CHAPTER
23

Diagnostic Value of Biochemistry

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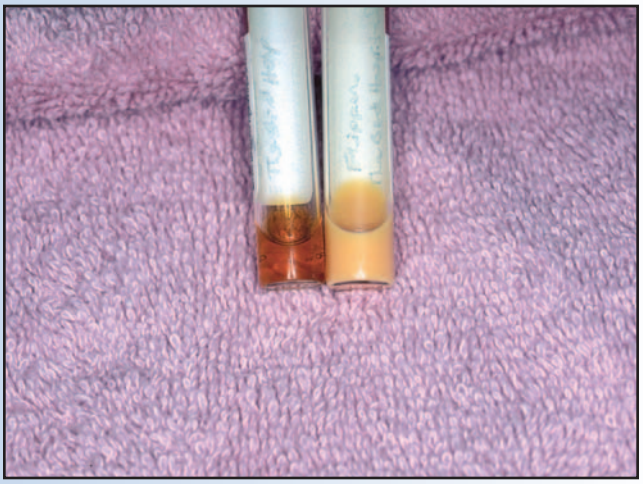

Fig 23.1 | A normal serum sample on the left and a lipemic sample on right.

Clinical chemistry, along with hematology and physical examination, is the cornerstone of medical diagnosis of disease in any species. Plasma biochemistry is especially important in avian species, which frequently show minimal overt clinical signs of disease, even when seriously ill. Veterinarians, therefore, need accurate and useful biochemical analysis to successfully diagnose and treat avian species. The Clinical Laboratory Improvement Amendments (CLIA) are the federal laws and regulations that govern human diagnostic laboratories. No such governing policy exists in veterinary diagnostic medicine. This results in variable methodology, protocol, and most importantly, quality control, between veterinary laboratories. It falls to the veterinary clinician to ensure that the diagnostic laboratory being used maintains a high standard of quality control and that results are accurate. The clinician should develop a working relationship with the laboratory of choice and must continually monitor results for possible inaccuracy and error. Feedback to the laboratory must occur to ensure that the laboratory personnel are aware of any errors and can correct them. The laboratory used should be familiar with handling avian samples. Modified techniques in the laboratory, such as the use of 10-μl microhematocrit tubes, pediatric sample cups and dilution, can extend the sample and allow more data to be collected, especially from the smaller avian patient. When choosing a laboratory, the clinician also should consider its location, transport issues and delayed processing of the sample. All of these can cause artifactual change in the sample and decrease ability to diagnose disease.

Concentrations of analytes represent a steady state of input, consumption and partitioning. The body is always in flux. Generally, pathologic conditions cause either an increased or decreased concentration of various analytes. However, if both input and consumption are

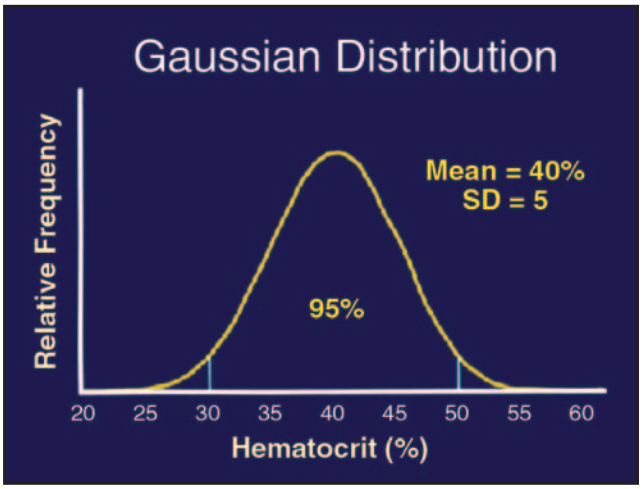

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Fig 23.2 | 2.5% of the population is eliminated on each side of the curve to generate a 95% reference interval. This increases the likelihood that diseased patients are flagged as abnormal. It also means that it is quite likely that a healthy patient will have one analyte that is mildly abnormal.

decreased or increased at the same time, the concentration may remain in the normal reference interval even though the patient is very ill. For example, the globulin fraction may remain within the normal reference interval in a bird with marked enteritis because of increased production of acute phase inflammatory proteins and antibodies with concurrent loss of protein through a compromised gastrointestinal tract. Interpretation of clinical chemistries must therefore be done on a case-by-case basis with knowledge of species-specific physiology.

In comparison to domestic species, it can be a challenge to simply ascertain normal reference intervals for avian patients. Reference intervals for biochemical analytes are highly dependent on the machines, reagents and methods used, and may vary significantly between different laboratories. According to CLIA, each human diagnostic laboratory must establish reference intervals for each methodology validated within that laboratory in order to create a diagnostic range that can be used medically. A minimum of 100 healthy individuals is sampled. A 95% reference interval is created for normally distributed analytes using the formula, mean +/- 1.96 standard deviations. Therefore, the normal medical reference interval used by clinicians excludes 2.5% of normal individuals at the high and low ends of the range (Fig. 23.2). Biochemical reference intervals for common species of psittacines (parrots), passerines (canaries and finches), and galliformes (turkeys and chickens) have been established by specialized laboratories, eg, California Avian Laboratory, Citrus Heights, CA. Each laboratory analyzing avian samples should develop species-specific and methodology-specific reference intervals. These are still lacking in some university and private laboratories.

Reference intervals generated by a laboratory represent population reference intervals that are broader than the reference interval generated when repeatedly sampling an individual. Some individuals will regularly have concentrations in the low end of the population's range and some individuals will regularly have concentrations in the high end of the population's range. Therefore, an individual may remain within the population reference range even though there are abnormalities in the patient.

In addition to accurate normal reference intervals, the clinician must have knowledge of the sensitivity/specificity and positive/negative predictive value of a test's ability to diagnose diseases specific to that species. Although a great deal is now known regarding avian medicine, research into the diagnostic application, sensitivity, specificity, and positive and negative predictive values of biochemical analytes is still needed.

MEASURES OF THE ACCURACY OF A TEST

Accuracy is how close the test approximates the true value in the body. Precision measures how far from the mean or average of replicate measurements a particular measurement lies (Fig. 23.3). Most laboratory techniques were designed for use in human medicine and are modified for use in birds. Assessment of analytic accuracy and precision of a technique is very important when assessing different mammalian species, not to mention different kingdoms of animals. Any instrument error such as old bulbs, slight variation in machine temperature, variation in reaction time or degraded reagents can cause decreased accuracy and precision.

Sensitivity, specificity and predictive values are the measures of diagnostic accuracy. In medicine, sensitivity is the likelihood that a diseased patient will have a positive test result in a population of individuals with the disease. Sensitivity is a measure of false negative values. To help remember the relationships note that the letter "N" is present in sensitivity and false negative. Specificity is the likelihood that a patient without the disease has a test value that remains within the reference interval in a population of healthy individuals. Specificity is a measure of false positive values. Note that the letter "P" is present in specificity and false positive. The predictive value of a test is determined by its measurement in a population of healthy and sick individuals. A positive test result is measured when the disease is present (positive predictive value) and a negative test result is measured when the disease is not present (negative predictive value). These can be mathematically determined using the formulas in Table 23.2. Tests that are highly sensitive frequently have a low specificity and vice versa. This does

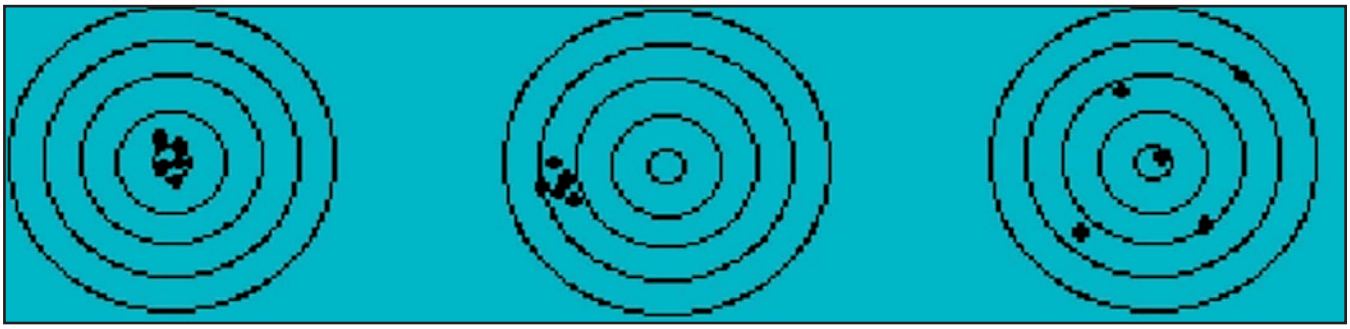

Fig 23.3 | Accuracy vs. Precision. When values are both precise and accurate, they are a tight cluster in the bull's eye. The precise values are all similar, but are some distance from the actual value. The accurate values are all within the third circle, with one almost approximating the actual value, but are scattered around the bull's eye.

Table 23.1 | Statistical Analysis of a Diagnostic Test

Formulas for the calculation of diagnostic sensitivity, diagnostic specificity, positive predictive value and negative predictive value. TP = true positive, TN = true negative, FP = false positive, FN = false negative.

- Diagnostic sensitivity = $\frac{TP}{TP+FN}$
- Diagnostic specificity = $\frac{TN}{TN+FP}$
- Positive predictive value = $\frac{TP}{TP+FP}$
- Negative predictive value = $\frac{TN}{TN+FN}$

not mean that the test is worthless. It simply means that it may have to be used with other tests to assess organ function and disease.

Sample Handling

HANDLING

In the USA, many exotic animal practitioners perform venipuncture using a syringe that has been coated with injectable sodium heparin to prevent clot formation. Experienced avian veterinarians working with experienced restrainers, who minimize trauma to the vessel wall, can collect a high-quality sample without the addition of sodium heparin. Reducing the amount of sodium heparin in the sample is desirable. If most of the heparin is expelled, it will minimally affect the sample. However, the amount of heparin actually retained may vary among samples. Any droplets remaining may cause dilutional effects as well as interfere with some analytical tests such as sodium and albumin. Samples for biochemical analysis should be placed into a lithium heparin microtainer rather than a red-topped tube to avoid variable clotting time and gelling of serum. Anticoagulant tubes must be filled to the appropriate volume. A plasma separator also can be used to increase plasma sample volume, though these tubes tend to be slightly more expensive.

Avian plasma samples are frequently yellow due to carotenoid pigments; rarely, in severe disease states, avian plasma may be truly icteric from bilirubin.[39,49] Pink or red plasma is usually indicative of hemolysis, though dyes from food should be ruled out. Green-tinged plasma is rarely observed, may be caused by biliverdin and is usually indicative of liver failure.[23,31] When working with smaller species of birds, tuberculin or insulin syringes are frequently used, however, not all of these syringes have detachable needles. Avian red blood cells are larger and deteriorate more quickly than mammalian erythrocytes. This can make accurate analysis difficult with ideal sample handling. Ejecting blood through a 25-gauge or smaller needle can cause moderate to marked hemolysis that will invalidate many biochemical analysis.[48] To avert this, attached needles can easily be cut from the syringe using a pair of large veterinary nail clippers or scissors before expelling the blood from the syringe.

ANTICOAGULANT

Prior to collection, the appropriate sample container is labeled with the names of the owner, patient and the signalment. Color-coded, rubber stoppered, evacuated tubes are well standardized. Green-topped tubes contain heparin, which should be used for plasma chemistry analysis in birds. Lithium heparin is the recommended anticoagulant, as sodium or potassium heparin can falsely increase the electrolyte values and skew anion gap and acid base analysis. Ammonium heparin should not be used, as it significantly increases ammonia and BUN concentrations. Heparin inhibits coagulation by binding to antithrombin III and greatly accelerates the inhibition of thrombin (factor II) by antithrombin III. Factors VII (proconvertin) and X (Stuart Prower factor) also appear to be inhibited by the heparin-antithrombin III complex.

The disadvantage of heparin is that leukocytes do not stain as well, and platelets and white blood cells clump much more than they do in blood collected in ethylene-diaminetetra-acetic acid (EDTA). This leads to decreased

accuracy and precision in the complete blood count (CBC) (see Chapter 22, Diagnostic Value of Hematology). Purple or lavender-topped tubes contain EDTA. Blue-topped tubes contain citrate and are used to harvest plasma for coagulation analysis. Both citrate and EDTA prevent coagulation by chelation of calcium (factor IV), an electrolyte essential to coagulation. Neither purple- nor blue-topped tubes are recommended for chemistry analysis because chelation of ions interferes with most reactions.

Red-topped tubes lack anticoagulant and are used to harvest serum required in antibody, hormone, and other protein analysis. At least 25% of avian serum samples will form a proteinaceous gel when separated, significantly decreasing sample volume and occasionally completely preventing biochemical analysis. Additionally, time to clot formation in avian species is variable, due in part to greater dependence on the extrinsic coagulation cascade. The use of heparinized plasma therefore decreases variability in time to sample separation and improves the chance of obtaining an adequate sample volume.

HEMOLYSIS

Hemolysis directly interferes with spectrophotometric absorbance readings and alters the pH of enzymatic reactions. Constituents that are found in higher concentrations within erythrocytes than in serum will be increased, eg, aspartate aminotransferase (AST) and, potentially, potassium. Alteration in the enzymatic reactions may appear randomly and cannot be predicted. Hemolysis can and should be monitored visually. Any sample that is more than very light pink should not be used diagnostically. Additionally, if the sample is analyzed by an automated hematology analyzer, a mean cell hemoglobin concentration (MCHC) that is greater than the reference range is an indication of possible hemolysis.

Artifactual change can vary between methods employed. Technical support and literature should be reviewed for each machine. Hemolysis in the sample falsely decreases bile acids measurement by the colorimetric assay, while radioimmunoassay (RIA) is unaffected. For many methods, hemolysis falsely increases alanine aminotransferase (ALT), aspartate aminotransferase (AST), lactate dehydrogenase (LDH), creatinine, calcium, albumin, potassium, amylase, creatine kinase (CK), hemoglobin, and MCHC. A false decrease in triglycerides can occur. Glucose, magnesium, phosphorus, cholesterol, alkaline phosphatase and lipase can be either increased or decreased depending on the methodology.[48]

LIPEMIA

Lipemia may be present in postprandial samples, but also may be indicative of underlying disease such as hypothyroidism, diabetes mellitus, hyperadrenocorticism, pancreatitis, or a primary lipid/lipoprotein disorder. Lipemia causes refraction of light and therefore causes error in many spectrophotometric and all refractometric methods (see Fig. 23.1).

Lipid can be partially cleared by ultracentrifugation or precipitating agents (polyethylene glycol, liposol, lipoclear). These techniques and clearing agents may themselves induce artifact. Additionally, the removal of lipid from a sample may in itself induce an artifact in analytes of interest. For example, lipoproteins bind bile acids, which would be discarded along with the lipid following ultracentrifugation. This may be one factor that contributes to the occasional measurement of decreased postprandial values in comparison to fasted values. The scattering of light due to lipemia will falsely increase the postprandial bile acid measurement. Varying technique can therefore significantly alter bile acid values. This underscores the importance of contact with your laboratory to determine which technique is used and that the techniques used are appropriate.

Again, technical support and literature should be reviewed for each machine. Wet chemistry analyzers are generally more impacted by lipemia than dry chemistry analyzers. Electrolytes measured by ion-specific electrodes are not affected by lipemia, but electrolytes measured by flame photometry are decreased.

Lipemia falsely increases all of the liver enzymes, alkaline phosphatase, hemoglobin, MCHC, bile acids, total bilirubin, glucose, calcium and phosphorous. Total protein measured by a refractometer is falsely increased, but the biuret method is minimally affected even by severe lipemia. BUN and gamma glutamyl transferase (GGT) may be increased or decreased depending on the methodology. Albumin is generally decreased using bromcresol green methodology.

Age

In general, non-protein nitrogen concentrations are lower in young, growing animals, as most nitrogen is being consumed by growth. In neonatal eclectus parrots *(Eclectus roratus)*, macaws and cockatoos, albumin, globulin and AST also have been found to be lower than in adults. This is likely due to decreased production of these analytes by the neonatal liver, combined with increased utilization in the tissues that is needed for growth. Additionally, it was found that calcium, sodium and chloride were decreased in chicks in comparison to adults. Alkaline phosphatase, a compound produced in

osteoblasts, is found in higher concentrations in growing animals. Blood phosphorus and potassium also are found in increased concentrations in young birds due partially to increased concentration of growth hormone, and mobilization for muscle and bone growth.[11,12,13]

Analytes

The following descriptions of analytes contain method, physiology and diagnostic value sections. The method sections are designed for practitioners who are running some values in their practice and, therefore, need to be familiar with the method that they are using to analyze plasma biochemical values. Different methods frequently will produce different results. The International Federation of Clinical Chemists (IFCC) has standardized some test methods and these should be used. Analyzer manufacturers may sell alternate methods at reduced prices to veterinarians, with the knowledge that they do not meet current standards. The veterinarian should be aware of the appropriate method to use and the limitations of interpretation in a species. Some artifacts and drug interactions are discussed in the method sections, though these should not be considered to be complete listings of those interactions. Product specification sheets for the methodology as well as technical support should be used as necessary. The sections on physiology discuss the function of the analyte in the body. The sections on diagnostic value discuss clinical utility in birds. See also the Differential Diagnoses in Table 23.2.

Acetoacetate, Acetone (Ketones)

Method

Common urine test strips present in most practices use the Rothera test in which alkaline nitroprusside turns purple in the presence of acetoacetate and, to a lesser extent, acetone. A third, relatively acutely produced ketone, 3-hydroxybutyrate, is not measured by this reaction. False negatives may occur if the patient is producing only 3-hydroxybutyrate. If diabetes is suspected and ketones are not measured, the urine should be rechecked in 48 hours. Some drug interactions may produce false positives, including penicillamine, levodopa and phenylketones.

Physiology

Decreased glucose availability to the tissues results in increased lipase activity in adipose tissue that catalyzes long-chain fatty acids. These are catabolyzed to acetyl CoA that is metabolized to the ketones: 3-hydroxybutyrate, acetoacetate and acetone. These compounds are excreted in urine and can be qualitatively measured in stressful physiologic or pathologic disease states.

Diagnostic Value

Healthy birds do not have ketones in their urine unless they have undergone strenuous activity, eg, migration. Measurement of ketone bodies in the urine is indicative of diabetes mellitus in most birds.

Albumin

Method

Most veterinary laboratories measure albumin using the dye bromcresol green (bcg), which has not been validated in companion avian species. Bromcresol green non-specifically binds protein. Binding of bcg causes increased color in the sample, which correlates with a higher reported albumin concentration. It has been demonstrated in dogs and humans that heparin can cause false increases in albumin concentration due to binding of fibrinogen.[59] Avian albumin is markedly different in structure than mammalian albumin and binds bcg with decreased affinity. Comparison of gel electrophoresis and bcg have revealed that bcg results in lower concentrations reported than actually exist in the patient.[58] This error is caused in part by use of human albumin standards and controls, which have different binding affinity for the dye than does avian albumin. This error in measurement may result in serious errors when assessing hypoproteinemic syndromes such as liver failure, protein-losing nephropathy and protein-losing enteropathy. At this time gel electrophoresis is the recommended method of albumin determination in avian species.[15,40,60] Bromcresol purple (bcp) also is commonly used in human laboratories and has different protein binding affinity for albumin.[2,5,64] Bromcresol purple may result in more accurate avian albumin measurement and better diagnostic acuity. Further study is needed.

Physiology

Albumin, a small, approximately 65-kD protein, is the most abundant protein found in plasma, most extravascular body fluid, CSF and urine. Albumin's synthesis by the liver is primarily controlled by plasma oncotic pressure. Albumin's main function is the maintenance of colloid oncotic pressure in the intravascular and extravascular spaces. Albumin also functions as a carrier protein to transport a large number of compounds including calcium and administered drugs. Albumin levels are lower in chicks than in adults.

Diagnostic Value

Accurate assessment of albumin enables the practitioner to assess hypoproteinemic disease such as liver failure, protein-losing nephropathy and protein-losing enteropathy. This diagnosis may lead to alternate fluid therapy such as colloid (hetastarch) administration to prevent edema, which is rarely seen in birds, and ascites. A true increase in albumin is pathognomonic for dehydration

Table 23.2 | Differential Diagnoses Based on Chemistry Abnormalities*

Albumin Increased	• Dehydration - generally accompanied by increased globulin and total protein • Reproductive - mild increase observed in females during egg formation
Albumin Decreased	• Liver failure - Cirrhosis/fibrosis - Neoplasia - Portosystemic shunt - Amyloidosis • Renal loss - Glomerulonephritis/sclerosis • Intestinal - Malabsorption/maldigestion · Mycobacterial disease · Endoparasites • Malnutrition (severe) • Exudative skin disease - Burns - Large wounds - Vasculitis - Frostbite • External blood loss (subacute to chronic) • Inflammatory disease state (seen with increased globulins) - Septicemia - Viremia • Neonates - normally lower than adults • Polyuria/Polydipsia
Alkaline Phosphatase Increased	• Bone isoenzyme - Trauma - Growth - Osteosarcoma - Osteomyelitis • No hepatic-associated increase currently documented
Ammonia Increased	• Hepatic disease/failure - Cirrhosis - Neoplasia - Polyoma • Artifactual - Hemolysis
Amylase Increased	• Pancreatic - Inflammation/Infection - Neoplasia - Necrosis - Pancreatic duct obstruction (eg, egg binding) • Zinc toxicity • Enteritis • Renal disease (decreased filtration) (mild to moderate increase)
Anion Gap Increased	• Respiratory acidosis - Anoxia (anesthetic induced, other) - Respiratory disease (chlamydia, aspergillosis, etc) • Hyperglobulinemia (reproductive, inflammatory) • Metabolic acidosis (increased lactic acid and other unmeasured anions) - Renal failure - Gastrointestinal bicarbonate loss - Hypoperfusion/reperfusion - Shock • Diabetic ketoacidosis • Toxic - Ethylene glycol (mammals) - Others in birds
Aspartate Aminotransferase (AST) Increased	• Muscle damage - Seizures - Trauma - Capture myopathy (exertional rhabdomyolysis) - Intramuscular injection • Hepatic damage - Drugs (cephalosporins, metronidazole, trimethoprim sulfa, dexamethasone) - Hemochromatosis (iron storage disease) - Endocrine disease (diabetes mellitus, hyperthyroidism) - Hypoxia (cardiopulmonary in origin) - Lipidosis (severe) - Inflammation/Infection
Aspartate Aminotransferase (AST) (cont.) Increased	• Hepatic damage (continued) - Infection [(bacterial, mycobacteriosis, chlamydophilosis, polyoma virus, Pacheco's disease - herpes virus, adenovirus, reovirus, duck virus hepatitis, *Plasmodium*, *Trichomonas*, *Histomonas* (turkeys), *Leucocytozoon* (ducks and geese), *Atoxoplasma*] - Toxic [(aflatoxin/mycotoxin, cottonseed (*Gossypium* sp.), *Crotalaria* sp., oleander (*Nerium* sp.), rapeseed (*Brassica napus*), ragwort (*Senecio jacobea*), castor bean (*Ricinus communis*)] - Neoplasia · Primary · Secondary - Artifactual · Erythrocyte leakage
Bicarbonate (CO_2) Increased	• Compensated respiratory acidosis - Respiratory disease - Drugs (anesthetics) • Small bowel obstruction • Gastric vomiting - Obstruction - Lead poisoning
Bicarbonate (CO_2) Decreased	• Metabolic acidosis - Dehydration - Renal failure • Respiratory alkalosis - Tachypnea/panting - Excess anesthetic ventilation • Artifactual - Delayed analysis
Bile Acids Increased	• Impaired liver function - Lipidosis - Biliary Stasis - Infection (see AST) - Inflammation - Neoplasia - Toxic (see AST) - Hemochromatosis - Cirrhosis/Fibrosis
Blood Urea Nitrogen Increased	• Prerenal azotemia - Dehydration (pigeons) - Postprandial (some raptors) - GI hemorrhage • Renal Failure
Blood Urea Nitrogen Decreased (Unlikely but can be measured in some species)	• Liver failure • Neonates • Diuresis - Iatrogenic - Physiologic - Pathologic
Calcium Increased	• Reproductive (may be marked) - Physiologic increase in females - Pathologic • Hypervitaminosis D • Primary hyperparathyroidism • Renal secondary hyperparathyroidism • Nutritional secondary hyperparathyroidism • Neoplasia - Lymphoma - Osteosarcoma • Osteomyelitis • Granulomatous disease
Calcium Decreased	• Nutritional - Excess dietary phosphorus (seed) - Hypovitaminosis D - Dietary deficiency (severe) • Chronic egg laying - Egg-bound hen • Hypomagnesemia • Hypoparathyroidism • Pancreatitis • Malabsorption • Alkalosis

Table 23.2 | Differential Diagnoses Based on Chemistry Abnormalities* (continued)

Chloride Increased	• Dehydration • Metabolic acidosis
Chloride Decreased	• Gastric vomiting - Obstruction - Lead poisoning • Metabolic alkalosis
Cholesterol Increased	• Reproductive - egg formation (cystic ovaries) • Nutritional/postprandial • Cholestasis • Obesity • Endocrine - Diabetes mellitus - Hypothyroidism - Hyperestrogenism • Nephrotic syndrome
Cholesterol Decreased	• Intestinal - Malabsorption/maldigestion • Liver failure • Starvation
Creatine Kinase Increased	• Muscle damage - Intramuscular injection - Seizures - Capture myopathy (exertional rhabdomyolysis) - Myositis · Sarcocystis · Toxoplasma · Other parasitic · Bacterial - Hyperthermia - Hypothermia - Vitamin E/Se deficiency - Trauma · Surgical - Ischemia
Fibrinogen Increased	• Bacterial infection • Other inflammation
Fibrinogen Decreased	• Liver failure • Coagulopathy
Gamma Glutamyltransferase (GGT) Increased	• Liver compromise - Cholestasis · Intrahepatic · Extrahepatic - Neoplasia · Biliary carcinoma • Other biliary compromise
Gamma Glutamyltransferase (GGT) Decreased	• Artifactual hemolysis
Globulin Increased	• Dehydration (concurrent increase in albumin) • Inflammation • Egg formation
Globulin Decreased	• Neonatal • Immunodeficiency • Blood loss (subacute to chronic) • Protein-losing enteropathy
Glucose Increased	• Endocrine - Diabetes mellitus • Pancreatitis • Stress • Drugs - Glucocorticoids - Progesterone
Glucose Decreased	• Liver failure • Starvation in small birds • Neoplasia • Septicemia
Iron Increased	• Hemochromatosis • Inflammation • Artifactual - Hemolysis
Iron Decreased	• Chronic blood loss • Nutritional dietary deficiency* **Ed. Note: Unlikely on formulated diet, but possible on a seed/fruit.*
Lipase Increased	• Enteritis • Pancreatitis • Pancreatic neoplasia • Renal disease (decreased loss)
Phosphorus Increased	• Renal disease • Neonates • Reproductive - Egg formation (concurrent rise in calcium) • Nutritional - Hypervitaminosis D - Increase dietary phosphorus • Toxic - Jasmine ingestion • Neoplasia - Osteosarcoma • Inflammation - Osteomyelitis • Artifactual hemolysis/delayed serum separation • Primary hyperparathyroidism • Nutritional secondary hyperparathyroidism • Neoplasia: PTH-like hormone
Phosphorus Decreased	• Diabetic ketoacidosis • Dietary deficiency
Potassium Increased	• Renal failure • Diabetic ketoacidosis • Severe muscle/tissue damage • Dehydration • Drugs - ACE inhibitors - Potassium-sparing diuretics • Artifactual - Collection in potassium heparin
Potassium Decreased	• Alkalosis • Drugs - Penicillins - Amphotericin B - Loop diuretics - Insulin therapy • Gastrointestinal loss • Renal disease (chronic)
Sodium Increased	• Gastric vomiting (water loss) • Intestinal fluid loss • Renal failure • Dehydration
Sodium Decreased	• Diabetes mellitus • Gastric vomiting • Intestinal sodium loss - Endoparasitism • Burns • Chronic effusions - Egg yolk • Psychogenic polydipsia • Renal failure (chronic) • Artifactual - Hyperlipidemia
Total Protein Increased	• Dehydration (albumin and globulin) • Artifactual - Hemolysis
Total Protein Decreased	• Hemorrhage (chronic) • Intestinal loss • Liver failure • Renal loss • Immune suppression
Uric Acid Increased	• Renal disease • Postprandial (carnivores) • Dehydration (severe)
Uric Acid Decreased	• Liver failure • Starvation

and would indicate the need for administration of IV crystalloid fluids.

Alkaline Phosphatase (ALP)

Method

Numerous methods have been developed to determine ALP activity. The IFCC recommended method uses 4-nitrophenyl phosphate (4-NPP) and 2A2M1P as a phosphate acceptor buffer at 37° C and absorbance at 405 nm. ALP catalyzes the hydrolysis of 4-NPP, forming phosphate and free 4-nitrophenol (4-NP) in an acidic solution. Alkalinization causes conversion of colorless 4-NP to 4-nitrophenoxide ion, which is an intense yellow color. As veterinary laboratories may employ different methods, normal reference intervals may be markedly different. Caution should be used when assessing a patient using reference intervals from a textbook. Laboratory-specific reference intervals should be generated.

Physiology

Alkaline phosphatase is a glycoprotein dimer with subunit masses ranging from 40 to 83 kD. The protein's exact function is unknown. Mammalian and avian isozymes of alkaline phosphatase have been identified in cell membranes in the liver (biliary epithelium), kidney, intestine, bone (osteoblasts), as well as a steroid-induced form in dogs. Isoenzymes from osteoblasts, duodenum and kidney have predominated in studies involving pigeons and domestic fowl.[28,43,45] Very low levels of alkaline phosphatase have been identified in the liver of pigeons and psittacines. Alkaline phosphatase levels are higher in chicks than in adults.

Diagnostic Value

In mammals, alkaline phosphatase is of particular interest in two specific disease states: biliary disease frequently associated with cholestasis and bone disease associated with increased osteoblastic activity. ALP does not increase with simple hepatocellular damage. In avian species at this time, marked increases in ALP have been associated only with increased osteoblastic activity including traumatic, neoplastic and infectious disease states. Further investigation into specific cholestatic and biliary disease such as biliary carcinoma is warranted to assess the sensitivity and specificity of ALP in these biliary diseases.

Ammonia

Method

Both enzymatic and chemical methods are used to measure ammonia. Enzymatic assay with glutamate dehydrogenase is the most frequently used method.[52] Glutamate dehydrogenase catalyzes the conversion of ammonium ion and 2-oxoglutarate to glutamate and water. This reaction oxidizes Nicoti Adenine Dinuleotide Hydrogen (NADH) to Nicotinamide Adenine Dinucleotide (NAD), which can be optically measured at 340 nm.

Meticulous precautions must be taken in sample handling to prevent false increases in ammonia concentration. Samples must be drawn cleanly, using an evacuated tube, and processed immediately for accurate results. Poor venipuncture technique or increased exposure to air may result in increased ammonia levels. Probing for a vein causes tissue damage that may elevate ammonia levels. Drawing blood into a syringe and transfer of that blood to a microtainer, or partial filling of an evacuated tube allows subsequent entry of air that may cause elevation of ammonia levels. Serum samples and ammonium heparin may cause falsely elevated levels. Production of ammonia by deamination of amino acids in the blood will occur once the specimen has been drawn. At 0° C, delays exceeding 15 minutes between blood sampling and centrifugation can increase ammonia concentrations.

Ammonia analysis are available on many dry chemistry analyzers used in practice. Machines-specific reference ranges should be established, as different methodologies will produce different reference intervals.

Physiology

The major source of ammonia is the gastrointestinal tract. It is derived from the hydrolysis of glutamine in the small and large intestine and from the action of bacterial proteases, ureases, and amine oxidases on digested food in the colon. Ammonia is converted to the less toxic uric acid and urea in the liver.

Diagnostic Value

Though plasma ammonia has not been validated in healthy or ill birds, some clinicians have observed up to a fourfold increase in blood ammonia values in birds with liver failure.

Amylase

Method

The three broad classifications of alpha-amylase (endoamylase) assays are saccharogenic, amyloclastic and chromogenic digestion of starch to glucose or maltose. The most commonly used assay in veterinary laboratories is the chromogenic alpha-amylase assay, which is the most appropriate assay in the canine.[35] This assay detects the release of dyes bound to synthetic starch substrates that are released as the starch is digested by amylase.

Physiology

Alpha-amylases are calcium-dependent metalloenzymes that catalyze hydrolysis of complex carbohydrates at

internal binding sites. The predominant sites of production are the pancreas and the duodenum. Trypsin in the small intestine degrades the enzyme, though some amylase frequently is still detectable in the feces. Urinary clearance of this small, 55- to 60-kD protein also has been documented in mammals.

Diagnostic Value

Urine-to-serum ratios are used in human medicine to diagnose acute pancreatitis.[26] Though this assay is frequently used for the diagnosis of pancreatitis in humans, it has decreased specificity and sensitivity in the dog. Validation of this assay for diagnosis of pancreatitis in birds is needed.

Anion Gap

Method

This number is calculated from the following formula:

Cations - Anions or
(Sodium + Potassium) - (Chloride + Bicarbonate)

Physiology

The gap is generally around 15 mEq/L (mmol/L) in most species with some variation, and represents unmeasured anions such as phosphate, sulfate, lactate, ketones, and drugs such as salicylates and ethylene glycol metabolites. If bicarbonate is not available, the total carbon dioxide value can be substituted. Generally, an increased anion gap indicates acidosis.

Diagnostic Value

Anion gap facilitates assessment of metabolic and respiratory acidosis. An increased anion gap should incite a search for the cause of increased numbers of unmeasured anions.

Acidemia causes an extracellular potassium shift as hydrogen ions enter the cells; the more chronic the disorder, the greater the intracellular potassium depletion. When correcting the acidosis, potassium moves back into the cell creating a potentially life-threatening hypokalemia. Accurate assessment of the acid base status of patients with chronic respiratory disease is essential to provide appropriate fluid therapy and electrolyte replacement.

Aspartate Aminotransferase (AST)

Method

The IFCC has standardized this reaction to some extent by limiting it to the rate-limiting reaction of L-aspartate and 2-oxoglutarate catalyzed by AST to form oxaloacetate and L-glutamate. Oxaloacetate is then reacted with NADH in the presence of malate dehydrogenase to form L-malate and NAD. Pyridoxal-5'-phosphate is a required coenzyme in the reaction and the IFCC recommends addition of this coenzyme in the reaction. The conversion of NADH to NAD can be optically measured at 340 nm. Reference intervals may vary slightly with variation of reagent concentration. AST is present in the cytosol of erythrocytes and extended red cell exposure can cause increased plasma AST concentration.

Physiology

The aminotransferases, including AST (formerly glutamate oxaloacetate transaminases, GOT) and alanine aminotransferase (ALT) (formerly glutamate pyruvate transaminases, GPT), are a group of enzymes that catalyze the interconversion of amino acids by transfer of amino groups. A variety of tissues, predominately liver and muscle, contain high aspartate aminotransferase. The mitochondrial and cytosolic isoenzymes of AST are approximately 90 kD in size.

Diagnostic Value

AST is not specific for hepatocellular damage, but is highly sensitive in detecting hepatocellular damage caused by ethylene glycol in pigeons.[40] Plasma AST activity returned to normal within 100 hours after doxycycline-induced muscle trauma in pigeons. AST activity is currently considered to be a very sensitive but nonspecific indicator of hepatocellular disease in other avian species as well, and is used with the muscle-specific enzyme creatine kinase (CK) to differentiate between liver and muscle damage.[16,30] ALT also is not liver-specific in birds. Prolonged postinjection increases in ALT decrease the diagnostic utility of this enzyme in the diagnosis of liver disease. ALT is therefore frequently omitted from avian chemistry panels.

Bicarbonate

Method

A common reaction used to measure bicarbonate is based upon phosphoenolpyruvate carboxylase (PEPC) utilizing bicarbonate present in the sample to produce oxaloacetate and phosphate. Malate dehydrogenase then catalyzes the reduction of oxaloacetate to malate, and the oxidation of NADH to NAD. NADH oxidation can be measured optically at 340 nm. Extended exposure of the sample to air (under-filled vials), late separation from the cell fraction or a dehydrated sample can introduce significant error in this measurement.

Physiology

Approximately 90% of carbon dioxide present in serum is in the form of bicarbonate. Therefore, measurement of total CO_2 is frequently used in place of bicarbonate measurement. This is different than pCO_2, which represents the remaining small percentage actually present in the gaseous form. The combination of water and CO_2 forms the weak acid carbonate, H_2CO_3, and its dissociated

Table 23.3 | Acid Base Imbalances and the Body's Compensation

	[H+]	pH	Imbalance	Compensation
Respiratory acidosis	↑	↓	↑ pCO_2	↑ $[HCO_3^-]$
Metabolic acidosis	↑	↓	↓ $[HCO_3^-]$	↓ pCO_2
Respiratory alkalosis	↓	↑	↓ pCO_2	↓ $[HCO_3^-]$
Metabolic alkalosis	↓	↑	↑ $[HCO_3^-]$	↑ pCO_2

forms, bicarbonate and hydronium ion, comprise one of the main buffering systems in animals. The Henderson Hasselbach equation, $pH = 6.1 + \log (HCO_3^- / H_2CO_3)$ where 6.1 = pK for the HCO_3^-/H_2CO_3 buffer pair, is used to quantitatively analyze buffering by carbonic acid.

Diagnostic Value

The measurement of bicarbonate, usually in conjunction with sodium, potassium and chloride, is used in the assessment of acid-base balance resulting from metabolic or respiratory disease. Respiratory acidemia is a common sequela in birds that have respiratory compromise or are anesthetized. Unfortunately, it is currently rarely assessed or treated. See the Anion Gap section for information and Table 23.3 for summaries of acid base assessment.

Bile Acids (BA)

Method

Radioimmunoassay (RIA) and enzymatic assay are the two commonly used methods for bile acid determination. Liquid chromatography also can be used in research settings. RIAs measure non-sulfated, conjugated bile acids.[29] Though less affected by hemolysis, RIA, an antibody-based assay, will measure only an unspecified proportion of bile acids in different species. The enzymatic BA method, validated for canine, feline and human samples, measures the 3-alpha-hydroxyl group present in most BAs. This test would be expected to best approximate total BA concentration in most avian species. The value generated by RIA is generally lower than the enzymatic measurement.

The pre- and postprandial sampling used in dogs and cats would likely be ideal for birds as well. However, the crop has varying emptying times in different species, and crop stasis is common in sick birds such that standardization of postprandial sampling is impossible. If possible, a fasted sample is preferred to eliminate random postprandial increases in BA concentration. Fasting is not necessary in species that do not have a gall bladder, such as pigeons, ostriches, and most parrots.[48]

Physiology

Bile acids are a group of amphipathic salts that act as detergent molecules both to facilitate hepatic excretion of cholesterol and to solubilize lipids for intestinal absorption. They promote formation of polymolecular aggregates known as micelles, which contain hydrophobic lipid in the center and have a hydrophilic outer surface. Bile acids are absorbed in the distal small intestine into the plasma and recycled from the blood by hepatocytes (enterohepatic circulation).

Diagnostic Value

Bile acids are used to assess liver function.[29,30,43] The clinician should be aware that RIA methodologies will generally produce significantly lower numbers than enzymatic methods. Laboratories should be questioned to determine which methodology they are using. Generally, using the enzymatic method, >75 μmol/L suggests hepatic insufficiency while >100 μmol/L is diagnostic for decreased liver function. Amazon parrots normally have slightly higher BA concentration than do other companion avian species.[29] Decreased bile acids may occur as a result of compromised intestinal absorption.

Bilirubin/Biliverdin

Method

The most commonly used method for bilirubin measurement are based on the diazo reaction, first developed by Ehrlich in 1883. Diazotized sulfanilic acid (diazo reagent) reacts with bilirubin to produce two azodipyrroles, which are reddish purple at neutral pH and blue at low or high pH values. The fraction of bilirubin that reacts with sulfanilic acid in the absence of alcohol is direct bilirubin (conjugated). Total bilirubin is determined after the addition of alcohol, and indirect bilirubin (unconjugated) is determined by subtracting direct bilirubin from total bilirubin.

At this time, biliverdin is measured only by high performance liquid chromatography (HPLC) for both clinical and research purposes.

Physiology

Bilirubin is the metabolic breakdown product of heme derived primarily from senescent erythrocytes. There are three types of bilirubin: unconjugated, conjugated and a fraction irreversibly bound to protein. The unconjugated portion of bilirubin is the most clinically important fraction, as this is most likely to cause tissue damage. Birds have heme oxygenase, which converts the protoporphyrin in heme to biliverdin;[4] however, birds and reptiles have significantly decreased hepatic production of biliverdin reductase that converts biliverdin to bilirubin. Bacteria in the intestine may produce biliverdin reductase and bilirubin may be absorbed from the GI tract. Additionally, though significantly decreased, hepatic biliverdin reductase is still present in some birds.[49,61] Bilirubin and biliverdin are detoxified via the glucuronic acid pathway in the liver and excreted in bile.

Diagnostic Value

Cholestasis and liver failure generally result in increased concentrations of biliverdin in birds. Bilirubin has been previously dismissed as unhelpful in the diagnosis of liver disease. However, there are reports of increased bilirubin in severe disease states.[49] Diagnostic sensitivity and specificity should be further assessed.

Blood Urea Nitrogen (BUN)

Method

There are numerous enzymatic, chemical and electrochemical methods for measurement of urea with good specificity for the compound. Reference intervals will vary with the methodology used. BUN levels are normally low in birds and may be below the detectable limit of some (but not all) assays used in the laboratory.

Physiology

During protein catabolism, nitrogen in amino acids is converted to urea in the liver by the action of the urea cycle enzymes. Though birds are predominately uricotelic, with urea being a minor component of nitrogen excretion, many still have functional hepatic enzyme action to drive the urea cycle. Additionally, bacteria in the gut may produce urea as well as ammonia, which can be absorbed from the intestinal lumen. The majority of urea is excreted through the kidneys, with some excreted through the GI tract in bile and through the skin. Urea is highly diffusible and, in addition to initial glomerular filtration, it moves passively through the renal tubules.

Diagnostic Value

Prerenal azotemia may be observed in dehydrated birds.[36,37] In penguins, it appears that BUN is not elevated postprandially, as was uric acid.[34] On the other hand, peregrine falcons (*Falco peregrinus*) had elevated BUN and uric acid when sampled 8 hours postprandially.[44] Renal disease also has been shown to cause azotemia.[40] BUN and uric acid may be used together — as separate pieces of the puzzle with history, physical exam, urinalysis and other more invasive diagnostic tests — to adequately assess prerenal versus renal disease. Using decreased BUN concentration as an indicator of liver failure has not been assessed in avian species, but may be possible in some species such as cockatoos.

Calcium (Ionized/Free)

Method

Ion-selective analyzers[b] capable of providing immediate whole-blood determinations of free calcium, electrolytes and blood gases are widely available. Calibration solutions, samples and wash solutions are pumped through a measuring cell containing calcium ion-selective, reference and pH electrodes. Sensitive potentiometers measure the voltage differential between electrodes, while the microprocessor calibrates the system and calculates calcium concentration and pH. Most of these units function at 37° C and so any significant temperature differential will make them inaccurate. These units must be maintained with regular calibration and assessment of controls.

Physiology

The ionized or free fraction of calcium is the freely diffusible, biologically active fraction. It is generally very tightly regulated by all species. It has been shown to increase mildly during active reproductive cycles in oviparous species.[33] Regulation of plasma calcium is achieved by interactions of parathyroid hormone, active vitamin D and calcitonin.

Diagnostic Value

A general reference interval is 1.0 to 1.3 mmol/L used in avian species at University of California Davis. Free calcium concentration in normal laying hens was found to be 1.3 to 1.6 mmol/L.[33] Species-specific values should be generated within the laboratory. There has been little work currently published on ionized calcium in disease in birds, but it will likely aid in differentiation of pathologic states such as renal disease, egg binding and malnutrition. Low ionized calcium in a symptomatic, possibly seizuring animal is an indication for well-monitored, intravenous calcium administration (see Chapter 5, Calcium Metabolism).

Calcium (Total)

Method

Photometric measurement of total calcium is generally used in veterinary diagnostic laboratories, though atomic absorption methods also may be used in research facilities. The two most common dyes used to bind calcium are o-cresolphthalein complexone (CPC) and arsenazo III. The sample is acidified to release protein-bound and complexed calcium. In alkaline buffered solution, CPC forms a red chromatophore when bound to calcium that can be measured at 580 nm. High magnesium concentration, lipemia and hemolysis will increase and invalidate results.

Physiology

The vast majority of calcium is stored in the skeleton as hydroxyapatite. In blood, a large portion of calcium is free, generally a smaller portion is protein bound and the smallest fraction is complexed to anions. Oviparous species have remarkable variability of the protein-bound and complexed portions due to estrogen-induced transport of calcium-bound yolk proteins to the ovary.[56]

Diagnostic Value

Calcium concentrations are dependent on the reproductive cycle, sex and possibly season; separate reference intervals for each of these variables should be established for accurate clinical evaluation of calcium values.

Absorption, excretion and compartmentalization all affect increases and decreases in plasma calcium. Disease states affecting the reproductive, renal, and digestive tract, as well as severe nutritive disorders may change calcium concentration.

The relationship between plasma total calcium concentration and total protein and albumin concentrations has been evaluated in several bird species.[3,38,46,62] Though correlations between calcium, total protein and albumin have been found in some species, they differ markedly between species. There are significant species differences in protein-calcium correlations such that generalized adjustment formulae will not be helpful in a clinical setting where many different species are evaluated. Total plasma calcium concentration, even if corrected for the effects of protein binding, does not provide information regarding ionized calcium concentration, the physiologically active fraction.

In oviparous species, increased phosphorus and calcium concentrations are observed during egg formation in females. Generally, these occur together and the calcium:phosphorus ratio stays above one in healthy individuals. If the calcium:phosphorus ratio is below one, renal disease should be investigated.

Chloride - See Electrolytes

Cholesterol

Method

There are numerous methods for lipid and cholesterol determination, with significant laboratory variation. Enzymatic methods are most commonly used in veterinary laboratories. Generally, cholesterol ester is reacted with water in the presence of cholesteryl ester hydrolase to form whole cholesterol and fatty acid. Cholesterol then reacts with oxygen in the presence of cholesterol oxidase to form cholest-4-en-3-one and hydrogen peroxide. Hydrogen peroxide is then measured in a peroxidase-catalyzed reaction that forms a dye that can be measured at approximately 500 nm.

Physiology

This 27-carbon, steroid alcohol is found in some concentration in almost all cells and body fluids in animals, and at a much lower concentration as phytosterols in plants. There is no cholesterol in plants. Cholesterol enters the intestine from three sources, the diet, bile and intestinal secretions, and sloughed cells. Cholesterol is metabolized by pancreatic secretions, intestinal secretions, and bile to micelles and then to chylomicrons that are absorbed into lacteals across intestinal villi. Transport of cholesterol in blood occurs via lipoproteins as high-density, intermediate-density, low-density, and very low-density lipoproteins. Higher density lipoproteins have increased concentrations of cholesterol. Cholesterol is synthesized and degraded in the liver (see Chapter 4, Nutritional Considerations, Section II Nutritional Disorders).

Diagnostic Value

Cholesterol levels may be altered in a normal bird due to oviparity, as well as postprandially. It should be noted that cholesterol can be elevated in oviparous reproductively active females before eggs can be visualized on a radiograph and may be accompanied by hyperostosis. In captive psitticines, cystic ovarian disease is a common syndrome that presents with the above mentioned clinical and laboratory findings in the absence of egg-laying (see Chapter 18, Evaluating and Treating The Reproductive System). When separated by plasma gel electrophoresis, the lipoproteins that transport cholesterol migrate predominately to the alpha and beta globulin regions. Cholesterol levels are rarely diagnostic but may be useful in determination of various disease syndromes (Table 23.3). HDL and LDL cholesterols are being investigated. Preliminary studies in birds show similar trends as those seen in humans (Stanford, 2004).

Creatine Kinase (CK)

Method

Bioluminescent methods are most sensitive and use the reaction of ATP (adenine triphosphate) with luciferin/luciferase. Spectrophotometric methods are used most commonly in veterinary laboratories, ATP also is used as the rate-limiting component of the reaction. CK catalyzes the reaction between creatine phosphate and ADP (adenine diphosphate) to form creatine and ATP at a pH of 6.7 and the reverse reaction at a pH of 9. This is coupled to a hexokinase-catalyzed reaction to form NADPH (reduced form of NADP). This conversion of NADP (nicotinamide adenine dinucleotide phosphate) to NADPH can be measured at 340 nm. Excess magnesium, manganese, calcium, zinc, copper, citrate, nitrate, iodide, bromide, malonate and L-thyroxine are inhibitory to the reaction and result in decreased values.

The addition of adenosine-5-monophosphate (AMP), N acetylcysteine, EDTA and diadenosine pentaphosphate (Ap5A) has been advocated to decrease changes in accuracy due to the above compounds. Addition of these components will change the value reported and result in significant laboratory variation.

Physiology

CK is a magnesium-dependent dimeric enzyme that catalyzes the reaction of ADP and creatine phosphate (CrP) to ATP and creatine in skeletal, cardiac and smooth muscle, as well as brain. It is present in the cytosol and mitochondria of myocytes.

Diagnostic Value

CK is increased for a short, <72-hour time period following myocyte damage or necrosis in birds.[47] Consideration should be given to the fact that all muscular structures in the body may be involved in CK elevation, including skeletal muscle, cardiac muscle and, less likely, muscle of the gastrointestinal tract. In mammalian species, hypothyroid patients frequently have marked (three- to five fold) increase in CK. This has not been reported in avian species at this time, but may exist.

Electrolytes - Chloride, Potassium, Sodium

Methods

The most common method for measuring electrolytes in veterinary laboratories is the ion-specific electrode (ISE). An ion-specific membrane is reacted either directly with plasma and serum or indirectly with a large volume of diluent, and then exposed to the membrane. The potentiometer measures the change in electromotive force (charge) in comparison to a reference electrode. The microprocessor then compares the unknown sample to a standard curve created from calibrators.

This method is very different from the spectrophotometric methods generally used in smaller machines in practitioner's offices. In this reaction, a specific ionophore changes color upon binding to the electrolyte. These dyes are measured by a spectrophotometer. Actual values that are generated by the two methods may be dramatically different and result in some difference between in-house and laboratory-generated reference intervals.

ISE is less affected by hemolysis; however, hemolysis will result in significant differences in potassium concentration (generally increased in most species) due to release from intracellular erythrocyte stores.

Physiology

Water homeostasis in any organism is a fundamental dynamic function. The four major electrolytes' primary functions are: maintenance of osmotic pressure, electroneutrality and water distribution. In addition to the main negatively charged ions (anions), bicarbonate and chloride, that are used to calculate anion gap, phosphates, sulfates, lactate, trace elements and proteins, all contribute to electrical neutrality and partitioning of water. The positively charged ions (cations) are predominately sodium and potassium, with contribution from divalent ions such as calcium and magnesium. The balance of cations and anions maintains pH and regulates nervous, cardiac and muscular function. Anions and cations also are essential cofactors in numerous enzymatic reactions.

Diagnostic Value

Any disease that disturbs water homeostasis will disturb electrolyte distribution and, therefore, plasma concentration. The success of electrolyte replacement therapy, fluid therapy or any attempt to restore nervous, muscular or cardiac function is completely dependent upon accurate assessment of electrolyte abnormalities.

Fibrinogen

Method

Heat precipitation is the most common method used in private practice settings and also is used commonly in commercial laboratories. It is the least accurate method for measuring fibrinogen in mammals, and is generally considered an estimate and not a true measurement. It is likely more inaccurate in birds. Protein is measured by refractometer in non-heated plasma and compared to a protein measurement in plasma heated to 57° C for 3 minutes. The difference in plasma protein represents the precipitated fibrinogen. This is obviously not specific to fibrinogen, as any refractile compound that precipitates will cause error. Birds, in comparison to mammals, frequently have increased quantities of refractile compounds, such as glucose and lipid, which will increase the error of this method.

In veterinary diagnostic labs, a modification of the thrombin test is used to more accurately measure fibrinogen. A known, relatively dilute concentration of thrombin is mixed with fibrinogen. Thrombin enzymatically cleaves fibrinogen to form fibrin, the final step in the coagulation cascade. The fibrin formation rate is proportional to fibrinogen concentration and can be calculated from tables. The tables were derived in humans, and though they work well in small animals, they markedly underestimate fibrinogen concentration in horses. The modified thrombin test has not been validated in avian species.

Physiology

Fibrinogen (coagulation factor I) is an acute-phase protein, made by the liver, that is digested by thrombin to form fibrin. Fibrin, when crosslinked, forms the backbone of the platelet clot.

Diagnostic Value

Fibrinogen is generally increased in non-specific inflammatory states. Fibrinogen concentration was increased above the reference interval in 77% of 89 birds, representing 20 species, with confirmed bacterial disease.[24] Decreased fibrinogen may be indicative of end-stage liver failure, but is most commonly detected in mammals with

disseminated intravascular coagulation (DIC). DIC is not commonly evaluated in birds at this time. Further investigation is warranted.

Galactose Clearance (GEC)

Method

Methods to determine GEC and blood galactose concentration in cockatoos have been described.[30] The birds were administered 0.5 g/kg of sterile galactose intravenously. Single-point galactose concentrations were best correlated with galactose clearance when sampled at 80 minutes postadministration. Blood samples were deproteinized within 90 minutes of collection by the addition of 1 ml of 0.3 M perchloric acid to a 0.2 ml aliquot of blood. After centrifugation at 1500 g, the clear supernatant was stored at -20° C for preservation until galactose was measured using a commercial ultraviolet method coenzyme assay kit[c]. Galactose clearance was calculated using the formula GEC (g/min)= (M-U)($t_{c=0}$ +7) where M is the amount of galactose injected, U is the amount of galactose excreted in urine, and $t_{c=0}$ is the extrapolated time when concentration equals zero. A standard value of 6% urinary excretion was used throughout, as the author determined previously in cockatoos. At 80 minutes, the normal reference intervals were 0.05 to 0.55 g/L for single-point galactose concentration and 0.86 to 1.52 g/min galactose clearance.

Physiology

Galactose is a monosaccharide isomer of glucose that is converted to glucose in the liver through gluconeogenesis pathways for use as energy. Approximately 90% of circulating galactose is filtered from the blood by a healthy mammalian liver during the first pass effect. As hepatic function decreases, the portion of galactose filtered decreases and so the concentration of galactose measured would increase in disease states.

Diagnostic Value

Galactose clearance and galactose single-point concentrations were evaluated during a prospective study on the effects of partial hepatectomy in cockatoos.[30] In the study, galactose clearance appeared to be a more sensitive indicator of hepatic insufficiency than plasma enzyme activities or BA levels, and were able to detect an 18% loss of hepatic mass. Though single-point concentration was never increased in animals with 18% hepatectomy, it is likely that this value would increase with more significant liver dysfunction. The authors concluded that GEC has the potential to be a simple, sensitive method of screening birds for decreased hepatic function.

Gamma Glutamyltransferase (GGT)

Method

The IFCC reference method uses L-gamma-glutamyl-3 carboxy-4-nitroanilide as the glutamyl donor and glycylglycine as the acceptor in a solution of hydrochloric acid. The nitrobenozoate produced is measured at 410 nm. Other methods are still in use and may produce different values, therefore reference intervals may vary from those values stated in available texts. Lipemia may increase or decrease GGT, depending on methodology used.

Physiology

GGT catalyzes the transfer of the gamma glutamyl group from a donor peptide to an acceptor compound. It is present in serum and in low levels in the cell membrane of all cells except muscle in mammals. It may be involved in glutathione metabolism and detoxification. The primary source of plasma GGT is the biliary system, while significant levels of GGT of renal epithelium origin are found in the urine.

Diagnostic Value

Significant increases in GGT are due to obstruction of or damage to the biliary tree including neoplasia, inflammation or cholelithiasis (stones). Hepatocyte damage alone will not significantly change plasma GGT concentration. In mammals, GGT is a more sensitive indicator of biliary damage than alkaline phosphatase. GGT has previously been thought to be insensitive in the diagnosis of "liver disease," which is likely due to the fact that it will not become elevated in cases where the biliary tree is not compromised. Increased plasma GGT activity was found in the majority of pigeons with experimentally induced liver disease.[47] Marked increases in GGT activity in birds with bile duct carcinoma also have been reported.[54]

Although reference intervals have not been established, GGT values of 0 to 10 U/L are considered normal at the Schubot Exotic Bird Health Center (College Station, Texas, USA). GGT values appear slightly higher in older Amazon parrots, which may have GGT values up to 16 U/L without other evidence of liver disease. These GGT values are higher than the reported reference intervals for GGT[47] of <3 or 4 U/L in most species except Amazon parrots, which had a high normal value of 10 U/L.[40] Differences in methodologies for measuring GGT may account for the marked differences in reference intervals.

There are numerous reports of birds with bile duct carcinoma or cholangiocarcinoma in which no concurrent increase in GGT activity was reported.[14,18,27,51] It is possible that GGT was not measured, since GGT activity would be expected to increase in these hepatic diseases. The authors did not indicate if GGT had been analyzed in these cases. The clinical utility of GGT in the diagnosis of biliary conditions in birds has not been adequately evaluated.

Globulin

Method

Globulin concentration is calculated by subtracting albumin from total protein. Any error in albumin or total protein measurement will cause error in globulin concentration. Globulins can be separated by plasma gel electrophoresis. When the exact size of the protein of interest is unknown, as is commonly the case in veterinary medicine, the proteins are classified in the alpha, beta or gamma globulin fraction, dependent upon where they band on the gel.

Physiology

Any plasma protein that is not albumin or, in birds, transthyretin (pre-albumin) is classified as a globulin. Plasma globulins that have been identified in the banding pattern of birds are alpha-1-antitrypsin (alpha-1 globulin fraction); alpha-2-macroglobulin (alpha-2 globulin fraction); fibrinogen, beta-lipoprotein, transferrin, complement, and vitellogenin (beta-globulin fraction); and immunoglobulins and complement degradation products (gamma globulin fraction).[15] In oviparous females, vitellogenin and other proteins used in egg formation can increase dramatically during reproductive activity. Thus, the globulin fraction increases more than the albumin fraction, causing the Albumin:Globulin (A:G) ratio to decrease physiologically in some female birds.

Diagnostic Value

In addition to egg formation causing increased globulins, inflammatory disease states frequently result in increased globulins. Acute-phase proteins such as alpha-2-macroglobulin are produced in the liver in response to inflammatory cytokines. Generally in inflammatory states, these acute-phase proteins and immunoglobulins increase while albumin, a negative acute-phase protein, decreases. This results in a decreased A:G ratio. Gel electrophoresis allows the practitioner to examine the banding pattern and determine if the decreased A:G ratio is due to acute or chronic inflammation or egg formation. Banding patterns cannot be used to diagnose specific disease such as aspergillosis or chlamydophila infection.

Decreased globulins result from decreased production such as in liver failure, or increased loss, most commonly due to protein-losing enteropathy.

Glucose

Method

There are several methodologies used to measure glucose that vary between the types of machines used. The normally high blood glucose level of many avian species may fall above the linear range of measurement of some handheld glucometers. The practitioner should know the linear limit of their glucometer, usually found in the manufacturer's insert. Results above that limit should be written as >linear limit (#). It should be noted that many small handheld units guarantee only 20% coefficient of variation, which means that the number obtained can vary as much as 20% from the actual glucose concentration in the sample.

Most veterinary laboratories use the hexokinase method on a wet reagent analyzer, where NAD is measured at 340 nm after two reactions using hexokinase and glucose-6-phosphate dehydrogenase. This method has an upper limit of 1000 mg/ml in undiluted samples and 2000 mg/ml with automatic dilution. It is therefore the preferred method in avian species.

Lipemia and hemolysis can both increase the measured plasma glucose concentration.

Physiology

There are species differences in the way that birds regulate blood glucose. The insulin content of the pancreas of granivorous species is about one-sixth that of the mammalian pancreas, while glucagon content is about 2 to 5 times greater.[25] Pancreatectomy induces hypoglycemic crisis in granivorous birds, but produces diabetes mellitus in carnivorous birds.[40] The finding suggests that while glucagon predominates in granivorous birds, insulin may predominate in carnivorous birds. Although diabetes mellitus in psittacines is attributed to increased glucagon secretion, there have been reports of decreased insulin concentration in a diabetic African gray *(Psittacus erithacus)* in comparison with a normal bird[9] and positive responses to insulin therapy. It is therefore possible that either glucagonemia or hypoinsulinemia are responsible for diabetes in psittacines and other species.

Stress hyperglycemia is induced in birds by high levels of endogenous or exogenous glucocorticoids.

Diagnostic Value

Stress hyperglycemia should be ruled out prior to a diagnosis of diabetes mellitus. Measurement of insulin and glucagon levels in comparison to a control bird should be attempted to determine etiology of diabetes on a case-by-case basis. Etiologies of hypoglycemia should be ruled out using additional diagnostics. Consider the use of a carbohydrate absorption test (xylene or glucose challenge) to rule out underlying malabsorption/maldigestion.

Iron

Method

Care must be taken to ensure that anticoagulant collection tubes do not contain iron, EDTA, oxalate or citrate that bind iron. Some iron methods require serum and

cannot assay plasma. Check with the laboratory prior to submission. There are a variety of methods, including coulometry, colorimetry and atomic absorption spectrophotometry, that are used to measure iron. Colometry involves applying a voltage to a reaction and measuring the amount of energy needed to drive the reaction. It can be performed as a titration with another ion and is generally quite accurate in the measurement of iron. Atomic absorption is unreliable due to matrix interference in serum. Through modification of the serum iron methods, total iron-binding capacity and serum transferrin also can be determined. Ferritin, the storage form of iron that is generally measured to assess total iron stores in mammals, is measured using antibody-based ELISA and RIA techniques that do not cross-react in avian species. Ferritin, therefore, cannot be measured at this time.

Physiology

All living organisms, except possibly *Lactobacillus* spp., require iron.[57] Aside from meat-based products, most ingested iron is in the less bioavailable ferric form. The ferric form (Fe^{3+}) can be reduced to the bioavailable ferrous form (Fe^{2+}) by intestinal bacteria. Free iron can catalyze free radical formation from oxygen and nitrogen, and can therefore cause marked cellular and tissue damage. For this reason, plasma and intracellular iron are protein bound. The majority of iron in the body is bound in hemoglobin; however, iron also is bound to transferrin for transport in the plasma, ferritin and hemosiderin for cellular storage, and myoglobin in muscle. Prior to egg laying, iron levels will increase 2 to 3 times normal in some species.[28]

Diagnostic Value

Serum chemistry results in birds with hemochromatosis may include increased liver enzyme activity, usually AST, which is believed to be due to iron-induced hepatocellular damage.[40] A few anecdotal case reports describe increased serum iron concentration in sick versus control birds.[40] Other studies found no significant correlation between serum chemistry values, serum iron concentration, total iron-binding capacity and unsaturated iron-binding capacity with hepatic iron accumulation, as assessed by histopathology and iron quantification.[66] However, the reference values used in one study were considerably higher than those used in domestic mammals and human beings. Additionally, birds with possible inflammation (eg, leukocytosis, heterophilia and monocytosis) were included in the study.[66]

Although serum ferritin concentration correlates significantly with non-heme iron in the liver and spleen of dogs, cats, horses and pigs, this correlation has not been explored in birds due to the species-specificity of antibody recognition and binding in ferritin ELISAs and RIAs.[21] Additionally, percentage iron saturation has not been evaluated in birds. Further study of iron status in companion avian species may have the clinical benefit of eliminating invasive liver biopsies as a screening modality for diagnosing hemosiderosis.

Lipase

Method

Many lipase methods including titrimetric, turbidimetric, spectrophotometric, fluorometric and immunological techniques have been described, with no one method recognized as a gold standard. Differences in laboratory values are likely due to differences in methodologies. Be cautious when using reference intervals from the literature to assess a patient.

Physiology

Lipase hydrolyzes glycerol esters of emulsified, long-chain fatty acids.[55] Most lipase produced in mammals is produced in the pancreas, however activity also is seen in gastrointestinal mucosa, leukocytes and adipocytes. Tissue enzyme contributions have not been investigated in birds.

Diagnostic Value

In mammals, lipase concentration in plasma and effusive fluid is most commonly used to investigate pancreatic disorders, usually pancreatitis. It is generally more useful in acute forms of the disease, as more chronic lesions are associated with increased parenchymal destruction that results in lower levels of lipase. Renal disease can result in mildly increased plasma levels due to decreased clearance, but increases are generally not as dramatic as with pancreatitic disease.

Phosphorous

Method

In the most common method used, phosphate reacts with ammonium molybdate to form a phosphomolybdate complex. The colorless phosphomolybdate can be measured photometrically at 340 nm or can be reduced to molybdenum blue and measured at 600 to 700 nm. Measurement in the 340-nm range is more likely to be affected by hemolysis, icterus and lipemia.

Common detergents are frequently contaminated with phosphate and it should be ensured that phosphate is measured using only new, unwashed equipment. Delayed plasma separation or hemolysis will significantly alter plasma phosphorus levels.

Physiology

Inorganic and organic phosphorus both have numerous vital roles in the body. Inorganic phosphate is complexed with calcium to form hydroxyapatite in beak and bone. This structural form also functions as a phosphate storage compartment. The dissolution of phosphoric

acid in plasma is an important buffering system that complements the carbonic acid buffering system. Chemical energy in all cells depends on the high energy bond in ATP and (guanosine triphosphate) GTP (both triple phosphate molecules). Organic phosphate is an essential component in phospholipid membranes and nucleic acids, and is critical for several important enzyme systems.

Diagnostic Value

In oviparous species, increased phosphorus and calcium concentrations are observed during egg formation in females. Generally, these occur together and the calcium:phosphorus ratio stays above one in healthy individuals. If the calcium:phosphorus ratio is below one, renal or other disease (ie, primary or secondary hyperparathyroidism) should be investigated. Both hyperphosphatemia and hypophosphatemia have been associated with renal disease in birds. This electrolyte flux may represent acute and chronic forms of renal disease as in mammals. Alkalosis will cause flux of phosphate into the cell and an apparent hypophosphatemia. Some acidosis, such as diabetic ketoacidosis, results in catabolism of phosphorylated compounds in the cell with excretion of phosphate at the kidney and whole-body phosphorus depletion.

Total Protein

Method

There have been several articles written on refractometer versus biuret analysis of total protein.[1,41,42,50] The biuret method is a more specific chemical reaction where peptide bonds are reacted with cupric ions to form a colored product measured spectrophotometrically at 540 nm. The total protein value determined using a refractometer is frequently inaccurate in companion avian species due to interference by high concentrations of other light-refractive compounds in plasma, such as chromagens, lipids and glucose. Studies in chickens, turkeys and ducks have shown good correlation between protein concentrations obtained by refractometer[d] and biuret methods.[1,50] These species tend to have lower blood glucose values than most psittacines and smaller birds. Correlation studies in avian species with high blood glucose levels, such as pigeons, have shown marked discrepancies between refractometer and biuret methods, however, a different brand of refractometer[e] was used, that may have contributed to the difference in results.[41,42] There is marked variation in normal blood glucose levels in avian species. The biuret method is the most accurate method to quantify total protein in the clinical setting, where samples from many different species may be evaluated.

Physiology

The body contains thousands of proteins each having one or more functions. An increase in plasma proteins may be observed in egg-laying females.

Diagnostic Value

Though not adding information about specific disease etiology, total protein is important information used to establish supportive care. Decreased total protein may indicate the need for colloid (hetastarch) supplementation and incite a search for an underlying protein-losing nephropathy, enteropathy or liver failure. In cases of increased total protein, reproductive activity should first be ruled out in females and then dehydration or an underlying inflammatory disease state should be investigated.

Uric Acid

Method

The most commonly used method is the uricase method where uric acid is catalyzed by uricase to allantoin. The decrease in concentration of uric acid is measured at approximately 285 nm.

Physiology

Uric acid is the major nitrogenous waste product of birds. It is hypothesized that this has evolved due to oviparity.[40] Embryonic and fetal development occur within a closed compartment, the egg, that lacks diffusion of nutrients and waste. Uric acid is relatively inert and substantially less toxic than ammonia or urea, thus ensuring a viable hatchling. Uric acid (an oxidized form of the purine, hypoxanthine) is synthesized predominantly in the liver from purine metabolism, with a small amount of synthesis occurring in the renal tubules. Approximately 90% of uric acid is secreted in the proximal convoluted tubules in the normal bird.[20] This percentage can be markedly altered in pathologic conditions. Uric acid is passed to the cloaca and then may be retropulsed to the rectum and ceca, where it may be broken down by bacteria and reabsorbed.[7,8]

Diagnostic Value

Due to active renal tubular secretion, blood uric acid levels are not notably affected by dehydration until GFR is decreased to the point that uric acid is not moved through the tubules, which may occur in severe dehydration. Raptors and penguins have higher reference values for uric acid, and marked increases in plasma uric acid concentration may be observed postprandially.[34,44] Therefore, sampling of carnivorous birds should be performed after a 24-hour fast. Fasting also will decrease the likelihood of lipemia, which is frequently observed in postprandial samples. Contamination of the blood sample with trace amounts of urates from the skin of birds may lead to extreme elevations in uric acid measurements. Questionable samples should therefore be redrawn and the testing repeated. Once the above etiologies are ruled

out, renal disease should be assessed with urinalysis, radiography, and/or renal biopsy (see Chapter 16, Evaluating and Treating the Kidneys).

Urinalysis

Osmoregulation is accomplished by contributions from the kidneys, intestinal tract, salt glands and, to some extent, the skin and respiratory tract.[19,20] Urine can be actively retropulsed from the urodeum to the coprodeum of the cloaca and then to the rectum and potentially the large intestine, where water can be reabsorbed and electrolytes can be modified. This results in a change in the specific gravity, electrolyte concentrations and bacterial contamination of urine.

Urinalysis is indicated when there is azotemia, uratemia, polyuria/polydipsia, hematuric abnormal urates, or genitourinary masses. Birds with renal pathology will frequently have polyuria, resulting in a urine sample of adequate volume for analysis. Avian urine is generally collected free catch by removal of cage paper and thorough cleaning of the cage surface. A needle and syringe or capillary tube can be used to aspirate urine from the cage bottom and minimize fecal contamination. Ureteral catheterization has been performed, but, requires anesthesia and is difficult.[65]

Normal urine has a clear fluid component. There is variation in normal urine volume among species that are adapted to different food sources and environments.[10,19] Specific gravity in most clinically normal birds has been reported as 1.005 to 1.020, and avian urine is generally acidic.[6] Normal urine sediment is generally composed of uric acid precipitates and crystals, sloughed squamous epithelial cells, <3 WBC/40x field and <3 RBC/40x field, and low quantities of predominantly gram-positive bacteria. Bacteria present in normal samples are attributed to fecal contamination.

The majority of uric acid in avian urine exists as a white to light yellow colloidal suspension made up of small, spherical conglomerates (urates) that range in diameter from 0.5 to 15 μm.[6] The urate precipitate is composed of uric acid, sodium and/or potassium, and protein. The precipitate is not measured in the specific gravity of the urine supernatant, and therefore urine specific gravity is lower in birds and reptiles than in mammals. Any protein not reabsorbed in the proximal tubule is generally precipitated with uric acid. Assuming there is no fecal contamination, normal avian urine should not contain protein that can be detected on a urine dipstick.[32]

Needle-shaped uric acid crystals also may be observed in normal urine. Uric acid crystals polarize and can be tested chemically using the murexide test. A drop of concentrated nitric acid is added to the crystals and heated to evaporation. A drop of ammonia is then added. If uric acid is present, the liquid will turn a mauve color. Adding several drops of sodium hydroxide to a urine sample will dissolve uric acid crystals. This can facilitate the identification of casts, bacteria and cells.

Biliverdinuria, grossly apparent as green urates, is not a normal finding and is most commonly caused by bile stasis due to liver compromise. It also may be seen in birds with hemolytic anemia. Biliverdinuria associated with nephrosis has been described histologically.[53] The clinical significance of the described nephrosis is unknown. Prior to diagnosing biliverdinuria, fecal contamination should be assessed by measuring urobilinogen with a urine dipstick. A positive urobilinogen result supports fecal contamination.

Hemoglobinuria has been documented in heavy metal poisoning, specifically lead toxicosis, in Amazon, Electus and African grey parrots secondary to intravascular hemolysis.[17] Ketonuria is not observed in normal birds. Ketones have been found in the urine of migratory birds, but otherwise support a diagnosis of diabetes mellitus.[9,63] (See acetoacetate, acetone in the Analytes section).

Even in free catch samples, culture and sensitivity are indicated when bacterial infection (eg, pyuria) is suspected based on clinical presentation or urinalysis results. Renal biopsy and culture also can be performed if inflammation or infection is believed to involve the kidney (see Chapter 16, Evaluating and Treating the Kidneys).

Products Mentioned in the Text

a. Vacutainer tubes, Becton Dickinson, Franklin Lakes, NJ, USA
b. I-STAT, Heska Corporation, Fort Collins, CO, USA
c. Lactose/D-galactose assay kit, Boehringer Mannheim, Mannheim, Germany
d. AO Goldberg Refractometer, American Optical Corporation, Buffalo, NY, USA
e. Atago Refractometer, Atago Corporation, Atago Ltd, Tokyo, Japan

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