.g. administration of intravenous fluids)
	Anesthetics (splenic storage), surgery
	Warfarin, arsenicals, estrogens, phenylbutazone, aspirin, sulphonamides, antihistamines, chloramphenicol, coal-tar derivatives, bracken fern poisoning, trichloroethylene-extracted feeds

5.1.2 Erythrocyte Morphology

Affected Organs: Marrow, G.I., kidneys.
Specimen Handling: Whole blood (preserved with EDTA) may be stored 24 h at room temperature, 48 h at 4 °C.
Supportive Tests: Complete Blood Count (CBC), M:E ratio.

Wet mounts are preferable to dry blood smears for evaluating erythrocyte morphology. Blood smears are prone to artifacts caused by drying, mechanical damage, and contact with glass surfaces. Some artifacts resemble abnormal erythrocytes. Dry erythrocytes have smaller diameters.

RBCs can vary widely in size, shape, volume, staining characteristics, fragility, and hemoglobin distribution. A normal RBC is a *Normocyte*. Any abnormal RBC is a *Poikilocyte*. While poikilocytes are often associated with abnormal erythrogenesis, circulatory trauma, specific diseases, toxicity, or blood smear artifact, they may be normal in some species. Table 5.1 illustrates and describes the morphology of the most commonly observed poikilocytes. Below Table 5.1 are additional poikilocyte terms with brief descriptions. All poikilocytes are further described in the hematology glossary (Chap. 8).

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Table 5.1 Erythrocyte morphology

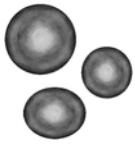
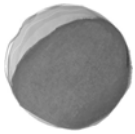
	Acanthocytes have projections of variable length that are unevenly spaced on the surface of the red cell. Acanthocytes may be seen as an incidental finding, as a consequence of a high-fat diet, with disorders of lipid metabolism, and with hemangiosarcoma. In the latter case, acanthocytes may form when 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 dacryocyes 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

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Table 5.1 (continued)

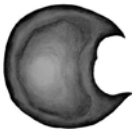
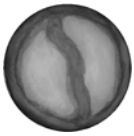
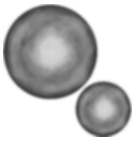
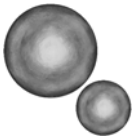
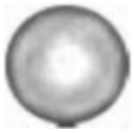
	Echinocytes III are spherical red cells with sharp projections of equal length that are evenly spaced on the surface of the red cell. They may be increased in animals with renal disease and/or electrolyte disturbances. They can also occur artifactually for similar reasons described for echinocytes I
	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
	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

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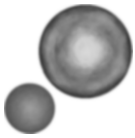
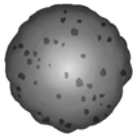
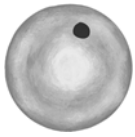
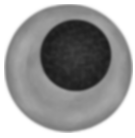
Table 5.1 (continued)

	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 cells fragments attributed to mechanical red cell injury/shearing (see karatocytes)
	**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
	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 basophila 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 ≥5NRBs/100 WBCs

(continued)

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Table 5.1 (continued)

Additional Poikilocytes	
Achromocyte—ghost cell	**Normocyte**—normal cell
Acuminocyte—fusiform	**Ovalocyte**—oval shape; elliptocyte
Burr cell—echinocyte	**Pappenheimer bodies**—iron granules
Cabot's rings—loops & figure 8's	**Poikilocyte**—any unusual RBC shape
Crenation—notched appearance (artifact)	**Punched-out cell**—torocyte
Cryohydrocyte—cold lysing	**Pyknocyte**—small, distorted, spiculed
Descicyte—xerocyte	**Pyropoikilocyte**—distorts on heating
Discocyte—normal cell shape	**Schistocyte**—schizocyte
Drepanocyte—sickle-shaped	**Selenocyte**—pale, crescent-shaped
Fusocyte—spindle-shaped	**Sickle cell**—drepanocyte
Gigantocyte—extremely large	**Siderocyte**—iron granules
Helmet cell—schistocyte	**Spur cell**—acanthocyte
Howell-Jolly bodies—nuclear material	**Stomatocyte**—mouth-like slit
Hydrocyte—swollen	**Stomatospherocyte**—nearly spherical
Knizocyte—triconcave	**Target cell**—codocyte
Megalocyte—unusually large	**Tear-shaped**—dacryocyte
Metarubricyte—immature RBC	**Torocyte**—donut-shaped
Microspherocyte—small, spherical	**Xerocyte**—flattened, dehydrated

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5.1.3 Hematocrit [HCT, Hct, Ht, PCV]

Affected Organs: Marrow.

Specimen Handling: Whole blood (preserved with EDTA) may be stored 6 h at room temperature, 48 h at 4 °C.

Supportive Tests: Complete blood count (CBC), M:E ratio, serum iron, total iron binding capacity, serum ferritin, iron-binding protein, marrow iron stores, fecal occult blood.

The hematocrit (also called packed cell volume, PCV) is the percentage of blood volume occupied by erythrocytes after the blood is centrifuged for approximately 5 min (10–20 min for sheep and goat blood because of small cell size). Hematocrit can be calculated by an electronic counter from the RBC count and MCV provided

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the counter has been calibrated for the erythrocyte size of the species. The microhematocrit method does not require expensive equipment, but it is prone to error when centrifugation fails to remove trapped plasma. The buffy coat layer of platelets and leukocytes is not included in the hematocrit measurement.

See Erythrocytes—Because erythrocytes, hematocrit, and hemoglobin usually rise and fall together, the interpretation of anomalies is the same for all three.

↑	**[Polycythemia, Hemoconcentration, Dehydration]**
↓	**[Hemodilution, Hydremia, Oligocythemia, Oligocytosis]**

5.1.4 Hemoglobin [Hb, HB, HGB, Hgb]

Affected Organs: Marrow.
Specimen Handling: Whole blood (preserved with EDTA) may be stored 24 h at room temperature, 48 h at 4 ° C.
Supportive Tests: Complete blood count (CBC), M:E ratio, serum iron, total iron binding capacity, serum ferritin, iron-binding protein, marrow iron stores, fecal occult blood.

Hemoglobins are the oxygen-carrying molecules in erythrocytes, so this parameter is a measure of the blood's ability to transport oxygen to the body. The cyanmethemoglobin method can measure all hemoglobin types. Other methods are less accurate. Excess anticoagulant causes hypochromic dilution. High altitudes and excitement caused by venipuncture and exercise can cause erythrocyte counts, hematocrit, and hemoglobin values to rise significantly due to splenic contraction. These values fluctuate widely in young animals, and are naturally high in ferrets (especially in males). Hb is reported in units of g/L (SI units) or g/dL (non-SI units).

NOTE: Hemoglobin is also measured in urine; see Chap. 7, Urinalysis Diagnosis.

↑	**[Hyperchromasia]**
	See Erythrocytes—RBC, HCT, and Hb usually rise and fall together. Since a cell's hemoglobin content cannot increase beyond normal levels, elevated hemoglobin must be attributed to polycythemia
↓	**[Hypochromasia]**
	See Erythrocytes—RBC, HCT, and Hb usually rise and fall together. Protein deficient diet

5.1.5 Mean Corpuscular Hemoglobin [MCH]

Affected Organs: Marrow.
Specimen Handling: Whole blood (preserved with EDTA) may be stored 6 h at room temperature, 24 h at 4 °C. Capillary blood may also be used.
Supportive Tests: Complete blood count (CBC), M:E ratio, serum iron, total iron binding capacity, serum ferritin, iron-binding protein, marrow iron stores, fecal occult blood.

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The MCH index is the calculated mass of hemoglobin in an average erythrocyte. The equation for calculating the MCH can be found in the Hematology Glossary. The MCH and MCV usually rise and fall together (i.e. as the cells get bigger, they will also contain more hemoglobin). The MCH can highlight errors in erythrocyte count, hematocrit, and hemoglobin measurements, and portray the course of anemia.

↑	**[Hyperchromasia]**
	Macrocytic anemia, postpartum (normal condition)
↓	**[Hypochromasia]**
	Iron deficient anemia, microcytic hypochromic anemia, chronic blood loss, copper or pyridoxamine deficiency

5.1.6 Mean Corpuscular Hemoglobin Concentration [MCHC]

Affected Organs: Marrow.
Specimen Handling: Whole blood (preserved with EDTA) may be stored 6 h at room temperature, 24 h at 4 °C. Capillary blood may also be used.
Supportive Tests: Complete blood count (CBC), M:E ratio, serum iron, total iron binding capacity, serum ferritin, iron-binding protein, marrow iron stores, fecal occult blood.

The MCHC is the calculated weight to volume proportion of hemoglobin in the average erythrocyte. The equation for calculating the MCHC can be found in the Hematology Glossary. The MCHC can highlight errors in erythrocyte count, hematocrit, and hemoglobin measurements, and portray the course of anemia.

↑	**[Hyperchromasia]**
	Macrocythemia (enlarged RBCs), xerocytosis
↓	**[Hypochromasia]**
	Iron deficiency anemia, lead poisoning, penicillamine poisoning

5.1.7 Mean Corpuscular Volume [MCV]

Affected Organs: Marrow, G.I., kidneys, liver, thyroid.
Specimen Handling: Whole blood (preserved with EDTA) may be stored 6 h at room temperature, 24 h at 4 °C. Capillary blood may also be used.
Supportive Tests: Complete blood count (CBC), M:E ratio, serum iron, total iron binding capacity, serum ferritin, iron-binding protein, marrow iron stores, fecal occult blood.

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The MCV is the calculated volume of an average erythrocyte. The equation for calculating the MCV can be found in the Hematology Glossary. The MCV and MCH usually rise and fall together (i.e. as the cells get bigger, they will also contain more hemoglobin). The MCV can highlight errors in erythrocyte count, hematocrit, and hemoglobin measurements, and portray the course of anemia.

↑	**[Macrocytosis, Macrocythemia]**
	Megaloblastic anemia—caused by drugs, alcohol, and vitamin deficiency
	Acute blood loss, aplastic anemia, chronic hemolytic anemia, pernicious anemia, reticulocytosis, liver disease, hypothyroidism
	Swelling of erythrocytes due to long sample storage
↓	**[Microcytosis, Microcythemia]**
	Hypochromic and microcytic anemias, lead poisoning, iron deficiency

Metarubricytes See Nucleated Erythrocytes
Morphology See Erythrocyte Morphology
Normoblasts See Nucleated Erythrocytes

5.1.8 Nucleated Erythrocytes [nRBC, NRBC, NucRBC]

Affected Organs: Marrow
Specimen Handling: Whole blood (preserved with EDTA) may be stored 24 h at room temperature, 48 h at 4 °C. Do not use heparin.
Supportive Tests: Complete blood count (CBC), M:E ratio, serum iron, total iron binding capacity, serum ferritin, iron-binding protein, marrow iron stores, fecal occult blood.

Nucleated erythrocytes (nRBCs), also known as nucleated red blood cells, normoblasts, and metarubricytes, are immature cells that are prematurely released from the bone marrow in response to anemia. They can also be a sign of extramedullary hematopoiesis. They are normal only in suckling pigs less than 3 months old. Reticulocytes generally accompany nRBCs. The presence of nRBCs in an anemic animal is a sign of a responsive marrow unless their numbers are great, or there is no evidence of maturation (i.e. a subsequent increase in reticulocytes and mature RBCs). Horses do not release nRBCs.

↑	**[Normoblastosis]**
	Early release of nucleated RBCs in response to regenerative anemias, severe bone marrow trauma, or extramedullary hematopoiesis
	Suppressed splenic function in dogs caused by corticosteroid therapy
	Marrow disorder (increased nRBCs in the absence of reticulocytes as in feline leukemia)
	Cushing syndrome in dogs, feline erythroleukemia
	Anoxia, lead poisoning, oncolytics
↓	Decrease or absence of normoblasts is not pathologic

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Nucleated Red Blood Cells	See Nucleated Erythrocytes
Packed Cell Volume	See Hematocrit
Polychromatic Erythrocytes	See Reticulocytes
Red Blood Cell Count	See Erythrocytes
Red Blood Cell Morphology	See Erythrocyte Morphology
Red Blood Cells	See Erythrocytes
Red Corpuscles	See Erythrocytes

5.1.9 Reticulocytes [Ret, Retics]

Affected Organs: Marrow, G.I., kidneys.
Specimen Handling: Whole blood (preserved with EDTA, heparin, or oxalates). Capillary blood may also be used.
Supportive Tests: Complete blood count (CBC), M:E ratio, serum iron, total iron binding capacity, serum ferritin, iron-binding protein, marrow iron stores, fecal occult blood.

Reticulocytes are large immature erythrocytes that contain polychromatophilic reticular material that appears as bluish stippling with Romanowsky stain. They are released into circulation in response to anemia (except in horses), and are therefore useful for assessing an animal's response to anemia. 'Shift' or 'stimulated' reticulocytes are released early in response to erythropoietin (EPO) and tend to be larger, less mature, and contain more reticular material. Reticulocytes are not visibly different from normal erythrocytes when stained with Wright's stain, so supravital staining is used (brilliant cresyl blue and new methylene blue). One thousand erythrocytes are examined to determine the number of cells that still contain reticular material.

Horses do not release reticulocytes in response to anemia. Reticulocytes are not found in cattle, goats, horses, and sheep in health. They are found in the blood of dogs, cats, pigs, and are common in guinea pigs, mice, rats, and rabbits. Ferrets have high reticulocyte counts. Excitement during sampling can stimulate a significant release of reticulocytes.

↑	**[Reticulocytosis]**
	Increased erythropoiesis due to hemolysis (hemolytic anemia), hemorrhage, blood loss anemia, and recovery from aplastic anemia
	Splenic contraction in response to excitement
	Epinephrine
↓	**[Reticulocytopenia]**
	Disorders of RBC maturation (e.g., chronic anemia, aplastic anemia, pernicious anemia, sickle cell anemia, bone marrow malignancies), renal disease, endocrine disease, iron deficiency anemia, impaired erythropoietin (EPO) production in the kidneys, vitamin B_9 or B_{12} deficiency
	Chemotherapy

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5.2 Leukocytes

5.2.1 Leukocytes, Total [WBC]

Affected Organs: Marrow, heart, spleen, kidneys.
Specimen Handling: Whole blood (preserved with EDTA) may be stored 24 h at room temperature, 48 h at 4 °C. Capillary blood may also be used. Do not use heparin.
Supportive Tests: Complete Blood Count (CBC), differential leukocyte count, M:E ratio.

Leukocytes, also known as white blood cells or WBCs, play a critical role in the immune system. The total leukocyte count is a manifestation of the absolute counts of the five major leukocyte specie—neutrophils (segmented and band), lymphocytes, eosinophils, basophils, and monocytes. An increase in a WBC count (leukocytosis) indicates a pathologic condition that is best assessed by evaluating the differential leukocyte count.

WBC counts can vary widely due to circadian effects and the choice of bleeding sites. A physiologic leukocytosis due to the release of epinephrine can result from exercise, stress, and the apprehension associated with venipuncture. This can lead to misdiagnosis. In small animals such as rats, mice, and rabbits with high lymphocyte counts, stress causes lymphopenia and neutrophilia with the marked decrease in circulating lymphocytes resulting in an overall leukopenia. Species with high neutrophil counts have leukocytosis, neutrophilia, and lymphopenia when stressed. The release (or administration) of corticosteroids causes neutrophilia, lymphopenia, and eosinopenia. This results in a leukocytosis in species with N:L ratios >1.0, and a leukopenia in species with N:L ratios <1.0.

↑	**[Leukocytosis]**
	Infection, various diseases, tumors, intoxication, leukemia, acute hemolysis, acute hemorrhage, acute myocardial infarct, burns, gangrene
	Noninfectious inflammatory diseases (e.g. stress and pancreatitis)
	Exercise, excitement, fever, pain, menstruation, exposure
	Oncolytics, steroid therapy, epinephrine
	Lead, mercury, black widow venom
↓	**[Leukopenia]**
	Severe infections (overwhelming tissue demands)
	Various diseases, pernicious anemia, lymphatic leukemia (due to lymphocyte fragility), aplastic anemia, endotoxic shock, anaphylactic shock, cachexia, hypersplenism, severe renal injury, panleukopenia (a viral disease in cats)
	Sulfonamides, antibiotics, antihistamines, analgesics, anticonvulsants, antithyroids, organic arsenicals, oncolytics, ionizing radiation

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5.2.2 Differential Leukocyte Count [Diffs]

A differential leukocyte count presents the proportion of each leukocyte species—total WBCs, neutrophils (segmented and band), lymphocytes, eosinophils, basophils, and monocytes—as counted on a Wright-stained blood smear. Differential counts should be evaluated as absolute cell counts (e.g. 1.2×10^3 neutrophils) rather than as percentages. When differential data are presented in percentages, absolute counts can easily be calculated using the following equation:

$$Total\ WBC \times \%\ Leukocyte\ Species = Absolute\ Count$$

Typically, 100–200 leukocytes per blood smear are classified for cell type and abnormalities. The use of blood several hours old (especially oxalated blood) may hinder evaluation since the nuclei of leukocytes degenerate with time. Further information on evaluating differential leukocyte count data can be found in the Hematology Highlights chapter.

White Blood Cells See Leukocytes
White Corpuscles See Leukocytes

5.2.3 Basophils [Basos, Bas]

Affected Organs: Marrow, kidneys, thyroid.
Specimen Handling: Whole blood (preserved with EDTA) may be stored 24 h at room temperature, 48 h at 4 °C. Capillary blood may also be used. Do not use heparin.
Supportive Tests: Complete Blood Count (CBC), differential leukocyte count, M:E ratio.

Basophils are involved in hypersensitivity reactions and inflammation. The basophil is a granular leukocyte with an irregular, pale-staining multilobed nucleus (2–3 lobes), and a small number of cytoplasmic granules whose size and staining characteristics vary between species. Basophils are morphologically similar to mast cells, and serve some of the same functions. They produce and store histamine, heparin, serotonin, hyaluronic acid, and other vasoactive substances, and release these chemicals upon stimulation. After leaving the bone marrow, basophils spend only a few hours in peripheral circulation before migrating into body tissues. Basophilia is rare, but may coincide with eosinophilia. Basophils are found in small numbers except in rabbits. As a rule, species with many basophils have few mast cells, and

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species with many mast cells, have few basophils. In those species that have very low basophil counts in health, it may be difficult to identify basopenia.

↑	**[Basophilia, Basocytosis]**
	Myelocytic leukemia, basophilic leukemia, chronic liver disease, ulcerative colitis, hyperlipoproteinemia, lipemia, nephrosis, chronic hemolytic anemias, hypothyroidism
	Long term hypersensitivity reactions to food, drugs, inhalants, and foreign protein injections
	Heart worm infestation in dogs
	Estrogens, antithyroid drugs, D-thyroxine
↓	**[Basopenia]**
	It is normal for most species to have no basophils in a differential count
	Hyperthyroidism, ovulation, pregnancy, stress, acute infection, myocardial infarct, bleeding peptic ulcer, anaphylactic shock, urticaria
	Adrenocorticotropin, corticosteroids, progesterone, thyrotropin, L-thyroxine, X-radiation, oncolytics

5.2.4 Eosinophils [Eosins, Eos]

Affected Organs: Marrow, lungs, skin, spleen.

Specimen Handling: Whole blood (preserved with EDTA) may be stored 24 h at room temperature, 48 h at 4 °C. Capillary blood may also be used. Do not use heparin.

Supportive Tests: Complete Blood Count (CBC), differential leukocyte count, M:E ratio.

Eosinophils mediate allergic and inflammatory reactions, and destroy parasites (e.g., helminths). The eosinophil is a granular leukocyte distinguished by its bright pink or orange cytoplasmic granules and a smooth polymorphic nucleus. The granules are rod-shaped in cats. Although their appearance varies widely between species, all eosinophils are parasitocidal, bacteriocidal, and phagocytic.

Eosinophils are produced mostly in the marrow, but also in the thymus, spleen, and cervical lymph nodes. They spend only a few hours (1 h in dogs) in the peripheral circulation before migrating into tissues. They tend to congregate near mast cells, and are found mostly in the bone marrow and ports-of-entry such as the skin, gastrointestinal tract, and lung. Thus, a CBC may not be a reliable measure of eosinophils in affected tissues. Most species have their lowest eosinophil counts in the morning. In those species that have very low eosinophil counts in health, it may be difficult to identify eosinopenia.

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↑	**[Eosinophilia]**
	Trichinosis, heartworm, and other parasites (nematodes, cestodes, and scabies)
	Bronchial asthma, nonspecific allergic bronchitis, allergy, drug sensitivity, various diseases, malignant neoplasms, myelocytic leukemia, eosinophilic leukemia, mast cell disorders and tumors, erythema, eczema, psoriasis, erythema multiforme, urticaria, scarlet fever, rheumatoid arthritis, tuberculosis, estrus
	Splenectomy, severe hypoxia, magnesium deficiency
	Smoking, penicillin, oncolytics, cephalosporins, sulfonamides, tetracyclines, anticonvulsants, antituberculars, phosphorus, black widow venom
↓	**[Eosinopenia]**
	Pyrogenic infections, labor, eclampsia, uremia, major surgery, shock, burns, acute infection, stress
	Corticotropin, costicosteroids, epinephrine

Granulocytes, Band See Neutrophils, Band
Granulocytes, Segmented See Neutrophils, Segmented
Granulocytes, Stab See Neutrophils, Band
Heterophil See Neutrophils, Segmented

5.2.5 Lymphocytes [Lymphs, Lym]

Affected Organs: Marrow, thymus, kidneys, heart, adrenals, thyroid.
Specimen Handling: Whole blood (preserved with EDTA) may be stored 24 h at room temperature, 48 h at 4 °C. Capillary blood may also be used. Do not use heparin.
Supportive Tests: Complete Blood Count (CBC), differential leukocyte count, M:E ratio.

Lymphocytes vary widely in size. They are round and have a single round or oval nucleus. Lymphocytes are produced mostly in the bone marrow, but also in lymphoid organs (including the lymph nodes, thymus, and spleen), and in the gut-associated lymphoid tissues (including tonsils, Peyer's patches in the small intestines, and the appendix). Immature lymphocytes are large, have dispersed chromatin, basophilic cytoplasm, and smooth chromatin. Mature lymphocytes are smaller and have smaller nuclei with chromatin clumping. When stimulated, mature B-lymphocytes and T-lymphocytes can revert to immature blast forms (blastogenesis), and can then divide and mature.

Lymphocytes are able to recirculate (mobilize); that is, they leave the blood, enter lymphatic tissues, and then return to the blood. This allows immunocompetent lymphocytes to distribute widely and yet target antigens in large numbers when needed. Immune competence is adequate at birth, complete within a few months, and gradually tapers off with age.

Changes in leukocyte counts generally reflect the status of cell production in the marrow, but this is <u>not</u> the case with lymphocytes. Mature lymphocytes are stored in

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a pool of unknown location, and their numbers are approximately 10-times that found in circulation. Lymphocytosis and lymphopenia are terms used to describe relative increases and decreases in circulating lymphocyte counts, which in turn are reflections of production, destruction, maturation, and recirculation (mobilization).

Corticosteroids inhibit lymphocyte production, especially in rats, mice, and rabbits. A physiologic lymphocytosis due to the release of epinephrine can result from exercise, stress, and apprehension associated with venipuncture, due to an increased number of circulating lymphocytes in cats, horses, and young animals.

↑	**[Lymphocytosis]**
	Chronic bacterial or viral infection, recovery from some infections, adrenocortical insufficiency, hyperthyroidism, lymphocytic leukemia, lymphosarcoma
	Vaccination, physiological leukocytosis due to stress (especially in cats and horses)
	Organic arsenicals, lead poisoning, I.V. heparin (in calves and rats)
↓	**[Lymphopenia, Lymphocytopenia]**
	Acute infections and illnesses, severe right heart failure, aplastic anemia, myelocytic leukemia, lymphatic leukemia (due to lymphocyte fragility), Hodgkin's disease, terminal carcinoma, lupus, thymic disorders, T-lymphocyte deficiency, gastrointestinal malabsorption syndrome, renal failure, canine distemper
	Oncolytics, corticosteroids, X-radiation

5.2.6 Monocytes [Monos, Mon]

Affected Organs: Marrow, G.I.

Specimen Handling: Whole blood (preserved with EDTA) may be stored 24 h at room temperature, 48 h at 4 °C. Capillary blood may also be used. Do not use heparin.

Supportive Tests: Complete Blood Count (CBC), differential leukocyte count, M:E ratio.

Monocytes are formed in the body marrow and are typically the largest of the leukocytes. They are found in small numbers in the blood. The nucleus can have a variety of shapes (generally ovoid or kidney shaped). The cytoplasm has a ground glass appearance and contains small purplish granules and large vacuoles. Monocyte nuclei degenerate quickly in old blood.

Monocytes are phagocytic and routinely attack the more difficult pathogens. After briefly circulating in the blood, monocytes enter body tissues and cavities where they become 'fixed' macrophages until they are stimulated by inflammation to become 'free' macrophages. The effectiveness of a monocyte increases when it becomes a macrophage. Unlike neutrophils, monocytes are capable of mitosis while residing in tissues. Corticosteroids inhibit the function of monocytes and macrophages. Since macrophages are rarely found in the blood, monocytes are counted instead. Monocytes are components of the Mononuclear Phagocyte System (MPS), which includes monocytes and monocyte precursors in the blood and marrow, and

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macrophages of the lymph nodes, spleen, lung, bone marrow, connective tissue, Kupffer cells (liver), and other organs. In those species that have very low monocyte counts in health, it may be difficult to identify monocytopenia.

↑	**[Monocytosis]**
	Recovery from acute infections, rickettsial diseases, and protozoal diseases
	Tuberculosis, syphilis, ulcerative colitis, regional enteritis, monocytic leukemia, multiple myeloma, recovery from aplastic anemia, rheumatoid arthritis, collagen diseases, tissue necrosis
	Stress (especially in dogs)
	Carbon disulfide, phosphorus, steroids, ACTH and corticosteroids (in dogs)
↓	**[Monocytopenia, Monopenia]**
	Generally not clinically significant
	Bone marrow injury, leukemia
	Glucocorticoids, ACTH, and corticosteroids (in mice, rats, guinea pigs, and humans)

5.2.7 Neutrophils [Neuts, Segs, N. Seg.]

Affected Organs: Marrow, G.I., pancreas, kidneys, liver.

Specimen Handling: Whole blood (preserved with EDTA) may be stored 24 h at room temperature, 48 h at 4 ° C. Capillary blood may also be used. Do not use heparin.

Supportive Tests: Complete Blood Count (CBC), differential leukocyte count, M:E ratio.

Segmented, or mature neutrophils are the predominant leukocyte in most species. Neutrophils are a type of granulocyte (the others being eosinophils, and basophils), but the term granulocyte is often used loosely to mean neutrophil. Neutrophils are both phagocytic and bacteriocidal, and are the first line of defense against microbial infection. In some species, particularly birds, they are called *heterophils* because of the diverse way they stain.

Band neutrophils (immature neutrophils) are in small numbers in the blood, but are released early in response to infection. The marrow contains an abundant storage pool of mature neutrophils (a 5 day supply in dogs) that allows for rapid replacement. The $t_{1/2}$ for circulating neutrophils is approximately 6 h.

Blood neutrophil counts reflect neutrophil production (granulopoiesis), release from the marrow, cell senescence, margination (adhering to vessel walls), and migration into the tissues. Fear, stress, and the release of epinephrine and norepinephrine can cause physiological neutrophilia due to a shift of the marginated neutrophils back into the circulating pool. This shift can result in a sudden 2–3 fold increase in neutrophils. This can lead to misdiagnosis in animals not conditioned to venipuncture. Corticosteroid administration prompts the marrow to release stored mature neutrophils.

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In response to infection, circulating neutrophils enter the tissues (*diapedesis*) causing an initial *neutropenia*. The marrow then releases stored neutrophils that marginate. The neutropenia persists despite the increased total blood granulocyte pool. As infection progresses, granulopoiesis steps up and new (as opposed to stored) neutrophils are released, resulting in neutrophilia. An animal with an acute inflammation will have neutropenia and a left shift (an increased release of band neutrophils). An animal with chronic inflammation will have marked neutrophilia with little or no left shift. Neutrophils are the major component in pus. An infection can cause neutrophils to produce significant quantities of histamine.

↑	**[Neutrophilia]**
	Bone marrow release in response to various diseases, inflammation, tissue damage, ischemic necrosis, colitis, pancreatitis, nephritis, hepatic necrosis, leukemia, neoplasms
	Heat, cold, exercise, pain, burns, pregnancy, labor, trauma, intense emotion
	Acute hemorrhage, uremia, diabetes, eclampsia
	Surgical trauma, splenectomy
	Corticosteroids, epinephrine, norepinephrine, histamine, heparin, lead, mercury, ethylene glycol, digitalis, CO
↓	**[Neutropenia]**
	Early stages of bacterial infection, overwhelming bacterial infections, viruses, anaphylactoid shock, endotoxic shock, bone marrow necrosis
	Various diseases, inadequate granulopoiesis, aplastic anemia, leukemia, megaloblastic anemia, iron deficiency anemia
	Hypothyroidism, hypopituitarism, cirrhosis
	Oncolytics, analgesics, barbiturates, tranquilizers, antidepressants, antithyroids, anticonvulsants, antihistamines, antimicrobials, diuretics

5.2.8 Neutrophils, Band [Bands, I. Neut., N-Band]

Affected Organs: Marrow, G.I., pancreas, kidneys, liver.
Specimen Handling: Whole blood (preserved with EDTA) may be stored 24 h at room temperature, 48 h at 4 °C. Capillary blood may also be used. Do not use heparin.
Supportive Tests: Complete Blood Count (CBC), differential leukocyte count, M:E ratio.

Band neutrophils are immature neutrophils. A small number of band neutrophils are always found in the blood. Whenever infection places a demand on the bone marrow to release more neutrophils, an increased number of immature neutrophils are released into the blood. This is called a **left shift**. As the released cells become more immature, the left shift is said to be more severe. The degree of left shift can be defined as follows:

Slight—release of band neutrophils
Moderate—release of many band neutrophils and some metamyelocyte neutrophils

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Marked—release of many myelocytes and some promyelocytes
Severe—release of myeloblasts

The left shift may be either regenerative or degenerative:

Regenerative—The left shift is due to increased granulopoiesis in healthy bone marrow. There is an increase in immature neutrophils, but they are outnumbered by mature neutrophils.

Degenerative—The left shift is due to inhibited granulopoiesis and septicemia, and is characterized by delayed maturation, so the immature neutrophils outnumber the mature neutrophils and WBC counts are normal or decreased.

In a **right shift**, there is an increased proportion of aged neutrophils in the blood as characterized by hypersegmentation of the nucleus (5 or more lobes). It is caused by corticosteroids, vitamin B_{12} and folate deficiency, and reduced cell mitosis in the marrow. It may also be an artifact in stored blood. Right shifts are seen in humans, but rarely in domestic animals.

↑	**[Left Shift]**
	A shift to the left (i.e., an increased proportion of immature to mature neutrophils) occurs in response to acute infections, metabolic acidosis, myocardial infarct, malignant tumors, hemolytic crisis, severe loss of blood, leukemia, and various diseases
↓	A decrease generally is not pathologic, but it can result from marrow suppression (e.g., oncolytics, or a shift to the right)

Neutrophils Immature See Neutrophils, Band
Neutrophils Mature See Neutrophils, Segmented

5.2.9 Neutrophil: Lymphocyte Ratio [N:L Ratio]

The Neutrophil: Lymphocyte Ratio compares the differential cell counts for these two major types of leukocytes. The ratio changes in response to disease, stress, and age. Lymphocyte counts are greatest in young animals, then taper off with age. Since neutrophil counts remain fairly stable over time, this means N:L ratios are lowest in youth and increase with age.

An animal's true leukocyte status can be misdiagnosed because of the stress of collecting blood. While species respond differently to stress, a rule of thumb is that stress causes leukopenia, lymphopenia, and neutrophilia in small animals such as rats, mice, and rabbits that have high lymphocyte counts. Species with high neutrophil counts have leukocytosis, neutrophilia, and lymphopenia when stressed. Stress causes lymphocytes to decrease more than neutrophils. In those animals with mostly lymphocytes, stress decreases the number of circulating lymphocytes so much that leukopenia results.

N:L ratios can be expressed either as differential percents (60:40), a calculated ratio (1.5:1), or a single number (1.5). Table 5.2 demonstrates the differences between species. These values should be used only as a rough guide since cell counts vary between laboratories.

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Table 5.2 Neutrophil: lymphocyte ratios for various species

Cat	1.8	Horse (cold-blooded)	1.7
Cattle	0.5	Horse (aged)	2.0
Dog	3.5	Human	1.5
Donkey	1.1	Monkey (newborn)	3.2
Goat (newborn)	1.6	Monkey (unconditioned)	0.4
Goat (1 week)	0.8	Monkey (conditioned)	1.3
Goat (1 month)	0.6	Mice (marked variance in cell counts due to diurnal, strain, and sex differences)	
Goat (3 months)	0.3	Mule	1.1
Goat (>2 years)	1.1	Pig (1 week)	1.0
Guinea pig (<30 day ♂/♀)	0.4/0.3	Pig (6 months)	1.2
Guinea pig (90 day ♂/♀)	0.5/0.4	Rabbit (2 months)	0.6
Guinea pig (1 year ♂/♀)	0.3/0.3	Rabbit (>1 year)	1.0
Guinea pig (2 year ♂/♀)	0.5/0.4	Rat (Sprague-Dawley)	
Hamster (1 month)	0.5	(1 month ♂/♀)	0.2/0.2
Hamster (6 months)	0.3	(1 year ♂/♀)	0.5/0.4
Hamster (12 months)	0.4	Rat (Long-Evans)	
Horse (day 1)	2.8	(1 month ♂/♀)	0.2/0.2
Horse (<2 month hot-blooded)	1.5	(1 year ♂/♀)	0.6/0.3
Horse (young hot-blooded)	1.1	Sheep	0.5
Horse (yearling hot-blooded)	0.9		

Segmented Granulocytes See Neutrophils

5.3 Hemostasis

5.3.1 Activated Partial Thromboplastin Time [APTT, aPTT, PTT]

Affected Organs: Liver, marrow, spleen, G.I.

Specimen Handling: Whole blood (preserved with oxalate or sodium citrate) is immediately centrifuged and the plasma removed. The plasma may be stored 1 h at 4 °C, 28 days at—20 °C.

Supportive Tests: Platelet count, prothrombin time, fibrinogen, bleeding time, clotting factors.

Activated partial thromboplastin time (APTT), also called partial thromboplastin time (PTT), is used to evaluate coagulation via the intrinsic and common pathways and to monitor heparin therapy. The addition of phospholipid, calcium, and an activator (silicate, kaolin, celite, or ellagic acid) to preserved plasma activates the intrinsic pathway. The APTT is the time it takes for a thrombus to form. Because this test

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is sensitive to the ratio of anticoagulant to blood, collection tubes that are underfilled or overfilled may give unreliable values.

↑	Decreased plasma levels of coagulation factors VIII, IX, XI, XII, prekallikren, high molecular weight kininogens, and fibrinogen (factor I)
	Hemophilia, sepsis, disseminated intravascular coagulation (DIC)
	Underfilled collection tube
	Anticoagulants, oncolytics
↓	Not clinically significant
	Increased coagulation factor VIII level

5.3.2 Bleeding Time [BT]

Affected Organs: Marrow, liver, spleen, G.I.
Supportive Tests: Platelet count, prothrombin time, activated partial thromboplastin time, fibrinogen, clotting factors.

Bleeding time is a timed measurement of hemostasis. It is the time required for bleeding to cease following a controlled skin incision of the inner thigh, lip, or tip of the ear. It is primarily a way to assess platelet function and diagnose von Willebrand's disease.

↑	Thrombocytopenia, abnormal platelet function, uremia, metabolic acidosis, capillary fragility, vascular lesions, von Willebrand's disease
	Obstructive jaundice, vitamin K deficiency
	Coumarin, warfarin, aspirin, nonsteroidal anti-inflammatory agents, ibuprofen, penicillin G
↓	Not clinically significant

Partial Thromboplastin Time See Activated Partial Thromboplastin Time
Platelets See Thrombocytes

5.3.3 Prothrombin Time [PT, Pro Time]

Affected Organs: Liver, G.I., marrow, liver, spleen.
Specimen Handling: Whole blood (preserved with sodium citrate in a 9:1 ratio) can be stored 2 h at room temperature, 4 h at 4 °C.
Supportive Tests: Activated partial thromboplastin time, platelet count, fibrinogen, bleeding time, clotting factors.

Prothrombin time is used to evaluate coagulation via the extrinsic and common pathways and to monitor warfarin therapy. Calcium and tissue factor (factor III) are added to plasma to activate the extrinsic pathway. The PT is the time it takes for a thrombus to form. Because this test is sensitive to the ratio of citrate to blood, collection tubes that are underfilled or overfilled may give unreliable values.

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↑	Decreased plasma levels of coagulation factors I (fibrinogen), II (prothrombin), V, VII, or X
	Vitamin K deficiency (may be due to G.I. trauma)
	Liver disease, severe liver damage, jaundice, intravascular coagulation
	Underfilled collection tube
	Acetaminophen, antibiotics, warfarin, aspirin, oncolytics, ethanol, halothane, heparin
↓	Not clinically significant

5.3.4 Thrombocytes (Platelets) [Thromb, Plate]

Affected Organs: Marrow, heart, liver, pancreas, spleen.
Specimen Handling: Whole blood (preserved with EDTA) may be stored 5 h at room temperature, 24 h at 4 °C. Capillary blood may also be used.
Supportive Tests: Complete blood count (CBC), M:E ratio, prothrombin time, activated partial thromboplastin time, fibrinogen, bleeding time.

Mammalian thrombocytes, also called platelets, are derived from cytoplasmic fragmentation of megakaryocytes that form in the bone marrow. Large platelets are an indication of increased marrow production, whereas small platelets are seen in idiopathic thrombocytopenia. Metakaryocytopoiesis is stimulated by thrombopoietin, which is produced in the kidneys and inhibited by a factor produced in the spleen.

Epinephrine, whether injected or released in response to stress, causes contraction of the spleen and the release of platelets, so platelet counts increase. Approximately 30–40 % of the platelets are stored in the spleen in health, and as much as 90 % can be stored in splenomegaly. Senescent and damaged platelets are removed by the spleen, liver, and bone marrow. Platelets maintain hemostasis. A failure to do this results in extended bleeding times and purpura (leakage of RBCs into tissues).

↑	**[Thrombocytosis, Thrombocythemia]**
	Myeloproliferative disorders (e.g. polycythemia vera, myelogenous leukemia, megakaryotic leukemia, malignancy), inflammatory disorders, splenectomy, splenic atrophy, myelosclerosis, cardiac disease, cirrhosis, chronic pancreatitis, iron deficiency anemia, high altitude, hypoxia, pregnancy, menstruation, hemorrhage, exercise
	Overcompensation after thrombocytopenia
	Epinephrine and oncolytics
↓	**[Thrombocytopenia]**
	Trauma with massive hemorrhage, bone marrow damage, aplastic anemia, congenital lack of megakaryocytes
	Menstruation, normal pregnancy, leukemia, platelet clumping, excessive destruction (e.g. autoimmune disease), numerous diseases and congenital disorders
	Filoviruses (e.g., Ebola, Marburg) and arboviruses in primates
	Clotting at any time in the sampling process
	Oncolytics and analgesics (aspirin, acetaminophen, codeine, phenacetin, etc.), estrogens, thalidomide

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5.4 Bone Marrow

5.4.1 Myeloid: Erythroid Ratio [M:E Ratio]

Affected Organs: marrow, kidney, G.I. tract.
Specimen Handling: Marrow is collected from an animal while living or within a few hours of death. Marrow is mixed with saline and EDTA.
Supportive Tests: Complete blood count (CBC), differential leukocyte count, bone marrow histopathology, in vitro bone marrow cultures.

Bone marrow is evaluated for the ratio of *myeloid* elements (promyelocytes, myelocytes, metamyelocytes, band granulocytes, and segmented granulocytes) to *erythroid* elements (prorubricytes, rubricytes, and metarubricytes). An evaluation can either be performed by microscopic examination of a bone marrow smear or by flow cytometry. It is important to realize that the M:E ratio may appear normal in cases when both the M and E constituents in the marrow are up-regulated or down-regulated.

↑	Either an increased myeloid production (as in non-anemic animals), or depressed erythroid production (as in anemic animals)
	Most infections, lymphocytic leukemia, myeloid leukemia, decreased nRBCs, kidney disease
↓	Either an increase in erythroid elements or a decrease in myeloid elements
	Pernicious anemia, recovery from iron deficiency anemia, hemorrhage, hemolysis, ulcerative colitis with blood loss

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Chapter 6
Clinical Chemistry

Abstract The three diagnosis chapters—Hematology, Clinical Chemistry, and Urinalysis—are intended to be the most informative and most used parts of this handbook. There is a lot of useful information packed into these chapters. Once animal clinical pathology data have been evaluated and anomalous values for several parameters have been identified, these chapters can help one ascertain what these anomalies signify. Next to the name of each parameter are its common abbreviations. For each parameter, there are listings for organs that may be affected, specimen handling information, and supportive tests that may be used to confirm a diagnosis. These are followed by a brief description of the parameter including its strengths and weaknesses and other need-to-know information. Next to up and down arrows are potential diagnoses for when a parameter's value is increased or decreased. When there is a name for an increase or decrease (e.g., hyperglycemia or hypoglycemia), that is also provided. The Clinical Chemistry Diagnosis chapter contains information for 35 parameters.

Activated Partial Thromboplastin Time See Hematology Diagnosis

6.1 Alanine Aminotransferase [ALT, ALAT, SGPT, GPT, PGPT]

Affected Organs: Liver (hepatocellular), cardiac muscle, skeletal muscle.
Specimen Handling: Serum may be stored 24 h at room temperature or 7 days at 4 °C. *Interferences*: Hemolysis, freezing
Supportive Tests: ALP, ALP isoenzyme study, AST, SDH (large animals), GGT, LDH, 5′-NT, bile acids, urobilinogen, bilirubin, albumin, total globulins, CBC, erythrocyte morphology, clotting factors, prothrombin time.

Alanine aminotransferase (ALT), also known as serum glutamic pyruvic transaminase (SGPT), is present in small amounts in muscle, pancreas, spleen, lung, and erythrocytes, but in large quantities in the liver of small animals. ALT and AST are

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J.E. Whalan, *A Toxicologist's Guide to Clinical Pathology in Animals*,
DOI 10.1007/978-3-319-15853-2_6

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usually measured together but ALT is superior to AST for assessing hepatocyte damage. Serum levels of ALT and AST are normally low, but leaky cell membranes release these enzymes into the blood when there is cell injury or death.

Little or no increase in ALT may be seen in severe hepatobiliary disease because so little ALT remains to enter the bloodstream. In myocardial infarction, AST is markedly increased while ALT increases only slightly. Sorbitol dehydrogenase (SDH) is used to assess liver damage in large animals such as pigs, sheep, cattle, and horses because their hepatocytes have negligible amounts of ALT,

↑	Hepatocyte leakage and necrosis, cirrhosis, cholestatic and obstructive jaundice, fatty degeneration of liver, infectious hepatitis, viral hepatitis, leptospirosis, hepatic hemangioma, liver tumors
	Myocardial infarction, myocarditis
	Skeletal muscle trauma (extensive), myositis, myopathies, dystrophies
	Prolonged fasting, infections, heat stroke, malaria, pancreatitis
	Carbon tetrachloride, chloroform, arsenic, phosphorus compounds, hepatotoxic drugs, drugs that cause cholestasis, morphine, heparin, diphenylhydantoin
↓	Not clinically significant
	Fasting

6.2 Albumin [Alb]

Affected Organs: Liver, skin, G.I., kidneys, pancreas, thyroid.

Specimen Handling: Serum. *Interferences*: Hemoglobin, bilirubin, lipemia. Decreased in supine position, and increased in upright position.

Supportive Tests: Globulin, total protein, fibrinogen, complete blood count (CBC).

Albumin (69,000 g/mol) is produced in the liver, and constitutes 35–50 % of the serum protein in animals and 60–70 % in humans. It accounts for 75 % of plasma oncotic (colloid osmotic) pressure. Albumin attracts interstitial water into the blood vessels; so as absolute albumin increases, so too does plasma volume. Decreases in serum albumin due to hepatocellular insult, severe hemorrhage, severe burns, or protein loss through the kidneys, skin, or G.I. tract cause water to move into interstitial spaces, resulting in edema. Serum albumin slowly decreases (~3 %/day) during hepatocellular damage. Albumin replacement is slower in large animals. Albumin levels decrease with age. Because albumin binds with many substances, it serves as a carrier for cations, calcium, fatty acids, bilirubin, and drugs. This binding slows excretion from the body and allows time for detoxification.

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↑	**[Hyperalbuminemia]**
	Dehydration (confirm with HCT and HGB), prolonged tourniquet use, shock
↓	**[Hypoalbuminemia]**
	Acute blood loss
	Decreased synthesis in the liver, increased catabolism, severe diffuse liver disease, subacute hepatitis, hepatocellular damage, ascites, cirrhosis, chronic alcoholism
	Starvation, protein malnutrition, severe malabsorption, nontropical sprue, intestinal obstruction, cachexia, peptic ulcer, ulcerative colitis, Crohn's disease, granulomatous enteritis, acute G.I. infections
	Chronic glomerulonephritis with protein loss, nephrotic syndromes
	Pancreatitis, diabetes mellitus
	Intestinal lymphangiectasia, Type II ostertagiasis (cattle), Johne's disease (cattle), neoplasia, lymphomas, carcinomatosis, severe infections, thyrotoxicosis (Grave's disease), heart failure, collagen diseases
	Pregnancy, egg laying, aging, surgery, prolonged immobility, overhydration, IV fluid therapy, skin trauma and burns (protein loss), parasitism
	Estrogen

6.3 Albumin: Globulin Ratio [A:G, A/G]

The Albumin: Globulin Ratio is a calculated value:

$$A:G\,Ratio = \frac{Albumin}{Globulins}$$

↑	Not clinically significant
↓	Same as for decreased albumin

6.4 Alkaline Phosphatase, Serum [ALP, AP, SAP, ALKP, Alk Phos]

Affected Organs: Liver (hepatobiliary), bone, thyroid, lung, adrenals, intestines, kidneys, and placenta; also sepsis and pregnancy.

Specimen Handling: Fasted animal. Serum must be refrigerated immediately, and should be analyzed the same day. ALP increases with storage at 4 °C, but is stable for 20 months at −20 °C. *Interferences*: Hemolysis, fluoride, citrate, oxalate, EDTA, nonfasted specimen.

Supportive Tests: ALP isoenzyme study, ALT, AST, SDH (large animals), GGT, LDH, 5′-NT, bile acids, urobilinogen, bilirubin, albumin, total globulins, CBC, erythrocyte morphology, clotting factors, prothrombin time.

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Distinct alkaline phosphatase isoenzymes are found in liver (especially the biliary tree), bile, bone, renal tubules, intestinal mucosa, lung, placenta, and leukocytes. The isoenzymes can be separated by electrophoresis to determine the source of increased ALP. Serum ALP, as reported in toxicology studies, is a mixture of all isoenzymes but mostly those from liver, bone, and intestines. Elevated ALP is primarily an indicator of cholestasis, liver disease, and osteoclast activity. The liver isoenzyme predominates in most species except during rapid bone growth (bone isoenzyme) and pregnancy (placental isoenzyme). Serum levels decrease after puberty. In rats, the intestinal and bone isoenzymes are the dominant isoenzymes.

Increases in serum ALP due to the bone isoenzyme are usually 2–3 fold, but increases due to the liver isoenzyme can be much greater. Marked increases in serum ALP can result from endogenous and exogenous corticosteroid induction in the liver (except in cats), and this effect can persist for weeks. In birds, most ALP consists of the intestinal isoenzyme. ALP increases due to liver disease in carnivorous birds but not in herbivorous birds. ALP is a poor indicator of cholestasis in cats. GGT is a more reliable indicator of cholestasis in large animals than ALP. The serum half-life of ALP ranges from 6 h in cats to 72 h in dogs. ALP has replaced BSP as an indicator of liver disease.

↑	Liver disease, hepatitis, cholestasis, extrahepatic biliary obstruction, hepatocellular jaundice, portal cirrhosis, liver abscess, and nodules, liver tumors
	Increased bone metabolism (healing fractures and hyperparathyroidism), bone disease, bone tumors, rickets, osteomalacia, Paget's disease
	Hyperthyroidism, acromegaly, chronic illness, chronic stress, increased endogenous corticosteroid production (hyperadrenocorticoidism), pregnancy (third trimester—placental isoenzyme), peptic ulcer, congestive heart failure, diabetes mellitus, pulmonary infarction, Fanconi syndrome, hepatic lipidosis in cats
	Hepatotoxic drugs, carbon tetrachloride, phenobarbital, estrogens, methyltestosterone, corticosteroids, anticonvulsants, drugs that cause cholestasis, erythromycin, chronic alcoholism
↓	Anorexia, food avoidance, fasting, malnutrition, hypophosphatemia, hypophosphatasia
	Hypothyroidism, dwarfism, pernicious anemia (vitamin B_{12} deficiency)
	Hypervitaminosis D

6.5 Aspartate Aminotransferase [AST, ASAT, SGOT, GOT, PGOT]

Affected Organs: Liver (hepatocellular), cardiac muscle, skeletal muscle, kidney, pancreas, lung, spleen, erythrocytes.

Specimen Handling: Serum may be stored 24 h at room temperature, 24 h at 4 °C, 1 month at −20 °C. *Interferences*: Hemolysis, freezing

Supportive Tests: ALT, ALP, ALP isoenzyme study, SDH (large animals), GGT, LDH, 5′-NT, urobilinogen, creatine kinase, bile acids, bilirubin, albumin, total globulins, CBC, erythrocyte morphology, clotting factors, prothrombin time.

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Aspartate aminotransferase (AST) is also known as serum glutamate oxalacetate transaminase (SGOT). AST is equipresent in liver and cardiac muscle, and minimally present in skeletal muscle, kidney, pancreas, lung, spleen, and erythrocytes. ALT and AST are usually measured together. AST is not as liver-specific as ALT. Serum levels of ALT and AST are normally low, but leaky cell membranes release these enzymes into the blood when there is cell injury or death.

In liver injury, ALT and AST are both increased with peak AST levels at 24–36 h. In myocardial infarction, AST is increased while ALT increases only slightly. AST is used to diagnose liver damage in large animals relative to creatine kinase (CK), an indicator of muscle damage.

↑	Hepatocyte necrosis, cholestatic and obstructive jaundice, chronic hepatitis, viral hepatitis, alcoholic hepatitis, cirrhosis, hepatic tumors, hemochromatosis, liver flukes
	Myocardial infarction, cardiac muscle trauma or necrosis, muscular dystrophy, myoglobinuria, white muscle disease, polymyositis, skeletal muscle trauma or necrosis, trichinosis
	Surgery, I.M. injections, infections, shock, septicemia, gangrene, intestinal complications, pancreatitis, hemolytic anemias
	Halothane, chloroform, carbon tetrachloride, copper, phosphorus compounds, opiates, salicylates, erythromycin, phenothiazines, progesterone, androgens, methyldopa, acetaminophen, hepatotoxic drugs, drugs that cause cholestasis
↓	Not clinically significant
	Vitamin B_6 deficiency, uremia

6.6 Bicarbonate [Bicarb, HCO_3^-]

Affected Organs: Blood, lungs, kidneys, CNS.
Specimen Handling: Arterial blood is drawn into a heparinized syringe anaerobically, chilled on ice, and analyzed expeditiously (i.e. <20 min).
Supportive Tests: pO_2, pCO_2, pH.

The body's acid-base balance is primarily regulated by the bicarbonate anion (HCO_3^-). Bicarbonate is produced endogenously. Bicarbonate levels are regulated by the lungs and kidneys. It can be lost in the urine and gastrointestinal secretions. Bicarbonate can be measured, or it can be calculated using the Henderson-Hasselbalch equation (pK is 6.1 for the bicarbonate buffer system, HCO_3^- is a salt, and pCO_2 is an acid):

$$pH = pK + log\left(\frac{HCO_3^-}{pCO_2}\right)$$

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↑	**[Metabolic alkalosis** or **compensated respiratory acidosis]**—see pH
↓	**[Metabolic acidosis** or **compensated respiratory alkalosis]**—see pH
	Prolonged exposure of a blood sample to air

6.7 Bile Acids, Total [TBA]

Affected Organs: Liver (hepatobiliary, hepatocellular), kidney.
Specimen Handling: Fasting serum is stable for 6 months at 4 °C.
Supportive Tests: ALP, ALT, AST, SDH (large animals), GGT, 5′-NT, urobilinogen, bilirubin, CBC, erythrocyte morphology.

The total bile acids test is a sensitive indicator of hepatobiliary function. The liver forms bile acids from cholesterol. Bile acids are stored and concentrated in the gall bladder and then secreted into the intestine to emulsify dietary fats. Approximately 90 % of the bile acids are reabsorbed from the ileum into portal circulation and then removed by the liver. Fasting serum levels of bile acids are low in animals with normal enterohepatic recirculation, but elevated when disease interferes with enterohepatic circulation or the hepatobiliary system.

↑	Cholestasis, cholestasis of pregnancy, acute hepatitis, viral hepatitis, chronic hepatitis, liver sclerosis, extrahepatic biliary obstruction, cholangiohepatitis, biliary cirrhosis, portal vein thrombosis, cholangitis, hemochromatosis, hepatic lipidosis, copper hepatopathy, portosystemic shunt, hepatic microvascular dysplasia, liver cancer, chronic kidney failure
	Methotrexate, cyclosporine, rifampin, isoniazid
↓	Not clinically significant
	Intestinal malabsorption
	Cholestyramine

6.8 Bilirubin, Conjugated (Direct) [C-Bili, CB, D-Bili]

Affected Organs: Liver (hepatobiliary).
Specimen Handling: Serum should be protected from light.
Supportive Tests: Total bilirubin, unconjugated bilirubin, ALT, ALP, AST, bile acids, GGT, 5′-NT, urobilinogen, CBC, erythrocyte morphology.

Conjugated (direct) bilirubin is water-soluble, and is eliminated in bile and through the kidneys. Bilirubin measurements in dogs lack sensitivity.

↑	**[Conjugated Hyperbilirubinemia]**
	Intrahepatic or extrahepatic biliary tree obstruction (cholestasis), hepatocellular damage (progressed)
	Blood transfusions
↓	Not clinically significant

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6.9 Bilirubin, Total [T-Bili, Bili]

Affected Organs: Liver (hepatobiliary).
Specimen Handling: Serum should be protected from light. *Interferences*: light, lipemia, turbidity.
Supportive Tests: Conjugated bilirubin, unconjugated bilirubin, ALP, bile acids, CBC, erythrocyte morphology.

Bilirubin is the breakdown product of hemoglobin. Macrophages release unconjugated, insoluble (indirect) bilirubin when they phagocytize senescent erythrocytes. Most bilirubin is bound to albumin for transport to the liver. This allows for iron to be recycled for more efficient erythropoiesis. The liver converts unconjugated bilirubin into bilirubin glucuronide that passes through the biliary system into the small intestines as conjugated bilirubin (direct), which is both water soluble and not bound to protein. Bacteria in the intestines break down unconjugated bilirubin into stercobilinogen that gives feces their characteristic brown color.

Total bilirubin is a measurement of both conjugated and nonconjugated bilirubin in the serum. Bilirubin measurements in dogs lack sensitivity. Serum bilirubin rarely increases in cattle except in cases of bile duct obstruction with decreased renal function and prolonged bile duct obstruction. Hyperbilirubinemia typically precedes bilirubinuria in most species but not in dogs.

↑	**[Hyperbilirubinemia]**
	Hepatocellular damage (inflammatory, toxic, or neoplastic), cirrhosis, excessive hemolysis, disseminated intravascular coagulation, septicemia, shock
	Intrahepatic and extrahepatic biliary tree obstruction due to parasites, calculi, or tumor, hemolytic diseases, fructose intolerance
	Prolonged fasting, blood transfusions
↓	**[Hypobilirubinemia]**
	Not clinically significant
	Prolonged exposure of blood samples to light
	Ethoxazine, phenazopyridine

6.10 Bilirubin, Unconjugated (Indirect) [U-Bili, UB, UCB, I-Bili]

Affected Organs: Liver (hepatocellular).
Specimen Handling: Serum should be protected from light.
Supportive Tests: Total bilirubin, conjugated bilirubin, ALP, ALT, AST, GGT, SDH (large animals), LDH, CBC, erythrocyte morphology.

Unconjugated (indirect) bilirubin binds to albumin for transport to the liver. It cannot pass through the kidneys into urine. Unconjugated hyperbilirubinemia occurs when the liver is unable to process the amount of unconjugated bilirubin

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provided to it, such as when there is excessive erythrocyte hemolysis. Unconjugated bilirubin is an indicator of very late liver damage. In the horse, most bilirubin is unconjugated.

↑	**[Unconjugated Hyperbilirubinemia]**
	Acute hepatitis, hepatocellular damage (progressed); excessive hemolysis, blood transfusion, impaired erythropoiesis due to vitamin B_{12} and folate deficiencies sepsis
↓	Not clinically significant

Bleeding Time See Hematology Diagnosis

6.11 Blood Urea Nitrogen [BUN, UN]

Affected Organs: Kidneys, heart, liver.

Specimen Handling: Serum or plasma may be stored for several hours at room temperature, or for longer periods at 4 °C. Avoid high sodium fluoride concentrations. *Interferences*: Citrate, EDTA, oxalate, fluoride, lithium heparin, ammonia, heavy metals.

Supportive Tests: Creatinine, bicarbonate, Ca, Cl, inorganic P, K, Na, blood pH, cholesterol, serum proteins, renal clearance tests, dye excretion tests, serum amylase, urine specific gravity.

Blood urea nitrogen is produced in the liver from ammonia, then eliminated in the kidneys by glomerular filtration. BUN levels increase and decrease with food intake and dietary protein. BUN is not a sensitive test of kidney damage since more than 75 % of the nephrons must be damaged for BUN values to increase significantly. Ideally, a series of BUN measurements should be taken since a single value can be misleading. Animals should be fasted prior to testing. BUN values increase with age. Creatinine is a more specific test than BUN for kidney damage caused by disease or toxicity, but BUN is useful for assessing reversibility of damage.

↑	**[Azotemia, Hyperuremia, Uremia]**
	Impaired kidney function due to chemically induced nephrotoxicity
	High protein diet, decreased body water (vomiting, diarrhea, starvation, vigorous exercise, sweating, hemorrhage, infection, burns)
	Shock, rcenal disease, postrenal obstruction, acute myocardial infarct, G.I. hemorrhage, infection, fever, toxemia, hypoadrenocorticism
	Corticosteroids, tetracycline
↓	**[Hypouremia]**
	Low protein and high carbohydrate diet, fasting
	Severe liver damage, hepatitis, hepatic insufficiency
	Drug poisoning, anabolic steroids

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6.12 Calcium [Ca, Ca^{++}, Ca^{2+}, Calc]

Affected Organs: Parathyroids, kidneys, pituitary, pancreas, liver, G.I., bone.
Specimen Handling: Plasma or whole blood may be used. Blood should be drawn anaerobically using heparin as an anticoagulant. Samples should be placed on ice, and analyzed promptly. For measurements of total calcium, serum must come from a fasted nonexercised animal. *Interferences*: EDTA, citrate, oxalates, fluoride, calcium salt of heparin, BSP dye, cork stoppers, precipitate.
Supportive Tests: Mg, Inorganic P, total protein, albumin, pH, BUN, creatinine.

Calcium exists as a cation (Ca^{2+}) in body fluids. Half of plasma calcium is metabolically active (ionized) and the other half is bound to albumin. Tetany can result from a decrease in ionized calcium. When there is an increase in total proteins in plasma (hyperproteinemia), more calcium is bound to protein and the calcium level increases. The opposite occurs during hypoproteinemia. Calcium is not excreted by the kidney except in horses.

↑	**[Hypercalcemia]**
	Primary hyperparathyroidism, pseudohyperparathyroidism (dogs), malignant diseases that involve bone (resorption) or marrow, acute osteoporosis, sarcoidosis, immobilization, renal failure (especially in horses), tuberculosis, hyperproteinemia
	Alkaline antacids, calcium salts, diethylstilbestrol (DES; in case of breast cancer), mercurials, thiazides, estrogens, oral contraceptives, progesterone, hypervitaminosis A and D
	Dehydration, prolonged use of a tourniquet
	Cork-stoppered test tubes
↓	**[Hypocalcemia]**
	Hypoparathyroidism, vitamin D deficiency, tetany, malabsorption of Ca, P, and vitamin D, late pregnancy, eclampsia, chronic renal disease with uremia and phosphate retention, Mg deficiency, acute pancreatitis with extensive fat necrosis, anterior pituitary hypofunction, proximal and distal renal tubular disease, alcoholism, hepatic cirrhosis, hypoproteinemia, hypoalbuminemia, hyperadrenocorticism, parturient paresis (milk fever) in dogs, sheep, cows, and horses, G.I. tract blockage in ruminants
	Anticonvulsants, calcitonin, corticosteroids, diuretics, mercurials, fluorides, glucagon, glucose, insulin, laxatives, Mg salts, phosphates, oxalate, sulfates, EDTA

Calculated Globulin See Globulin

6.13 Carbon Dioxide, Partial Pressure [pCO_2, $p(a)CO_2$]

Affected Organs: Blood, lungs, kidneys, CNS.
Specimen Handling: Arterial or free-flowing capillary blood is anaerobically drawn into a heparinized syringe, chilled on ice, and analyzed expeditiously (i.e. <20 min).
Supportive Tests: pO_2, pH, bicarbonate.

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This parameter measures carbon dioxide dissolved in plasma (i.e., not in hemoglobin). See pH.

↑	**[Hypercapnia]**
	Respiratory acidosis, metabolic alkalosis with compensatory hypoventilation, obstructed airways, increased atmospheric pCO_2, pulmonary diseases as during rebreathing, asthmatic shock, chronic bronchitis, pulmonary tuberculosis, polio, emphysema, severe electrolyte disturbance, respiratory center depression, reflex bradypnea, hypothermia
	Exposure of drawn blood to air
	Opiates, barbiturates, diazepam, curare, succinyl choline
↓	**[Hypocapnia]**
	Respiratory alkalosis, hyperventilation due to mechanical ventilation, hypoxia, pulmonary emboli, anxiety, hyperthermia, respiratory center disturbance from head trauma
	Salicylates, trimethomine

6.14 Chloride [Cl, Cl^-]

Affected Organs: Kidneys, parathyroids, adrenals.

Specimen Handling: The tubes used to obtain serum or plasma must be free of contaminants. The serum should be rapidly separated, and hemolysis must be avoided.

Supportive Tests: Na, K, Ca, inorganic P, Mg, BUN, creatinine, urine specific gravity.

Chlorine exists as chloride (Cl^-) in body fluids and is the predominant anion electrolyte in extracellular fluid (ECF). Very little chloride is found in intracellular fluid (ICF). Chloride and sodium are essential in regulating the osmolality and acid-base balance of ECF. Sodium and chloride levels tend to rise and fall together. Chloride readily passes through the kidney's renal glomeruli and is then passively reabsorbed in the proximal convoluted tubules and actively reabsorbed in the loop of Henle. Because a slight increase is normal after eating, blood should be drawn from fasted animals.

↑	**[Hyperchloremia, Hyperchloridemia]**
	A slight increase is normal after eating
	Dehydration, respiratory alkalosis, renal tubular acidosis, acute renal failure, diabetes insipidus, prolonged diarrhea with loss of $NaHCO_3$, hyperparathyroidism, adrenocortical hyperfunction
	Salicylates, androgens, corticosteroids, estrogens
↓	**[Hypochloremia, Hypochloridemia]**
	Sweating, prolonged vomiting, persistent gastric secretion, salt-losing nephritis, burns, open wounds, metabolic acidosis, potassium depletion due to alkalosis, respiratory acidosis, adrenal insufficiency, water intoxication
	Bromides (displacing chlorides), bicarbonates, corticosteroids, diuretics, mercurials, mannitol, laxatives, theophylline

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6.15 Cholesterol, Total [Chol]

Affected Organs: Liver, kidneys, thyroid, heart, pancreas.
Specimen Handling: Serum or plasma (preserved with EDTA or heparin) collected from a fasted animal may be stored 7 days at room temperature or 4 °C or >6 months at −20 °C. Oxalates, fluoride, and citrate must not be used as preservatives. *Interferences*: Hemolysis, acetic acid, surfactants, and detergents.
Supportive Tests: glucose (to rule out diabetes), thyroid panel, ALT, ALP, AST, SDH (large animals), GGT, LDH, bile acids, creatinine, BUN, bilirubin, albumin, total globulins.

Some cholesterol comes from the diet but most cholesterol is produced endogenously in the liver where it serves as a precursor for bile acids and steroid hormones. Hypercholesterolemia is primarily associated with hypothyroidism but it can also be caused by a high fat diet or disease. Total cholesterol levels tend to fluctuate, especially in dogs; and they increase with age. Blood samples should be obtained after a fast of approximately 12 h. Gerbils have high total cholesterol levels.

↑	**[Hypercholesterolemia, Hypercholesteremia]**
	Hepatic disease, intrahepatic and extrahepatic cholestasis
	Chronic renal failure, kidney diseases
	Pancreatic and prostatic neoplasms, hypothyroidism, gout, ischemic heart disease, myocardial infarct
	Pregnancy, diabetes mellitus, alcoholism, anorexia nervosa, protein rich diet, high cholesterol diet
	Failure to fast animals before sampling
	Androgens, corticosteroids, corticotropins, disulfiram, epinephrine
↓	**[Hypocholesterolemia, Hypocholesteremia]**
	Hepatic dysfunction, hepatocellular necrosis, malignant liver neoplasms, hyperthyroidism (except in dogs), malabsorption, malnutrition, severe illness or trauma
	Colchicine

6.16 Cholinesterase [ChE, CHE]

Affected Organs: Nervous system, liver, heart, kidneys.
Specimen Handling: Serum may be stored 6 h at room temperature, 7 days at 4 °C, or 6 months at −70 °C. Hemolysis and repeated freezing and thawing should be avoided.
Supportive Tests: Plasma ChE, RBC ChE, brain ChE, clinical signs of ChE inhibition, BUN, creatinine, ALT, AST, ALP, SDH, GGT.

Cholinesterase (ChE) is found in RBCs, plasma, brain, and rat liver. Because organophosphate (OP) and carbamate insecticides cause cholinesterase inhibition, ChE measurements are used to diagnose pesticide poisoning. Because of the nervous

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system's redundancy and adaptability, plasma, RBC, and brain ChE inhibition may be substantial (>90 % in a subchronic exposure) without any adverse effect to the animal. Nevertheless, a 20 % inhibition is generally considered to be biologically significant.

When an OP inhibits cholinesterase from breaking down acetylcholine at the synapses, the parasympathetic nervous system is continuously stimulated, resulting in an exaggerated parasympathetic response. Clinical manifestations include tightness of the chest, wheezing due to airway constriction, increased airway secretions, ptyalism (salivation), lacrimation, sweating, nausea, vomiting, abdominal cramps, diarrhea, involuntary defecation and rectal straining, frequent and involuntary urination, slow heartbeat that can progress to heart block, pinpoint pupils, easy fatigue, mild weakness, involuntary twitching, and cramps. Muscle weakness can result in abnormal breathing and bluish appearance of the skin and mucous membranes (cyanosis) due to low blood oxygen.

Central nervous system effects include tension, anxiety, restlessness, insomnia, headache, emotional instability and neurosis, excessive dreaming and nightmares, apathy, confusion, slurred speech, tremors, generalized weakness, incoordination, convulsions, depression of respiratory and circulatory centers, and coma. The cause of death in OP poisoning is asphyxiation due to respiratory failure as a result of airway constriction, increased airway secretions, paralysis of the respiratory muscles, and depression and paralysis of the respiratory center in the brain.

Localized effects at the point of contact involve the smooth muscles. Eye exposure causes pinpoint pupils and blurred vision. Respiratory tract exposure causes bronchoconstriction, watery nasal discharge, swelling of nasal blood vessels, a sensation of tightness in the chest, and wheezing. Dermal exposure can result in localized sweating and fasciculations.

The route of exposure determines which toxic signs will be seen first. Respiratory and eye symptoms are the first signs of exposure to vapors and aerosols. Gastrointestinal symptoms are the first signs of ingestion. Localized sweating is the first sign of dermal exposure.

Some OPs—the phosphate triesters—can induce delayed nerve damage that begins in the lower limbs of humans and animals. Mild sensory disturbances, muscle weakness, and incoordination are the first signs of toxicity. Over several days or weeks, these symptoms worsen, and other parts of the body may be affected before a slow recovery occurs.

↑	Hyperlipoproteinemia, nephrosis
↓	Organophosphate and carbamate poisoning
	Hepatitis, cirrhosis, hepatic metastases, heart failure (liver congestion), myocardial infarct, malnutrition, anemia, chronic renal disease, low serum albumin
	Oncolytics, estrogens, succinyl choline, oral contraceptives, testosterone

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6.17 Creatine Kinase [CK, CPK]

Affected Organs: Cardiac and skeletal muscle, thyroid, lungs, brain, nerves.
Specimen Handling: Serum may be stored at 4 °C or −20 °C. CK activity diminishes significantly over a few hours, even at freezing temperatures. Avoid hemolysis and UV light.
Supportive Tests: AST, ALT, SDH, myoglobin, urine color, myosin light-chain assays, troponin I assays.

Creatine kinase (CK), also known as creatine phosphokinase (CPK), is found in intracellular fluid (ICF). CK is found in skeletal muscle (CK_3), cardiac muscle (CK_2), and brain (CK_1; not found at significant levels in serum). Following muscle trauma, serum CK and AST levels increase. Serum CK levels peak within a few hours of muscle trauma but AST levels peak after 24–36 h; and the levels of both CK and AST return to normal after several days. CK elevations that are moderate and prolonged indicate ongoing muscle damage.

↑	Muscle trauma, rhabdomyolysis, bite wounds, myocardial infarction, arrhythmia, congestive heart failure, tachycardia, loss of blood supply to muscle, myopathic disorders, myoglobinuria, muscular dystrophy, Reye's syndrome, alcoholism, poisoning related coma, hypothermia, hyperthermia, infectious diseases, hypothyroidism, pulmonary embolism, repeated convulsions or seizures, selenium deficiency, vitamin E deficiency, hemolysis
	Severe exercise, I.M. injections, surgery, venipuncture
	Halothane plus succinyl choline, ethanol, barbiturates
↓	Not clinically significant
	Sample damage due to hemolysis, UV light, or loss of CO_2

Creatine Phosphokinase [CPK] See Creatine Kinase [CK]

6.18 Creatinine [Creat, Cre, Cr]

Affected Organs: Kidneys, heart.
Specimen Handling: Serum or plasma may be stored for 24 h at 4 °C, or frozen.
Supportive Tests: BUN, bicarbonate, Ca, Cl, inorganic P, K, Na, blood pH, cholesterol, serum proteins, renal clearance tests, dye excretion tests, serum amylase, urine specific gravity.

Creatinine is non-protein nitrogenous substance that is constantly produced from muscle creatine and phosphocreatine during muscle metabolism. Because creatinine is filtered almost entirely by the glomerulus, creatinine levels can be used to assess glomerular filtration. It is also be used to diagnose impaired renal blood flow. Any condition that decreases the glomerular filtration rate (GFR) will cause an increase in serum creatinine. A 50 % decrease in GFR will double a serum creatinine

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level. Unlike BUN, creatinine is not affected by food intake, dietary protein intake, infection, fever, or toxemia. Creatinine levels also fluctuate less than BUN levels. Thus, creatinine is a more specific test than BUN for kidney damage caused by disease or toxicity. Creatinine levels increase with age.

↑	Renal function impairment, postrenal obstruction, inadequate renal blood flow due to congestive heart failure, dehydration, shock
	Cachexia, burns, high fever, corticosteroid therapy
	Nephrotoxic drugs, ascorbic acid, glucose, fructose
↓	Not clinically significant
	Normal debilitation due to age or decreased muscle mass, pregnancy, malnutrition

Electrolytes See Bicarbonate, Calcium, Chloride, Magnesium, Potassium, Sodium

6.19 Fibrinogen [F, PF, FBG]

Affected Organs: Marrow, liver, spleen.
Specimen Handling: Plasma may be mixed with sodium citrate or oxalates. *Interferences*: heparin, fibrin degradation products (PDPs)
Supportive Tests: Total protein, albumin, platelet count, prothrombin time, partial thromboplastin time, bleeding time, coagulation factors.

Fibrinogen (coagulation factor I) is a plasma protein produced by the liver. It is essential for clotting. It enters extravascular spaces in response to inflammatory disease. Fibrinogen is a good indicator of inflammatory disease because increases are seen sooner than are increases in leukocyte and neutrophil counts. Persistent high values indicate serious disease. Fibrinogen levels may not increase during mild inflammation, however. It is measured by the heat precipitation method (fibrinogen precipitates at 56–58 °C) and the refractometer. Measurements are affected by the presence of heparin and fibrin degradation products (FDPs) and are inaccurate at high levels.

↑	**[Hyperfibrinogenemia]**
	Inflammation, suppurative diseases, necrosis, dehydration
↓	**[Hypofibrinogenemia]**
	Massive liver disease, protein loss

6.20 Gamma-Glutamyl Transferase [GGT, GTP]

Affected Organs: Liver (hepatobiliary), pancreas, kidneys, prostate, heart.
Specimen Handling: Serum may be stored 1 month at 4 °C, 1 year at −20 °C.

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Supportive Tests: ALT, ALP, ALP isoenzyme study, AST, SDH (large animals), LDH, 5′-NT, bile acids, urobilinogen, bilirubin, albumin, total globulins, CBC, erythrocyte morphology, clotting factors, prothrombin time.

Gamma-glutamyl transferase (also known as gamma glutamyl transpeptidase) is primarily found in liver (hepatobiliary) but also in kidney, pancreas, and prostate. Although GGT levels parallel alkaline phosphatase levels, GGT is more sensitive for liver disease and cholestasis. Bone damage is indicated when alkaline phosphatase is elevated but GGT is normal.

↑	Obstructive liver disease, cholestasis, posthepatic obstruction, cirrhosis, infectious mononucleosis, liver inflammation, acute and chronic hepatitis, hepatotoxicity, fatty liver, pancreatitis, diabetes mellitus, renal disease, acute myocardial infarction
	Absorption of GGT in colostrum in nursing neonates (e.g., dogs, cattle, sheep)
	Acetaminophen, phenytoin, barbiturates and other antiepileptic drugs, ethanol poisoning (moderate to heavy consumption)
↓	Not clinically significant

Gamma-Glutamyl Transpeptidase [GGTP] See Gamma-Glutamyl Transferase

6.21 Globulins [GLOB]

Affected Organs: Liver, lungs, kidneys, skeletal system.
Supportive Tests: Total protein, albumin.

The globulins are nonwater soluble proteins that fall into three categories—alpha (α_1, α_2), beta (β_1, β_2), and gamma (γ). These proteins can be separated electrophoretically. Molecular weights can range from 40,000 to 1,000,000 g/mol. Most globulins are synthesized in the liver but immunoglobulins are produced by plasma cells. Globulin levels increase with age. Instead of being measured, a value for globulins is usually calculated:

$$Globulins(approximate) = Total\,Protein - Albumin$$

↑	**[Hyperglobulinemia]**
	Third trimester of pregnancy, egg laying
	Acute inflammatory disease, nephrotic disease, iron deficiency, atopic dermatitis, disseminated intravascular coagulation (plasminogen), sarcoidosis, cirrhosis, hepatitis, hepatic failure, neoplasia
	Allergy, anaphylaxis, myeloma
↓	**[Hypoglobulinemia]**
	Severe liver disease (α and β), autoimmune disease, chronic pulmonary disease, hemolytic anemia, iron storage disease, agammaglobulinemia, disseminated intravascular coagulation (fibrinogen), nephrotic diseases, lymphoma, multiple myeloma and skeletal destruction
	Acute blood loss, exudative skin disease
	Colostrum deprivation

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6.22 Glucose [GLU, Gluc]

Affected Organs: Pancreas, kidneys, liver, heart, pituitary, adrenal cortex, G.I.
Specimen Handling: Cells must be separated promptly to avoid glucose metabolism. Serum or plasma preserved with sodium fluoride may be stored 8 h at room temperature, 3 days at 4 °C. *Interferences*: Hemolysis, lipemia, EDTA.
Supportive Tests: ALT, ALP, AST, bile acids, 5′-NT, urobilinogen, BUN, creatinine, lipase, amylase, glucose, Ca.

Nearly all body glucose is in extracellular fluid (ECF). Blood glucose levels are regulated by the liver, the pancreas (via insulin), the anterior pituitary, the adrenal cortex, and the thyroid. The liver maintains glucose homeostasis by converting the majority of dietary glucose (via portal circulation) into glycogen, which is stored in the liver and muscles. Blood samples should be obtained after a fast of approximately 12 h since serum glucose is elevated for 2–4 h after eating. In vitro erythrocytes metabolize 10 % of glucose/h (≤10 mg/dl/h) at room temperature, but sodium fluoride prevents this metabolism. Glucose levels are higher for serum and plasma than for whole blood.

↑	**[Hyperglycemia]**
	Failure to fast an animal before sampling, increased production of glucose by the liver
	Diabetes mellitus, pancreatitis, pancreatic carcinoma acute myocardial infarction or angina, chronic liver disease, chronic kidney disease, acromegaly, hyperpituitarism, milk fever in ruminants, vitamin B_1 deficiency
	Strenuous exercise, strong emotion, convulsions, anoxia, increased epinephrine due to injection, shock, burns, exposure to cold, following general anesthesia
	Caffeine, morphine, epinephrine, streptozotocin, thiazides, ACTH, corticosteroids, thyroxine, oral contraceptives, diuretics, sugars
↓	**[Hypoglycemia]**
	Fasting, starvation, severe exertion, failure to use an enzyme inhibitor (e.g. sodium fluoride)
	Islet cell tumors, carcinoma of the adrenal gland or stomach fibrosarcoma, decreased glucose production in the liver hepatoma, severe liver disease, liver circulatory deficiency, cirrhosis, glucagon deficiency, hypopituitarism, hypothyroidism, autonomic nervous system disorders, ketosis in ruminants, G.I. malabsorption, pregnancy toxemia in sheep, renal glycosuria in dogs, functional hypoglycemia in dogs
	Iatrogenic hyperinsulinism
	Liver poisoning due to arsenic, carbon tetrachloride, chloroform, alcohol, salicylates, antihistamines, cirrhosis, hepatitis, metastatic tumor

6.23 Glutamate Dehydrogenase [GLDH, GLD, GDH]

Affected Organs: Liver, kidney.
Specimen Handling: Serum or plasma preserved with EDTA may be stored for 48 h at 4 °C or for 2 weeks at −20 °C. *Interferences*: Hemolysis, lipemia.
Supportive Tests: ALP, AST, SDH (large animals).

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Glutamate dehydrogenase is an enzyme found in mitochondria throughout the body. High levels of GLDH are found in the centrilobular region of the liver and lesser levels are found in the kidney. Because GLDH is in mitochondria, it is not released during inflammatory liver diseases. Rather, it is released during hepatocellular necrosis. This test is often used in ruminants and horses as an alternative to ALT. GLDH is elevated in juvenile rats with mitochondrial damage but tends to be variable in old rats.

↑	Hepatocellular necrosis, hypoxic liver disease
	Ethanol, acetaminophen, streptokinase, lead nitrate, isoniazid, methapyrilene, dexamethasone, cyproterone
↓	Not clinically significant

Glutamic Oxaloacetic Transaminase [GOT] See Aspartate Aminotransferase
Glutamic Pyruvic Transaminase [GPT] See Alanine Aminotransferase
Iditol Dehydrogenase See Sorbitol Dehydrogenase

6.24 Lactate Dehydrogenase [LD, LDH]

Affected Organs: Liver, gall bladder, blood cells, cardiac and skeletal muscle.
Specimen Handling: Serum or plasma (preserved with heparin) may be stored at room temperature, and should be analyzed promptly after removal from the clot. *Interferences*: Hemolysis, oxalate, citrate, heparin.
Supportive Tests: ALT, ALP, ALP isoenzyme study, AST, SDH (large animals), GGT, 5′-NT, bile acids, bilirubin, albumin, total globulins, BUN, creatinine, creatine kinase, CBC.

An increase in total serum LD is of little diagnostic value because it is found in most organs and because tissue distribution differs between animals. Of greater value are measurements of the isozymes found in skeletal muscle (mostly LD_5), cardiac muscle (LD_1, LD_2), kidneys (LD_1, LD_2), RBCs (LD_1, LD_2), and liver (LD_5). WBC precursors contain the LD_3 isozyme. LD retains its activity over several weeks. The LD isoenzymes are separated by electrophoresis.

LD increases following liver damage but other enzymes are more sensitive and liver-specific than LD. Artifactually elevated LD values are found when RBCs hemolyze. The following diagnoses are for total serum LD:

↑	Megaloblastic anemia, extensive carcinomatosis, viral hepatitis, shock, hypoxia, extreme hyperthermia, cirrhosis, obstructive jaundice, renal diseases, skeletal muscle diseases, congestive heart failure, loss of cellular cytoplasm, myocarditis, myocardial and pulmonary infarct, pulmonary embolism, leukemia, hemolytic anemia, hepatitis, lymphoma, carcinoma, muscular dystrophy, myoglobinuria, lactic dehydrogenase virus in mice
	I.M. injections, surgery
	Hemolysis
	Anesthetics, ethanol, fluorides, oncolytics, narcotic analgesics
↓	Not clinically significant
	X-ray irradiation

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6.25 Magnesium [Mg, Mg^{++}]

Affected Organs: Kidneys, bone, adrenals, thyroid, pancreas, parathyroids, G.I.
Specimen Handling: Serum from a fasted animal is collected in a metal-free container. Hemolysis and impaired venous flow must be avoided. The RBCs should be removed promptly. *Interferences*: Hemolysis, EDTA, citrate, oxalate.
Supportive Tests: Ca, inorganic P, BUN, creatinine.

Magnesium exists as a cation electrolyte (Mg^{2+}) in body fluids. It is absorbed from food in the small intestines. Most magnesium is bound in bone but it can be mobilized when there is a demand for it for enzyme reactions and neuromuscular activity. Magnesium is also found in intracellular fluids (ICF). Tetany can result when fluid surrounding the neuromuscular junction lacks magnesium. Magnesium is excreted through the kidneys, intestinal tract, and mammary glands. Plasma levels are regulated within a narrow range.

↑	**[Hypermagnesemia]**
	Dehydration, renal insufficiency, adrenocortical insufficiency, tissue trauma, hypothyroidism, diabetic coma
	Aspirin, lithium, Mg containing products (e.g. antacids), progesterone
↓	**[Hypomagnesemia]**
	Inadequate intake or absorption of Mg, diet low in Mg and calories
	Tetany, acute pancreatitis, hypoparathyroidism, thyrotoxicosis, chronic alcoholism, delirium tremens, loss of body fluids, pregnancy, lactation
	Weakness, irritability, delirium, convulsions

Metabolic Acidosis and **Metabolic Alkalosis** See pH

6.26 Methemoglobin [metHb]

Affected Organs: Organs with high oxygen demand (CNS, cardiovascular system).
Specimen Handling: Whole blood preserved with heparin, EDTA, or ACD; chilled on ice and analyzed within a few hours to avoid false negatives (methemoglobin levels tend to increase with storage).
Supportive Tests: Blood gases.

Methemoglobin is an inactive, oxidized form of hemoglobin with its iron in the ferric (Fe^{3+}) state rather than the ferrous (Fe^{2+}) state. Oxygen can only bind to ferrous iron. It is normal for 1–3 % of total hemoglobin to be methemoglobin. Arterial blood that contains a significant percentage of methemoglobin has a chocolate-brown appearance. Methemoglobin is not capable of carrying oxygen. Methemoglobinemia may rarely be inherited, but it is usually acquired due to exposure to drugs or chemicals, especially those with nitro and amino groups. The clini-

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Table 6.1 Clinical signs associated with methemoglobinemia in humans

metHb (%)	Clinical signs
1–3	None (normal level)
3–15	Slight skin discoloration; asymptomatic
15–25	Cyanosis likely; relatively asymptomatic
25–50	Fatigue, weakness, lightheadedness, dizziness, headache, tachycardia, dyspnea, confusion
50–70	Skin discoloration, cyanosis, acidosis, bradycardia, arrhythmia, cardiac or neurologic ischemia, hypoxia, delirium, seizures, coma
>70	Death

cal signs associated with methemoglobinemia in humans are presented in Table 6.1. Although animals cannot complain of some signs of methemoglobinemia (e.g., weakness, lightheadedness, dizziness, and headache), one may note behavioral changes that are consistent with these signs.

↑	**[Methemoglobinemia]**
	Inherited methemoglobinemia (rare)
	Benzocaine, lidocaine, prilocaine, amyl nitrite, chloroquinone, dapsone, ferrous sulfate, nitrates, nitrites, nitroprusside, nitroglycerin, phenacetin, phenazopyridine, primaquine, quinones, sulfonamides
	Aniline dyes, aromatic amines, arsine, butyl nitrite, chlorates, chlorobenzene, chromates, combustion products, dimethyltoluidine, isobutyl nitrite, naphthalene, nitroaniline, nitrobenzene, nitrofurans, nitrophenol, nitrosobenzene, resorcinol, silver nitrate, trinitrotoluene
	Food and well water containing nitrates or nitrites
↓	Not clinically significant

6.27 5′ Nucleotidase [5′-NT]

Affected Organs: Liver (hepatobiliary, hepatocellular).
Specimen Handling: Serum or plasma preserved with heparin. Stable at 4 °C for 4 days. May be frozen for 4 months.
Supportive Tests: ALT, ALP, AST, bile acids, conjugated bilirubin, GGT, urobilinogen.

The enzyme 5′ nucleotidase is produced solely in the liver. It is elevated in animals with cholestasis and other liver diseases. Because it is liver specific, 5′-NT may be used to confirm that an elevation in alkaline phosphatase is indeed liver disease. Conversely, if 5′-NT is normal and alkaline phosphatase is elevated, then the liver is not affected. GGT may be used similarly because it is also more specific for liver disease than alkaline phosphatase.

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↑	Cholestasis, bile duct obstruction, cirrhosis, hepatitis, hepatic tumor, hepatic ischemia, hepatic necrosis
	Pregnancy (3rd trimester), preeclampsia, inflammatory arthritis
	Hepatotoxic chemicals, acetaminophen, aspirin, drugs that cause cholestasis, phenytoin and other anticonvulsants, halothane, methyldopa, nitrofurantoin, isoniazid, carbenoxolone, hemolysis, Mg^{2+}
↓	Not clinically significant
	EDTA, Ni^{2+}

6.28 Oxygen, Partial Pressure [pO_2, $p(a)O_2$]

Affected Organs: Blood, lungs, kidneys, CNS.
Specimen Handling: Arterial or free-flowing capillary blood is drawn anaerobically into a heparinized syringe, chilled on ice, and analyzed expeditiously (i.e. <20 min).
Supportive Tests: pCO_2, pH, bicarbonate.

This parameter measures oxygen dissolved in plasma (not in hemoglobin). See pH.

↑	**[Hyperoxemia]**
	Hyperbaric oxygen, hyperventilation
↓	**[Hypoxemia]**
	Reduced alveolocapillary membrane surface area or pulmonary diffusing capability, low atmospheric pressure, bronchitis, pneumonia, asthma, emphysema, pulmonary infarction, airway obstruction, neoplasm, phrenic nerve paralysis, tetanus, head injury, pulmonary edema, shock, reflex bradypnea, hypothermia
	Anesthetics, CO, barbiturates, diazepam, opiates

Partial Pressure Carbon Dioxide [pCO2] See Carbon Dioxide, Partial Pressure
Partial Pressure Oxygen [pO2] See Oxygen, Partial Pressure
Partial Thromboplastin Time See Hematology Diagnosis

6.29 pH

Affected Organs: Blood, lungs, kidneys, G.I. CNS, adrenals, pancreas, thyroid.
Specimen Handling: Arterial blood is drawn into a heparinized syringe anaerobically, chilled on ice, and analyzed expeditiously (i.e. <20 min).
Supportive Tests: pO_2, pCO_2, bicarbonate.

Blood pH can be measured, or it can be calculated using the Henderson-Hasselbalch equation (pK is 6.1 for the bicarbonate buffer system, HCO_3^- is a salt, and pCO_2 is an acid):

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Table 6.2 Imbalances in blood pH

	pH	HCO_3^-	pCO_2
Metabolic alkalosis	↑	↑	N
Respiratory alkalosis	↑	N	↓
Metabolic acidosis	↓	↓	N
Respiratory acidosis	↓	N	↑

↑—increased, ↓—decreased, *N*—normal

$$pH = pK + log\left(\frac{HCO_3^-}{pCO_2}\right)$$

Cellular metabolic processes require that blood pH be regulated between 7.3 and 7.5. The pH of blood is primarily a function of the quantities of dissolved CO_2 (pCO_2) and bicarbonate (HCO_3^-) as regulated by the lungs, kidneys, and the respiratory center in the medulla oblongata. Toxins, pharmaceuticals, trauma, disease, and altered physiology can cause alterations in the blood's acid/base status. These alterations include respiratory alkalosis and acidosis—a result of increased or decreased CO_2 elimination in the lungs—and metabolic alkalosis and acidosis—a result of elimination or retention of bicarbonate or nonvolatile acids in the kidneys. Since small changes can be lethal, the body regulates the pH by pulmonary excretion of CO_2, and renal excretion of bicarbonate. This regulation is called *compensation*. Table 6.2 summarizes the imbalances that can occur:

Example In aspirin poisoning (salicylism), a loss of CO_2 due to hyperventilation causes an increased blood pH; that is, respiratory alkalosis. The kidneys compensate by excreting base (potassium and sodium bicarbonate) to lower the blood pH. In cases of severe aspirin poisoning, this lowering of blood pH coupled with stimulated metabolism and a release of acid metabolites results in a simultaneous metabolic acidosis.

↑	**[Alkalosis, Alkalemia]**
	Refer to pO_2 (increased) and pCO_2 (decreased)
↓	**[Acidosis, Acidemia]**
	Refer to pO_2 (decreased) and pCO_2 (increased)

Further diagnostic information for each of the four blood pH imbalances is provided below:

6.29.1 Metabolic Alkalosis: pH ↑ HCO_3^- ↑ pCO_2 Normal

An alkaline shift in the blood's acid/base status due to a retention of base by the kidneys, or loss of nonvolatile acids.

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Plasma base increase caused by:

Excessive alkali administration, loss of gastric HCl, potassium depletion or reduced intake, chronic diarrhea and/or vomiting, diuresis, hyperadrenocorticism, nephropathy.
Adrenal steroids, licorice, acetates, tubocurarine, diuretics, mercurials, antacids, carbenicillin.

Compensation: Decreased CO_2 elimination by the lungs.

6.29.2 Respiratory Alkalosis: pH ↑ HCO_3^- Normal pCO_2 ↓

An alkaline shift in the blood's acid/base status due to an excess loss of CO_2 by the lungs.

Decreased dissolved CO2 caused by:

Hysteria, hyperventilation, reflex bradypnea.
Stimulation of the respiratory center by brain trauma, hypoxia, fever, asthma, pulmonary emboli, liver disease, bacteremia.
Salicylates (early response).

Compensation: Retention of acid or excretion of base by the kidneys.

6.29.3 Metabolic Acidosis: pH ↓ HCO_3^- ↓ pCO_2 Normal

An acidic shift in the blood's acid/base status due to a loss of base (e.g. bicarbonate) through the kidneys, or retention of nonvolatile acids.

Loss of base caused by:

Increased acid formation as in diabetes mellitus and lacticacidosis, starvation, hyperthyroidism, diets high in fats and low in carbohydrates, and trauma.
Cellular hypoxia, decreased excretion of H^+, renal failure, renal tubular acidosis, decreased cardiac output, hypercalcemia, hyperkalemia; massive rhabdomyolysis, and loss of alkaline body fluids from intestine, gall bladder, pancreas, and kidney; massive rhabdomyolysis.
Salicylates (late response), methanol, ethanol, ether, acetone, toluene, ethylene glycol, fluorides, tetracycline, tubocurarine, metformin (Glucophage®), aminobenzoic acid, ammonium chloride, arginine, formaldehyde, paraldehyde, acetazolamide.

Compensation: CO_2 elimination by the lungs.

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6.29.4 Respiratory Acidosis: pH ↓ HCO_3^- Normal pCO_2 ↑

An acidic shift in the blood's acid/base status due to decreased CO_2 elimination.

Increased dissolved CO2 caused by:

Decreased alveolar ventilation caused by pulmonary edema, airway obstructions, bronchoconstriction, depression of the respiratory center.

Chronic obstructive or restrictive pulmonary disease resulting in bicarbonate increase.

Compensation: Retention of HCO_3^- by the kidneys.

6.30 Phosphorus, Inorganic [P, Phos, P_i]

Affected Organs: Kidneys, parathyroids, bone, G.I., pituitary

Specimen Handling: Cells must be separated promptly, and hemolysis must be avoided. *Interferences*: Hemolysis, oxalate, citrate, EDTA.

Supportive Tests: Mg, Ca, K, inorganic P, ALP, ALP-2 isoenzyme (bone), BUN, creatinine.

Phosphorus exists as an organic ester in erythrocytes. It exists in plasma as inorganic phosphorus, which is routinely measured in toxicity studies, and as a phospholipid. Phosphorus levels are higher in young animals. Serum levels are controlled by the kidneys. Hemolyzed blood should not be used because phosphorus is released from erythrocytes. Phosphorus levels are also elevated in standing blood samples due to prolonged contact with RBCs.

↑	**[Hyperphosphatemia]**
	Starvation, protein-rich diet, increased dietary phosphates, normal in young growing animals
	Multiple myeloma, osteolytic metastatic tumor in bone, acromegaly, gigantism, leukemia, excess vitamin D intake, healing fractures, renal failure (accompanied by hyperparathyroidism in dogs), reduced glomerular filtration rate in dogs and cats, hypoparathyroidism, diabetic ketosis
	Androgens, oral contraceptives, I.V. phosphate therapy
	Artifactual hemolysis of blood, elevated temperature, prolonged RBC contact in samples
↓	**[Hypophosphatemia]**
	Malnutrition, high carbohydrate diet, vomiting, diarrhea, rickets, malabsorption syndrome, vitamin D or calcium deficiency, hyperinsulinism, primary hyperparathyroidism, pseudohyperparathyroidism (dogs), osteomalacia, Fanconi's syndrome, renal leakage, steatorrhea, acute alcoholism, gram negative bacterial septicemia, hypokalemia, diabetes mellitus, acidosis, hypomagnesemia, parturient paresis (milk fever) in dogs, sheep, cows, and horses
	Antacids containing aluminum, salicylates, amino acids, anesthetics, anticonvulsants, calcitonin, epinephrine, androgens, estrogens, steroids, insulin, fructose, I.V. glucose, oral contraceptives

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Plasma Fibrinogen See Fibrinogen
Plasma Glutamic Oxaloacetic Transaminase [PGOT] See Aspartate Aminotransferase
Plasma Glutamic Pyruvic Transaminase [PGPT] See Alanine Aminotransferase

6.31 Potassium [K, K^+]

Affected Organs: Kidneys, G.I., heart, muscle, bone marrow, adrenals
Specimen Handling: Cells should be removed from serum using care to avoid hemolysis. Plasma may be preserved using ammonia heparin.
Supportive Tests: Na, Cl, BUN, creatinine.

Potassium exists as a cation electrolyte (K^+) in body fluids. Serum potassium is a poor indicator of total body potassium since 90 % is in intracellular fluid (ICF). Both carnivores and herbivores are able to consume sufficient levels of potassium in their diets. Potassium is removed by the kidneys, G.I. tract (digestive fluids and feces), and sweat glands. Kidney excretion is regulated by aldosterone, which is produced in the adrenal cortex. Serum potassium levels increase as a result of prolonged RBC contact, heel stab sampling, and forceful transfer of blood into a specimen tube.

↑	**[Hyperkalemia, Potassemia, Hyperpotassemia]**
	Increased supply of potassium
	Redistribution within the body due to massive hemolysis, severe tissue damage, necrosis, acute starvation, anorexia nervosa, hyperkinetic activity, thrombocytosis, leukocytosis
	Reduced renal excretion (oliguria, anuria) due to acidosis, chronic renal failure, sodium depletion, hyperglycemia, insulin deficiency
	Abnormal electrocardiograms (elevated T waves and depressed P waves), cardiac arrest (K^+ >10–12 mEq/l)
	Oncolytics, epinephrine, heparin, histamine, mannitol, penicillin, succinylcholine, tetracycline, digitalis
	Poor handling or storage of samples, hemolysis
↓	**[Hypokalemia]**
	Reduced intake due to chronic starvation
	Loss from the body due to prolonged vomiting, diarrhea, renal tubular acidosis, renal tubular failure, metabolic alkalosis, acute myeloid leukemia
	Neuromuscular disorders (weakness to paralysis), GI disorders, abnormal electrocardiograms (depressed T waves and elevated U waves)
	Corticosteroids, diuretics, EDTA, glucose, glucagon, ethylene glycol, insulin, licorice, salicylates, gentamycin

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Protein, Total	See Total Protein
Respiratory Acidosis and **Respiratory Alkalosis**	See pH
Serum Alkaline Phosphatase [SAP]	See Alkaline Phosphatase
Serum Glutamic Oxaloacetic Transaminase [SGOT]	See Aspartate Aminotransferase
Serum Glutamic Pyruvic Transaminase [SGPT]	See Alanine Aminotransferase

6.32 Sodium [Na, Na^+]

Affected Organs: G.I., kidneys, liver, adrenals.
Specimen Handling: Serum or heparinized plasma (lithium heparin or ammonia heparin are preferred) are used. Blood should be promptly centrifuged, and hemolysis should be avoided.
Supportive Tests: K, Cl, BUN, creatinine, ALT, AST, ALP, SDH, GGT.

Sodium exists as a cation electrolyte (Na^+) in body fluids. Approximately half of the body's sodium is sequestered in bone and most of the rest is found in extracellular fluid (ECF). Very little sodium is found in intracellular fluid (ICF) because the 'sodium pump' actively removes it. Sodium and chloride have a major effect on ECF osmolality and acid-base balance. The excretion of excess sodium in the kidneys is regulated by aldosterone, which is produced in the adrenal cortex. Carnivores consume sufficient sodium in their diet, but the diet of herbivores can result in a deficiency. Sodium is lost through sweating and digestive tract secretions.

↑	**[Hypernatremia]**
	Water loss through sweating, vomiting, diarrhea, or urination
	Inadequate water intake, excessive salt in diet
	Renal failure
	Hypertonic I.V. sodium bicarbonate or sodium chloride
	Androgens, estrogens, corticosteroids, ACTH, licorice, lithium, oral contraceptives, butazones, anticoagulants containing Ca or Na
↓	**[Hyponatremia]**
	Low sodium intake
	Sodium losses (without salt replacement) due to vomiting, diarrhea, sweating, burns, open wounds, renal tubular damage, metabolic acidosis (increased excretion of cations)
	Adrenocortical insufficiency (Addison's disease), ascites, cardiac failure, hepatic cirrhosis, hepatic failure, renal failure, malnutrition, hypothyroidism, hyperglycemia, hyperlipidemia, hyperproteinemia
	Sodium-free I.V. fluids
	Diuretics, mercurials, mannitol, heparin, vasopressin, barbiturates, opiates, nicotine, acetaminophen, chlorpropamide, oncolytics

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6.33 Sorbitol Dehydrogenase [SDH]

Affected Organs: Liver (hepatocellular)
Specimen Handling: Serum or plasma (preserved with heparin) is used. Hemolysis must be avoided, and samples should be analyzed promptly (<12 h) due to instability.
Supportive Tests: Liver—ALT (except large animals), ALP, ALP isoenzyme study, AST, GGT, GLDH, 5′-NT, bile acids, bilirubin, albumin, total globulins, lactate dehydrogenase, clotting factors, prothrombin time.

Sorbitol dehydrogenase (also called iditol dehydrogenase) is found at high levels in hepatocytes but at low levels in other tissues. It is a liver-specific substitute for ALT (SGPT) in pigs, sheep, cattle, and horses. It can also be used to support a diagnosis of hepatotoxicity in dogs, rats, and nonhuman primates.

↑	Hepatocyte leakage and necrosis, infectious hepatitis, fatty degeneration of the liver, cirrhosis, obstructive jaundice, liver tumors
	Prolonged fasting, glucocorticoid induced hepatopathy
	Carbon tetrachloride, halothane
↓	Not clinically significant

Total Bile Acids See Bile Acids, Total
Total Bilirubin See Bilirubin, Total

6.34 Total Protein [TP, T. PROT]

Affected Organs: Kidneys, liver, G.I.
Specimen Handling: Serum, fasted sample. Sample should be analyzed while fresh or stored at 4 °C for <3 days or −20 °C for 6 months. *Interferences*: Hemolysis, lipemia, BSP dye, and lactescence (milkiness) causes increased values.
Supportive Tests: Albumin, fibrinogen, globulins, Ca, bilirubin, ALT, ALP, AST, bile acids, cholesterol, GGT, 5′-NT, urobilinogen, LDH, BUN, creatinine.

The primary proteins found in plasma are albumin, fibrinogen, the globulins, clotting factors, gamma globulins (including antibodies), and hormones; most of which are produced in the liver. Fibrinogen and clotting factors are found in plasma but not in serum because they are removed during clotting.

Because the molecular weights of plasma proteins range from approximately 40,000 to 20,000,000 g/mol, they are too large to pass through blood vessel membranes. Their presence in plasma causes an oncotic (colloid osmotic) pressure that affects distribution of extracellular fluid between the blood vessels and the interstitial fluid. A reduction in total plasma protein results in reduced oncotic pressure and a loss of plasma from the capillaries.

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Albumin, the major plasma protein, binds with many substances, and serves as a carrier for cations, calcium, fatty acids, bilirubin, and drugs. This binding slows excretion from the body and allows time for detoxification. Decreases in total protein values are usually due to hepatocellular insult, severe hemorrhage, or protein loss through the kidneys, skin, or G.I. tract. Protein values increase with age.

↑	**[Hyperproteinemia]**
	Marked dehydration, venous stasis, hyperimmunoglobulinemia, chronic liver disease, cirrhosis, chronic infection, autoimmune disease, sarcoidosis, tumor
	Hypercholesterolemia, hyperglycemia
	Exercise
	Prolonged use of a tourniquet
	Anabolic steroids, corticosteroids, androgens, epinephrine, progesterone, insulin
↓	**[Hypoproteinemia]**
	Severe G.I. or skin hemorrhage, gastroenteropathies, acute burns, nephrotic syndrome, chronic liver disease, cirrhosis, malnutrition, malabsorption, agammaglobulinemia, pregnancy (third trimester)
	Low protein diet, excessive water intake
	Allopurinol, estrogens, dilution with I.V. fluids

6.35 Triglycerides (Serum Lipoproteins) [TG, TRIG]

Affected Organs: Liver, kidneys, heart, brain, thyroid, pancreas, lungs.

Specimen Handling: Serum or plasma (preserved with EDTA) is prepared from a fasted animal. Stable for 4–7 days at 4 °C. *Interferences*: Sodium citrate, heparin, fluoride, glycerol, marked hemolysis, and icterus.

Supportive Tests: Fasting glucose, TSH, liver panel, lipid analysis, chylomicron determination, urinalysis.

Triglycerides are a type of lipid (neutral fat) used as a source of metabolic energy. Triglycerides are esters with three long-chain fatty acids (LCFAs) attached to glycerol. The liver and adipose tissue are the primary sites of triglyceride metabolism. Triglycerides are stored in adipose tissue and released in response to bodily energy needs. They are transported in the blood bound to albumin. Blood samples should be obtained after a fast of approximately 12 h. Hyperlipidemia may cause milky, turbid plasma. The terms *hyperlipidemia* and *hypolipidemia* are often used to refer to abnormal triglyceride levels, but they may also be used to refer to abnormal levels of one or more lipids and/or lipoproteins.

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↑	**[Hypertriglyceridemia, Hyperlipidemia]**
	Hyperlipoproteinemia, viral hepatitis, alcoholism, alcoholic cirrhosis, biliary cirrhosis, extrahepatic obstruction, acute and chronic pancreatitis, chronic renal failure, hypertension, acute myocardial infarction, ischemic heart disease, cerebral thrombosis, hypothyroidism, diabetes mellitus, pregnancy, hypercalcemia, anorexia nervosa, obesity, glycogen storage disease
	Stress, high carbohydrate diet
	Cholestyramine, corticosteroids, estrogens, diuretics, ethanol, tobacco smoking, oral contraceptives
↓	**[Hypotriglyceridemia, Hypolipidemia]**
	Hypolipoproteinemia, lipoproteinemia, chronic obstructive lung disease, brain infarction, hyperparathyroidism, lactosuria, malnutrition, malabsorption syndrome, end-stage parenchymal disease
	Ascorbic acid, heparin, niacin, statins

Urea See Blood Urea Nitrogen
Urea Nitrogen See Blood Urea Nitrogen

6.36 Uric Acid [UA]

Affected Organs: Kidneys, marrow.
Specimen Handling: Serum is used. Use EDTA or heparin. *Interferences*: Hemolysis, lipemia, turbidity, bilirubin, ascorbic acid, oxalate, citrate, fluoride.
Supportive Tests: Liver panel, glucose, triglycerides, cholesterol, BUN, creatinine, uric acid in urine.

Uric acid (2,6,8-trioxypurine) is the end-product of purine metabolism in humans, great apes, and some other animals, and is often associated with gout. It exists in plasma as sodium urate and is eliminated in the urine. This test is of value for Dalmatians, great apes, and humans. Other mammals metabolize uric acid to the more soluble end product, allantoin.

↑	**[Hyperuricemia]**
	Renal failure, polycystic kidney disease, chronic lead nephropathy, gout, polycythemia, leukemia, lymphoma, multiple myeloma, neoplasms, toxemia of pregnancy
	Purine-rich diet, antileukemics, diuretics, ethanol, epinephrine, norepinephrine, mercurials, salicylates, nicotinic acid, corticosteroids
↓	**[Hypouricemia]**
	Multiple myeloma, Hodgkins disease, Fanconi syndrome, Wilson's disease, bronchogenic carcinoma
	Low purine diet

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Chapter 7
Urinalysis Diagnosis

Abstract The three diagnosis chapters—Hematology, Clinical Chemistry, and Urinalysis—are intended to be the most informative and most used parts of this handbook. There is a lot of useful information packed into these chapters. Once animal clinical pathology data have been evaluated and anomalous values for several parameters have been identified, these chapters can help one ascertain what these anomalies signify. Next to the name of each parameter are its common abbreviations. For each parameter, there are listings for organs that may be affected, specimen handling information, and supportive tests that may be used to confirm a diagnosis. These are followed by a brief description of the parameter including its strengths and weaknesses and other need-to-know information. Next to up and down arrows are potential diagnoses for when a parameter's value is increased or decreased. When there is a name for an increase or decrease (e.g., hypersthenuria or hyposthenuria), that is also provided. Diagnosis information is provided for 13 urinalysis tests.

The primary function of the kidneys is the excretion of metabolic waste products (urea, electrolytes, creatinine, hemoglobin, hormone byproducts) and other toxic chemicals from the blood stream. The kidneys are key to whole-body homeostasis including the regulation of blood pH to within a very narrow range, arterial pressure, blood volume, and water and electrolyte levels. It should not be surprising that 22 % of an animal's cardiac output goes to the renal arteries when at rest. The kidneys also produce several hormones. Most notably, kidneys undergoing hypoxia release erythropoietin (EPO) to stimulate the bone marrow to produce more erythrocytes. The character of urine is the result of three renal functions:

- *Glomerular filtration*—A passive process regulated mainly by arterial hydrostatic pressure. Substances with molecular weights >68,000 g/mol (e.g. cells, lipoproteins, and most proteins) cannot pass through the glomerulus.
- *Tubular Reabsorption*—Useful compounds (e.g. glucose, water, electrolytes, amino acids, and vitamins) are efficiently reabsorbed from the glomerular filtrate by the renal tubules.
- *Tubular Secretion*—Body homeostasis is maintained by the secretion of water, electrolytes and other substances in the renal tubules.

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J.E. Whalan, *A Toxicologist's Guide to Clinical Pathology in Animals*,
DOI 10.1007/978-3-319-15853-2_7

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Time, alkalinity, bright light, acids, alkalis, moisture, medication, and high temperatures can result in faulty urinalysis measurements. Urine stored in the urinary bladder for several hours may differ from freshly-formed urine with respect to pH, cells, casts, crystals, and bacterial growth. The pH of stored urine increases (i.e., becomes more alkaline) due to urease-producing bacteria and the escape of dissolved CO_2. Alkalinity promotes lysis of blood cells and casts, and may also affect crystals. Cell lysis can occur in urine with a specific gravity of <1.008. Ideally, samples should be analyzed within 30 min. Urinary nitrates from dietary metabolites are converted to nitrite by bacteria in the urinary tract. A bacterial infection can result in elevated nitrite levels.

Urine content is affected by diet, water intake, activity, and body temperature. Morning urine is more concentrated, and is more likely to contain cells, casts, and abnormal constituents, but it is unaffected by activity or feeding. Normal urine can range from clear to dark yellow. Darker urine is usually, but not always, more concentrated. Some foods can affect the color of urine, such as carrots, blackberries, beets, and rhubarb. Drugs that can color urine include chloroquine, iron supplements, levodopa, riboflavin, nitrofurantoin, phenazopyridine, phenothiazines, phenytoin, and triamterene. Turbidity or cloudiness indicates the presence of crystals, cells, mucus, bacteria, casts, or fluids from the reproductive tract. Cat urine tends to be cloudy because of the presence of lipid droplets. Test strips are a simple way to evaluate multiple parameters including specific gravity, pH, protein, glucose, ketone, bilirubin, urobilinogen, leukocytes, nitrite, and hemoglobin.

Acetoacetic Acid	See Ketones
Acetone	See Ketones
Appearance	See Color and Turbidity
Beta-Hydroxybutyric Acid	See Ketones
Bile Pigments	See Bilirubin

7.1 Biliburin [Bili]

Affected Organs: Liver, RBCs, marrow.

Specimen Handling: Fresh random urine may be stored 24 h at 4 °C away from light.

Supportive Tests: Serum conjugated bilirubin, ALT, ALP, AST, bile acids, GGT, 5′-NT.

Bilirubin is the breakdown product of hemoglobin. The form of bilirubin found in urine is conjugated (direct) bilirubin. It is water-soluble and able to pass freely through the glomerulus without being reabsorbed. Unconjugated (indirect) bilirubin is not found in urine. When freshly voided urine is shaken, a yellow-green or brown foam indicates the presence of bilirubin. Normal urine has a lesser white foam when shaken.

The concentration of urinary conjugated bilirubin reveals the extent of intra- and extrahepatic biliary tract obstruction. The liver may be markedly diseased before

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bilirubinuria occurs. Urinary bilirubin should always be compared with serum bilirubin levels. Serum hyperbilirubinemia typically precedes bilirubinuria but not in dogs.

Bilirubin is not typically found in the urine of horses, pigs, or sheep; but it is found in the urine of cats with liver disease. It is normal for cattle and some dogs to have mild bilirubinuria. Urine exposed to light may give a false-negative reading because of photoconversion to biliverdin. If measurements cannot be made within 30 min, samples should be refrigerated in the dark. Bilirubinuria is rare in cats.

↑	**[Bilirubinuria]**
	Intrahepatic and extrahepatic biliary tract obstruction, increased serum conjugated bilirubin, hepatitis, hepatocellular damage (progressed), abnormal RBC destruction, starvation, fever, feline leukemia
	Phenothiazines (large doses), salicylates, blood transfusions
↓	Ascorbic acid, nitrites

Blood Pigments See Hemoglobin
Clarity See Turbidity
Cloudiness See Turbidity

7.2 Color

Affected Organs: Kidneys, pancreas, adrenals.
Specimen Handling: Fresh random urine is used. Urine should be evaluated quickly since color can change on standing.
Supportive Tests: Turbidity, specific gravity, serum conjugated bilirubin, ALT, ALP, AST, bile acids, GGT, 5′-NT.

The color of urine can be affected by many things including diet, fluid balance, diseases, and medicines. Normal urine color may range from very pale yellow (practically colorless) to dark amber depending on the amount of yellow urochromes it contains. While urine tends to be pale when diluted and dark when concentrated, this is not always the case. The only sure way to gauge urine concentration is by measuring specific gravity. Abnormal colors of red or green may denote the presence of blood, bile pigments, pigmented drugs, or their breakdown products. Urine may change colors on standing. Cloudy urine, due to mucus and calcium carbonate, is pathologic except in horses. Cat urine is darker when it has jaundice. Normal rabbit urine ranges from yellow to red/brown. Ferrets have dark urine that interferes with colorimetric techniques.

Pale yellow—Urine is diluted and typically has a low specific gravity due to increased water consumption, diabetes mellitus, diabetes insipidus, toxic nephrosis (diuretic phase), nephrosis, advanced amyloidosis, hyperadrenocorticism, pyelonephritis, primary renal glucosuria, pyometra; IV fluids, administration of corticosteroids or ACTH.

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Dark yellow—Urine has high levels of yellow urochromes, usually has a high specific gravity, and is concentrated due to dehydration, fever, toxic nephrosis, terminal renal disease, low blood pressure, circulatory dysfunction.
Bright yellow—Vitamin B supplements.
Yellowish brown (foams on shaking)—Bilirubin and urochrome.
Deep brown/black—Blood, hemoglobin (urine is translucent), myoglobinuria, melanin from malignant melanoma.
Red/brown—Hemoglobin (urine is translucent), hematuria (RBCs in urine cause urine to be cloudy), myoglobinuria (urine is translucent), food dyes (carrots, blackberries, beets, rhubarb), phenolphthalein, phenothiazine, azosulfamide, urates, bile, drug metabolites.
Green/blue—Oxidation of bilirubin to biliverdin in poorly preserved specimens, drug metabolites, methylene blue, acriflavine, dithiazanine iodide.
White cloud—Excessive oxalic acid and glycolic acid in urine.

7.3 Glucose [GLU, Gluc]

Affected Organs: Pancreas, kidneys, adrenals.
Specimen Handling: Urine should be stored in a dark container on ice. It can be stored 24 h when mixed with 5 mL of glacial acetic acid or 5 g of sodium benzoate or sodium fluoride. The sample should be measured at room temperature. *Interferences*: ascorbic acid, bilirubin, ketones, fluoride, or formaldehyde.
Supportive Tests: Serum glucose, ALT, ALP, AST, bile acids, 5′-NT, urobilinogen, BUN, creatinine, lipase, amylase, glucose, Ca.

Glucose should be nonexistent in urine. Glucose readily passes through the glomerulus but is completely reabsorbed in the proximal tubules, even when the kidneys are damaged. Colorometric test strips are typically used to detect and quantify glucose. Faulty colorometric measurements can result from the presence of ascorbic acid in dog urine, bilirubin, ketones, fluoride, or formaldehyde.

NOTE: Glucose is also measured in serum or plasma; see Chap. 6, Clinical Chemistry Diagnosis.

↑	**[Glucosuria, Glycosuria]**
	Kidney disease or damage, impaired renal tubular reabsorption, high carbohydrate diet, increased blood glucose, uncontrolled diabetes mellitus, acute pancreatitis, hyperthyroidism, chronic liver disease, milk fever in ruminants, adrenal cortical hyperplasia, fear, excitement, restraint
	I.V. glucose therapy
	Corticosteroids, ACTH, D-thyroxine, morphine, glucagon, diuretics, penicillin, tetracycline, streptomycin, chlortetracycline, chloramphenicol, lactose, maltose, pentose, ascorbic acid, salicylates, EDTA, lead
↓	Not clinically significant

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7.4 Hemoglobin [Hb, HGB, Hgb]

Affected Organs: Blood, kidneys, skin.
Specimen Handling: Fresh random urine should be examined immediately. The lysing of RBCs in urine can lead to erroneous results.
Supportive Tests: BUN, creatinine, erythrocyte morphology (ghost cells), urine pH, CBC.

Hemoglobinuria, an increased level of hemoglobin in the urine, typically indicates a systemic disease or poisoning that results in intravascular hemolysis. It should not be confused with *hematuria*, which is blood in the urine due to genitourinary tract disease. Still, RBCs in dilute or alkaline urine can lyse, releasing their hemoglobin and leaving behind ghost erythrocytes.

NOTE: Hemoglobin is also measured in blood; see Chap. 5, Hematology Diagnosis.

↑	**[Hemoglobinuria]**
	Intravascular hemolysis, hematuria, pregnancy, extensive burns, autoimmune hemolytic anemia, transfusion reactions, kidney infarction, thrombotic thrombocytopenia, purpura (bruises), bacillary hemoglobinuria (in cattle, sheep, and occasionally dogs)
	Sulfonamides, quinine, phenylhydrazine, hemolytics
	Copper poisoning, fava beans, poisonous snakes and spiders
↓	Not clinically significant

Ketone Bodies See Ketones

7.5 Ketones

Affected Organs: Pancreas, G.I., pituitary.
Specimen Handling: Urine is stored in a tightly sealed container and refrigerated to avoid microbial decomposition and the loss of acetone.
Supportive Tests: Diabetic panel, lipid panel.

The ketones (ketone bodies) found in the urine include acetoacetic acid, acetone, and beta-hydroxybutyric acid, which are intermediates of lipid metabolism. Acetoacetic acid and acetone are detected by urinalysis reagent strips but beta-hydroxybutyric acid is not. The ketone bodies are filtered by the glomeruli and almost totally resorbed by the tubules. An elevated urinary ketone level is not a sign of kidney damage, but rather an exceedance of the tubular resorption threshold. Ketone levels increase sooner in the urine than in the blood. *Ketonuria*, an elevated level of ketones in the urine, is typically due to diabetes mellitus, starvation, or a diet high in fats and low in carbohydrates. Acetone can often be smelled in exhaled breath.

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↑	**[Ketonuria]**
	Diabetes mellitus, uncontrolled diabetic ketoacidosis, starvation, prolonged fasting (ketotic hypoglycemia), low carbohydrate/normal fat intake, high fat diet, persistent vomiting, markedly increased metabolic rate, excess of growth hormone, corticotropin, or glucocorticoids, hyperinsulinism, excess of catecholamines causing hyperglycemia, decreased insulin secretion, pregnancy diseases in sheep, ketosis in lactating cattle
	Aspirin, alcohol, BSP injection
↓	Not clinically significant

7.6 Occult Blood in Urine

Affected Organs: Kidneys, urinary tract, skeletal muscle.
Specimen Handling: Fresh random urine is used. Urine should be tested quickly to minimize the lysing of RBCs. If urine pH cannot be measured immediately, it can be stored in a container at 4 °C. Interferences: The presence of hemoglobin from lysed RBCs.
Supportive Tests: Plasma hemoglobin, erythrocyte morphology (ghost cells), urine myoglobin, urine pH.

The presence of lysed erythrocytes, hemoglobin, and myoglobin in the urine will give positive reactions in occult blood tests. Urine test strips are often used to detect hemoglobin or myoglobin. A positive test should be followed by a measurement of free hemoglobin in plasma. If no free hemoglobin is found in the plasma, then the positive finding can be attributed to myoglobin in the urine. Faulty tests can result when urine sits too long because RBCs lyse and release hemoglobin. This is a particular problem with alkaline urine.

↑	**POSITIVE [Hematuria]**
	Trauma to the kidneys or urinary tract, bladder catheterization, tumor, glomerulonephritis, calculus, acute infection, tuberculosis, infarction
	Hemoglobinuria (due to intravascular hemolysis and autoimmune hemolytic anemia) Myoglobinuria (due to muscle trauma, exertional myopathy in horses)
	Salicylates, sulfonamides, oncolytics, anticoagulants
↓	**NEGATIVE**
	Not clinically significant

Osmolality [Osmol] See Specific Gravity

7.7 pH

Affected Organs: Kidneys, bladder, pancreas, G.I.
Specimen Handling: Fresh random urine is used. If urine pH cannot be measured immediately, it can be stored in a container with little free air space at 4 °C.
Supportive Tests: Ketones, diabetic panel, K, Na, Cl, Ca.

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The pH of urine can range from 4 (strongly acidic) to 7 (neutral) to 9 (strongly basic). Standing urine will become basic due to loss of CO_2 and bacterial production of ammonia from urea. The kidneys regulate blood pH by excreting bicarbonate, ammonium ion, and phosphates. Diet, time of day, and systemic disease affect urine pH. It is normal for animals that eat animal protein or a cereal diet to have acidic urine, while animals that eat fruit and vegetables have alkaline urine. A nursing animal will always have acidic urine. Urine pH reveals more about an individual's metabolic status and systemic health than about the kidneys.

NOTE: Blood pH is also measured; see Chap. 6, Clinical Chemistry Diagnosis.

↑	**[Alkalinuria, Basic]**
	Acidic diet (e.g. fruit and vegetation), metabolic alkalosis (with normal potassium), respiratory alkalosis
	Prolonged vomiting, urinary tract infection, renal tubular acidosis, retained urine in the bladder due to cystitis or an obstruction
	Epinephrine, potassium citrate, sodium citrate, sodium bicarbonate, sodium lactate, nitrate
↓	**[Aciduria, Acidic]**
	Diet high in meat protein, cereals, and/or cranberries, metabolic acidosis, metabolic alkalosis (with potassium depletion), respiratory acidosis, starvation
	Diabetes mellitus, urinary tract infection, fever, severe diarrhea, ketosis, urine retention
	Sodium chloride, ammonium chloride, calcium chloride, sodium acid phosphate, ascorbic acid, corticotropin

7.8 Sediment

Affected Organs: Kidney, liver, bone, genitourinary tract, heart, thyroid, pancreas.

Specimen Handling: Fresh random urine should be examined immediately, but it may be preserved with 1 drop of 40 % formalin/10 mL or 1 crystal of thymol/10-15 mL. Interferences: Thymol interferes with the protein acid precipitation test. Urine must not be refrigerated.

Supportive Tests: urine specific gravity and pH, BUN, creatinine, diabetic panel, ALP-2 isoenzyme (bone), uric acid, total urinary protein.

Microscopic examination of urine sediment can reveal a wide variety of cells and other materials including: casts, RBCs, WBCs, tubular epithelial cells, bladder cells, transitional or squamous epithelial cells, spermatozoa, bacteria, ova, parasites, yeast, tumor cells, and crystals. These are described below along with possible diagnoses.

There is no standardized method for these examinations but typically a urine sample is centrifuged and all but 0.5 mL of the supernatant is decanted. The sediment at the bottom of the tube is resuspended in the remaining supernatant and a few drops are put on a glass slide and a cover slip applied. Most sediment is unstained but staining may be used to aid cell identification. Typical magnification is 100× for casts, crystals, and cells; and 400× for cells and bacteria.

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Bacteria—Gram-negative organisms are typically seen in urinary tract infections. Infection is indicated when bacteria are plentiful in urine collected aseptically via cystocentesis or catheterization.

Diagnosis: Urinary tract infection, cystitis, pyelonephritis, urine contamination from genital tract infection.

Casts—Casts are cylindrical plugs of material that form in the distal and collecting tubules and the ascending loop of Henle where urine is the most concentrated and acidic. They are called casts because they take the shape of the tubules where they are formed before being flushed into the urine. They may contain blood cells, kidney cells, fatty or waxy substances, or protein. There are two main types of casts—*hyaline* and *cellular*. Hyaline casts contain protein and mucoprotein, and indicate proteinuria. Some hyaline casts are found in health, especially after strenuous exercise. Cellular casts contain degenerated cells. The presence of more than 2–4 casts per low-power field (LPF) may be considered *cylindruria*, and the presence of numerous casts is a sign of renal disease. Casts are decreased in hypotonic (diluted) urine. Casts slowly dissolve in alkaline urine. Each cast type is described below along with its possible diagnosis:

Epithelial casts—Casts that form from desquamated tubule epithelial cells. They can be oval, flat, or elongated, and can be difficult to distinguish from WBC casts.

Diagnosis: Acute nephritis, renal tubular necrosis, transplant rejection, viral disease.

Fatty casts—When epithelial cells desquamate into the renal tubule, fatty deposits within the cells bind with proteins to form fatty casts.

Diagnosis: Degenerative tubular disease with deposition of lipoidal material in the tubules, nephritis, nephrotic syndrome, bone fracture. Commonly seen in cats with renal disease. May be found in dogs with diabetes mellitus.

Granular casts—Hyaline casts contain fine or coarse granules derived from the disintegration of tubular epithelium or aggregates of serum protein. Commonly found in domesticated animals. Not a reliable indicator of kidney lesion severity.

Diagnosis: Nonspecific indication of kidney disease, amyloidosis chronic nephritis, normal following strenuous exercise.

Hyaline casts—Homogeneous, colorless, semitransparent, cigar-shaped structures containing protein and mucoprotein. Found in acidic urine. Not typically found in large domestic animals.

Diagnosis: Dehydration, strenuous exercise, mild renal irritation, diuretic drugs.

RBC (erythrocytic) casts—Homogeneous cylindrical structures having a deep yellow to orange color.

Diagnosis: Hemorrhage into the kidney tubule that originates from the glomerulus or tubule, glomerular diseases including lupus nephritis, IgA nephropathy, Goodpasture syndrome, and Wegener's granulomatosis.

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Renal failure casts—Renal failure casts resemble granular casts but they are larger because they originate in collecting ducts or dilated tubules of the nephron.
Diagnosis: Obstruction or loss of nephrons.

Waxy casts—A further degenerated granular cast that results from diminished urine flow through the tubule. It resembles a hyaline cast but is more opaque, appears dull and waxy, has a broken end, and contains some granules and whole cells.
Diagnosis: Advanced kidney disease, degeneration, chronic kidney failure, malignant hypertension, diabetic nephropathy, glomerulonephritis, amyloid degeneration of the kidney.

WBC (leukocytic) casts—Hyaline casts with pus cells within the hyaline matrix.
Diagnosis: Interstitial cell kidney diseases such as pyelonephritis, kidney abscesses, parenchymal infection, and interstitial inflammation.

Crystals—Crystals are generally of no clinical concern. Many crystals form when urine cools. The most commonly seen crystals are urates, oxalates and phosphates. Urine pH and crystal solubility and concentration impact the presence of crystals. For example, triple phosphate, amorphous phosphate, and calcium carbonate crystals are found in alkaline urine; and amorphous urates and uric acid are found in acidic urine.
Diagnoses:
Ammonium biurate—liver diseases, portacaval shunt.
Calcium oxalate—diets high in rhubarb, cabbage, or asparagus; severe chronic renal disease, ethylene glycol poisoning, methoxyflurane toxicity.
Cholesterol—Nephritis, severe urinary tract infection.
Cystine—Homocystinuria, cystinuria.
Leucine and tyrosine—Severe liver disease, abnormal protein metabolism.
Carbon tetrachloride, chloroform, or phosphorus poisoning.
Triple phosphate—Indicates a high fruit diet.
Uric acid—Gout, leukemia, malignant lymphoma. Uric acid crystals are commonly seen in Dalmatians.

Epithelial cells—The extent of epithelial cells in urine increases during disease.
Diagnosis: cystitis, inflammation of the genitourinary tract, tubular degeneration, acute tubular necrosis.

Fat droplets—The presence of fat droplets in urine is called *lipuria*. It may be difficult to distinguish fat droplets from erythrocytes, but droplets stained with Sudan III will stain orange-red. Catheter lubricant is a common source of lipid. Lipuria is normal in cat urine.
Diagnosis: Fatty metamorphosis of the renal tubules, rupture of a lymphatic vessel, diabetes mellitus, high-fat diet, obesity, hypothyroidism.

Fungi—Fungi are a common contaminant in animal urine, but they are generally not of concern.

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Ova—The ova of many parasites can be found in urine. Fecal contamination is a common source of ova.

RBCs—*Hematuria* is the presence of RBCs in urine. RBCs may enter urine at any point in the genitourinary tract. The kidney is the source of RBCs when hematuria is accompanied by RBC casts, but the absence of cast or proteinuria suggests the source is distal to the kidney.

Diagnosis: Urinary tract disease, urinary tract stones, neoplasms, infections, tuberculosis, trauma, cystitis, prostatitis, gout, colon cancer, rectal cancer, hemoglobinopathies, heart failure, coagulation disorders.

Allopurinol anticoagulants, colchicine cyclophosphamide, aspirin, penicillins, sulfonamides.

Spermatozoa—It is not unusual to find spermatozoa in the urine of male dogs. The presence of spermatozoa may cause an increase in urinary protein.

Tubular epithelial cells—The presence of tubular epithelial cells in urine indicate acute tubular damage.

Diagnosis: Acute tubular necrosis, pyelonephritis, necrotizing papillitis.

Heavy metals, salicylate poisoning, ethylene glycol poisoning.

WBCs—*Pyuria* is the presence of WBCs or pus cells in the urine. Pyuria occurs with most diseases of the genitourinary tract. The pH, temperature, and specific gravity of urine, as well as bacteriauria, proteinuria, the voiding interval, and the time to examination all affect the number and appearance of WBCs in urine.

Diagnosis: Genitourinary tract disease, lupus nephritis, tubulointerstitial nephritis, pyelonephritis, exercise, fever.

Yeast—The presence of yeast cells in urine is usually not a concern except in diabetic or immunocompromised animals, in which case a yeast infection is usually accompanied by pyuria.

7.9 Specific Gravity [SG]

Affected Organs: Kidneys, pancreas, pituitary, hypothalamus, heart, liver.

Specimen Handling: Random or timed fresh urine is used. It must not be refrigerated.

Supportive Tests: Volume, BUN, creatinine, Na, total protein, glucose.

Specific gravity, also called *osmolality*, is mostly a measure of dissolved salts and, to a lesser extent, proteins and glucose. Urine specific gravity can vary widely in healthy animals—from dilute to isosthenuria (about the same specific gravity as protein-free plasma) to concentrated—based on fluid intake and the state of hydration. Specific gravity shows the ability of the renal tubules to concentrate glomerular filtrate. Measurements should be corrected for proteins and glucose.

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Generally, as urine volume increases, specific gravity decreases due to dilution; and as volume decreases, specific gravity increases due to urine concentration. High urine volume and low specific gravity are often, but not always, evidence of kidney disease. Decreases in specific gravity may not occur until approximately 70 % of the nephrons are impaired. *Isosthenuria* is a condition in chronic renal disease in which excreted urine has the same specific gravity as plasma (about 1.010) due to an inability to concentrate urine. A faulty water supply can result in high specific gravity and low urine volume. Specific gravity is measured as followed:

$$Specific\ Gravity = \frac{Weight\ of\ urine}{Weight\ of\ water}$$

↑	**[Hypersthenuria]**
	Dehydration, diarrhea, vomiting, decreased renal perfusion with intact concentrating mechanism, diabetes mellitus, proteinuria, glomerulonephritis, obstructive uropathy, polyuria, hypovolemic shock, circulatory failure with edema, high fever, burns with a loss of extracellular fluid (ECF), toxemia of pregnancy, jaundice in cats
	Refrigeration of urine
↓	**[Hyposthenuria]**
	Reduced water intake, renal tubular damage, chronic renal tubule insufficiency, end-stage renal disease (urine volume will be low), nephrogenic diabetes insipidus, diabetes mellitus, hyperadrenocorticism, pyometra, toxic nephrosis, nephritis, advanced renal amyloidosis, chronic general pyelonephritis, malignant hypertension, isosthenuria
	Corticosteroids, diuretics, ACTH, IV fluids

Transparency See Turbidity

7.10 Total Protein [TP, T. Prot]

Affected Organs: Kidneys, heart, CNS.
Specimen Handling: Urine is collected over 24 h and stored on ice with no preservatives.
Supportive Tests: BUN, creatinine, sediment (RBCs, WBC, spermatozoa).

Normal urine contains only traces of protein due to normal leakage or the presence or spermatozoa (dogs). Protein that passes through the glomeruli is reabsorbed in the tubules. It is normal to find brief *proteinuria* (elevations in urinary protein) in newborn animals, during estrus, at parturition, or following exercise; otherwise, proteinuria is considered pathologic.

NOTE: Total protein is also measured in serum; see Chap. 6, Clinical Chemistry Diagnosis.

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↑	**[Proteinuria]**
	Glomerular damage (some RBCs and WBCs will be present), glomerular nephritis, pyelonephritis, Bence-Jones proteins, urinary or genital tract inflammation (associated with hematuria and pyuria), fever, shock, emotional stress, heart disease, amyloidosis, renal infarction, CNS disease, arboviruses in primates, estrus, pregnancy, parturition, convulsions, excessive protein ingestion, urinary tract hemorrhage, renal trauma, renal neoplasms
	Post-renal proteinuria (after the kidneys) can be caused by vaginal or preputial discharge, spermatozoa, prostatitis, cystitis, urethritis, pyelitis, urolithiasis (stones)
	Exercise
	Arsenic, lead, mercury, turpentine, phenol, phosphorus, ether, sulfonamides
↓	Not clinically significant

7.11 Turbidity

Affected Organs: Kidney, genitourinary tract.
Specimen Handling: Fresh random urine is used. Urine should be tested quickly before it has a chance to cool.
Supportive Tests: Occult blood in urine, sediment, urinary hemoglobin and myoglobin.

Freshly voided urine is transparent, but becomes turbid (cloudy) on cooling due to the precipitation of salts. Turbidity can be due to blood cells (RBC, WBC), epithelial cells, semen, mucus (especially in horses), crystalline precipitates, fecal contamination, lipids, yeasts, or dirty collection equipment. Urine containing RBCs (hematuria) is turbid, but urine containing hemoglobin (hemoglobinuria) or myoglobin (myoglobinuria) is translucent. A fine diffuse cloudiness may be due to bacteria. Increased turbidity is usually due to pus cells. Concentrated urine is more turbid than dilute urine. The urine of healthy cats tends to be cloudy due to lipid droplets.

Urine Volume See Volume

7.12 Urobilinogen [Urobl]

Affected Organs: Liver, heart, gall bladder, biliary tract, kidneys.
Specimen Handling: A urine sample is collected over a 2-h period, and is stored on ice in a dark container. It should be measured within 30 min or frozen for storage. Samples collected over 24 h should also be preserved with 5 g of sodium bicarbonate and 100 mL of toluene.
Supportive Tests: ALT, ALP, AST, bile acids, conjugated bilirubin, GGT, 5′-NT, BUN, creatinine, urine pH.

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The urobilinogens are a group of pigmented substances found in urine. Bilirubin conjugates eliminated into the G.I. tract are converted to urobilinogen by gut bacteria. Some of this urobilinogen is reabsorbed by the small intestines, passes through the liver, and is eliminated by the kidneys; and the rest is eliminated in feces as stercobilin. The presence of urobilinogen in urine indicates that the bile duct is open and that enterohepatic circulation is normal. It is not unusual for urobilinogen levels to decrease for a day, but a decrease of several days is of concern. Oral antibiotics can impede bacterial conversion of bilirubin to urobilinogen. Diuresis can dilute urobilinogen levels to the extent that it cannot be measured. When a diseased liver is unable to excrete urobilinogen into bile, a high concentration of urobilinogen will pass into circulation and be excreted by the kidneys into urine.

Alkaline urine contains higher urobilinogen levels. Exposure to fluorescent light or prolonged standing of samples results in an orange-brown coloration caused by conversion to urobilin, which may interfere with bilirubin measurements.

↑	**[Urobilinogenuria]**
	Intravascular hemolysis, hemorrhage into tissues, constipation, hepatitis, congestive heart failure, hepatotoxic drugs, portal cirrhosis, biliary obstruction <u>with</u> biliary tract infection, pernicious anemia
	Bananas
	Drugs causing hemolysis, lead, sodium bicarbonate
↓	Biliary tract obstruction <u>without</u> biliary tract infection, massive hepatocellular damage, renal insufficiency, acid urine
	Exposure to light
	Drugs that cause cholestasis, antibiotics that reduce G.I. bacterial flora, ascorbic acid, carbon tetrachloride

7.13 Volume

Affected Organs: Kidneys, pancreas, pituitary, hypothalamus, heart, liver.
Specimen Handling: Urine is collected over a set period, such as 24 h.
Supportive Tests: Specific gravity, BUN, creatinine, Na, total protein, glucose.

Urine volume is primarily a factor of water intake, environmental conditions, and an animal's activity level and health status. Generally, as urine volume increases, specific gravity decreases due to dilution; and as volume decreases, specific gravity increases due to urine concentration. High urine volume and low specific gravity are often, but not always, evidence of kidney disease. Low urine volume and high specific gravity in toxicity studies can result from a faulty water supply. Gerbils conserve water and electrolytes by excreting a small volume of concentrated urine.

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↑	**[Polyuria]**
	Diabetes insipidus: central (pituitary), nephrogenic (acquired renal disease)
	Diabetes mellitus (there will also be a high specific gravity), excess protein, sodium, amino acids, or glucose in the diet
	Chronic renal failure, toxic nephrosis, pyrometra, advanced renal amyloidosis, adrenal insufficiency, polydipsia
	Excessive fluid intake (polydipsia) due to drug, iatrogenic, psychogenic, or hypothalamic effects
	I.V. mannitol or urea, ACTH, corticosteroids, diuretics, radiographic contrast media, caffeine, ethanol, aspirin, chlorpromazine, digitalis, tetracycline
↓	**[Oliguria, Anuria]**
	Reduced water intake, panting (dogs), hot weather, prerenal perfusion impairment due to dehydration, diarrhea, vomiting, low blood pressure, fever, blood loss, shock, congestive heart failure with edema, cirrhosis, edema, peritonitis, bowel obstruction, nephrotic syndrome, acute arterial or renal vein obstruction
	Renal parenchymal disease due to acute tubular necrosis, glomerulonephritis, acute vasculitis, hypercalcemic or analgesic nephropathy, chronic renal failure, septicemia, crush injuries, protein malnutrition, end-stage renal disease (specific gravity will be low)
	Nephrotoxic drugs

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Chapter 8
Hematology Glossary

Abstract It can be annoying and time-consuming having to search for information in multiple places, which is why this handbook includes a glossary of more than 400 frequently used hematology terms. It includes descriptions of dozens of cell types including normal, abnormal, hematopoietic, and premature blood cells. The shapes and staining characteristics of 60 poikilocytes (RBCs with abnormal morphology) are described. Because many cells and many terms have more than one name, these are cross-referenced to avoid confusion. Equations for calculating MCH, MCV, and MCHC values are included; and there is a table of coagulation factors and their roles in maintaining hemostasis. There are also descriptions for hematologic diseases such as Von Willebrand disease, thalassemia, hemophilia, and pernicious anemia.

Absolute Erythrocytosis Excessive erythropoiesis and increased erythrocyte mass in the blood due to primary or secondary causes. See also *Primary Erythrocytosis* and *Secondary Erythrocytosis*.

Absolute Polycythemia Excessive polycythemia and increased erythrocyte mass in the blood due to primary or secondary causes. See also *Primary Polycythemia* and *Secondary Polycythemia*.

Acanthocyte An erythrocyte with abnormal membranes that has multiple irregularly-spaced thorny projections. They are found in healthy cows and in dogs with severe liver disease. They can be evidence of a high fat diet, disorders of lipid metabolism, and hemangiosarcoma. They should not be confused with Echinocytes (burr cells).

Achromocyte An artifact caused by the loss of hemoglobin due to intravascular hemolysis. Also called *Ghost Corpuscle* or *Ghost Erythrocyte*.

Acuminocyte A fusiform erythrocyte similar to the fusocyte. It is found in goats, including Angoras.

Agglutination A clumping of erythrocytes resembling a bunch-of-grapes caused by an antibody reaction. It should not be confused with rouleaux, which resembles a stack-of-coins.

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J.E. Whalan, *A Toxicologist's Guide to Clinical Pathology in Animals*,
DOI 10.1007/978-3-319-15853-2_8

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Agranulocyte A leukocyte with a single-lobed nucleus that lacks well-defined granules in the cytoplasm.

Agranulocytosis A deficiency of granulocytes (neutrophils, eosinophils, and basophils) from the blood and/or marrow. Also called *Granulocytopenia* and *Schultz's Disease*.

Amphophil A cell that may be stained with either an acid or basic dye.

Anemia A significant decrease in the erythrocyte count, hematocrit, hemoglobin concentration, and/or quantity of oxygen carrying hemoglobin due to blood loss or impaired blood production (secondary to a disease state). Anemias are morphologically classified according to erythrocyte size (based on MCV values) and hemoglobin content (based on the MCHC values) as described in Table 2.1. See also *Aplastic Anemia (Refractory Anemia)*, *Hemolytic Anemia*, *Hemorrhagic Anemia*, *Hypochromic Anemia*, *Hypoplastic Anemia*, *Macrocytic Anemia*, *Megaloblastic Anemia*, *Microcytic Hypochromic Anemia*, *Myelophthisic Anemia*, *Non-Regenerative Anemia (Non-Responsive Anemia)*, *Normochromic Anemia*, *Normocytic Hypochromic Anemia*, *Pernicious Anemia*, *Regenerative Anemia (Responsive Anemia)*, *Splenic Anemia.*

Anisocytosis An excessive variation in RBC size.

Antibody An immunoglobulin molecule that interacts with an antigen.

Anticoagulant Any chemical that inhibits blood coagulation in vivo or in vitro.

Antigen A substance (foreign proteins, toxins, bacteria) that induces a specific immune response and that is acted upon by a specific antibody and/or sensitized T-lymphocyte.

Aplastic Anemia A form of anemia in which the marrow is neither acellular nor hypoplastic, but there is a lack of cell maturation. There is often a decrease in granulocyte and thrombocyte counts. It is unresponsive to treatment. Also called *Refractory Anemia*.

Azurophil Cells or granules that readily stain with blue aniline dyes. Azurophilic granules are found in the cytoplasm of lymphocytes, monocytes, monoblasts, mature megakaryocytes, promyelocytes, and in abnormal granulopoiesis. Azurophilic granules in neutrophils are called toxic granules and are found in sheep, cattle, and horses.

Avian Leukosis See *Leukosis*.

Band Cell See *Band Granulocyte*.

Band Granulocyte An immature granulocyte (neutrophil, heterophil, eosinophil, basophil) that has an unsegmented, curved nucleus. Also called *Stab Granulocyte*, *Immature Granulocyte*, and *Band Cell*.

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Barr Body An appendage to the neutrophil nucleus in females of some species. It is composed of extra chromatin and resembles a drumstick.

Basket Cell A degenerated cell in which the nucleus is swollen and the chromatin is separated such that it resembles a basket.

Basocytosis See *Basophilia*.

Basopenia A decrease in the absolute or differential basophilic count.

Basophil
1. A granular leukocyte that has an irregular, pale-staining multilobed nucleus (2–3 lobes) and a small number of cytoplasmic granules whose size and staining characteristics vary between species. Basophils are involved in hypersensitivity reactions and inflammation. Basophils are morphologically similar to mast cells, and serve some of the same functions. They produce and store vasoactive substances and release these chemicals upon stimulation. Basophils are produced in the bone marrow, spend only a few hours in peripheral circulation, and then migrate into body tissues. They are found in small numbers, except in rabbits.
2. A cell or element that readily stains with basic dyes.

Basophilia
1. An increase in the absolute or differential basophilic count. Also called *Basocytosis*.
2. The tendency of immature erythrocytes to stain bluish either diffusely, or with stippling or granules.
3. An increase in the number of circulating basophilic erythrocytes.

Basophilic Cells or cell structures that readily stain with basic dyes.

Basophilic Erythrocyte An immature erythrocyte that readily stains with basic dyes.

Basophilic Stippling The blue-staining granules of ribosomal material found in immature erythrocytes. It may be found in lead poisoning when anemia is mild.

B-Cell See *B-Lymphocyte*.

Berry Cell See *Echinocyte*.

Blast An immature cell in the lineage of a cell type.

Blastogenesis The process by which mature (small) lymphocytes revert to a blast (immature) form, divide, and differentiate as in the production of plasma cells. Blastogenesis is stimulated by an antigen to which an individual has been immunized.

Bleeding Time The time required for bleeding to stop following a controlled skin incision.

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Blister cell A feline erythrocyte that appears to have a hole punched in its periphery; caused by oxidative injury.

Blood A fluid tissue consisting of erythrocytes (red blood cells) that transport oxygen and carbon dioxide throughout the body; several types of leukocytes (white blood cells) that serve an immune function; thrombocytes (platelets) that are required for clotting; and plasma, the liquid portion that suspends the blood cells and carries proteins, hormones, all nutrients needed by cells, and cellular wastes for elimination.

Blood Dyscrasia An abnormal or pathological change in the blood or hematopoietic tissues.

Blood Film The technique of spreading a thin film of blood onto a glass slide for observation and cell counting. Also called *Smear*.

B-Lymphocyte A short lived lymphocyte that originates in the bone marrow of mammals and birds that is not acted upon by the thymus. In birds, B-lymphocytes differentiate in the bursa of Fabricius, which is located near the cloaca. B-lymphocytes are involved in antibody production (humoral immunity). When mature B-lymphocytes become sensitized, they can revert to a blast (immature) form, divide, and differentiate into plasma cells. Also called *B-Cell*, *Bursa-Derived Lymphocyte* (in birds), *Thymus Independent Lymphocyte*, *Bursa Equivalent Lymphocyte*, and *Bone Marrow Derived Lymphocyte* (in mammals).

Bone Marrow The soft material in the cavities of bones where blood cells are formed via hematopoiesis.

Bone Marrow Derived Lymphocyte See *B-Lymphocyte*.

Buffy Coat Layer A buff-colored to pinkish layer of leukocytes, thrombocytes, nucleated erythrocytes, and poikilocytes that settles above the erythrocyte mass following centrifugation.

Bursa-Derived Lymphocyte See *B-Lymphocyte*.

Bursa Equivalent Lymphocyte See *B-Lymphocyte*.

Burr Cell See *Echinocyte*.

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Burst Forming Unit A cell found in small numbers in the bone marrow that is derived from the multipotent hematopoietic stem cell that gives rise to the colony forming unit. It forms large colonies known as bursts.

Cabot's ring bodies Mitotic spindle remnants found in erythrocytes that appear as loops and figure 8's. They are seen in severe anemia.

Cell Mediated Immune Response An acquired immunity controlled by T-lymphocytes as in pathogen resistance (bacteria, virus, fungi), autoimmunity, graft and tumor rejection, response to malignancies, and delayed hypersensitivity reactions.

Chemotaxis The immunologic response that draws leukocytes to a reaction site.

Clot See *Thrombus*.

Clotting Factors See *Coagulation Factors*.

Clotting Time (CT) A timed measurement of whole blood coagulation in a glass tube. This test is prone to error due to contamination with tissue fluids. It is rarely performed.

Coagulation The process whereby leakage from a blood vessel is stopped. Coagulation requires platelets and coagulation factors.

Coagulation Factors With the exception of calcium ion (factor IV), the coagulation factors are a group of proteins required for blood clotting. Many of the coagulation factors are listed by Roman numeral in the order in which they were discovered. Most factors must be activated. The activated form is designated by adding a lower case 'a' after the factor number. For example, the activated form of factor XII is XIIa. All factors are found in the plasma except for thromboplastin (factor III), which is released from injured tissue. Serum contains reduced quantities of factors I, II, V, VIII, and XIII. There are extravascular pools of factors II, VIII, IX, and X. There is no factor VI. Table 8.1 list the coagulation factors and identifies them as being part of the intrinsic, extrinsic, and/or common pathways. See also *Platelet Factors*.

Coagulation Time See *Clotting Time*.

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Table 8.1 Coagulation factors

I	**Fibrinogen**—A glycoprotein produced in the liver that is part of the common pathway. Fibrinogen is converted by proteolytic action into soluble fibrin (factor Ia) by thrombin (factor IIa) in the presence of Ca^{++}, and then into stable fibrin in the presence of Ca^{++} and factor XIIIa
II	**Prothrombin**—A serine protease produced in the liver (vitamin K-dependent) that is part of the common pathway. Prothrombin is converted to thrombin (factor IIa) by Ca^{++} and platelet phospholipid (PF-3), and facilitated by factors Xa and Va (an accelerator). Thrombin then converts fibrinogen (factor I) to soluble fibrin (factor Ia)
III	**Thromboplastin (Tissue Factor)**—A protein phospholipid that forms a complex with factor VII and Ca^{++} to initiate coagulation by the extrinsic pathway. Thromboplastin is not found in the blood, but rather is released from injured tissue
IV	**Calcium Ions (Ca^{++})**—Calcium ions are necessary throughout the intrinsic, extrinsic, and common pathways
V	**Proaccelerin (Accelerator Globulin, AcG, Labile Factor)**—Proaccelerin is produced in the liver, and serves as an accelerator in converting prothrombin (II) to thrombin (IIa). It is inactivated by EDTA. It is a part of the common pathway
VI	*There is no clotting factor VI*
VII	**Proconvertin (Serum Prothrombin Conversion Accelerator, SPCA, Convertin, Stable Factor)**—A serine protease that is produced in the liver (vitamin K-dependent) and is part of the extrinsic pathway
VIII	**Antihemophilic Factor (Antihemophilic Globulin Cofactor I, AHG, Hemophilia A Factor)**—A factor produced in the liver that is involved in the intrinsic pathway. The sex-linked recessive deficiency trait is classical hemophilia (hemophilia A)
IX	**Christmas Factor (Plasma Thromboplastin Component, PTC, Hemophilia B Factor)**—A serine protease produced in the liver (vitamin K-dependent) that is involved in the intrinsic pathway. A deficiency results in Hemophilia B
X	**Stuart Factor (Stuart-Prower Factor, autoprothrombin C, Prower Factor)**—A serine protease produced in the liver (vitamin K-dependent). It is the first step in the common pathway. It can be activated to factor Xa by both the intrinsic and extrinsic pathways. It can also be activated in vitro with Russell's viper venom and trypsin. Xa activates prothrombin (II) to become thrombin (IIa)
XI	**Plasma Thromboplastin Antecedent (PTA, Antihemophilic Factor C)**—A serine protease produced in the liver that is part of the intrinsic pathway
XII	**Hageman Factor (Glass Factor)**—Factor XII, a serine protease of unknown origin that is converted to factor XIIa in vivo upon exposure to collagen at the site of injury, and in vitro upon exposure to glass, kaolin, asbestos, diatomaceous earth, fatty acids, and other negatively charged surfaces in what is called the contact reaction. Factor XIIa activates factors IX and XI to IXa and XIa. It is the first stage in the intrinsic pathway, and is also involved in disseminated intravascular coagulation
XIII	**Fibrin-Stabilizing Factor (Fibrinase, Fibrinoligase)**—A factor produced by megakaryocytes, the liver, and the placenta that is involved in the common pathway. It converts soluble fibrin (factor Ia) into stable fibrin in the presence of Ca^{++}. Factor XIII is activated to XIIIa by factor IIa (thrombin)
–	**High Molecular Weight Kininogen (HMW Kininogen, KMWK, Fitzgerald Factor, Williams Factor, Flaujeac Factor)**—A factor that is probably produced in the liver, and is a part of the intrinsic pathway. It is involved in contact activation of factor XII, and it enhances the activation of factor XI by factor XIIa

(continued)

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Table 8.1 (continued)

–	**Prekallikrein (Fletcher Factor)**—A serine protease produced in the liver that is involved in the intrinsic pathway. It is involved in contact activation of factor XII.
–	**Platelet Factors**—Proteins and lipoproteins found in platelets that participate in blood coagulation, designated PF-1, PF-2, PF-3, and PF-4
–	**Vitamin K**—A group of lipid soluble vitamins found in many foods and synthesized in the gut. Although these are not coagulation factors, they are co-factors that are necessary for the production of coagulation factors II (prothrombin), VII, IX, and X in the liver. Vitamin K can be administered to treat poisonings caused by anticoagulant rodenticides (e.g. warfarin) that interfere with prothrombin synthesis

Codocyte A *Target Cell* or *Mexican Hat* erythrocyte; a type of leptocyte. It is a thin cell with concentrations of hemoglobin in the center and the outer edge, and a pale area in between. It is found in human and canine blood during liver disease, obstructive jaundice, postsplenectomy, hemoglobinopathies, thalassemia, and congenital and acquired anemias. It is rare in other species. It may be artifactual in hypertonic plasma.

Codocytosis The presence of codocytes.

Colony Forming Unit (CFU) A large cell found in the bone marrow that is derived from the burst forming unit, and is found in large numbers. It forms small colonies that give rise to rubriblasts, and is responsive to erythropoietin (EPO).

Common Lymphoid Progenitor A cell derived from the multipotent stem cell. It is a committed cell that gives rise to B-lymphocytes, T-lymphocytes, and plasma cells. Also called *Lymphoid Stem Cell*.

Common Myeloid Progenitor A cell derived from the multipotent stem cell. It is a committed cell that gives rise to erythrocytes, neutrophils, heterophils, eosinophils, basophils, macrophages, and thrombocytes. Also called *Myeloid Stem Cell*.

Common Pathway Blood coagulation is promoted via the intrinsic or extrinsic pathway and is then completed via the common pathway.

Complete Blood Count (CBC) An evaluation of anticoagulated blood that includes counts of the cellular components, erythrocyte morphology, and measurements of hematocrit, hemoglobin, plasma fibrinogen, total protein, and erythrocyte fragility.

Contact Reaction See *Coagulation Factor XII*.

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Crenation The notched appearance in an erythrocyte caused by delayed drying, shrinking in a hypertonic solution, or extended standing of the sample. Crenation is an artifact, not a type of poikilocyte (abnormally shaped erythrocyte).

Cryohydrocyte Erythrocytes that lyse when stored at 4 °C for no apparent pathologic reason.

Cyanosis Low blood oxygen that results in the skin and mucous membranes having a bluish tinge.

Cytotoxic T-Cell A type of T-lymphocyte with previous antigenic exposure that can recognize and destroy foreign cells.

Dacryocyte A tear-shaped erythrocyte found in dogs and cats with myeloproliferative disorders. They are found in healthy goat kids. They may be artifactual in the feathered edge of a blood smear (all points are pointing the same way).

Descicyte See *Xerocyte*.

Desiccytosis See *Xerocytosis*.

Diapedesis The passage of leukocytes from intact blood vessels into the tissue.

Differential Leukocyte Count The proportion of each type of leukocyte counted on a Wright-stained blood smear. Usually presented in percentages and/or absolute values.

Discocyte A normal biconcave erythrocyte. Also called *Normocyte*.

Disseminated Intravascular Coagulation (DIC) A coagulation disorder in which coagulation elements are reduced due to widespread clotting in the blood vessels and massive consumption of thrombocytes and coagulation factors. It is a secondary response to snake bite, neoplasia, surgery, heartworm, pancreatitis, hemorrhagic enteritis, polycythemia, and obstetric complications.

Dohle Bodies RNA-derived rough endoplasmic reticulum that appears as round or oval blue-staining inclusions in the cytoplasm of neutrophils. They are found in cases of burns, infections, aplastic anemia, pregnancy, and poisoning.

Drepanocyte A sickle-shaped erythrocyte that contains polymerized hemoglobin S. It is found in humans (sickle cell disease) and in sheep, goats, and deer.

Dyscrasia See *Blood Dyscrasia*.

Dyserythropoiesis Abnormal erythrocyte production.

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Eccentrocyte An erythrocyte with condensed hemoglobin in one part of the cell due to oxidative damage to the cell membrane. It is found in dogs with hemolytic anemia caused by onion and acetylphenylhydrazine poisoning.

Echinocyte An erythrocyte with many evenly arranged small projections. Echinocytes are classified as I, II, or III based on the extent of abnormality. Echinocyte III cells are spherical and have pronounced projections (also called *Burr Cell* or *Berry Cell*). Echinocytes are found in bleeding peptic ulcers, gastric carcinoma, uremia, and azotemia. They may be artifactual. They should not be confused with acanthocytes that have uneven projections.

Echinoelliptocyte An oval erythrocyte with evenly spaced projections found in cats with liver disease. They are rarely seen in other species.

Echinosis An erythrocyte with a spiny appearance.

Effector Cell Any activated lymphocyte or plasma cell that can destroy antigen coated cells and foreign material.

Elliptocyte An oval-shaped erythrocyte found in the *Camellidae* family, deer, and Angora goats, and in dogs and cats with hematopoietic malignancies and some anemias. Also called *Ovalocyte*.

Embolus A thrombus that travels from one part of the body to another.

Eosinopenia A decrease in the absolute or differential eosinophil count.

Eosinophil A granular leukocyte that is distinguished by its bright pink cytoplasmic granules and a smooth polymorphic nucleus. Eosinophils mediate allergic and inflammatory reactions, and destroy parasites. Although their appearance varies widely with different species, all eosinophils are parasitocidal, bacteriocidal, and phagocytic. Eosinophils are produced mostly in the marrow, but also in the thymus, spleen, and cervical lymph nodes. They spend only a few hours in the peripheral circulation before entering tissues. They tend to congregate near mast cells, and are found mostly in the bone marrow and ports of entry such as the skin, gastrointestinal tract, and lung.

Eosinophilia An increase in the absolute or differential eosinophil count.

Erythemic Myelosis A myeloproliferative disorder involving the erythropoietic tissue that is characterized by marked anemia, anisocytosis, and rubricytes in the circulation. Erythemic myelosis is seen in cats infected with feline leukemia virus, and rarely in other species.

Erythroblast See *Nucleated Erythrocyte*. Also called *Erythrocytoblast*.

Erythroblastosis The presence of erythroblasts in circulating blood.

Erythroblastemia The presence of erythroblasts in circulating blood.

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Erythrocyte A blood cell that contains hemoglobin and transports oxygen to the body. Erythrocytes are anuclear in mammals, marsupials, and monotremes, but nucleated in all other vertebrates (e.g., birds and reptiles). A mammalian erythrocyte is a mature cell (i.e. not nucleated) unless specified otherwise. Also called *Red Blood Cell* and *Red Corpuscle*.

Erythrocyte Count Erythrocytes per unit volume of blood.

Erythrocyte Fragility The propensity of erythrocytes to lyse when subjected to physical, osmotic, or mechanical stress.

Erythrocyte Indices Calculated values of erythrocyte status including Mean Corpuscular Hemoglobin (MCH), Mean Corpuscular Hemoglobin Concentration (MCHC), and Mean Corpuscular Volume (MCV). They are calculated from the erythrocyte count, hematocrit, and hemoglobin concentration. The indexes are used to classify anemia and assess the status of erythropoiesis.

Erythrocyte Refractile Bodies See *Heinz Bodies*.

Erythrocyte Fragmentation The normal destruction of erythrocytes. It is also the way that abnormal erythrocytes are removed. See *Keratocyte and Knizocyte*.

Erythrocyte Sedimentation Rate (ESR) A measure of the amount of erythrocyte settling over an hour in a tube of anticoagulated whole blood.

Erythrocyte Stimulating Factor (ESF) Plasma levels of erythrocyte stimulating factor are controlled by the kidneys. ESF stimulates stem cells to increase production by as much as 4–5 fold in response to anoxia.

Erythrocytoblast See *Nucleated Erythrocyte*.

Erythrocytopenia A decrease in circulating erythrocytes.

Erythrocytosis An increased number of circulating erythrocytes, or an increase in erythrocyte mass in the blood. Also called *Polycythemia*. Erythrocytosis can be further characterized as follows:

- **Absolute Erythrocytosis** Excessive erythropoiesis and increased erythrocyte mass in the blood due to primary or secondary causes as described below.
- **Familial Erythrocytosis** Hereditary erythrocytosis.
- **Primary Erythrocytosis** An increase of the total erythrocyte cell volume due to a dramatic increase in erythrocyte production while serum EPO levels are low. It is accompanied by splenomegaly and increased production of granulocytes and thrombocytes. It is caused by a myeloproliferative disease and is

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seen in dogs, cats, horses, and cattle. Also called *Primary Polycythemia*, *Polycythemia Vera*, and *Polycythemia Rubra Vera.*

- **Relative Erythrocytosis** — An apparent excess of circulating erythrocytes (i.e. elevated RBCs and hematocrit) due to a decrease in plasma volume. Also called *Relative Polycythemia*.
- **Secondary Erythrocytosis** — An increase in the erythrocyte count in the circulating blood because of excessive EPO production; either due to systemic hypoxia (appropriate) or an EPO secreting tumor in the kidney (inappropriate). Unlike primary erythrocytosis, there is no increased production of granulocytes and thrombocytes. Also called *Secondary Polycythemia.*

Erythrogenesis — Erythrocyte production. Also called *Erythropoiesis*.

Erythroid — Any of the developing cells that mature into erythrocytes.

Erythroleukemia — Neoplastic proliferation of erythroblastic and myeloblastic elements with the release of atypical erythroblasts and myeloblasts.

Erythron — Any cell that can be characterized as an erythrocyte or erythrocyte precursor.

Erythropenia — A decrease in circulating erythrocytes.

Erythropoiesis — Erythrocyte production. Also called *Erythrogenesis*.

Erythropoietin (EPO) — A glycoprotein hormone released by adult kidneys and fetal liver that stimulates erythropoiesis. In response to hypoxia, EPO binds to receptors on BFU-E and CFU-E cells in the bone marrow. This shortens cell cycle and maturation times and allows for an increased release of erythrocytes into circulation.

Extramedullary Hematopoiesis — The production of blood cells outside of the bone marrow such as in the spleen, liver, and lymph nodes.

Extrinsic Pathway — The initiatory coagulation pathway involving coagulation factors VII and III (thromboplastin from the trauma site) that then progresses to the common pathway.

Familial Erythrocytosis — Hereditary erythrocytosis.

Familial Polycythemia — Hereditary polycythemia.

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Ferritin The iron-apoferritin complex that stores iron in the body, particularly in the liver, gastrointestinal mucosa, bone marrow, spleen, and reticuloendothelial cells.

Fibrin The insoluble fibrous protein that is the end product of coagulation. Fibrin forms a meshwork that snares blood cells to form a clot.

Fibrinogen A plasma protein produced by the liver that is essential for clotting. It enters extravascular spaces in response to inflammatory disease, and is thus an excellent means of assessing inflammatory disease. Also called *Coagulation Factor I*.

Fragility Test A test that measures the resistance of erythrocytes to hemolysis when placed in hypotonic saline solutions ranging from 0.28 % to 0.50 %, and allowed to stand for 2 h.

Fusocyte A spindle-shaped erythrocyte similar to the acuminocyte that is found in goats, including Angoras.

Ghost Cell Achromocyte. An artifact caused by the loss of hemoglobin due to intravascular hemolysis. Also called *Ghost Erythrocyte* or *Ghost Corpuscle*.

Gigantocyte An erythrocyte that is 1.5-2.0 times the normal diameter. It is larger than the *Macrocyte* and the *Megalocyte*. They are normal in goats, and are seen in other species following severe anemia.

Granule A nonmembranous particle found in cytoplasm.

Granulocyte A leukocyte (including neutrophils, heterophils, eosinophils, and basophils) at any stage of maturation that contains well-defined cytoplasmic granules. Granulocytes undergo three phases—the intermedullary (i.e. in bone marrow), intravascular, and tissue phases. The term granulocyte is often used to mean neutrophil.

Granulocytopenia See *Agranulocytosis*.

Granulocytopoiesis The production of granulocytes (neutrophils, heterophils, eosinophils, and basophils). Also called *Granulopoiesis*.

Granulocytosis An increased number of granulocytes in the blood.

Granulopoiesis The production of granulocytes (neutrophils, heterophils, eosinophils, and basophils). Also called *Granulocytopoiesis*.

Heinz Bodies Small irregular masses of denatured protein associated with hemolytic anemias, abnormal hemoglobin, and erythrocyte enzyme deficiencies following toxicity. They indicate oxidative damage to RBCs and may be seen along with eccentrocytes. Healthy non-anemic cats can have Heinz bodies in high numbers. Also called *Erythrocyte Refractile Bodies*.

Helmet Cell See *Schistocyte*.

Hemacytometer An instrument used to count blood cells. Also called *Hematocytometer* and *Hemocytometer*.

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Hematocrit (Hct) The percentage of whole blood occupied by erythrocytes following centrifugation. Also called *Packed Cell Volume* (PCV).

Hematopoiesis The production of blood cells. Hematopoiesis primarily occurs in bone marrow, but can also occur in the spleen (especially in rats and mice) and the liver.

Hematopoietic Stem Cell (HSC) The hematopoietic stem cells are found primarily in the bone marrow but also in the liver and spleen. These cells give rise to all the blood cells. Only 0.01 % of myeloid cells in the bone marrow are hematopoietic stem cells.

Hematuria Blood in the urine.

Hemoconcentration An increase in hematocrit due to a decrease in blood fluid content. Also called *Dehydration* and *Polycythemia*.

Hemoblast See *Multipotent Stem Cell*.

Hemocytoblast See *Multipotent Stem Cell*.

Hemodilution A decrease in hematocrit due to an increase in blood fluid content. Also called *Hydremia*, *Oligocythemia*, and *Oligocytosis*.

Hemoglobin The iron containing pigments that impart the red color to erythrocytes and that are capable of reversible oxygenation. They are conjugated proteins consisting of heme and globin. Hemoglobin is responsible for the transport of oxygen and carbon dioxide throughout the body.

Hemoglobinemia An excess of hemoglobin in the plasma.

Hemoglobinopathy An inherited genetic defect that results in the abnormal structure or production of either the α or β globin in the hemoglobin molecule. Common examples are thalassemia and sickle cell anemia. See also *Thalassemia*.

Hemogram A tabulation of blood cell counts. Also called *Complete Blood Count* (CBC).

Hemolysis The lysing (bursting) of erythrocytes with the release of hemoglobin into plasma or serum. Hemolysis occurs naturally in the spleen, liver, and lymph nodes, which have abundant macrophages, and also in peripheral blood. The major causes of hemolysis are presented in Table 2.2.

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Hemolytic Anemia Anemia caused by RBC destruction due to lysis. It may be caused by toxins, infectious agents, physical agents, autoimmune diseases, and hereditary factors.

Hemophilia Hemophilia is a rare genetic disorder in which blood does not clot normally due to a lack of clotting factors.

Hemophiliac An individual that has hemophilia, a rare genetic disorder. Hemophiliacs have extended bleeding time following injuries and internal bleeding that can damage the organs.

Hemopoiesis See *Hematopoiesis*.

Hemorrhage A loss of blood from a blood vessel. The opposite of hemostasis.

Hemorrhagic Anemia Anemia resulting from an acute loss of blood.

Hemosiderin An insoluble intracellular form of storage iron (~33 % iron) that can be seen microscopically. It accumulates when the ferritin storage pool is saturated.

Hemosiderosis Local iron deposits in tissues without any deleterious effect as in the lungs, kidneys, and liver. In hepatic hemosiderosis, there is an abnormal quantity of hemosiderin found in Kupffer cells. Pulmonary hemosiderosis, caused by bleeding into the lungs (as occurs in severe congestive heart failure), results in the deposition of abnormal amounts of hemosiderin in the lungs.

Hemostasis The stoppage of bleeding through clotting, vasoconstriction, or surgical means. The opposite of hemorrhage.

Heterophil A granular leukocyte identified as the neutrophil in humans and many animals, but called the heterophil in other species (including rats, mice, guinea pigs, gerbils, rabbits, birds, and lemurs) because they stain differently.

Histiocyte See *Macrophage*.

Histiocytosis An abnormal number of histiocytes (macrophages) in the blood.

Histocyte Histiocyte (macrophage). See *Macrophage*.

Howell-Jolly Bodies Bluish-spherical remnants of nuclear material found in any part of an erythrocyte. They are found following splenectomy and in cases of severe anemia, including megaloblastic anemia and hemolytic anemia.

Humoral Immunity An acquired immunity involving antibodies produced by B-lymphocytes and plasma cells.

Hydremia A marked decrease in hematocrit due to an increase in blood fluid content. Also called *Hemodilution*, *Oligocythemia*, and *Oligocytosis*.

Hydrocyte A swollen erythrocyte that resembles a stomatocyte as a result of hydrocytosis.

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Hydrocytosis The swelling of erythrocytes due to an accumulation of cellular sodium that can result in hemolytic anemia.

Hyperchromasia See *Hyperchromatic*.

Hyperchromatic Excessive erythrocyte hemoglobin pigmentation due to increased erythrocyte thickness. The term 'hyperchromic' should not be used.

Hyperfibrinogenemia An increased fibrinogen level in the blood. Also called *Fibrinogenemia*.

Hyperlipemic Plasma or serum with an excess of lipid has a milky appearance and is said to be *Hyperlipemic* or *Lipemic*.

Hypersegmentation The extensive segmentation of the granulocyte nucleus as seen in old circulating neutrophils (right shift), in vitamin B_{12} and folate deficiency, and as an artifact. True hypersegmentation is seen in humans but is rare in domestic animals.

Hypersplenism A reduction in peripheral blood cells due to exaggerated destruction function in the spleen, sometimes accompanied by splenomegaly.

Hypertonic A solution with high tonicity that may cause erythrocyte crenation.

Hypervolemia An increased volume of circulating plasma.

Hypochromasia An increased area of central pallor in erythrocytes due to decreased hemoglobin content. It is caused by iron deficiency. MCH and MCHC are decreased.

Hypochromia See *Hypochromasia*.

Hypochromic Pertaining to erythrocytes with increased central pallor due to decreased hemoglobin content.

Hypochromic Anemia Anemia in which erythrocytes have decreased hemoglobin content and increased area of central pallor.

Hypofibrinogenemia A decreased fibrinogen level in the blood.

Hypoplastic Anemia Erythrocyte hypoplasia (decreased erythropoiesis) concurrent with normal leukocyte and thrombocyte counts.

Hypotonic A solution with low tonicity that may cause blood cell lysis.

Hypovolemia A decrease in plasma volume. Also called *Oligemia*.

Icterus Index The color of the plasma is compared to 1 % potassium dichromate standards to assess the amount of bilirubin in the blood. Hemolysis, lipemia, and lactescence (milkiness) interfere with these measurements.

Idiopathic A self-originated condition of unknown cause.

Immature Granulocyte See *Band Granulocyte*.

Immunoglobulin Y-shaped glycoproteins, also known as antibodies, produced by plasma cells. Immunoglobulins target antigens on foreign material including bacteria and viruses for destruction.

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Indices See *Erythrocyte Indices*.

Inflammatory Macrophage *See Macrophage*.

Intrinsic Pathway The initiatory coagulation pathway involving (in order) coagulation factors XII, XI, IX, and VIII that then progresses to the common pathway. Blood exposed to glass (e.g. a collection tube) will clot by the intrinsic system.

K-Cell See *Killer Cell*

Keratocyte A fragmented erythrocyte with one or more complete cuts. A kerocyte may have a crescent shape due to mechanical shearing. They are found in hemolytic anemias. RBC fragments called schizocytes accompany keratocytes.

Killer Cell A type of null cell that resembles a mature lymphocyte and is capable of antibody-dependent cell-mediated cytotoxicity (ADCC). Killer cells attack cells coated with specific IgG antibody. Also called *K Cell* and *Killer Lymphocyte*.

Killer Lymphocyte See *Killer Cell*.

Knizocyte A triconcave erythrocyte seen in hemolytic anemias in dogs and humans.

Kupffer Cell Macrophages that differentiate from monocytes and a part of the mononuclear phagocyte system (MPS). They reside in liver sinusoids and eliminate bacteria, particulate matter, foreign proteins, and senescent erythrocytes.

Kurloff Cell As many as 4 % of guinea pig lymphocytes are Kurloff cells that contain one or more cytoplasmic inclusions called Kurloff bodies, which are rich in neutral mucopolysaccharides. Kurloff cells may function as natural killer cells.

Labrocyte See *Mast Cell*.

Left Shift An increased proportion of immature neutrophils (and rarely eosinophils) in the circulating blood. As released cells become more immature, the left shift is said to be more severe. The left shift may be either regenerative or degenerative. A *regenerative* left shift is due to increased granulopoiesis, so mature neutrophils outnumber immature neutrophils. A *degenerative* left shift is due to inhibited granulopoiesis and septicemia and is characterized by delayed maturation, so immature neutrophils outnumber mature neutrophils and WBC counts are normal or decreased.

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Leptocyte A thin, large diameter erythrocyte with increased surface area and normal cell volume (e.g. target cells and transverse folded cells) that readily distorts and gives a pinkish hue to the buffy coat layer. Leptocytes tend to become knizocytes. They are found in chronically diseased animals and in hepatic disease, obstructive jaundice, regenerative anemia, and iron deficiency anemia.

Leptocytosis The presence of thin, large diameter erythrocytes with increased surface area and normal cell volume.

Leukemia A malignancy of the hematopoietic organs expressed as abnormal proliferation and development of leukocytes and leukocyte precursors.

Leukemogen Any chemical that induces leukemia.

Leukemoid Any condition that closely resembles true leukemia.

Leukon Any cell that can be characterized as a leukocyte or leukocyte precursor.

Leukocyte A white blood cell (white corpuscle) that can be characterized as either granular (neutrophil, heterophil, eosinophil, and basophil) or nongranular (lymphocyte and monocyte). Leukocytes are a key part of the body's immune system.

Leukocyte Count See also *Differential Leukocyte Count*.

Leukocytopenia See *Leukopenia*.

Leukocytosis An increase in the circulating leukocyte count (all leukocyte species combined).

Leukogenesis Leukocyte production. Also called *Leukopoiesis*.

Leukogram A tabulation of a differential leukocyte count.

Leukopenia A decrease in the total circulating leukocyte count (all leukocyte species combined).

Leukopoiesis Leukocyte production. Also called *Leukogenesis*.

Leukosis
1. A proliferation of leukocyte producing tissue as seen in leukemia, myelosis (proliferation of granulocyte-producing tissue), and lymphadenosis (lymphoid tissue proliferation).
2. Virus-induced diseases in birds in which there is proliferation of immature erythroid, myeloid, and/or lymphoid cells, and solid tumors. Also called *Avian Leukosis*.

Lipemia Excessive lipid in the blood. Lipemia interferes with some tests.

Lipemic Plasma or serum with an excess of lipid has a milky appearance and is said to be *Lipemic* or *Hyperlipemic*.

Lymphoblast A lymphocyte precursor. Also called *Lymphocytoblast*.

Lymphoblastosis An excess number of lymphoblasts in the blood.

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Lymphocyte A type of leukocyte that is round and has a single round or oval nucleus. Lymphocytes are produced mostly in the bone marrow, but also in lymphoid organs and in the gut-associated lymphoid tissues. The three major types are B-lymphocytes, T-lymphocytes, and Null cells (also called non-T, non-B lymphocytes). B-lymphocytes are involved in antibody production (humoral immunity). They can become plasma cells in a process called blastogenesis. T-lymphocytes kill cells in cell mediated immune responses (i.e. pathogen resistance, autoimmunity, graft and tumor rejection, response to malignancies, and delayed hypersensitivity reactions). They also regulate antibody response by stimulating and suppressing antibody production by B-lymphocytes. There are several types of T-lymphocytes including cytotoxic T cells, T helper cells, T suppressor cells, and T memory cells. Both B- and T-lymphocytes secrete lymphokines in response to antigenic stimulation. Null cells have surface antigens that differ from those seen on B- and T-lymphocytes. Two major types of null cells are the killer cells and the natural killer cells. Lymphocytes are able to recirculate (mobilize). That is, they leave the blood, enter lymphatic tissues, and return to the blood. This allows immunocompetent lymphocytes to distribute widely and yet home in on antigens in large numbers when needed.

Lymphocytoblast A *Lymphoblast*.

Lymphocytopenia A decrease in the absolute or differential lymphocyte count.

Lymphocytopoiesis Lymphocyte production. Also called *Lymphopoiesis*.

Lymphocytosis An increase in the absolute or differential lymphocyte count.

Lymphoid Stem Cell A cell derived from the multipotent stem cell. It is a committed cell that gives rise to B-lymphocytes, T-lymphocytes, and plasma cells. Also called *Common Lymphoid Progenitor*.

Lymphokines Soluble protein mediators that have a variety of significant effects on target cells, macrophages, and other leukocytes. Approximately 100 lymphokines have been identified. The best known include interferons, interleukin-2, macrophage activating factor (MAF), transfer factor, migration inhibiting factor (MIF), transfer factor, mitosis stimulating factor, and lymphotoxin. T-lymphocytes, and B-lymphocytes to a lesser degree, secrete lymphokines in response to antigenic stimulation.

Lymphopenia A decrease in the absolute or differential lymphocyte count.

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Lymphopoiesis Lymphocyte production. Also called *Lymphocytopoiesis*.

Lymphoma Lymphoid tissue neoplasia that is generally malignant.

Lysis Destruction of a cell, such as the bursting of erythrocytes in a hypotonic solution.

Macrocyte An unusually large erythrocyte with elevated MCV. It is smaller than the *Megalocyte* and the *Gigantocyte*.

Macrocythemia See *Macrocytosis*.

Macrocytic Anemia Anemia with an increased MCV value that may be either hypochromic or normochromic.

Macrocytosis Large RBCs due to regenerative anemia (accelerated erythropoiesis), vitamin B_{12} or folic acid deficiency, and bone marrow disorders. Macrocytosis is common in miniature poodles.

Macrophage A large, mononuclear phagocytic cell derived from the monocyte. 'Fixed' macrophages are found in the sinusoids of the spleen, liver (known as Kupffer cells), lymph nodes, bone marrow, the lining of the gastrointestinal tract, and in loose connective tissues. They become 'Free' or 'Wandering' macrophages when stimulated by inflammation, and can be found at the site of infections in the alveoli and in the pleural, synovial, and peritoneal cavities. They generally do not divide, and are rarely found in the blood. Also called *Histiocyte* and *Histocyte*. See also *Microglia* and *Kupffer Cell*.

Margination Adhesion of leukocytes to vascular walls.

Marrow See *Bone Marrow*.

Mast Cell A connective tissue cell of uncertain function that is widely distributed throughout the body. It is involved in hypersensitization reactions, and is morphologically related to the basophil. It contains cytoplasmic granules containing histamine, heparin, serotonin, and other vasoactive substances. Mast cells are probably produced in the connective tissue from undifferentiated mesenchymal cells. Also called *Mastocyte* and *Labrocyte*.

Mastocyte See *Mast Cell*.

Mean Corpuscular Hemoglobin (MCH) The calculated mass of hemoglobin in an average erythrocyte. The MCH and MCV usually rise and fall together; i.e. as the cells get bigger, they typically contain more hemoglobin. This calculated erythrocyte index can highlight errors in erythrocyte count, hematocrit, and hemoglobin measurements, and portray the course of anemia. It is calculated as follows:

$$MCH(picograms) = \frac{Hb(g/dL)\times 10}{RBCs(million/\mu L\ or \times 10^{12}/L)}$$

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Mean Corpuscular Hemoglobin Concentration (MCHC) The calculated weight to volume proportion of hemoglobin in the average erythrocyte. This calculated erythrocyte index can highlight errors in erythrocyte count, hematocrit, and hemoglobin measurements, and portray the course of anemia. It is calculated as follows:

$$MCHC\left(g\,of\,Hb\,/\,dL\,of\,RBCs\right)=\frac{Hb\left(g\,/\,dL\right)\times 100}{Hct\left(\%\right)}$$

Mean Corpuscular Volume (MCV) The calculated volume of an average erythrocyte. The MCV and MCH usually rise and fall together (i.e. as the cells get bigger, they will also contain more hemoglobin). This calculated erythrocyte index can highlight errors in erythrocyte count, hematocrit, and hemoglobin measurements, and portray the course of anemia. It is calculated as follows:

$$MCV\left(femtoliters\right)=\frac{Hct\left(\%\right)\times 10}{RBCs\left(millions\right)\,/\,\mu L\;or\times 10^{12}\,/\,L)}$$

Medullary Hematopoiesis The production of blood cells in the bone marrow.

Megakaryocyte A giant polyploidy cell found in the bone marrow of mammals. Mature blood platelets are fragments of its cytoplasm.

Megakaryocytopoiesis The production of megakaryocytes in the bone marrow.

Megakaryocytosis
1. The presence of megakaryocytes in the blood.
2. Excess megakaryocyte production in the bone marrow.

Megaloblast An abnormal erythrocyte precursor that is large, nucleated, and developmentally corresponds to the metarubricyte.

Megaloblastic Anemia An anemia in which megaloblasts are present in the bone marrow, such as in pernicious anemia.

Megalocyte An unusually large erythrocyte that is larger than a *Macrocyte*, but smaller than a *Gigantocyte*.

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Metamyelocyte A granulocyte precursor between the myelocyte and the band forms at which stage division ceases and the nucleus becomes bean shaped.

Metarubricyte An erythrocyte precursor between the polychromatic rubricyte and the reticulocyte. The nucleus is extruded at the end of this stage.

Methemoglobin The compound that results from the irreversible oxidation of the iron in hemoglobin from the ferrous (2^+) to the ferric (3^+) state with ionic bonds. Methemoglobin is useless for oxygen and carbon dioxide transport.

Methemoglobinemia An excessive level of methemoglobin in the blood that can lead to cyanosis. Methemoglobinemia may result from exposure to certain chemicals. It also may be a genetic defect (e.g. a defect in the enzyme NADH methemoglobin reductase, or a defect in hemoglobin M). Arterial blood that contains a significant percentage of methemoglobin has a chocolate-brown appearance.

Mexican Hat See *Codocyte*.

Microcyte An unusually small erythrocyte with a decreased MCV, reduced hemoglobin content, and increased central pallor. Microcytes are caused by iron deficiency and inflammatory disease anemias.

Microcythemia Small RBCs due to iron deficiency or chronic blood loss.

Microcytic Hypochromic Anemia An anemia in which erythrocytes are smaller than normal, and hematocrit, hemoglobin, MCV, and MCH values are reduced. It is usually caused by chronic blood loss, or deficiencies in iron, copper, or pyridoxine (a B_6 vitamin). MCHC values may be within a normal range.

Microcytosis See *Microcythemia*.

Microglia A type of glial cell that serves as the resident macrophage in the central nervous system (CNS). Microglia are the primary form of active immune defense. They originate from progenitor cells in the embryonic yolk sac and are self-renewing within the CNS; i.e., they are not produced in the bone marrow.

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Microspherocyte See *Spherocyte*.

Mitogen A substance that induces lymphocyte transformation.

Mobilization The ability of lymphocytes to leave the blood, enter lymphatic tissues, and return again to the blood. This allows immunocompetent lymphocytes to distribute widely and yet home in on antigens in large numbers when needed. Also called *Recirculation*.

Monoblast A monocyte precursor between the colony forming unit-granulocyte, monocyte (CFU-GM) and the promonocyte that is found only in the bone marrow, and which becomes more plentiful in monocytic leukemia.

Monocyte The largest of the leukocytes. The nucleus can have a variety of shapes (generally ovoid or kidney shaped). The cytoplasm has a ground glass appearance and contains small purplish granules and large vacuoles. Monocytes are formed in the body marrow and are found in small numbers in the blood. They are phagocytic and routinely attack the more difficult pathogens. After briefly circulating in the blood, monocytes enter body tissues and cavities where they become 'fixed' macrophages until they are stimulated by inflammation to become 'free' macrophages. Monocytes and macrophages are part of the Mononuclear Phagocyte System (MPS).

Monocytopenia A decrease in the absolute or differential monocyte count. Also called *Monopenia*.

Monocytopoiesis Monocyte production. Also called *Monopoiesis*.

Monocytosis An increase in the absolute or differential monocyte count.

Mononuclear Phagocyte System (MPS) A widely distributed system of phagocytic cells composed of macrophages of the lymph nodes, spleen, lung, bone marrow, connective tissue, liver (Kupffer cells), and other organs, and monocytes and monocyte precursors in the blood and marrow. The MPS cells serve many functions including immune response, countering microbial infection, the breaking down of hemoglobin, iron storage, and the phagocytizing of old erythrocytes, cellular debris, and foreign matter. Formerly called the *Reticuloendothelial System*.

Monopenia A decrease in the absolute or differential monocyte count. Also called *Monocytopenia*.

Monopoiesis Monocyte production. Also called *Monocytopoiesis*.

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Multipotent Stem Cell An undifferentiated hematopoietic cell capable of producing all blood cell lines (erythrocytes; neutrophils, heterophils, eosinophils, and basophils; monocytes, macrophages; lymphocytes and plasma cells; and thrombocytes). They are first found in the embryonic yolk sac, and later in the fetal liver, spleen, and bone marrow. Also called Hemocytoblast or Hemoblast.

Myeloblast A granulocyte precursor between the colony forming unit and the promyelocyte. It is a large cell with finely stippled chromatin and no cytoplasmic granules.

Myeloblastemia The presence of myeloblasts in the blood.

Myeloblastosis Excess myeloblasts in the blood.

Myelocyte A granulocyte precursor between a promyelocyte and a metamyelocyte at which stage cytoplasmic granules can be clearly seen.

Myelocythemia An excess of myelocytes in the blood.

Myelocytoma
1. Chronic myelocytic leukemia.
2. A tumor of bone marrow cells.

Myelocytomatosis
1. A leukosis involving mostly myelocytes.
2. A disease in birds in which tumors are composed of myeloid cells.

Myelocytosis The proliferation of granulocyte producing marrow tissues as seen in myelocytic leukemia. Also called *Myelosis*.

Myelogenous
1. Derived from or pertaining to the bone marrow.
2. Non-lymphocyte cells in the bone marrow that develop into granulocytes, monocytes, and platelets. Also called *Myeloid*.

Myeloid
1. Derived from or pertaining to the bone marrow.
2. Non-lymphocyte cells in the bone marrow that develop into granulocytes, monocytes, and platelets. Also called *Myelogenous*.

Myeloid: Erythroid Ratio The ratio of myeloid elements (promyelocytes, myelocytes, metamyelocytes, band granulocytes, and segmented granulocytes) to erythroid elements (pro-rubricytes, rubricytes, and metarubricytes) in a bone marrow sample.

Myeloid Stem Cell A cell derived from the multipotent stem cell. A myeloid stem cell is a committed cell that gives rise to erythrocytes, neutrophils, heterophils, eosinophils, basophils, macrophages, and thrombocytes. Also called *Common Myeloid Progenitor*.

Myeloma A tumor of bone marrow cells.

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Myelopathy A pathologic change in the bone marrow.

Myelophthisic Anemia Anemia resulting from a reduction in the ability of bone marrow to produce erythrocytes.

Myelopoiesis Production of bone marrow cells, erythrocytes, leukocytes, or thrombocytes.

Myeloproliferative Disorder (MPD) Various diseases marked by medullary and extramedullary proliferation of one or more blood cell lines (erythroblastic, myelocytic, and megakaryocytic). Examples are polycythemia vera, acute and chronic myelocytic leukemia, agnogenic myeloid metaplasia, and erythremic myelosis.

Myelosis The proliferation of granulocyte producing marrow tissues as seen in myelocytic leukemia. Also called *Myelocytosis*.

Myelosuppressive Characterized by decreased production of all types of blood cells formed in the bone marrow.

Myelotoxic Characterized by damage to the bone marrow.

Myelotoxin Any chemical that damages bone marrow.

Natural Killer Cell An immature (large) null cell lymphocyte with broad activity that does not require antibody to interact with foreign tissue such as bacteria, viruses, tumors, and transplanted tissue. Natural killer cells provide the first response to tumors and viruses. They are activated in response to interferons and macrophage-derived cytokines. Also called *NK Cell*.

Neutropenia A decrease in the absolute or differential neutrophil count.

Neutrophil The predominant leukocyte in most species. Neutrophils are a type of granulocyte (the others being eosinophils, and basophils), but the term granulocyte is often used loosely to mean neutrophil. Neutrophils are both phagocytic and bacteriocidal, and are the first line of defense against microbial infection. In some species, they are called *Heterophils*.

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Neutrophilia An increase in the absolute or differential neutrophil count, typically in response to various diseases, inflammation, tissue damage, neoplasia, and trauma.

Non-Regenerative Anemia Anemia in which the bone marrow is unable to increase erythrocyte production to replace lost cells. The erythrocytes are typically normocytic (normal size). Also called *Non-Responsive Anemia*.

Non-Responsive Anemia See *Non-Regenerative Anemia*.

Normoblast Any of the four nucleated erythrocyte precursor cell types including the pronormoblast, basophilic normoblast, polychromatic normoblast, and orthochromatic normoblast (seen in cats and horses).

Normoblastosis An excessive production of normoblasts by the bone marrow.

Normochromasia Normal erythrocyte staining characteristics, i.e. without basophilic material.

Normochromic Containing a normal amount of hemoglobin.

Normochromic Anemia Anemia in which MCHC is in the normal range. Normochromic anemia can be either macrocytic or microcytic. See Table 2.1.

Normocyte An erythrocyte having normal color and morphology.

Normocytic Hypochromic Anemia An anemia in which the MCV value is normal, but the MCHC is decreased because the erythrocyte count, hematocrit, and hemoglobin value are decreased. It is a sign of early iron deficiency, depressed erythropoiesis, and disease in domestic animals.

Nucleated Erythrocyte (nRBC) An immature erythrocyte that has not yet lost its nucleus. It is never released in health except in young pigs (<3 month old). It is confined to the bone marrow and is only released along with reticulocytes in response to demands on blood forming tissues. They are counted per 100 WBCs (sic). Bone marrow damage or hypoxia is generally indicated when an nRBC count is ≥5 nRBCs/100 WBCs. Also called *Normoblast*, *Erythroblast*, *Erythrocytoblast*, and *Nucleated Red Blood Cell*.

Nucleated Red Blood Cell See *Nucleated Erythrocyte*.

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Null Cell A type of lymphocyte with surface antigens that differ from those seen on B and T-lymphocytes. They are seen in systemic lupus erythematosus. Two major types are the killer cells and the natural killer cells. Also called *Non-T, Non-B Lymphocyte*.

Oligemia A decrease in plasma volume. Also called *Hypovolemia*.

Oligocythemia Reduced erythrocyte mass in the blood. Also called *Hydremia*, *Hemodilution*, and *Oligocytosis*.

Oligocytosis Reduced erythrocyte mass in the blood. Also called *Hydremia*, *Hemodilution*, and *Oligocythemia*.

Ovalocyte An elliptically-shaped erythrocyte. Also called *Elliptocyte*.

Packed Cell Volume See *Hematocrit*.

Pancytopenia A decrease in all blood cell elements (i.e. erythrocytes, leukocytes, and thrombocytes) in the circulating blood. See also *Aplastic Anemia*.

Pappenheimer Bodies Basophilic staining erythrocyte granules that contain iron.

Pelger-Huet Anomaly Defective nuclear lobulation of neutrophils and eosinophils resulting in cylindrical, spherical, or dumbbell-shaped nuclei. It may be due to a genetic defect or some types of anemia or leukemia.

Pernicious Anemia A megaloblastic anemia caused by malabsorption of vitamin B_{12} in the intestine due to a lack of availability of intrinsic factor.

Plasma The cell-free portion of blood that contains suspended particulate components including fibrinogen. It is collected with an anticoagulant to prevent clotting. Compare to *Serum*.

Plasma Cell Plasma cells are the most prolific producers of antibodies (immunoglobulins) with each plasma cell producing only one type of antibody. Sensitized mature B-lymphocytes can revert to a blast form, divide, and differentiate into plasma cells in a process called blastogenesis. Plasma cells have a large cytoplasm volume that is basophilic. They form in the lymph nodes, spleen, and bone marrow, and disseminate to all parts of the body but concentrate in the lymph nodes, spleen (white pulp and perivascular sheaths), and connective tissue. They are rarely found in the blood.

Plasma Protein: Fibrinogen Ratio (PP:F) The PP:F ratio is used to distinguish between actual fluctuations in plasma protein (PP) and fibrinogen (F) and fluctuations caused by dehydration (hemoconcentration).

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Plasma Erythropoietin See *Erythrocyte Stimulating Factor*.

Platelets See *Thrombocytes*.

Platelet Factors Proteins and lipoproteins found in platelets that participate in blood coagulation. There are four platelet factors designated PF-1, PF-2, PF-3, and PF-4.

Poikilocyte A nonspecific name for any erythrocyte with an abnormal shape (morphology) or size. Poikilocytes may be an indication of abnormal erythrogenesis, circulatory trauma, disease, toxicity, or a blood smear artifact; or they may be normal for some species. Table 5.1 provides illustrations and descriptions of the various poikilocytes.

Poikilocytosis The presence of abnormally-shaped erythrocytes of any kind.

Polychromasia
1. Variations in erythrocyte hemoglobin content.
2. Having the staining characteristics of reticulocytes due to the bluish-staining of basophilic erythrocytic cytoplasm along with the pinkish color of hemoglobin.

Polychromatic Having the staining characteristics of reticulocytes due to the bluish-staining of basophilic erythrocytic cytoplasm along with the pinkish color of hemoglobin.

Polychromatic Erythrocyte A reticulocyte.

Polychromatophilia Having the staining characteristics of reticulocytes due to the bluish-staining of basophilic erythrocytic cytoplasm along with the pinkish color of hemoglobin.

Polycythemia An increased number of circulating erythrocytes, or an increase in erythrocyte mass in the blood. Also called *Erythrocytosis*. Polycythemia can be further characterized as follows:

- **Absolute Polycythemia** Excessive polycythemia and increased erythrocyte mass in the blood due to primary or secondary causes as described below.
- **Familial Polycythemia** Hereditary polycythemia.
- **Primary Polycythemia** An increase of the total erythrocyte cell volume (hematocrit) due to a dramatic increase in erythrocyte production while serum EPO levels are low. It is accompanied by splenomegaly and increased production of granulocytes and thrombocytes. It is caused by a myeloproliferative disease and is seen in dogs, cats, horses, and cattle. Also called *Primary Erythrocytosis*, *Polycythemia Vera*, and *Polycythemia Rubra Vera.*

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- **Relative Polycythemia** An apparent excess of circulating erythrocytes (i.e. elevated RBCs and hematocrit) due to a decrease in plasma volume. Also called *Relative Erythrocytosis*.
- **Secondary Polycythemia** An increase in the erythrocyte count in the circulating blood because of excessive EPO production either due to systemic hypoxia (appropriate) or an EPO secreting tumor in the kidney (inappropriate). Unlike primary polycythemia there is no increased production of granulocytes and thrombocytes. Also called *Secondary Erythrocytosis.*

Polycythemia Vera See *Primary Polycythemia*.

Polymorphonuclear (PMN) Having a multilobed nucleus that may appear to be multiple nuclei. Polymorphonuclear leukocytes include neutrophils, eosinophils, and basophil.

Primary Erythrocytosis An increase of the total erythrocyte cell volume due to a dramatic increase in erythrocyte production while serum EPO levels are low. It is accompanied by splenomegaly and increased production of granulocytes and thrombocytes. It is caused by a myeloproliferative disease and is seen in dogs, cats, horses, and cattle. Also called *Primary Polycythemia*, *Polycythemia Vera*, and *Polycythemia Rubra Vera.*

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Proerythroblast The earliest erythrocyte precursor. It is between the multipotent stem cell and the prorubricyte. Also called *Rubriblast*.

Progranulocyte See *Promyelocyte*.

Promonocyte A monocyte precursor between the monoblast and the mature monocyte found only in the bone marrow, and which becomes more plentiful in monocytic leukemia.

Promyelocyte A granulocyte precursor between the myeloblast and the myelocyte at which stage cytoplasmic granules cannot be found. Also called *Progranulocyte*.

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Prorubricyte An erythrocyte precursor between the rubriblast and the basophilic rubricyte. Also called *Basophilic Erythroblast* and *Basophilic Normoblast*.

Prothrombin Coagulation Factor II. A serine protease produced in the liver (vitamin K-dependent) that is part of the common pathway. Prothrombin is converted to thrombin (factor IIa) by Ca^{++} and platelet phospholipid (PF-3) and facilitated by factors Xa and Va (an accelerator). Thrombin then converts fibrinogen (factor I) to soluble fibrin (factor Ia).

Punched-Out Cell See *Torocyte*.

Pyknocyte A small, distorted erythrocyte that may be spiculed. It is seen in hemolytic disorders.

Pyropoikilocyte An erythrocyte with a defective cell membrane that deforms at 45 °C instead of the usual 50 °C.

Pyropoikilocytosis A congenital erythrocyte membrane abnormality that results in erythrocyte deformation upon heating. It is associated with hemolytic anemia and elliptocytosis.

Recirculation The ability of lymphocytes to leave the blood, enter lymphatic tissues, and then return to the blood. This allows immunocompetent lymphocytes to distribute widely and yet home in on antigens in large numbers when needed. Also called *Mobilization*.

Red Blood Cell See *Erythrocyte*.

Red Blood Cell Indices See *Erythrocyte Indices*.

Red Corpuscle A red blood cell or erythrocyte.

Refractory Anemia See *Aplastic Anemia*.

Regenerative Anemia Anemia in which the bone marrow increases erythrocyte production to replace lost cells. The erythrocytes are typically macrocytic (enlarged and immature) and may show polychromasia, except in horses. Also called *Responsive Anemia*.

Regenerative Left Shift See *Left Shift*.

Relative Erythrocytosis An apparent excess of circulating erythrocytes (i.e. an elevated hematocrit) due to a decrease in plasma volume. Also called *Relative Polycythemia*.

Relative Polycythemia An apparent excess of circulating erythrocytes (i.e. an elevated hematocrit) due to a decrease in plasma volume. Also called *Relative Erythrocytosis*.

Responsive Anemia See *Regenerative Anemia*.

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Reticulocyte Reticulocytes are large, immature, non-nucleated erythrocytes that contain some bluish stippling (polychromatophilic reticular material). They are erythrocyte precursors between the metarubricyte and the mature erythrocyte. They are released into circulation in response to anemia, and are therefore useful for assessing an animal's response to anemia. 'Shift' reticulocytes or 'stimulated' reticulocytes are released early in response to erythropoietin (EPO) and tend to be larger, less mature, and contain more reticular material. Horses rarely release reticulocytes. They are not found in cattle, goats, horses, and sheep in health. They may be found in the blood of dogs, cats, pigs, and are common in guinea, pigs, mice, rats, and rabbits. Ferrets have high reticulocyte counts. Also called *Polychromatic Erythrocyte*.

Reticulocyte Shower A significant increase in circulating reticulocytes, usually in response to acute blood loss.

Reticulocytopenia A decreased reticulocyte count.

Reticulocytosis An increased reticulocyte count.

Reticuloendothelial System (RES) See *Mononuclear Phagocyte System*.

Right Shift An increased proportion of aged neutrophils in the blood as characterized by hypersegmentation of the nucleus (five or more lobes). It is caused by corticosteroids, vitamin B_{12} and folate deficiency, and reduced cell mitosis in the marrow. It may also be an artifact in stored blood.

Ringed-Sideroblast A sideroblast with a ringed structure encircling the nucleus. The ring is made up of mitochondria that contain iron.

Rouleaux A grouping of RBCs that resembles a stack-of-coins. Rouleaux should not be confused with agglutination that resembles a bunch-of-grapes. It is an indicator of hyperproteinemia and inflammatory or neoplastic disease in dogs. Rouleaux is found in healthy horses and cats. Also called *Rouleaux Formation*.

Rubriblast The earliest erythrocyte precursor. It is between the multipotent stem cell and the prorubricyte. Also called *Proerythroblast*.

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Rubricyte An erythrocyte precursor between the prorubricyte and the metarubricyte. Most hemoglobinization of the cytoplasm occurs in the rubricyte stage.

Schistocyte A 'helmet cell' erythrocyte. A fragmented erythrocyte with a complete cut caused by collisions with intravascular fibrin. Schistocytes are evidence of disseminated intravascular coagulopathy, hemolytic anemias, myelofibrosis, diseases of the spleen and liver with fibrin deposition, glomerulonephritis, congestive heart failure, and neoplasms.

Schizocyte See *Schistocyte*.

Secondary Erythrocytosis An increase in the erythrocyte count in the circulating blood because of excessive EPO production either due to systemic hypoxia (appropriate) or an EPO secreting tumor in the kidney (inappropriate). Unlike primary erythrocytosis there is no increased production of granulocytes and thrombocytes. Also called *Secondary Polycythemia.*

Secondary Polycythemia An increase in the erythrocyte count in the circulating blood because of excessive EPO production either due to systemic hypoxia (appropriate) or an EPO secreting tumor in the kidney (inappropriate). Unlike primary polycythemia there is no increased production of granulocytes and thrombocytes. Also called *Secondary Erythrocytosis.*

Sedimentation Rate The rate at which erythrocytes settle in standing anticoagulated blood over a 1 h period. The preferred term is *Erythrocyte Sedimentation Rate (ESR)*.

Segmented Granulocytes A leukocyte that, when mature, has a nucleus divided into definite lobes. Neutrophils, eosinophils, and basophils are segmented granulocytes. They are involved in the nonspecific mechanisms of host resistance and are phagocytic toward foreign material. Sometimes the term granulocyte is used to mean neutrophil. In some species, especially birds, neutrophils are called heterophils due to their staining characteristics.

Selenocyte A poorly staining crescent-shaped erythrocyte seen in hemolysis, toxemia, lipemia, and infections.

Serum The cell-free portion of blood from which fibrinogen has been removed by clotting. Compare to *Plasma*.

Shift See *Left Shift* and *Right Shift*.

Shift Reticulocyte A large, less mature reticulocyte that contains more reticular material than usual. They are released early in response to erythropoietin (EPO). Also called *Stimulated Reticulocyte*.

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Sickle Cell See *Drepanocyte*.

Sideroblast A nucleated (i.e. immature) erythrocyte that contains cytoplasmic iron granules (crystalized ferritin aggregates). The counts are increased in sideroblastic anemia, refractory anemia, thalassemia, and lead poisoning and decreased in iron deficiency anemia.

Siderocyte An erythrocyte that contains cytoplasmic iron granules (crystalized ferritin aggregates). The counts are increased in sideroblastic anemia, refractory anemia, thalassemia, and lead poisoning and decreased in iron deficiency anemia.

Siderophilin See *Transferrin*.

Smear The technique of spreading a thin film of blood onto a glass slide for observation and cell counting. Also called *Blood Film*.

Smudge Cell An artifactual leukocyte that results from making a smear.

Spherocyte A small dark-staining spherical erythrocyte with reduced membrane and no central pallor. Their intravascular life span is short because of resistance to deformation. They are frequently found in dogs and cats with autoimmune disease, blood transfusions, hereditary spherocytosis, and acquired hemolytic anemia. They are also found in stored blood used for transfusion due to erythrocyte swelling. Also called *Microspherocytes*.

Spherocytosis The presence of spherocytes in the blood. Hereditary spherocytosis is a congenital, familial type of hemolytic anemia in humans.

Spiculed Erythrocyte A general term for an erythrocyte with surface spicules. These include acanthocytes, dacryocytes, drepanocytes, echinocytes, keratocytes, and schistocytes.

Splenic Anemia Congestive splenomegaly that results in pancytopenia because of cell destruction. Also called *Banti's disease*.

Spur Cell See *Acanthocyte*.

Stab Cell See *Stab Granulocyte*.

Stab Granulocyte An immature granulocyte (neutrophil, heterophil, eosinophil, or basophil) with an unsegmented, curved nucleus. Also called *Band Cell*, *Band Granulocyte*, and *Immature Granulocyte*.

Stem Cell A cell derived from the multipotent stem cell (hemocytoblast). It is a committed cell, and may be either a myeloid stem cell that gives rise to erythrocytes, neutrophils, heterophils, eosinophils, basophils, macrophages, and thrombocytes or a lymphoid stem cell that gives rise to B-lymphocytes T-lymphocytes, and plasma cells.

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Stimulated Reticulocyte See *Shift Reticulocyte*.

Stippling The blue-stained polychromatophilic reticular material found in reticulocytes.

Stomatocyte An erythrocyte with a uniconcavity, or a clear 'mouth-like' slit in the area of the central pallor. It is seen in chronic anemia in dogs, and in liver disease and hemolytic anemias.

Stomatospherocyte A nearly spherical erythrocyte that has a small amount of central pallor. It is produced by the same processes that produce spherocytes.

Supravital Staining Stains used to stain Heinz bodies and reticular material in reticulocytes, such as new methylene blue and brilliant cresyl blue.

T-Cell See *T-Lymphocyte*.

T-Helper Cell A type of T-lymphocyte that stimulates B-lymphocytes to produce antibodies. Also called *Th-Cell*.

T-Lymphocyte A type of lymphocyte that can kill cells in cell mediated immune responses (i.e. pathogen resistance, autoimmunity, graft and tumor rejection, response to malignancies, and delayed hypersensitivity reactions), and can regulate antibody response by stimulating and suppressing B-lymphocyte antibody production. T-lymphocytes secrete lymphokines in response to antigenic stimulation. Disruption in the immunoregulatory function of T-lymphocytes can result in autoimmune disease. There are several types of T-lymphocytes including cytotoxic T-cells, T-helper cells, T-suppressor cells, and T-memory cells. Also called *T-Cell*, *Thymus-Derived Lymphocyte*, and *Thymus-Dependent Lymphocyte*.

T-Memory Cell A very long-lived T-lymphocyte that allows for rapid antigen response because of its ability to retain antigen experience over long periods of time.

T-Suppressor Cell A type of T-lymphocyte that regulates T-helper cells and the antibody production of B-lymphocytes. It is involved in autoimmune diseases.

Target Cell See *Codocyte*.

Th-Cell See *T-Helper Cell*.

Thalassemia An inherited autosomal recessive hemoglobinopathy in which the hemoglobin in erythrocytes is defective due to reduced production of the alpha globin (α-thalassemia) or the beta globin (β-thalassemia). The erythrocytes are small, abnormally shaped, and susceptible to hemolysis. Patients with a thalassemia may have fatigue due to anemia and an enlarged spleen. It is treated with folate and periodic blood transfusions. See also *Hemoglobinopathy*.

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Thrombin An enzyme derived from prothrombin that converts fibrinogen to fibrin.

Thrombocyte Thrombocytes are produced in the bone marrow. They play a major role in blood coagulation and are critically important for maintaining hemostasis. Also called *Platelets*.

Thrombocythemia An increase in the circulating thrombocyte count in response to blood loss.

Thrombocytopenia A decrease in the circulating thrombocyte count.

Thrombocytopoiesis The production of platelets (thrombocytes) in mammals and nucleated thrombocytes in non-mammals. Also called *Thrombopoiesis* (meaning 1.)

Thrombocytosis An increase in the circulating thrombocyte count.

Thrombogenesis The formation of blood clots (not to be confused with thrombocytopoiesis).

Thrombon Any cell that can be characterized as a thrombocyte (platelet) or thrombocyte precursor.

Thromboplastin Coagulation Factor III.

Thrombopoiesis
1. The production of platelets (thrombocytes) in mammals and nucleated thrombocytes in non-mammals. Also called *Thrombocytopoiesis*.
2. The formation of blood clots.

Thrombopoietin A glycoprotein hormone produced in the kidney and liver that regulates megakaryocytopoiesis and the production of platelets.

Thrombus A fibrous plug that stops the loss of blood from a damaged blood vessel. A blood clot. A thrombus that travels from one part of the body to another is called an *Embolus*.

Thymus-Dependent Lymphocyte See *T-Lymphocyte*.

Thymus-Derived Lymphocyte See *T-Lymphocyte*.

Thymus Independent Lymphocyte See *B-Lymphocyte*.

Torocyte An erythrocyte with a central hole so that it resembles a donut. They are seen in iron deficiency anemias, and may be artifactual. Also called *Punched-Out Cell*.

Total Plasma Protein A measure of all proteins in the plasma including albumin, fibrinogen, and globulins.

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Toxic Granules See *Azurophil*.

Transferrin A beta globulin found in the small intestines that combines with iron in the gut and releases it into the blood plasma. Also called *Siderophilin*.

Vacuolation The presence of small spaces in a cell.

Vitamin K A group of lipid soluble vitamins found in many foods and synthesized in the gut. It is necessary for the production of coagulation factors II (prothrombin), VII, IX, and X in the liver. Vitamin K is used to treat poisonings caused by anticoagulant rodenticides (e.g., warfarin) that interfere with prothrombin synthesis.

Von Willebrand Disease An inherited bleeding disorder seen in humans and dogs caused by a lack of von Willebrand factor (vWF) that leads to abnormal platelet function and prolonged bleeding times. Dogs are prone to bleeding episodes (e.g., nose bleeds) and excessive bleeding during surgery or trauma.

White Blood Cell A leukocyte.

White Corpuscle A leukocyte.

Xerocyte A flattened erythrocyte that results from a loss of cellular potassium and water due to abnormal membrane permeability. See *Xerocytosis*. Formerly call *Descicyte*.

Xerocytosis An autosomal dominant hemolytic anemia characterized by the flattening of erythrocytes due to the loss of cellular potassium and water. MCHC is increased. Formerly call *Desiccytosis*.

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Chapter 9
Clinical Pathology Panels

Abstract A clinical pathology *panel* (also known as a *battery* or *profile*) is a collection of tests routinely performed to confirm good health, or to diagnose a disease or toxic effect. If, for example, one wishes to determine whether an animal has liver disease, some or all of the parameters in a hepatic panel might be performed. Because each parameter in a panel has its own advantages and specificities for diagnosing disease, the measurement of multiple parameters increases the likelihood of a correct diagnosis through a weight-of-the-evidence approach. A panel may be modified as prudence and costs allow. There are panels for a wide variety of health conditions ranging from acute cholecystitis to acute pancreatitis to a pancreas panel.

Abnormal bleeding panel	
Prothrombin time (PT)	Thrombin time
Partial thromboplastin time (PTT)	Fibrinogen
Platelet count	Tourniquet test
Bleeding time	

Acute cholecystitis panel	
Total and differential WBC	AST
Serum bilirubin	Serum amylase
Serum alkaline phosphatase	

Acute myocardial infarction panel	
Creatine phosphokinase (CPK)	Lactate dehydrogenase (LDH)
Creatine phosphokinase isoenzymes (CPK-MB)	Lactate dehydrogenase isoenzymes (LDH-1 & 2)

Acute pancreatitis panel	
Serum amylase	Serum trypsinogen
Urine amylase	Calcium
Serum lipase	

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J.E. Whalan, *A Toxicologist's Guide to Clinical Pathology in Animals*,
DOI 10.1007/978-3-319-15853-2_9

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Acute renal failure panel	
Blood urea nitrogen (BUN)	Fractional excretion of Na
Serum creatinine	Urine to plasma ratio for
Urinary sediment	Osmolality, creatinine and urea
Urine specific gravity	
Urine sodium	

Anemia panel	
RBC, HCT, Hb	RBC morphology (peripheral blood smear)
MCV, MCH, MCHC	WBC, total
Reticulocytes (except horses)	Thrombocytes (platelets)
nRBC	

Arthritis panel	
Complete blood count (CBC)	Serum rheumatoid factor (RF)
Erythrocyte sedimentation rate (ESR)	Serum antinuclear antibodies (ANA)
Serum uric acid	Serum complement
Serum protein electrophoresis	Streptozyme and ASO tests
Quantitation of immunoglobulins	

Blood gas panel	
Partial pressure oxygen (pO_2)	Bicarbonate (HCO_3^-)
Partial pressure carbon dioxide (pCO_2)	Oxygen saturation
pH	

Bone panel	
Alkaline phosphatase	Serum calcium
Hydroxyproline	Serum phosphorus

Coagulation panel	
Thrombocytes (platelets)	Thrombin time
Prothrombin time (PT)	Bleeding time
Partial thromboplastin time (PTT)	Clot urea solubility
Fibrinogen	Clot retraction time

Coma panel	
Complete blood count (CBC)	pH
Glucose	pO_2
Calcium	pCO_2
Magnesium	Acetone
AST	Creatine phosphokinase (CPK)
Total bilirubin	Blood urea nitrogen (BUN)
Direct bilirubin	Creatinine

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Sodium	Osmolality
Potassium	Urinalysis
Chloride	Drug screen
CO_2 content	Volatiles
Anion gap	

Complete blood count (CBC)	
RBC, HCT, Hb	Thrombocytes (platelets)
MCV, MCH, MCHC	Total plasma protein
Reticulocytes (except horses)	Plasma fibrinogen
Erythrocyte sedimentation rate (cats & dogs)	Icterus index
nRBC	RBC fragility test
WBC, total	RBC diameter
WBC, differential	

Connective tissue disease panel	
Erythrocyte sedimentation rate (ESR)	MCHC
C-Reactive protein	Thrombocytes
WBC	Coombs test
RBC	Blood urea nitrogen (BUN)
MCH	Creatinine
MCV	Creatinine clearance

Diabetic panel	
Glucose (blood)	Chloride
Glucose (urine)	CO_2
Ketones	Anion gap
Serum osmolality	Acetoacetate
pH	Acetone
pCO_2	β-Hydroxybutyrate
HCO_3^-	Lactic acid
Base excess	BUN
Potassium	Phosphorus
Sodium	Urine output

Disseminated intravascular coagulopathy (DIC)	
Thrombocytes (platelets)	Thrombin time (TT)
Prothrombin time (PT)	Serum fibrin degradation products (FDP)
Partial thromboplastin time (PTT)	Euglobulin lysis time
Plasma fibrinogen	Peripheral smear

(continued)

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Easy bruising panel	
Prothrombin time (PT)	Bleeding time
Partial thromboplastin time (PTT)	Tourniquet test
Platelet count	Platelet adhesiveness
	Platelet aggregation

Electrolytes	
Na^+	Cl^-
K^+	HCO_3^- (bicarbonate)
Ca^{2+}	Mg^{2+}

Gastrointestinal hemorrhage panel	
Blood in stool	Fibrinogen level
Complete blood count (CBC)	Thrombocytes
Urinalysis	Total bilirubin
Blood urea nitrogen (BUN)	Direct bilirubin
Sodium	AST
Potassium	Alkaline phosphatase
Chloride	Serum albumin
CO_2 content	Serum amylase
Prothrombin time (PT)	Urine amylase
Partial thromboplastin time (PTT)	Blood in gastric aspirate

Hepatic panel	
ALT (small animals)	Bile acids
AST	5′-NT
SDH (large animals)	Urine bilirubin
AP	Urine urobilinogen
GGT	Total protein (TP)
GLDH	Albumin
SDH	Protein electrophoresis
Total bilirubin	Prothrombin time (PT)
Direct bilirubin	Partial thromboplastin time (PTT)

Lipids	
Cholesterol, total	Cholesterol, HDL
Cholesterol, VLDL	Triglycerides
Cholesterol, LDL	

Metabolic panel—basic (BMP)	
Sodium	CO_2
Chloride	BUN
Potassium	Creatinine
Magnesium	Glucose
Calcium	

(continued)

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Metabolic panel—comprehensive (CMP)	
Sodium	AST
Chloride	ALT
Potassium	GGT
Magnesium	Alkaline phosphatase
Calcium	Albumin
CO_2	Bilirubin-total
BUN	Bilirubin-direct
Creatinine	
Glucose	

Pancreas panel	
Lipase	Glucose
Amylase	Ca^{++}

Pituitary panel	
Serum prolactin	Thyroid stimulating hormone (TSH)
Serum growth hormone (GH)	Total thyroxine (T-4)
Serum adrenocorticotropin (ACTH)	Triiodothyronine resin uptake (T-3RU)
Follicle stimulating hormone (FSH)	Cortisol
Luteinizing hormone (LH)	Estradiol

Platelet disorder panel	
Platelet count	Tourniquet time
Platelet factor 3	Platelet adhesiveness
Bleeding time	Platelet aggregation

Pre-operative coagulation panel	
Prothrombin time (PT)	Platelet count
Partial thromboplastin time (PTT)	Bleeding time

Proteins panel	
Total protein	Fibrinogen
Albumin	Globulin

Renal panel	
NGAL	P, Inorg
BUN	Mg
Creatinine	pCO_2
TP	Urine specific gravity
Alb	Urinary sediments
Chol.	Urine sodium

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Na	Fractional excretion of sodium
K^+	Urine to plasma ratio for urea, osmolality, and creatinine
Cl^-	
Ca^{++}	

Skeletal muscle panel	
Creatine phosphokinase (CPK)	Serum aldolase
and isoenzymes	Urine myoglobin

Thyroid panel	
Total thyroxine (T-4)	TSH following thyroid releasing hormone (TRH)
T-3 resin uptake (T-3RU)	Thyroid anti-thyroglobulin antibody
Free thyroxine Index (FTI) or free thyroxine	Thyroid anti-microsomal antibody
Thyroid stimulating hormone (TSH)	Thyroglobulin
Triiodothyronine (T-3)	Thyroxine binding proteins (TBP)
	Radioactive iodine uptake (RAIU)

Von Willebrand's panel	
Partial thromboplastin time (PTT)	Platelet count
Factor VIII panel	Bleeding time
Factor VIII related antigen	Platelet aggregation

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About the Author

John E. Whalan has had a rich and varied 41 year career in toxicology. He began as an inhalation toxicologist at Hazleton Laboratories (now Covance) where he had hands-on experience with animals, inhalation chambers, instrumentation, necropsies, data evaluation, and the implementation of Good Laboratory Practice regulations. His next position was with Battelle Columbus Laboratories where he monitored preclinical animal studies of many investigational cancer drugs on behalf of the National Cancer Institute. For the past 30 years, he has served as a senior inhalation toxicologist and risk assessor at the United States Environmental Protection Agency (US EPA). For 19 of those years, he evaluated animal toxicology data and performed risk assessments for numerous pesticide registrations. He has drafted test guidelines and guidance documents for the US EPA and the OECD, and has had an opportunity to work with expert toxicologists and pathologists around the world. He is currently working in the EPA's National Center for Environmental Assessment in the Office of Research and Development. He is a Diplomate of the American Board of Toxicology.

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J.E. Whalan, *A Toxicologist's Guide to Clinical Pathology in Animals*,
DOI 10.1007/978-3-319-15853-2

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