CHAPTER

11

BIOCHEMISTRIES

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Managing abnormalities in birds requires an understanding of how disease processes change the biochemical functions of the body. Because the clinical signs of illness in birds are frequently subtle, clinical chemistries are necessary to evaluate cellular changes. Properly evaluating a biochemical profile requires knowledge of the diagnostic sensitivities and specificities of tests, correct intervals for a specific test in a given species and a list of diseases that can induce the observed changes.

Adding clinical pathology data to the anamnesis and physical examination findings is important for diagnosing most organopathies. There is a need for further documentation of the clinical and pathologic changes induced by specific diseases of all avian organ systems. Many functional disorders can be diagnosed in birds for which an exact pathomorphologic or pathophysiologic explanation has yet to be reported. Many disease reports based on postmortem findings frequently lack clinicopathologic data that would be of value to the clinician.

With many diseases, a clinician will be able to demonstrate disruption of functional integrity of an organ by means of associated clinicopathologic changes. Supportive therapy, aimed at reestablishing homeostasis, is often lifesaving and enables the body to restore normal organ function. Sometimes a cause for the organ dysfunction can be found for which a specific treatment can be given. Only when distinct diseases can be diagnosed clinically will it be possible to rationally evaluate the effects of a specific therapy.

Laboratory Considerations

Nearly all routine hematologic and biochemical investigations can be performed with blood placed in lithium heparin, the anticoagulant of choice when dealing with most avian blood samples. The ability to use one single sample for numerous different tests limits unnecessary blood wastage, which can be an important consideration when dealing with small birds. The amount of blood needed to perform a specific diagnostic test depends on the equipment and technical capacity of the laboratory. When dealing with small birds, the use of micromethods is a necessity.

A blood smear should be made immediately after the blood is collected. A hematocrit-capillary tube is filled and the amount of blood needed for a total white cell count is collected in a diluting pipette. Immediately thereafter, the sample is centrifuged to separate the plasma. Any delay in separation may cause artificial changes of several plasma chemical variables. For example, if whole pigeon or chicken blood is stored at room temperature, a rapid decline (10% in 10 minutes, 30% in 30 minutes, up to 65% in two hours) in plasma potassium concentration occurs due to a shift of potassium ions from the plasma into the red blood cells.[47]

Many reference values for avian blood chemistries are based on values determined using serum instead of plasma, or plasma from blood samples that were not centrifuged immediately. When serum is prepared for blood chemistry, it is inevitable that the blood has to stand for a certain period to allow coagulation, which can cause changes in the sample. Some authors believe that plasma is superior to serum for blood chemistry in birds.[47]

Analytic Goals

Clinical laboratory data is used by the veterinarian to answer specific questions about a patient's metabolic state. The analytic sensitivity of the test, precision with which the test is performed and the way the sample is handled during collection and processing will affect the validity of the test.

The questions that laboratory results can answer generally fall into one of five categories:

1. Is an unapparent disease present? (screening)
2. Is a particular disease process occurring? (pathophysiology)
3. Is a tentative diagnosis correct? (confirmation)
4. What is the severity of a disease process? (prognosis)
5. Has therapy favorably altered the disease process? (monitoring)

Any disease should be considered an evolving process and not a fixed condition. Diagnostic considerations include the cause (etiology), the destructive and reparative processes involved (pathogenesis), the abnormalities observed (diagnostic criteria) and the probable outcome (prognosis). With the complexity of these considerations, rarely does a single test provide a definitive understanding of the clinically apparent disease process, not to mention any subclinical changes that occur at a cellular level.

Accuracy and Precision

The two most important concepts for evaluating the analytic performance and thus the validity of any test are: 1) analytic accuracy, which is the agreement between the best estimate of a quantity and its "true" value; and 2) analytic precision, which is the agreement between replicates. Different results from the same sample may be produced by different analyzers. Likewise, repeat analysis of the same sample by the same analyzer may provide different results. This is true for all laboratory equipment including inexpensive dry chemistry units and high quality computerized analyzers.

Other considerations in interpreting test results include analytic sensitivity (the ability of an analytic method to detect small quantities of the measured component) and analytic specificity (the ability of an analytic method to determine solely the component it is designed to measure).[7,39,41,68]

Human clinical pathology laboratories have found that day-to-day variabilities in an individual patient make it difficult to accurately predict certain biochemical levels. For example, calcium is measured with an average precision of 2.0%, but the day-to-day variation in humans and mammals is around 0.9%. This means that normal analytic variations in the test can be interpreted as abnormal. Creatinine kinase, on the other hand, is usually measured with a

precision of 9.0%, which is much better than its day-to-day variation, at least in humans, of 13.0%.

In birds, some blood chemistry variables may show a circadian rhythm (ie, plasma corticosterone) or a circannual rhythm (ie, plasma thyroxine).[38a,49a] Because of these inherent problems in determining specific serum chemistry values it is important to have a basic knowledge of the technical and statistical methods used in establishing the value of these tests. Developing this working knowledge is further complicated in avian medicine due to a lack of knowledge concerning the day-to-day variations that occur in different biochemical parameters in different avian species.

To be of optimum use, clinical chemistry data must be evaluated based on the values in healthy individuals, the precision of quantitative measurements and the clinical chemistry changes characteristically expected in various pathologic states.

Reference Values – Reference Intervals

Values for any set population of living organisms will have a range that have high, median and low values. For this reason, "normal" is a state of the individual and is not a term that can be easily extrapolated from any given individual as a comparison to others. *The terminus technicus* is to compare the values of an individual to the reference intervals established to define normal limits for a healthy population.[24,65]

Reference ranges established for a population of animals are statistically reduced to reference intervals to allow discrimination between health and disease. Reference intervals of plasma chemical variables are highly dependent on the materials and methods used in the determination, and can vary among different laboratories. At best, reference intervals can be defined for a set population of birds on a given diet, maintained in a given geographic location at a given time of year.

In mammalian medicine, reference intervals are of statistical significance because of the substantial studies that have been performed to evaluate the biochemical changes that occur in various states of disease. Until reference intervals are established for birds free from subclinical infections (particularly viral diseases) and maintained on adequate diets for long periods, it will be impossible to define precise reference intervals on a population basis. Many normal values published in the literature have been collected by diagnostic laboratories, which generally receive samples from abnormal birds.

In addition to varying among populations, reference intervals may also vary among laboratories because of variation in test methods. A laboratory must be able to provide a reference interval established in that laboratory for the species and variables concerned, or the results from the laboratory will be of little value.

In interpreting clinicopathologic data, it should be noted that:

1. There are subtle changes that exist between health and disease. The concept of normality rarely exists.
2. Not all values from healthy individuals will fall within a normal reference interval (usually encompasses 95% of healthy individuals).
3. Some values from abnormal individuals will fall within the reference interval.

Reference intervals are established statistically to produce a 95% confidence interval. Because many biological data do not have a Gaussian distribution, it is often incorrect to define the reference range as the mean plus or minus two standard deviations. In most cases non-parametric statistics must be used to establish reference intervals for clinicopathologic tests because the data are not normally distributed.

If data is normally distributed, 5% of the healthy population with values that are higher or lower than the defined 95% intervals are considered abnormal. With this test evaluation system, it is accepted that there are 2.5% (one out of 40) of the normal population that fall above or below the normal range even though they are clinically healthy.

Further, reference values established for a species may not be normal for an individual. The individual may regularly have a test value that is in the lower part of the normal range. If such a bird developed pathology, the test parameter could stay within the normal range for the species, even though it is elevated for the individual. Consequently, reference values established for an individual bird are more sen-

sitive in detecting subtle abnormalities than comparing test results to reference intervals for a population. These idiosyncrasies in interpreting data confirm the importance of using laboratory tests as only one part of the patient evaluation process (in conjunction with physical examination, clinical changes, radiography) and not as diagnostic panacea.

Units of Measurement, SI Units

To be meaningful, a measurement must be expressed with both a number and a unit. The unit identifies the dimension (eg, mass, volume, concentration) of a measured property. The number indicates how many units are contained within a given sample size. Traditionally, measurements in clinical laboratories are expressed in metric units.

An International System of Units, the so-called SI Unit System (Systeme International d'Unites) was adopted in the 1970's to standardize measurements.

Standardization created a change in the numerical values of many frequently used tests. The mol, for example, indicates the amount of a substance in terms of molecules. The concentration of all substances is now expressed in terms of liters. For biochemical substances, the molar concentration per liter, which is expressed in sub-multiples (millimoles per liter - mmol/l or micromoles per liter - µmol/l) is the preferred standard of measurement.

The advantages of an accepted, worldwide standardized system are obvious. Unfortunately, the standardized system is not always used to report data. Many refereed journals continue to use the conventional rather than SI units. All publications before 1975 used the conventional units, and even in countries that are committed to SI units there are laboratory instruments still in use that produce results in conventional units. Most enzyme activities are still expressed in terms of international units per liter (IU/l or U/l) because the SI unit, the catal, has not been widely adopted. It is often necessary to convert values expressed in conventional units to SI units. Conversion tables are provided in the Appendix.

Types of Testing

Enzymology

Each cell within an organ has a specific function and contains enzymes designed to perform those functions. In some situations, enzymes are unique to specific cells within an organ, and in other cases, enzymes are found in numerous cells from various organs. When the integrity of a cell is disrupted, enzymes escape into the surrounding fluid compartment, where their activities can be measured as an index of cellular integrity.

An enzyme that is released into the serum/plasma must be easy to assay in order to be of diagnostic value. In addition, the assay must be economically feasible and indicate pathologic changes in a specific organ, or a defined small group of organs. The enzyme must also be stable in the serum/plasma for a sufficient time to permit its detection.

It is important to realize that cells must be damaged before they release enzymes into the serum/plasma. Therefore, enzymatic-based tests are a measure of cell damage, and not necessarily a measure of organ function. Anoxia causes the cell membrane to lose its integrity so that soluble enzymes from the cytosol can leak into the serum/plasma.

With liver disease, it is common to have normal histology with marked biochemical changes.This loss of integrity may be observed histologically as a swelling of the cell. Anoxic red blood cells, for example, leak cytosolic LDH into serum/plasma, causing an increase of LDH activity in a sample. Combining the values obtained for several enzymatic assays will increase the diagnostic value of the biochemical evaluation of a patient.

Enzyme activities in tissue or serum/plasma are usually in such low concentrations that it is not practical to quantitate the enzyme directly. Therefore, enzymes are measured indirectly based on their *in vitro* activity under controlled or specific conditions at which their activity is proportional to enzyme concentration. There are a multitude of methods used by different laboratories for detecting enzyme activities, and the reference intervals will vary among these laboratories despite all results being expressed in U/l. Test

values will vary depending on the substrate, buffer and incubation temperature used by the laboratory.

Metabolites

Metabolites can be measured to provide information about the functional capacity of the organs that are involved in a particular metabolic pathway. Tests are usually designed to provide measurements of endpoint metabolites. Commonly measured metabolites include: plasma ammonia, enzymes, bile acids, bilirubin, calcium, cholesterol, creatinine, glucose, inorganic phosphate, iron, total protein, urea, uric acid and triglycerides.

Electrolytes

Electrolytes may be positively charged (cations) or negatively charged (anions). Balances of these electrolytes are essential for all living matter, and commonly measured electrolytes include potassium, chloride and sodium. Trace elements including magnesium may also be determined. The major electrolytes occur primarily as free ions. The trace elements exist primarily in combination with proteins.

Hormones

It has been suggested that hormone concentrations may be good indicators of disease in humans or mammals, but their analytic accuracy and precision are difficult to evaluate in birds.[50,62,37] Hormones are usually detected using a radio-immunoassay (RIA) or an ELISA, both of which require an antigen/antibody reaction. Nonspecific cross-reactions that occur when tests designed for mammalian hormones are used for bird plasma can lead to questionable results.

Assay Methods

Historically, wet chemistry systems have been used for evaluation of blood parameters. Wet chemistry means that liquid reagents act with a certain volume of sample under strictly defined constant conditions (eg, temperature, pH, time) and produce a change of color that is proportional to the concentration of substances or the activity of enzymes. As the indicator dye changes color, the reaction is read spectrophotometrically. Because the reagents can be prepared in the laboratory, the cost for frequently used tests is inexpensive on a per test basis. The minimum sample size often depends on whether reagents are added by hand (older systems that may require 100 to 200 µl/parameter) or automatically (Autoanalyzer Two only requires 20 µl/parameter).

With dry chemistry systems,[30,35,42] test reagents are dried in layers and are dissolved by the fluid in a sample. Incubation steps, reaction time and factors for calculation of the results are all contained within the reagent strip or slide. The technician need only apply the sample and wait for the result. Like a wet chemistry test, the reaction causes a change in color that is measured photometrically by light reflectance. Specific strips or slides are needed for each test, and these are available only from the manufacturer. When compared to wet chemistry tests, dry chemistry assays are more expensive.

Indices

Biochemical tests that can be used to evaluate avian patients will be discussed in alphabetical order within three specific groups: enzymes (see Table 11.2), metabolites (see Table 11.3) and electrolytes.

The discussion of each test will include:

- ***Sample:*** Recommendations for the best sample to collect for testing are listed in Table 11.1. Specific concerns with respect to sample handling are discussed with all indices.
- ***Method:*** An overview of the common assay techniques designed to show why results will vary between different laboratories.
- ***Physiology:*** The physiologic role of the parameter in the bird.
- ***Diagnostic Value:*** The validity of a parameter in suggesting or confirming the presence of disease.
- ***Physiologic Influence:*** The influence of physiologic conditions on a test assay.
- ***Pathologic Changes:*** The effect that pathologic changes have on test values with reference to special literature.

Reference intervals for different avian species using various testing methods are provided in the Appendix.

TABLE 11.1 Recommended Samples for Biochemical Tests[41]

Tissue Enzymes	Sample
ALT	Hemolysis-free plasma or serum
AP	Heparinized plasma or serum
AST	Heparinized plasma or serum
CPK	Serum is preferred. Citrate and fluoride inhibit CK activity.
GGT	EDTA plasma or serum (see text)
GLDH	Heparinized plasma or serum
LDH	Hemolysis-free plasma or serum
Metabolites	**Sample**
Plasma Ammonia	EDTA (see text)
Amylase	Heparinized plasma or serum
Bile Acids	Heparinized plasma or serum
Bilirubin	Heparinized plasma or serum
Calcium	Heparinized plasma or serum (see text)
Cholesterol	Heparinized plasma or serum
Creatinine	Heparinized plasma or serum
Glucose	Heparinized plasma or serum (see text)
Iron	Heparinized plasma or serum
TIBC	Heparinized plasma or serum
Lipase	Heparinized plasma or serum
TP	Heparinized plasma or serum
Triglycerides	Heparinized plasma or EDTA plasma
Urea	Heparinized plasma or serum
Uric Acid	Heparinized plasma or serum
Electrolytes	**Sample**
Chloride	Heparinized plasma or serum
Potassium	Heparinized plasma or serum
Sodium	Heparinized plasma or serum

Enzymes

Alanine Aminotransferase ALT (GPT)

- ***Method:*** It is not possible to monitor transaminase (ALT and aspartate aminotransferase AST) reactions directly; however, continuous monitoring assays can be performed by coupling the transaminase reactions to specific dehydrogenase reactions. Because of the value of AST and ALT activities in diagnosing disease, standardization of reference methods for these two enzymes have been given priority by national and international groups. These groups have chosen a coupled-reaction with malate or lactate dehydrogenase as the indicator enzymes. These methods differ with respect to substrate concentration, nature of buffer and assay temperature.[68]

- ***Physiology:*** Alanine aminotransferase and AST belong to a group of enzymes that catalyze interconversion of amino acids and oxoacids by the transfer of amino groups. While there are numerous enzymes involved in the conversion cascade, AST and ALT are the two enzymes of greatest clinical importance.

TABLE 11.2 Causes of Tissue Enzyme Increased Activities[41]

Enzyme	Activity	Causes of Increases
ALT	Present in most tissues 1.6 times higher in RBCs than plasma	Cell damage (nonspecific)
AST	Liver, heart, skeletal muscle, brain, kidney	Mainly liver or muscle disease Vitamin E/Se deficiencies
AP	Mainly duodenum and kidney Low activity in liver	Increased cellular activity (not damage) Higher in juveniles Egg-laying
CK	Skeletal muscle, heart muscle, brain	Mainly muscle damage IM injections Neuropathies Vitamin E/Se deficiencies Lead toxicity
GGT	Biliary and renal tubular epithelium	Hepatocellular damage Some renal diseases
GLDH	Mitochondrial enzyme found in most tissues Liver, kidney, brain	Hepatocellular necrosis
LDH	Skeletal muscle, cardiac muscle, liver, bone, kidney, RBCs	Hemolysis Hepatic necrosis Muscle damage

- ***Diagnostic Value:*** Alanine aminotransferase activity occurs in many different tissues. Specific diagnostic value of these enzymes in birds is poor. In many cases, patients with severe liver damage have had normal ALT activities, reflecting a low level of enzyme activity in liver cells from certain species. Alanine aminotransferase activities often increase due to damage in many different tissues. In some avian species, normal ALT activities are below the sensitivity of many analyzers.[35,36]

- ***Pathologic Changes:*** Elevated activities are difficult to interpret, and this enzyme has limited usefulness in birds because it can be increased by pathologic changes in almost all tissues. Activity in erythrocytes is 1.6 times higher than in plasma, and hemolysis will cause elevated activities.[50]

- ***Physiologic Influence:*** Age-dependent elevation (increased activity with aging) of enzyme activity has been described in birds.[25] In raptors, seasonal variation in ALT activities has been reported. These changes were independent of reproductive activity.[22]

Alkaline Phosphatase - AP

- ***Method:*** Numerous methods of determining AP activity are currently used. The variety of methods in

use make it difficult to compare AP activities between laboratories or reference literature.

- ***Physiology:*** Alkaline phosphatase operates at an alkaline pH and is possibly involved in energy transfer for exchange of ions across the cell membrane. Alkaline phosphatase activity has been found to occur predominantly in the duodenum and kidney. Low AP activities were reported in the liver with no activity in other organs of pigeons.[50,51] Similar findings have been described in chickens[8] and turkeys.[9] Most enzyme assays are used to document damage to cells resulting in enzyme release. In contrast, plasma AP activity is induced by increased cellular activity (increased synthesis) rather than cell damage.

- ***Diagnostic Value:*** Alkaline phosphatase activities may be elevated due to irritation of the cells in different tissues. Increased activities have no specific importance.

- ***Physiologic Influence:*** Juvenile birds have significantly higher AP activities from bone growth and development than adults.[15,16,17,32] In hens, activities are elevated prior to egg laying.[28] Seasonal variations in activities have been described.[4]

- ***Pathologic Changes:*** Elevations are most common with liver disease even though the level of activity in this organ is low. Hyperparathyroidism-induced stimulation of osteoplastic activity may also cause increased AP activity. Enteritis has been described as a cause of higher AP activities but activity of this isoenzyme is labile and difficult to measure.[36] Aflatoxin B_1 intoxication with massive liver destruction in pigeons, cockatiels, Red-tailed Hawks and Great Horned Owls was not found to significantly increase AP activity.[13] Low AP activities have been linked to dietary zinc deficiencies.

Aspartate Aminotransferase - AST (GOT)

- ***Method:*** See ALT.

- ***Physiology:*** See ALT.

- ***Diagnostic Value:*** High AST activity has been described in liver, skeletal muscle, heart, brain and kidney cells. The distribution of AST in avian tissues varies among the species.[43,25,50] Elevated activities are usually indicative of liver or muscle damage. Aspartate aminotransferase activity provides the best information when combined with other more specific tests.[49,36] Creatinine kinase activity can be used to exclude muscle damage as a cause of increased AST activity.

- ***Physiologic Influence:*** Aspartate aminotransferase values are age-dependent to varying degrees among different species.[25,15,16,17] The cause of this age-dependent increase in activity has not been defined. Gender differences have not been described.[4,32]

- ***Pathologic Changes:*** In general, AST activities in birds greater than 230 U/l are considered abnormal. Abnormal activities have been linked to vitamin E, selenium or methionine deficiencies,[25] liver damage (particularly psittacosis or Pacheco's disease virus),[23,61,63] pesticide and carbon tetrachloride intoxication[43] and muscle damage. Intramuscular injections of irritating substances may cause elevation of CK with no increases in AST activity. In other patients, both the CK and AST activities will increase post-injection.

Creatinine Kinase - CK (CPK)

- ***Method:*** Numerous colorimetric, fluorimetric and coupled enzyme assays have been developed to detect CK activity.[68]

- ***Physiology:*** Creatinine kinase functions in skeletal muscle, heart muscle and brain tissue. In muscle, this enzyme makes ATP available for contraction by the phosphorylation of ADP from creatinine phosphate. There are three isoenzymatic forms of CK that can be separated by electrophoresis. In mammals, quantization of isoenzymes can be used to determine the tissue source of the enzyme.[39,68] There have been no reported attempts to separate tissue-specific creatinine kinases in birds.

- ***Diagnostic Value:*** Elevations in activities are mostly seen because of muscle cell damage. This enzyme has value in distinguishing muscle from liver cell damage. However, the clinician should consider that muscle and liver cell damage can occur simultaneously from the same or different pathologic processes.

- ***Physiologic Influence:*** In mammals, CK activity is subject to a number of physiologic variations (eg, muscle mass of an individual, gender, age, physical activity).[39,68] Physiologic changes of CK are well known and are also described in avian species. CK activity in healthy turkeys is extremely sensitive to physical stress and exercise.[50] Neither gender[32] nor age[15,16,17] has been shown to significantly affect CK activity.

- ***Pathologic Changes:*** Increase in CK activity has been linked to muscle cell necrosis, convulsions, intramuscular injections (depending on the volume and degree of irritation), vitamin E and selenium defi-

ciencies, neuropathies, lead toxicity and occasionally chlamydiosis.[36,43]

Gamma Glutamyl Transferase - GGT

- ***Sample:*** EDTA plasma or serum can be used to determine GGT activity. Heparin will interfere with the test reactants causing turbidity; citrate, oxalate and fluoride may artificially depress activity.[68]

- ***Method:*** Reagent kits for GGT determination use different substrates that have different sensitivities. Results are totally dependent on the assay used.[68]

- ***Physiology:*** Peptidases constitute a broad group of enzymes of varied specificity, and some individual enzymes catalyze the transfer of amino acids from one peptide to another amino acid or peptide. Gamma glutamyl transferase cleaves the gamma-glutamyl group from peptides and moves them to an appropriate accepter. This is primarily a brush border enzyme with greatest activity in the biliary and renal tubular epithelium. Serum activity is from biliary origin.

- ***Diagnostic Value:*** Little is known about the significance of plasma GGT activity for the diagnosis of hepatobiliary disease in birds. In racing pigeons GGT has been found to be a specific indicator for liver disease. One investigator reported measurable activities in the kidney and brain of pigeons, and the kidney and duodenum of budgerigars.[50] Another investigator[13] concluded that GGT is not a sensitive test for the detection of liver disease in different avian species. Enzyme activity in normal birds typically falls below the sensitivity range of most analyzers.

- ***Pathologic Changes:*** Elevations in GGT activity have been described in association with liver disease, but not on a regular basis.[36] The highest levels of activity have been reported in the kidneys. However, elevations do not always occur with renal disease, probably because the enzyme is excreted in the urine.

Glutamate Dehydrogenase - GLDH

- ***Method:*** Methods for the determination of GLDH can be based upon both forward and reverse reactions, and results are dependent on the temperature of the reaction.[68]

- ***Physiology:*** Glutamate dehydrogenase is a mitochondrial enzyme found in numerous tissues.

- ***Diagnostic Value:*** Significant amounts of this enzyme have been found in the liver, kidney and brain of chickens, ducks, turkeys and racing pigeons.[50,51] In budgerigars, the highest enzyme activity has been reported in the kidney.[50] Significant elevations have been observed in birds with liver disease, but few reference intervals are available for avian species.[36]

- ***Physiologic Influence:*** Glutamate dehydrogenase is present in normal serum only in trace amounts. No physiologic variations have been described for this enzyme.

- ***Pathologic Changes:*** Activity in plasma or serum is increased in all conditions in which hepatocellular damage is present. As an exclusive mitochondrial isoenzyme, GLDH is released from cells that are necrotic or markedly injured. Therefore, activities are lower in inflammatory processes that do not result in cellular necrosis.

- ***Reference Intervals:*** Hyacinth Macaw - 0 to 1 U/l (method, temperature not described)[54]; Psittacines - < 2 U/l (German Society of Clinical Chemistry, 25°C).[36]

Lactate Dehydrogenase - LDH

- ***Sample:*** Heparinized plasma or serum are satisfactory if hemolysis is not present. Serum must be separated from the clot immediately to prevent LDH contamination of the sample caused by damaged erythrocytes. Plasma containing other anticoagulants, especially oxalate, should not be used.

- ***Method:*** Numerous LDH assays have been introduced over the last 25 years. Procedures use the forward (lactate to pyruvate) or the reverse (pyruvate to lactate) reactions in almost equal numbers. Methods using the forward reaction are more expensive and less precise, but have fewer problems with substrate inhibition of the test.

- ***Physiology:*** Lactate dehydrogenase functions in glycolysis. Erythrocytes contain high activities of LDH, and *in vitro* hemolysis will result in falsely elevated values. There are five LDH isoenzymes, each of which occurs in a wide variety of tissues, in particular skeletal muscle, cardiac muscle, liver, kidney, bone and red blood cells. Electrophoretic separation of the isoenzymes can help establish the source of increased activity, but is seldom used in veterinary laboratories.

- ***Diagnostic Value:*** Although this enzyme is not specific for any organ, elevations are most common with hepatic disease in psittacines. Lactate dehydrogenase activities are thought to rise and fall more quickly than AST activities in birds with liver disease.[61] These differences may provide information on the chronicity of liver disease.

- ***Physiologic Influence:*** Seasonal variations[43] and gender differences[32] in LDH activities have been described. The highest physiologic activities have been reported in canary finches.[64]

- ***Pathologic Changes:*** Elevated enzyme activity can be observed due to liver and muscle damage.

Nutrients and Metabolites

Plasma Ammonia

- ***Sample:*** EDTA is the anticoagulant of choice. Lithium heparin can be contaminated with ammonium heparin, which will lead to falsely elevated values. Samples must be analyzed immediately because ammonia is released through the catabolism of various substances (eg, urea).[11] Ammonia levels in serum are significantly but variably higher than corresponding plasma values.[68]

- ***Method:*** There are several techniques for the determination of ammonia. In private practice, the dry chemistry method used by the Kodak Ektachem System can be used. This assay measures creatinine and ammonia in two different steps.

- ***Physiology:*** Blood ammonia is principally absorbed from the intestines, although some is derived from protein catabolism, particularly in the skeletal muscles. Normally, ammonia absorbed from the bowel is converted into uric acid and urea in the liver, and blood concentrations of ammonia are maintained at a low level.

- ***Diagnostic Value:*** Little data is available on the use of ammonia concentrations as a diagnostic test in birds.

- ***Pathologic Changes:*** High blood ammonia concentrations may indicate reduced liver function or ammonia poisoning. Ammonia toxicity usually occurs from buildup of ammonia gases in poultry houses and has rarely been reported in companion birds. Atmospheric ammonia can contaminate a blood sample that is left open in room air.

- ***Reference Values:*** Budgerigar - 7-141 μmol/1 (Kodak Ektachem, 25°C).[32]

Amylase

- ***Method:*** Some 20 methods have been described for assaying amylase activity. These tests are based on nine different principles, and various substrates and reference intervals are dependent on the detection method used. With dry chemistry units, the amylase

TABLE 11.3 Causes of Increases in Metabolic Tests[41]

Metabolite	Comments	Causes of Increases
Ammonia	Absorbed from intestines Released through catabolism	Old sample Decreased liver function Ammonia poisoning
Amylase	Derived from pancreas, liver, small intestine	Pancreatitis Enteritis
Bile acids	Indicator of liver function and enterohepatic circulation	Reduced liver function
Biliverdin	Major bile pigment	Liver disease
Calcium		Hyperproteinemia (dehydration) Ovulating hens Osteolytic bone Hypervitaminosis D
Cholesterol	Precursor of steroid hormone Precursor of bile acids Component of cell membranes	Lipemia (high fat diet) Fatty liver degeneration Males > females Liver disease Hypothyroidism Bile duct obstruction Starvation
Creatinine	Derived from catabolism of creatine	Low sensitivity Severe renal disease Decreased filtration rate Egg-related peritonitis Septicemia Nephrotoxic drugs Renal neoplasias
Glucose	Energy source	May be higher in neonates Variation in age, time of day, stress Diabetes
Phosphorus	Diagnostic value poor	Renal disease Secondary hyperparathyroidism Hypoparathyroidism Hemolysis
Iron	Unknown	Pre-ovulatory period
TIBC		Iron deficiency
Lipase	Produced in pancreas	Possibly with pancreatitis
TP		Advancing age Pre-ovulatory period Immune stimulation Dehydration Chronic infections
Triglycerides		Egg-related peritonitis Hyperadrenocorticism? Exercise
Urea		Low urine flow Dehydration Bilateral ureteral obstruction
Uric acid	Synthesized mainly by the liver Excreted by the renal tubules	Postprandial Renal disease Ovulation Decreased glomerular filtration Tissue damage Starvation Hepatocellular disease

activity of approximately 30% of avian samples will exceed the upper range limit of the test. Samples that exceed the test limit must be diluted and reanalyzed.

- ***Physiology:*** Amylase occurs in plasma as a number of isoenzymes that are principally derived from the pancreas, liver and small intestine. In birds, the isoenzymes have not been separated, making it impossible to determine which specific tissues are responsible for increased plasma amylase activity.

- ***Diagnostic Value:*** Little information is available on amylase activity in birds. In some cases it has been found to be useful in the diagnosis of neuropathic gastric dilatation.[36]

- ***Pathologic Changes:*** Increased enzymatic activity can be seen with acute pancreatitis. In these cases enzyme activity may exceed three times the upper limit of the reference interval. Activities less than twice the upper limit of the reference interval are sometimes seen in macaws with severe enteritis in the absence of pancreatic lesions. In most cases of neuropathic gastric dilatation, amylase activity is normal or only slightly elevated.[36]

- ***Reference Values:*** Budgerigars (187-582 U/1); African Grey Parrots (211-519 U/1); Amazon parrots (106-524 U/1); macaws (276-594 U/1) (Kodak Ektachem, Amylopectin, 25°C).[33]

Bile Acids

- ***Method:*** Several assays have been used to quantitate either total or individual bile acids. The most frequently used assays are gas liquid chromatography, high performance liquid chromatography, enzymatic assays and immunoassays (RIA, ELISA). Among these, RIA and enzymatic methods are mainly used by commercial laboratories. RIA-derived values are not comparable to those detected using other methods. Nonspecific cross reactions occur when human anti-bile acid antibodies are used to detect bile acids in bird plasma; therefore, enzymatic methods seem to be the assay of choice for use in birds.

- ***Physiology:*** The liver synthesizes the primary bile acids (cholic acid and chenodeoxycholic acid). It then excretes these acids as sodium salts into the bile. With the ingestion of food, bile is carried via the bile duct into the small intestine where the bile acids act principally as emulsifying agents in fat digestion and absorption. Most bile acids that enter the gastrointestinal tract are reabsorbed in the distal small and large intestines where they return, via the portal circulation, to the liver. They are then extracted from the blood and recycled. Only a small percentage of the total pool of bile acids is lost in the feces each day. A small quantity of the total bile acids reabsorbed from the gastrointestinal tract is not removed from the blood by the liver and reaches the general circulation. It is this fraction of unextracted bile acids that is measured. The quantity of bile acids in the plasma normally increases following the ingestion of food.

- ***Diagnostic Value:*** If liver function is impaired, bile acids are not properly reabsorbed from the blood, and consequently the proportion of excreted bile acids reaching the peripheral circulation increases. Circulating bile acids can therefore be used as a sensitive indicator of liver function, and of the integrity of the circulation through the liver, biliary tract and intestines. It has been suggested that chronic liver disease that results in cirrhosis may decrease the production of bile acids with a subsequent decrease in the plasma. This may be particularly true in a postprandial sample. Further investigations are needed to determine if decreased bile acid concentrations are a reasonable indicator of a loss of functional liver mass. Low bile acid concentrations are common in birds with microhepatia (as detected radiographically), poor feather formation and an overgrown, malformed beak.

- ***Physiologic Influence:*** A significant postprandial increase of bile acids has been documented in racing pigeons and the Mallard Duck. Healthy birds with a gall bladder may not have significantly different postprandial bile acid concentrations when compared to species that do not have a gall bladder.[55]

- ***Pathologic Changes:*** Elevations in bile acids have been shown to correlate with liver disease in pigeons,[55] chickens[10] and African Grey Parrots.[50] With further research, bile acid assays may prove to be one of the best tests for liver function in birds.[38] Bile acids are stable in plasma for prolonged periods, allowing shipment of specimens to distant laboratories for analysis.

- ***Reference Intervals:*** African Grey Parrots (18-71); Amazon parrots (19-144); cockatoos (23-70); macaws (25-71).

Bilirubin

- ***Method:*** Most methods for measuring bilirubin are based on the diazo reaction, in which diazotized sulfanilic acid reacts with bilirubin to produce two azodipyrroles. These products are reddish purple at neutral pH, and blue at low or high pH.

- ***Physiology:*** In birds, the major bile pigment is biliverdin. The enzyme biliverdin reductase is absent, and biliverdin is not converted into bilirubin.[44,45]

- ***Diagnostic Value:*** Low concentrations of bilirubin were detected in the sera of healthy ducks. Concentrations increased following infection with duck hepatitis virus.[1] The diagnostic value of bilirubin appears to vary among species. It has no value in chickens that cannot form bilirubin, but may be of value in other species.

- ***Pathologic Changes:*** Bilirubin cannot normally be detected in plasma of normal psittacines. With severe hepatic disease (eg, chlamydiosis or Pacheco's disease virus) bilirubin concentrations up to 44.5 µmol/l have been reported. A slight yellow coloration (icterus) could be seen in the facial skin of two macaws with bilirubin concentrations exceeding 40 µmol/l.

Calcium

- ***Sample:*** Heparinized plasma or serum can be used. Some calcium-binding anticoagulants, like EDTA, citrate and oxalate (fluoride oxalate is used for determining glucose levels in mammals) will cause falsely low values. For the determination of ionized calcium levels, whole blood, heparinized plasma or serum can be used, but the pH of the specimen must be the same as that of the patient's blood at the time of sampling. This is most readily achieved by collecting and processing the specimen quickly and anaerobically.

- ***Method:*** Total calcium concentrations include the sum of biologically active ionized calcium, protein bound calcium (which is bound mainly to albumin) and calcium chelated with anions, like phosphate or citrate. Bound calcium is biologically inactive and can be decreased (thus decreasing the measurement of total calcium) without causing any clinical effects. Of the many methods described to measure total calcium, atomic adsorption spectrophotometry and spectrophotometry of calcium-dye complexes are most often used. Ionized calcium levels have been shown to be clinically valuable; however, this is not a commonly available assay.

- ***Physiology:*** As a major constituent of bone, calcium plays a vital role in the structure of the body. It also has important physiologic functions involving the transmission of nerve impulses, the permeability and excitability of all membranes, the activation of enzyme systems (eg, blood clotting), calcification of egg shells and contraction of the uterus during oviposition.

- ***Diagnostic Value:*** Total calcium should always be interpreted along with albumin concentrations. Hypoalbuminemia will reduce the quantity of bound calcium and result in a decreased total calcium concentration without reducing biologically active calcium (ionized fraction).[31,36,53] The hyperproteinemia that occurs with dehydration may result in an increased total calcium concentration.

- ***Physiologic Influence:*** Ovulating hens have significantly higher calcium levels than non-reproductively active females. Female budgerigars were found to have significantly higher calcium concentrations than males. Young birds generally have lower calcium concentrations than adults.[32,27]

- ***Pathologic Changes:*** Decreased calcium concentrations are common in seizuring African Grey Parrots. This hypocalcemia syndrome has been described as a unique form of hypoparathyroidism in which calcium is not properly released from bone.[31,33,36] Glucocorticoid therapy will decrease total calcium concentrations. Increased calcium concentrations have been reported with dietary excesses of Vitamin D, osteolytic bone tumors and dehydration. Even in cases of severe dietary calcium deficiency, parahormone will normally mobilize bone to maintain calcium blood concentrations within physiologic limits.

Cholesterol

- ***Method:*** Cholesterol consists of both free cholesterol and cholesterol esters, which are measured together as total cholesterol. Either enzymatic or chemical methods can be used for quantification. Enzymatic procedures have virtually replaced chemical methods in the clinical laboratory. The initial reaction steps are common to all enzymatic procedures. These include the hydrolysis of cholesterol esters to form free cholesterol, which is measured after a subsequent oxidation step utilizing O_2 to produce H_2O_2.

- ***Physiology:*** Cholesterol is a major lipid that is a precursor of all the steroid hormones and bile acids as well as a component of the plasma membrane of cells. It is obtained from the animal protein sources in the diet as well as being synthesized by the liver.

- ***Diagnostic Value:*** Elevated and decreased cholesterol concentrations may occur from a number of physiologic influences and different diseases; however, the diagnostic value of this test in birds appears to be poor. Very high cholesterol concentrations usually accompany lipemia, especially in Amazon parrots, macaws and Rose-breasted Cockatoos with fatty liver degeneration.

- ***Physiologic Influence:*** Cholesterol concentrations will vary with a bird's diet. Carnivorous birds have higher concentrations, whereas fruit- or grain-eating birds have lower concentrations.[43] Male budgerigars were found to have significantly higher cholesterol concentrations than females.[32]

- ***Pathologic Changes:*** Elevations can occur because of hypothyroidism, liver disease, bile duct obstruction, starvation or high fat diets.[2,25,73,36] High cholesterol concentrations have been reported in budgerigars with xanthomatosis.[36] Decreased cholesterol levels have been associated with some cases of liver disease, aflatoxicosis,[73] reduced fat in the diet, *Escherichia coli* endotoxemia and spirochetosis.[25]

Creatinine

- ***Method:*** Most currently used assays are based on the Jaffe reaction.[68] This reaction occurs between creatinine and the picrate ion formed in an alkaline medium.

- ***Physiology:*** Blood creatinine is derived mainly from the catabolism of creatine found in muscle tissue. Phosphocreatine is used to store energy in muscle, and its catabolism to creatinine occurs at a steady rate. Excretion of creatinine is solely via the kidneys. It is freely filtered and reabsorbed in the tubules.[25] In birds, creatine is excreted in urine before it has been converted to creatinine.[6] The urinary excretion of creatine may be one reason that creatinine levels do not provide an accurate assessment of avian renal function.

- ***Diagnostic Value:*** There is a slim margin between the physiologic and pathologic levels of creatinine. For many analyzers, physiologic values are below the detectable range. This test parameter is very insensitive and is a relatively poor diagnostic test in birds.

- ***Physiologic Influence:*** Normally, creatinine production is relatively constant and is minimally affected by catabolism of dietary or tissue proteins. Theoretically, the pool of creatine from which creatinine is liberated depends on the total muscle mass. However, in all avian species that have been investigated, the reference interval for creatinine has been between 0.1-0.4 mg/dl, with no significant differences between species.

- ***Pathologic Changes:*** Severe kidney damage can lead to increased creatinine levels, especially if the filtration rate is decreased. Elevations have also been described in connection with egg-related peritonitis, septicemia (eg, chlamydiosis), renal trauma and nephrotoxic drugs.[43]

Glucose

- ***Sample:*** Heparinized plasma or serum can be used. For reliable glucose determination in avian blood, it is not necessary to prevent glycolysis as long as the blood is not stored for more than two hours.[57] This is contrary to the situation in mammals in which sodium fluoride is often used to ensure accurate glucose determinations. This is because avian erythrocytes consume very little, if any, glucose, and depend primarily on fatty acid metabolism for energy.

- ***Method:*** Glucose levels may be determined using enzymatic (eg, hexokinase) or colorimetric (eg, toluidine) techniques. There is a reasonable agreement in the values among the most commonly used methods.[68] Simple colorimetric tests in the form of a dip stick have been used with some success in birds. Lipemia or hemolysis of the sample can interfere with photometric methods of measurement, giving falsely elevated values.[11] This is less likely to occur with kinetic assays that evaluate a change in optical density over time and are therefore self-blanking.

- ***Physiology:*** Glucose is continuously required as an energy source by all the body cells and must be maintained at adequate levels in plasma. Glucose levels are maintained principally through the conversion of liver glycogen, with some being derived from non-carbohydrate sources (hepatic gluconeogenesis). In periods of starvation, glucose is increasingly derived from the breakdown of fats and proteins, primarily from muscle tissue, through gluconeogenesis in the liver and the kidneys. All plasma glucose is filtered from the blood through the renal glomeruli and then totally reabsorbed in the tubules.

  Interestingly, 73 hours of starvation in pigeons induces hyperglycemia rather than starvation hypoglycemia.[57] This finding has important consequences for avian anesthesia and gastrointestinal surgery, as presurgical fasting varying from four hours (emptying of the crop) to 24 hours (emptying of the entire gastrointestinal tract) can be advantageous. Prolonged fasting is not recommended in birds that weigh less than 100 grams.

- ***Diagnostic Value:*** Glucose is often a part of a laboratory panel[25,43,73] even though pathologic changes in birds are seldom detected.[36] Glucose should be evaluated in convulsing birds or those with glucosuria.

- ***Physiologic Influence:*** Plasma glucose levels are higher in juvenile than adult budgerigars.[32] Variations also occur due to time of day and amount of environmental stress.[43] Plasma glucose concentrations in fasted birds are subject to a circadian rhythm. A rise in plasma glucose concentration starts during the scotophase, reaching peak values early during the photophase. Subsequently, a gradual increase can be observed with the lowest values at the end of the photophase. Afternoon plasma glucose concentrations in birds that are fed early during the photophase are significantly higher when compared to fasted birds.[57]

- ***Pathologic Changes:*** Increases in plasma glucose levels are due to increased glucose production or release. For example, increases occur after meals, with excitement or stress or because of decreased glucose usage (diabetes mellitus).[2,25,50,73] Diabetes mellitus has been confirmed in budgerigars, cockatiels, Amazon parrots, Scarlet Macaws, Umbrella Cockatoos and a Toco Toucan.[43] Transient elevations in glucose have been reported in cockatiels with egg-related peritonitis.[63] Decreases in plasma glucose levels can be due to hepatic dysfunction (eg, Pacheco's disease virus), impaired glucose production or its excessive utilization (eg, septicemia, neoplasia, aspergillosis).[61,63] In young birds of prey, hypoglycemia can cause convulsions.[36] Starvation of up to four days' duration will not cause hypoglycemia in all birds, but in some (particularly raptor neonates), hypoglycemia can occur after a few days of anorexia.[50] Glucose concentrations can be artificially decreased during storage if the blood sample is contaminated with bacteria.[34]

Phosphorus

- ***Sample:*** Heparinized plasma or serum is suitable. Anticoagulants such as citrate, oxalate or EDTA should not be used because they interfere with the formation of the phosphomolybdate complex. Hemolysis must be avoided, because the phosphate concentration of erythrocytes is higher than that of plasma, and hemoglobin interferes with the colorimetric detection reaction used to determine phosphorus levels.

- ***Method:*** Most assays for inorganic phosphate rely on the formation of a complex of phosphate ion with a molybdate compound.[20]

- ***Physiology:*** Inorganic phosphorus is derived from the diet. It is a major constituent of bone and a vital cellular component, playing important roles in the storage, release and transfer of energy and in acid-base metabolism.

- ***Diagnostic Value:*** Changes in inorganic phosphorus concentration can occur with several diseases, but not on a consistent basis. The diagnostic value is poor.

- ***Physiologic Influence:*** Diets that consist mostly of seeds may lead to increased phosphorus levels. Juvenile budgerigars were found to have higher concentrations than adults.[32] No changes in inorganic phosphorus levels were noted in laying hens.[43]

- ***Pathologic Changes:*** Increased plasma inorganic phosphate levels can be seen in some cases of severe kidney damage [2,36,73] due to vitamin D hypervitaminosis,[2] nutritional secondary hyperparathyroidism[43,73] and hypoparathyroidism.[31,33] False elevations will occur if samples are hemolyzed. Occasionally, decreased plasma inorganic phosphate levels may occur from hypovitaminosis D (calcium level also decreased), malabsorption because of phosphate binding agents in the diet (calcium normal) and long-term glucocorticoid therapy.

Iron

- ***Sample:*** Heparinized plasma or serum can be used. Plasma specimens collected with EDTA, oxalate or citrate are unsatisfactory, because they bind iron. Markedly hemolyzed specimens are nondiagnostic because free hemoglobin will increase the total serum iron levels.

- ***Method:*** For iron level assays, reduced Fe (II) is complexed with a chromogen. This complex has a high light absorbance that is proportional to the iron concentration.[68] Most assays require a large sample size (200 µl).

- ***Physiology:*** Iron is an essential constituent of the heme portion of hemoglobin. As the hemoglobin in aged erythrocytes is broken down, iron is recycled and fresh hemoglobin is synthesized. Iron is transported in the plasma attached to a β-1-globulin known as transferrin.

- ***Diagnostic Value:*** The value of determining iron in different avian species has not been thoroughly investigated. A recent report shows a failure to correlate serum iron levels with liver biopsy and subsequent toxicologic analysis for iron.[75]

- ***Physiologic Influence:*** Prior to egg laying, iron levels will increase two to three times normal in some species.[25] Raptors maintained in captivity have significantly lower values than their free-ranging counterparts.[36] Captive toucans have approximately three

times higher reference values of iron than psittacine birds (see Chapter 47).[74]

- ***Pathologic Changes:*** Severe and chronic loss of blood will increase iron values. Iron deficiency anemia has been described in raptors.[40] Changes in plasma iron levels in mynah birds and toucans with iron storage disease are described in Chapter 47.

Total Iron-Binding Capacity (TIBC)

- ***Method:*** An excess amount of ferric ammonium citrate is added to serum to saturate the transferrin iron-binding sites. The unbound Fe (III) is removed and the iron content of the supernatant is assayed as described for iron.[68]

- ***Physiology:*** Normally, only one-third of the iron-binding sites of transferrin are occupied by Fe (III), creating a reserve of iron-binding sites. The total iron-binding capacity (TIBC) is a measurement of the maximum concentration of iron that serum proteins, principally transferrin, can bind. A urine iron-binding capacity (UIBC) test is also available.[75]

- ***Diagnostic Value:*** Abnormalities in TIBC occur with some disorders of iron metabolism. Very little data from birds is available. This parameter appears to have little importance in diagnosing hemochromatosis, but insufficient research has been performed.[74,75]

- ***Pathologic Changes:*** TIBC may be increased with iron deficiency and decreased in chronic inflammatory disorders.

Lipase

- ***Method:*** There are various methods for determination of lipase activity, and the reference ranges depend on the method used.

- ***Physiology:*** Lipase measured in plasma or serum is produced in the pancreas. This enzyme functions in the digestion of fat in the diet.

- ***Diagnostic Value:*** Lipase and amylase activities were high in a caique with clinical signs of pancreatic exocrine insufficiency when compared to the activities of these enzymes in the mate (Ritchie BW, unpublished). Hemolysis inhibits enzyme activity.

- ***Pathologic Changes:*** Although no reference values are currently available, birds do exhibit high lipase activity in severe cases of acute pancreatitis. For diagnostic purposes, a blood sample from a representative of the same species should be included for comparison.

Total Protein (TP)

- ***Sample:*** When only small amounts of blood can be collected, it may be advantageous to use plasma instead of serum to determine the TP concentration. In pigeons, the concentration of TP in plasma is about 1.5 g/l higher than in serum, because the former contains fibrinogen.[58a]

- ***Method:*** Total protein levels may be determined using a chemical method or a refractometer. The chemical method of choice is the biuret method, which measures the TP in fluids colorimetrically using the formation of a blue peptide (copper complex in alkaline solution). This method is extremely accurate for the protein levels typically found in plasma or serum (1 to 10 g/dl), but is not precise enough to determine the low concentrations of proteins that are found normally in other body fluids. Both wet and dry chemistry methods use this technique, but the results vary with the instrument used.

  Most commercial laboratories use a human standard when determining TP and albumin concentrations, and various studies have shown that there are significant differences between TP concentrations when different standards are used (eg, human, bovine, pigeon, chicken). Because it is impossible to have a species-specific standard for all species presented to the avian practitioner and because there is a high correlation between the results obtained with the various standards, it seems wise to establish reference values for the various species using the human standard.[53]

  The refractometer is widely used by veterinarians to measure change in the refractive index of a solution, which is caused mainly by the proteins in solution and is proportional to the concentration of total solids or protein. Most refractometers are temperature-compensated and already calibrated in scales expressing TP concentration (g/dl) and specific gravity of urine.

  Information on the reliability of the refractometric method to determine TP concentrations in avian blood is conflicting. One study indicated that temperature compensated refractometers provide reliable results when compared to non-temperature-compensated devices.[3] In another study, temperature-compensated and non-compensated refractometers yielded higher values than the biuret method, with the temperature-compensated instrument being consistently higher in readings than the non-temperature-compensated refractometer. In juvenile

Eclectus Parrots,[15] macaws[16] and cockatoos,[17] proteins measured by refractometer were consistently higher than those measured by the biuret method.

Due to its dependence on the transmission of light, it is important that a refractometer be used only for clear, non-turbid and non-lipemic fluids. A moderate degree of hemolysis or icterus should not alter the values.[39] In mammals, hyperglycemia (> 700 mg/dl) affects the accuracy of a refractometer for determining TP.[68] At protein concentrations < 3.5g/dl, refractometric results are likely to be inaccurate.[68] Hemolysis causes the release of hemoglobin and intracellular proteins that will increase the refractometry reading. Because of the higher glucose and lower TP concentrations in birds, correlation of results from the refractometer and the biuret methods may not be possible in some species.[3,15-17,47,48,51,52,53,72] Refractometry should be considered a rapid method for determining an estimate of the body fluid protein. Ideally, total protein concentrations have the most value when considered with the results of plasma protein electrophoresis.

- ***Physiology:*** Most plasma proteins, with the exception of immunoglobulins and protein hormones, are synthesized in the liver. They form the basis of organ and tissue structure, operate as catalysts (enzymes) in biochemical reactions, are regulators (hormones) and are transport and carrier compounds for most of the constituents of plasma. The biological activity of proteins for these various functions is dependent upon their primary and secondary structure.

  In female birds, a considerable increase in plasma TP concentration occurs just prior to egg laying, which can be attributed to an estrogen-induced increase in globulins. The proteins are the yolk precursors (vitellogenin and lipoproteins), which are synthesized in the liver and transported via the plasma to the ovary where they are incorporated in the oocyte.

- ***Diagnostic Value:*** Total protein is often used as an indicator for the health status of a patient. Determination of plasma protein concentrations may be of value in diagnosing gastrointestinal, hepatic or renal diseases. Furthermore, plasma proteins will be abnormal in infectious diseases that cause a stimulation of the immune system. Although determination of plasma proteins seldom leads to a specific diagnosis (eg, in the case of monoclonal gammopathies), it will help the clinician to evaluate the severity and progression of a disease.[49] Changes in protein concentration can occur passively due to dehydration (hyperproteinemia) or over-hydration (hypoproteinemia) or actively due to dysproteinemias.

- ***Physiologic Influence:*** Changes in TP must be interpreted with respect to physiologic influences disassociated with disease. Age and stage of development will influence the concentration of TP in birds. Advancing age has been associated with increases in TP in several species.[15-17,25] Hormones can have either an anabolic or catabolic effect on TP. In general, hormonal effects on TP are minimal. However, testosterone, estrogen and growth hormone were found to increase TP in chickens; thyroxine decreased concentrations.[39] The effects that diet has on the total protein concentrations are subtle and difficult to detect or interpret. Temperature stress (hypothermia or hyperthermia) is associated with nitrogen loss, increased adrenal activity and increased protein turnover, resulting in a decrease in TP. Similar findings are observed following crushing injuries, bone fractures and extensive surgery.[39]

- ***Pathologic Changes (Dysproteinemia):*** Hypoproteinemia can reflect reduced synthesis caused by chronic hepatopathies, malabsorption caused by chronic enteropathies (enteritis, tumors, parasitism), increased loss caused by proteinuria due to renal disease, blood loss and malignant tumors (rarely seen in birds) or starvation and malnutrition. Hyperproteinemia may be induced by chronic infectious diseases that stimulate production synthesis of gamma globulin. It also has been seen with chronic lymphoproliferative disease that resembles leukosis in chickens[43] and myelosis in budgerigars.[36] As mentioned previously, dehydration should always be ruled out as a cause of hyperproteinemia.

Electrophoresis

- ***Sample:*** Serum is most commonly used for protein electrophoresis in mammals, so fibrinogen is not included in the sample. Hemolysis will affect electrophoresis results, and heparinized plasma is often used to prevent this problem.[50,54,72]

- ***Method:*** Electrophoresis is used to separate different types of plasma proteins, making it possible to determine their relative proportion in a particular sample. At a neutral or alkaline pH, serum or plasma, supported on a specific matrix, is placed in an electrical field, causing the different protein fractions to migrate at varying speeds toward the anode based on their relative charge. Following staining, these fractions appear as bands of varying intensity, which can be scanned by a densitometer to produce an electro

phoretic tracing. The length and height of each peak ithin the pattern indicates the relative amount of a particular protein or group of proteins. This can be translated into percentage readings and, by combining this information with the TP concentration, absolute values for the concentration of each protein, or protein group, can be calculated.

- ***Physiology:*** Most frequently used electrophoresis methods identify five main protein fractions in birds: albumin, α1-, α2-, β- and γ-globulins.[66] A pre-albumin fraction has been described in pigeons and some parrot species.[15,16,17,50,54] The α-globulins are acute phase proteins that typically increase with acute inflammation; β-globulins are composed of complement, hemopexin, ferritin, fibrinogen and lipoproteins.[39] Some immunoglobulins, including IgM and IgA, also migrate in the β-globulin range. The β-globulins are also acute phase proteins. The γ-globulin fraction is mainly composed of immunoglobulins (IgA, IgM, IgE and IgG).[66]

- ***Diagnostic Value:*** In healthy birds the albumin fraction is the largest protein fraction. An inflammatory process will cause a rise in TP because of increased concentrations of α, β or γ globulin fractions. Often albumin concentrations are decreased in these situations. The combined effect of these changes is a decrease in the albumin/globulin (A/G) ratio. Often the TP concentration is within the reference range, while the A/G ratio is decreased. Therefore, the A/G ratio is of greater clinical importance than the TP concentration. Examples of diseases with a decrease in the A/G ratio are egg-related peritonitis, and chronic infectious diseases such as aspergillosis, psittacosis and tuberculosis.

  Serum or plasma protein electrophoresis can be used to monitor response to treatment. When the bird responds favorably, an increase in the albumin concentration and a decrease in the globulin concentration can be observed, which leads to normalization of the A/G ratio. In birds with liver failure, extremely low plasma protein concentrations can occur in combination with a decreased A/G ratio. Gastrointestinal and renal diseases can also lead to severe hypoproteinemia. These changes are caused by a loss of albumin. Elevated TP concentrations with a normal A/G ratio can be expected in dehydrated birds.

- ***Physiologic Influence:*** Physiologic factors that may change the protein concentration and therefore affect protein electrophoresis results include gender, age, dietary protein, temperature stress, state of hydration, hemorrhage and inflammation.[66]

- ***Pathologic Changes:*** Decreases in albumin concentration can occur from decreased synthesis due to chronic liver disease or chronic inflammation, increased albumin loss due to renal disease, parasitism or over-hydration.[72] A decrease in albumin causes edema because of a decrease in oncotic pressure. Increases are seen because of dehydration.

  Increases in α- and β-globulins may be caused by acute nephritis, severe active hepatitis, systemic mycotic diseases (γ-) and the nephrotic syndrome.[72] Increases in γ-globulins occur with acute or chronic inflammation, infection, chronic hepatitis and immune mediated disorders.[72]

Triglycerides

- ***Sample:*** Serum and lipemic specimens should be warmed to 37°C and vigorously mixed prior to analysis.

- ***Method:*** Usually, triglycerides are enzymatically detected by breaking down the triglycerides and measuring the glycerol that is liberated.

- ***Physiology:*** Triglycerides are the major storage form of lipids, and are a major energy source. Each molecule of triglyceride consists of three fatty acid molecules attached to a molecule of glycerol. They are synthesized in the intestinal mucosa and liver from the components of fat digestion and absorption.

- ***Diagnostic Value:*** Triglyceride values have been insufficiently evaluated in birds. Several factors can influence the blood concentration and increases may not be of clinical importance.

- ***Physiologic Influence:*** Triglyceride levels may vary based on climate, hormone influence, diet and gender. Increases may occur during starvation, particularly in obese birds. Estrogen injections have been shown to elevate triglyceride concentrations in some species.[25]

- ***Pathologic Changes:*** Egg-related peritonitis has been associated with high concentrations of triglycerides.[73] High concentrations (2000-5000 mg/dl) were reported in Amazon parrots showing signs of hyperadrenocorticism. Because triglyceride values are determined based on enzymatically released glycerol, these values may be falsely elevated after exercise or following any event that causes increased levels of blood glycerol (eg, catching birds in an aviary).

Urea

- ***Method:*** Both indirect methods (based on preliminary hydrolysis of urea with urease) and direct methods (based on variations in the thiazide reaction) are used for urea determination. This reaction involves the condensation of diacetyl with urea to form the chromogen diazine.[68]

- ***Physiology:*** In the liver, protein breakdown to amino acids releases urea, which is excreted by glomerular filtration in the kidney. Tubular reabsorption can occur and is dependent on the state of hydration. In dehydrated birds, nearly all of the filtered urea is reabsorbed. If properly hydrated, almost all of the filtered urea is excreted.

- ***Diagnostic Value:*** Urea is present in very small amounts in avian plasma, and determining urea levels has generally been considered of little value. However, recent investigations have shown good correlation between increased plasma urea concentrations and renal disease in pigeons.[50] In other avian species, urea may have little value in detecting renal disease but can be used as a sensitive indicator of dehydration.

- ***Physiologic Influence:*** Physiologic conditions are known to change urea concentrations in mammals, but similar effects have not been documented in birds.

- ***Pathologic Changes:*** High urea plasma levels can occur in all conditions that cause low urine flow, such as dehydration or bilateral ureteral obstruction.[50]

Uric Acid

- ***Method:*** Both wet and dry chemistry systems use oxidation of urates by uricase as a detection method. Most uricase methods are extremely specific and only a few structural analogues to uric acid will interfere with the test. In general, the concentrations of these analogues are low in biological fluids.[68]

- ***Physiology:*** In birds, uric acid is the major product of the catabolism of nitrogen. Synthesis occurs mainly in the liver[50] and in the renal tubules.[14] Approximately 90% of blood uric acid is eliminated by secretion into the lumen of the tubules. Only 50% of the healthy avian kidney is actually used for excreting protein waste, providing a large functional reserve.[25]

- ***Diagnostic Value:*** The evaluation of uric acid concentrations in plasma or serum is widely used in birds for the detection of renal disease. Species differences in the ability of the avian kidney to compensate for damage before uric acid levels are elevated reduces the diagnostic value for this test. However, if reference intervals are available, hyperuricemia is a good indicator of renal disease. Normal uric acid concentrations do not guarantee that the kidneys are healthy.

- ***Physiologic Influence:*** Age and diet may influence the concentration of blood uric acid in birds. Juvenile birds have lower concentrations than adults.[15,16,17,32] Hyperuricemia has been documented during ovulatory activity.[43] Grain-eating birds have approximately 50% lower uric acid concentrations than do carnivorous birds.[25] Uric acid levels are higher shortly after food consumption. Gender differences have not been reported.[5]

- ***Pathologic Changes:*** Hyperuricemia can be expected if the glomerular filtration is decreased more than 70 to 80%. Decreased filtration may occur from hypovitaminosis A-induced damage to renal epithelial cells, dehydration, intoxications or from some bacterial and viral (Newcastle disease) infections.[2,5,21,25,36,73] Uric acid levels may also be increased from the release of nucleic acids caused by severe tissue damage or starvation. If a toenail clip is used for blood collection and urates from the droppings contaminate the sample, the uric acid levels may be falsely elevated.[21,43]

  If the blood uric acid concentration exceeds its solubility it will be deposited in different locations in the body. High plasma or serum concentrations of uric acid are a prognostic indicator that gout may occur. Use of nephrotoxic drugs may also lead to hyperuricemia. Hypervitaminosis D_3-induced renal damage is frequently associated with gout and extremely high uric acid levels. This problem is particularly common in macaws. This has been described for aminoglycosides (gentamicin),[2,25,43] and allopurinol in Red-tailed Hawks.[56] Interestingly, in most species, allopurinol is effective in treating, not inducing gout.

  Hypouricemia is much less common in birds than hyperuricemia. Severe hepatocellular disease with reduced synthesis of uric acid has been suggested as one etiology.

Electrolytes

Chloride

- ***Method:*** Different methods are in use, but ion-selective electrode methods are most common.

- ***Physiology:*** Chloride is the major extracellular anion. Sodium and chloride together represent the majority of the osmotically active constituents of plasma.

- ***Diagnostic Value:*** Elevations in chloride concentrations rarely are detected.

- ***Physiologic Influence:*** In budgerigars, no gender or other physiologic variables have been observed.[32]

- ***Pathologic Changes:*** Hyperchloridemia can occur with dehydration.[25,36] The role of chloride in maintaining acid-base balance has not been sufficiently evaluated in birds.

Potassium

- ***Sample:*** Either heparinized plasma or serum is appropriate for detecting potassium. If ion-selective electrode methods are used, whole blood is also an effective sample. Differences in the electrolyte concentrations in serum and plasma must be considered when interpreting results. Potassium levels are usually higher in serum due to the release of potassium from thrombocytes damaged in the coagulation process. Hemolysis will elevate the plasma concentration of potassium (500 to 700%).[39] Potassium concentrations were found to rapidly decline in pigeon and chicken plasma allowed to sit for two hours.[46] For accurate results, plasma should be separated within minutes of collection. Hyperproteinemia and hyperlipemia will result in falsely low potassium levels caused by a decreased aqueous fraction of the total plasma volume.

- ***Method:*** Potassium may be determined by atomic adsorption spectrophotometry, flame emission spectrophotometry or electrochemically with a sodium ion-selective electrode. The last two systems are most commonly used.[30,68]

- ***Physiology:*** Only two percent of the body's potassium is in the extracellular fluid. The other 98% is kept within the cells by "potassium pumps" in the cell membranes.

- ***Diagnostic Value:*** Alternatives in potassium homeostasis have serious consequences. Decreased extracellular potassium is characterized by muscle weakness, paralysis and cardiac effects. Many potassium abnormalities are the result of hemolytic samples.

- ***Physiologic Influence:*** High amounts of potassium in the diet can elevate plasma concentrations.

- ***Pathologic Changes:*** Hyperkalemia can be caused by severe tissue damage, reduced potassium excretion by diseased kidneys,[25,73] adrenal disease[73] or because of redistribution of potassium from the intracellular to the extracellular fluid (acidosis).[25] Dehydration[25,73] and hemolytic anemia[25] can also cause hyperkalemia.

  Hypokalemia may be caused by decreased potassium intake, increased potassium loss due to chronic diarrhea or diuretic therapy (seldom used in birds)[73] and the shift of potassium from the extracellular to the intracellular fluid (alkalosis).[25]

Sodium

- ***Sample:*** Either heparinized plasma or serum is appropriate for sodium assays. With ion-selective electrodes, whole blood may be used. Electrolyte concentrations are different between serum and plasma. Hyperlipemia and hyperproteinemia will cause falsely low potassium levels by a mechanism similar to that described for potassium.

- ***Method:*** Sodium may be determined by atomic adsorption spectrophotometry, flame emission spectrophotometry or electrochemically with a sodium ion-selective electrode. The last two systems are most commonly used.[30,68]

- ***Physiology:*** Sodium is present mainly in the extracellular fluid and is primarily responsible for determining the volume of the extracellular fluid and its osmotic pressure. Intracellular sodium levels are kept low by a relatively impermeable cell membrane and a sodium pump which removes sodium from the cell. The amount of sodium in the body is regulated by the kidney. In addition, many avian species have a specialized nasal gland (salt or supraorbital gland) that is able to secrete large quantities of sodium in response to osmotic changes, thus allowing these birds to drink salt water. When sea birds are kept in fresh water for a period of time the gland shrinks so that when returned to salt water the birds can no longer tolerate high sodium levels.This mechanism of decreasing sodium concentration in the serum and urine of birds is mediated by a pituitary-adrenal response.[66]

- ***Diagnostic Value:*** Abnormal sodium levels that are not caused by technical failures are rarely seen in birds. If they do occur, they are good indicators of a pathologic situation. Salt poisoning, mainly from high salt foods, may occur more frequently in companion birds than is documented.

- ***Physiologic Influence:*** Sodium plasma levels are maintained within narrow limits, despite wide fluctuations in dietary intake.
- ***Pathologic Changes:*** Hypernatremia can occur from increased sodium intake (peanuts, crackers), excessive water loss or decreased water intake.

  Hyponatremia may be due to increased sodium loss as in kidney disease[73] or severe diarrhea.[25,73] It may also be caused by over-hydration as in psychogenic polydipsia or after intravenous fluid therapy with sodium-free or low sodium solutions. The relative over-hydration, which follows a reduction in renal perfusion possibly because of decreased colloid osmotic pressure, may also cause hyponatremia.

Total Carbon Dioxide Content (Bicarbonate)

- ***Method:*** Heparinized plasma or serum can be used. Bicarbonate levels are determined by mixing the sample with a strong acid and measuring the carbon dioxide (CO_2) release. Most of the carbon dioxide produced is derived from bicarbonate, but a small amount is generated from dissolved carbonic and carbamino acids.
- ***Physiology:*** Alterations of bicarbonate and CO_2 dissolved in plasma are characteristic of acid-base balance. For clinical purposes, the total CO_2 content is the same as the bicarbonate content.[11]
- ***Diagnostic Value:*** Bicarbonate levels are useful for establishing whether or not acidosis or alkalosis is present and, if so, how severe it is.
- ***Pathologic Changes:*** Increases are mainly due to metabolic alkalosis and decreases due to metabolic acidosis. Reference intervals for most avian species are not available.
- ***Reference Values for Adult Budgerigars:*** 21 to 26 mmol/l.[32]

Blood Gases - pCO_2, pO_2 and pH

- ***Sample:*** Venous heparinized blood is the most likely specimen that will be collected for blood gas analysis. Determination should be performed as quickly as possible (in house).[68] When measuring blood gases and acid-base status in birds, it is necessary to collect blood samples in pre-cooled syringes and store the samples on ice to stop the metabolism of the erythrocytes. The nucleated avian erythrocytes possess virtually all the enzymes typical of metabolically active cells and consume oxygen seven to ten times faster than mammalian erythrocytes. Even during analysis, which occurs at 37°C, the values are being influenced by temperature.
- ***Method:*** An expensive blood gas instrument is necessary.
- ***Diagnostic Value:*** Clinical significance in companion birds has not been thoroughly investigated.
- ***Pathologic Changes:*** Acidemia (decrease in blood or plasma pH) has been reported in some birds with renal disease.
- ***Reference Values for Budgerigars:***[32] pH (7.334 to 7.489); pCO_2 (30.6 to 43.2 mm Hg) (see Chapter 39).

Other Tests

Delta-Aminolevulinic Acid Dehydratase

- ***Method:*** Plasma or serum can be used to measure delta-aminolevulinic acid dehydratase colorimetrically.
- ***Diagnostic Value:*** Delta-aminolevulinic acid dehydratase can be used to detect lead intoxication, and decreased plasma activity is pathologic.
- ***Pathologic Changes:*** The activity can decrease depending on the dosage of lead and the species up to 50% of the normal value.[18,19,25] Central nervous system changes have been reported if plasma activity is below 86 U/l (see Chapter 37).

Acid Phosphatase

This enzyme consists of a number of isoenzymes in a variety of organs. The activity is much lower than that of alkaline phosphatase. Ovulation has been shown to increase acid phosphatase activities.[25]

Copper

- ***Method:*** Atomic adsorption spectrophotometry after direct dilution is the method of choice for determining serum copper.[68]
- ***Physiology:*** Copper is a component of several major enzymes and plays a vital role in hemapoiesis. It is involved in the absorption and the transfer of iron and hemoglobin synthesis. In the plasma it is mainly bound to ceruloplasmin.
- ***Diagnostic Value:*** Elevation occurs with copper intoxication. In postmortem specimens, copper concentration in the liver provides the best diagnostic sample.[25]
- ***Physiologic Influence:*** Copper levels are generally higher in female mammals under the influence of

estrogens. In birds, the effect of estrogens on copper levels has not been investigated.

- ***Pathologic Changes:*** Copper intoxications will increase the serum level.

Plasma Dye Clearance Test

In many animal species, the hepatic uptake and excretion of different organic dyes injected intravenously has been used for diagnosis of liver disease. Indocyanine green has been successfully used to detect liver disease in three raptor species.[70] The dye was non-irritating if accidentally injected perivascularly and clearance occurred.

In contrast, Bromsulphalein must be injected with care, because perivascular injection causes severe pain. In chickens, the clearance is markedly influenced by age and gender.[6,58] The clinical value of these two tests has been insufficiently studied in birds.

Urinalysis

Urinalysis is indicated if renal disease is suspected. Polyuria is a common clinical presentation in companion birds.[71] It may be caused by excitement, in which the content of the cloaca is shed before the water is reabsorbed; by the intake of large amounts of fluids (fruits, vegetables); by renal disease, neoplasia, diabetes, sepsis, toxins, adrenal disorders or gout; after administration of some medications; and with impending egg laying. In all of these cases, it is relatively easy to separate the urine from the feces via aspirating the liquid deposited on a water-resistant surface. Transient polyuria can be induced by administering water by crop tube. This will usually result in urine production within 30 minutes after administration.[73] In pigeons, urine for analysis has been collected directly from the cloaca using a cannula.[26] Urine samples can be collected from individual ureters of anesthetized parrots using a speculum.

Volume, Color and Consistency[73,25]

Urine evaluation should include a measurement of volume, a record of appearance (color, consistency) and determination of specific gravity. Normal companion birds produce a small quantity of urine, and if it can easily be collected it is generally abnormal (stress or disease). The urine is usually clear in most companion bird species, but in other birds, such as ratites and Anseriformes, it is normally opaque, cloudy or slightly flocculent.

Many factors can influence the color of avian urine. It can change with the ingestion of water-soluble vitamins (especially Vitamin B), the amount of uric acid and feces mixed with the urine, the specific gravity and certain diseases (see Color 8). Macaws often have very dark yellow urine, which is not normal.

The white crystalline portion of the urine in birds is seldom evaluated except for color. Birds that are in a negative nitrogen balance (severe cachexia, catabolic disease) usually have an increased quantity of urates.

- ***Pathologic Changes:*** Lead intoxication in some species may result in chocolate milk-colored urine and urates. This hemoglobinuria is common and normal for some nervous birds. Severe liver disease, like that induced by chlamydia or Pacheco's disease virus, can increase the secretion of biliverdin, which results in yellow-green or mustard-colored urine and urates. Because many other severe clinical diseases cause this color to be present, it is not pathognomonic.

Specific Gravity

- ***Normal:*** The specific gravity varies with the state of hydration and with the individual bird. In the polyuric bird, values from 1.005 to 1.020 are considered normal. A refractometer can be used for this determination. Water deprivation should be used to evaluate the kidney's ability to concentrate low levels, often due to psychogenic polydipsia.

- ***Pathologic Changes:*** Increased loss of water without an increased loss of solute will create a low specific gravity. This situation can be caused by intravenous fluid therapy, hyperthyroidism, liver disease, pituitary neoplasia, progesterone or glucocorticoid therapy. Any disease that causes polyuria and polydipsia can cause a low specific gravity. A reduced ability to concentrate or dilute the glomerular filtrate will lead to an increased specific gravity and severe renal pathology.

Specific Evaluation

Substances filtered by the normal kidney generally have a molecular weight of less than 68,000 (eg, water, uric acid, urea, glucose, electrolytes). Two substances that are on the border of this molecular weight cutoff are hemoglobin and albumin. Most other physiologic proteins have higher molecular weights. Most substances that are filtered by the kidneys are critical to normal bodily functions and are completely reabsorbed (eg, amino acids, glucose, vitamins). The excretion or retention of other substances are regulated according to the body's needs.

Urinary pH and the concentration of some chemical constituents in the urine can be measured using commercial test strips designed for use with human urine. It should be noted that the sensitivity of these tests has been adjusted to detect what would be regarded as abnormal levels of certain substances in human urine. These sensitivities are not necessarily applicable to birds and the fact that a "higher" reading is obtained on an area of the test strip does not necessarily imply an abnormality. For example, alkaline urine can produce falsely elevated protein levels. The color of the urine sample may also affect the results of some test parameters.

- ***Normal pH:*** Most pet birds have a pH between 6.0 to 8.0, which is largely related to the diet. Birds fed large amounts of protein (carnivores) have an acidic urine, while grain-eating birds have more alkaline urine.

- ***Pathologic Changes:*** Companion birds with urine pH lower than 5.0 are considered acidotic.[73] Increased protein catabolism will cause a lower pH. Bacterial metabolism tends to cause an alkaline pH. Companion birds with papillomatosis and other disorders that typically cause tenesmus may have acidic urine. Presumably this is caused by excretion of fluids from the upper intestinal tract.[8] It has been suggested that the cloacal mucosa of a normal companion bird is neutral to slightly alkaline when measured with litmus paper (Harrison GJ, unpublished).

Urinary Protein

- ***Normal:*** Trace amounts of protein can be detected in the urine of 90% of birds tested.[73]

- ***Pathologic Changes:*** Many renal disorders will result in a mild to moderate proteinuria. Non-renal sources of proteinuria include hematuria, hemoglobinuria and hyperproteinemia, which are usually caused by an increase in the production of immunoglobulins. Inaccurate protein levels will be detected if the urine is alkaline or if the strip is soaked in urine (instead of briefly dipped), which leaches out the citrate buffer.

Glucose

- ***Normal:*** Avian urine normally contains no glucose. In healthy pigeons, reference values between 0 and 3.2 mmol/l were established by the hexokinase reaction.[50] Trace glucose readings may be detected in normal avian urine by using dip sticks.[73]

- ***Pathologic Changes:*** The threshold for glucosuria to occur varies with the species.[73] Glucosuria will occur in most birds when the blood glucose level exceeds 600 mg/dl. In diabetes mellitus, birds may have blood glucose concentrations above 800 mg/dl.

Ketones

Ketones should be absent from the urine of birds. Any significant shift in energy production from carbohydrates to fats results in the increased oxidation of fatty acids and the production of intermediate metabolites that accumulate faster than they can be oxidized by the tissues. Catabolic processes such as severe hepatitis in combination with low blood glucose concentrations and diabetes mellitus can cause ketonuria.

Bilirubin

Bilirubin is not normally present in birds. Biliverdin is the major bile pigment, but will not react with the bilirubin portion of a mammalian urine dip stick.

Urinary Urobilinogen

Normal readings are 0.0 to 0.1 in healthy birds. Pathologic changes would be expected in cases of intravascular hemolysis and severe liver disease, but are seldom reported. Falsely high levels of urobilinogen in urine can be due to drugs which appear red in acid urine (eg, Vitamin B_{12}) or if sulphonamides are present.

Blood

Commercial strip tests are available that can distinguish hematuria (ie, an abnormally large number of intact RBCs in the urine) and hemoglobinuria (ie, hemoglobin that is free within the urine and not contained within cells). With hematuria, individual erythrocytes lyse on the test area, giving individual spots of color. If there is free pigment, the color change is uniform throughout.

Normal readings are negative or trace. Blood in the urine may originate from the cloaca or from the urinary, reproductive or gastrointestinal tracts. Hemoglobinuria can be due to intravascular lysis of RBCs (rare) or lysis of RBCs present in the urine.

Urinary Nitrite

This test is included on many commercial test strips and is used to screen for bacteriuria. It is an unreliable test for avian urine.

Urinary Sediment

Examination of the urinary sediment is a valuable part of urinalysis but one that is often omitted. A fresh or refrigerated sample is required. With time,

there is increasing alkalinity causing progressive lysis of blood cells and casts. Usually centrifugation is used to concentrate the sediment to approximately ten percent of its original urinary volume.

White and Red Blood Cells

The number of RBCs and WBCs in the sediment is reported as the number per high power field (HPF). Normal urine contains 0 to 3 RBCs/HPF and 0 to 3 WBCs/HPF.[73] More than 6 white or red blood cells per HPF is a cause for concern. All cells noted within the urine sediment may have origins within the cloaca or the urinary, reproductive or gastrointestinal tracts.

Epithelial Cells

Normal urine contains no epithelial cells. The presence of any epithelial cells (eg, renal tubular cells) should be considered abnormal.[73]

Casts

Casts are cylindrical structures molded into the shape of the renal tubules. Normally no casts are seen in avian urine. Casts are frequently noted in cases of renal disease. Granular casts are most common. Cellular casts (which incorporate cells like RBCs, WBCs or tubular epithelial cells) and hyaline casts (consisting of mucoprotein gel) may also be seen.

Bacteria

In mammals, it is believed that bacteria in excess of 3 x 10^4/ml of urine must be present before they are detectable in urinary sediment.[68] Gram-positive cocci and rods may be noted in the avian urinary sediment if the sample has been contaminated with fecal material.

- ***Pathologic Changes:*** Reports of bacteria that are "too numerous to count" or numerous cocci and rods in reasonably clean urine samples should be viewed with suspicion.[73] Avian urine is sterile leaving the kidneys, and Gram's stains or cultures comparing stool and urine flora may be helpful in documenting bacteria that originate from the urinary tract. Bacteria may multiply en route to the laboratory, which will lead to high counts in the sample.

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