
T. W. Campbell

Department of Clinical Sciences, College of Veterinary Medicine & Biomedical Sciences, Colorado State University, Fort Collins, CO, USA.

Introduction

Blood biochemistry profiles are often used to assess the physiologic status of lower vertebrate patients, such as fish, amphibians, reptiles, and birds. However, there is a general lack of controlled studies designed to clarify the meaning of changes in the blood chemistries of these animals compared to those of domestic mammals. Therefore, the clinical chemistry of lower vertebrates has not achieved the same degree of critical evaluation as demonstrated in domestic mammalian medicine. Currently, interpretations of blood biochemistries of these animals are considered the same as for domestic mammals with the consideration that external factors, such as environmental conditions, have a greater influence on the normal physiology and health of the ectothermic vertebrates [ectotherms] compared to endotherms. Species, age, gender, nutritional status, season, and physiological status influence blood biochemistries of the lower vertebrates, especially the ectothermic species [1-3]. This makes interpretation of blood biochemistry results challenging. Normal reference values for specific blood biochemical tests for a few species of lower vertebrates have been reported. Environmental conditions and physiological parameters such as nutritional status, gender and age often have not been taken into consideration when establishing reference intervals, making them less meaningful. Methods of sample collection, handling, and biochemical analysis are additional sources of variation in the published reference values. Therefore, published references are generally used as a broad guide to interpretation of blood biochemical results in lower vertebrates. Because of the difficulty in obtaining meaningful reference intervals for these exotic species seen in clinical practice, most clinicians utilize decision levels when assessing their lower vertebrate patients. Decision levels are threshold values above or below which a decision is made to respond to a value of an analyte. The response may vary from repeating the test or ordering additional tests to treatment of the patient. Decision levels may be obtained by utilizing published reference intervals and applying the values to those obtained in the laboratory used by the hospital. Decision levels may vary to some degree among clinicians, depending upon laboratory results and experience. Values suggested in this text are general guidelines that can be used as decision levels when evaluating each analyte in the avian and reptilian blood biochemical profile. The process of evaluating the blood chemistries of a avian or reptilian patient can be refined by obtaining a set of normal values from that patient housed under a given set of environmental and nutritional parameters. Therefore, when that patient becomes ill, a more meaningful set of reference values specific for that patient can be used to evaluate the chemistry results.

Sample Collection and Handling

Blood samples for biochemical studies can be collected from lower vertebrates using a variety of methods depending upon the species, volume needed, size of the patient, physiological condition of the patient, and preference of the collector [4]. Depending upon the site of blood collection, blood samples collected from some species, such as reptiles, are often contaminated with lymphatic fluid. Most of the analytes such as glucose, calcium, phosphorus, sodium, urea, and enzymes in lymph are comparable with that of plasma or serum in reptiles; however, there is a significantly lower concentration of total protein and potassium in lymph compared to blood [5,6]. Therefore, the amount of lymph contamination of the blood sample should be considered when interpreting blood biochemical parameters of reptiles and other patients. Many clinicians prefer to collect blood using an anticoagulant, such as lithium heparin, for blood biochemical testing of lower vertebrates, primarily because a greater sample volume can be achieved for plasma compared to serum. Collection of blood into lithium heparin also allows for the evaluation of the hemogram and blood biochemistries using one sample. Plasma is preferred over serum.
because clot formation in some animals, such as birds and reptilian blood is unpredictable and often prolonged, resulting in significant changes in some of the chemistries such as serum electrolytes. The blood of some animals, such as reptiles, clots slowly because of low intrinsic thromboplastin activity and a strong natural circulating antithrombin factor, which compensates for sluggish blood flow. Often, the sample size collected from small patients is sufficient enough for only a few tests rather than a complete panel. Therefore, the clinician must decide which tests would be most beneficial in the evaluation of the patient. Blood biochemical tests that appear to be most useful in the evaluation of lower vertebrates include total protein, glucose, uric acid, aspartate aminotransferase [AST], creatine kinase [CK], calcium, and phosphorus. Other tests that may also be helpful include creatine, sodium, potassium, chloride, total CO₂, and protein electrophoresis. Many modern blood chemistry analyzers require a small sample size [10 - 30 ul] to perform many of these tests. Commercial veterinary laboratories often offer chemistry profiles that require a minimal amount of serum or plasma [0.5 ml]. Blood chemistry analyzers that utilize dry reagents and reflectance photometry for "in-house" testing may be used for these small samples.

The plasma of most animals is colorless; however, it may be orange to yellow owing to carotenoid pigments in the diets of herbivores [7]. The plasma of some carnivores, such as pythons, may be a greenish yellow owing to carotenoids and riboflavin from their prey. Some lizards normally have green plasma because of high plasma concentrations of biliverdin [7].

**Laboratory Evaluation of the Kidneys**

The reptilian renal cortex contains only simple nephrons [cortical nephrons] that have a tubular system devoid of loops of Henle; therefore, reptiles are unable to concentrate their urine. The avian kidney has a mixture of cortical [reptilian] and medullary [mammalian] nephrons. Nitrogenous wastes excreted by the reptilian kidney include variable amounts of uric acid, urea, and ammonia, depending upon the animal's natural environment. Freshwater turtles that spend much of their life in water excrete equal amounts of ammonia and urea, whereas those with amphibious habits excrete more urea [8]. Sea turtles excrete uric acid, ammonia, and urea and alligators excrete ammonia and uric acid [8]. Terrestrial reptiles such as tortoises must conserve water. Ammonia, urea, and other soluble urinary nitrogenous wastes require large amounts of water for excretion; therefore, terrestrial reptiles produce more insoluble nitrogenous waste in the form of uric acid and urate salts, which are eliminated in a semisolid state. The same is true with birds. Blood biochemical detection of renal disease in lower vertebrates is more difficult than that of mammals because of the physiological differences in their kidneys; for example, blood urea nitrogen [BUN] and creatinine concentrations are generally poor indicators of renal disease in these animals. The normal BUN value of most birds and reptiles is low [<10 mg/dl]. Plasma urea nitrogen may be more useful in the evaluation of renal disease in aquatic reptiles that excrete primarily urea. Because terrestrial reptiles and birds are primarily uricotelic, normal urea nitrogen concentration in these species is less than 10 mg/dl, with the exception of terrestrial chelonians, especially desert species, that typically have plasma urea nitrogen concentrations that can normally vary from 30 to 100 mg/dl. This is considered to be a mechanism to elevate the plasma osmolarity to reduce water loss from the body [9]. The plasma osmolarity of freshwater turtles and crocodilians is approximately the same as that of common domestic mammals but is higher in terrestrial reptiles. An increase in plasma urea nitrogen concentration may be suggestive of severe renal disease, prerenal azotemia, or high dietary urea intake in birds and reptiles. However, blood urea nitrogen does not reliably increase under these conditions. Creatinine is a normal constituent of the urine of mammals, but the amount formed in most birds and reptiles is negligible [<1 mg/dl]. Plasma creatinine concentration is generally considered to be of poor diagnostic value in the detection of renal disease in birds and reptiles. While blood creatine concentration may be of diagnostic value in the detection of renal disease in some of these animals; however, the test is unavailable from most veterinary laboratories. Uric acid is the primary catabolic end product of protein, nonprotein nitrogen, and purines in terrestrial reptiles and birds. It represents 80 to 90% of the total nitrogen excreted by the kidneys [10]. The normal blood uric acid concentration for most birds and reptiles is less than 10 mg/dl. Hyperuricemia is indicated by uric acid values greater than 15 mg/dl and is usually associated with renal disease, such as those caused by severe bacteremia, septicemia, nephrocalcinosis, and nephrotoxicity. Plasma uric acid is neither sensitive nor specific for renal disease in birds and reptiles. Hyperuricemia associated with renal disease most likely reflects loss of two-thirds or more of the functional renal mass and hyperuricemia can also be associated with gout or recent ingestion of a high protein diet. Carnivorous birds and reptiles tend to have higher blood uric acid concentrations than do herbivorous birds and reptiles. Carnivorous reptiles, such as snakes, exhibit a postprandial hyperuricemia that will generally peak the day following a meal resulting in a 1.5 to 2.0 fold increase in uric acid [10]. Gout can result from an overproduction of uric acid [primary gout] or an acquired disease that interferes with the normal production and excretion of uric acid [secondary gout]. Conditions that result in secondary gout in birds and reptiles include starvation, renal disease [especially tubular damage], severe prolonged dehydration, and excessive dietary purines [i.e. herbivorous reptiles fed diets rich in animal proteins]. Hyperuricemia associated with renal disease and gout will often result in greater than two fold increases in uric acid concentrations.
Water Balance
The species, diet, and environmental conditions, such as temperature and humidity influence water consumption of animals. For example, desert reptiles require less water than temperate and tropical species. Some reptiles have developed methods for conserving water [11]. For example, tortoises and some lizards store water in the urinary bladder. Many reptiles can achieve water uptake through the cloaca by soaking [8]. Water is also conserved in birds and reptiles by the elimination of nitrogenous waste in the form of uric acid and urate salts, which are excreted in a semisolid state.

Sodium and Chloride
Dietary sodium is absorbed in the intestines and transported to the kidneys where it is excreted or resorbed depending upon the animal's need for sodium. In birds and reptiles, sodium and potassium metabolism involves an active renin-angiotensin system with direct action upon osmoregulation. Some birds and reptiles have nasal salt glands that participate in the regulation of sodium, potassium, and chloride in the blood. Therefore, disorders of the salt gland may affect electrolyte balance.

The normal serum or plasma sodium concentration of most birds and reptiles ranges between 130 and 160 mEq/L. There are exceptions, for example, sea turtles tend to have higher normal sodium plasma concentrations that range between 150 and 170 mEq/L. Hyponatremia can result from excessive sodium loss associated with disorders of the gastrointestinal tract [i.e. diarrhea], kidneys, or possibly the salt gland. Iatrogenic hyponatremia can occur with overhydration of the patient with intravenous or intracoelomic fluids that are low in sodium. Hypernatremia results from dehydration, either from excessive water loss or inadequate water intake, and excessive dietary salt intake.

Chloride is the principle anion in the blood and, along with sodium, represents the primary osmotically active component of plasma in most birds and reptiles. Normal serum or plasma chloride concentration of birds and reptiles varies among species, but generally ranges between 100 and 120 mEq/L. Hypochloremia is rare and suggests excessive loss of chloride ions or overhydration with fluids low in chloride ions. Hyperchloremia is associated with dehydration and possibly renal tubular disease or disorders of the salt glands.

Potassium
Normal serum or plasma potassium concentrations also vary with species. Plasma potassium of reptiles generally ranges between 2 and 6 mEq/L. Plasma potassium ranges between 2 and 4 mEq/L in most birds. False elevations in plasma potassium concentration occur with hemolysis or failure to quickly separate the plasma from the cells. Hyperkalemia is associated with renal failure, acidosis, excessive dietary potassium intake, and severe tissue necrosis. Hypokalemia occurs with chronic diarrhea, prolonged anorexia, alkalosis, and use of potassium poor fluid therapy.

Acid/Base
The normal blood pH of birds is maintained between 7.33 and 7.45, and the buffering systems that regulate blood pH in mammals appear to be present in birds. The normal blood pH of turtles and most other reptiles ranges between 7.5 and 7.7 at 23 to 25 ºC [8]. Normal blood pH of some snakes and lizards may fall below 7.4. The blood pH of reptiles and other ectotherms is labile and changes with temperature fluctuations. An increase in temperature or excitement can cause blood pH to decrease. The blood pH of reptiles may increase during anesthesia from a normal pH of 7.5 to 7.6 to a pH of 7.7 to 7.8. As in mammals, the oxygen dissociation curve for reptilian hemoglobin shifts to the left as the pH increases, resulting in an increase in the affinity of hemoglobin for oxygen but decrease release to tissues. The buffering systems that regulate the blood pH in mammals are most likely the same in reptiles with the bicarbonate/carbonic acid buffer system being the most important because of the rapid rate of CO₂ elimination by the lungs following the conversion from H₂CO₃. Total plasma CO₂ or bicarbonate concentrations are rarely reported in reptiles and other ectotherms; however, normal total CO₂ values for most reptiles would be expected to range between 20 and 30 mmol/L. A marked fasting physiologic metabolic alkalosis occurs in postprandial alligators owing to an anion shift with bicarbonate replacing chloride in the blood, as chloride is lost as HCl in gastric secretions [12]. Therefore, there is a postprandial decrease of chloride and increase of bicarbonate concentrations in some ectotherms, such as alligators [12].

Calcium and Phosphorus
Blood calcium metabolism and the amount of ionized calcium in the plasma of birds and reptiles is mediated by parathormone [PTH], calcitonin [CT], and activated vitamin D₃ [1,25 dihydrocholecalciferol]. Other hormones, such as estrogen, thyroxin, and glucagon may also influence calcium metabolism in these animals. The primary function of PTH is to maintain normal blood calcium levels by its action on bone, kidneys, and intestinal mucosa. Low blood ionized calcium
stimulates the release of PTH, which results in the calcium mobilization from bone, increased calcium absorption from the intestines, and increased calcium reabsorption from the kidneys. The exact role of calcitonin in lower vertebrates is unknown, but it most likely has a physiological role opposite that of PTH. Increases in blood calcium stimulate the release of calcitonin from the ultimobranchial gland, which inhibits calcium reabsorption from bone. The active form of vitamin D₃ stimulates calcium and phosphorus absorption by the intestinal mucosa. Photochemical production of the active form of vitamin D₃ by exposure to ultraviolet [UV] radiation [290 to 320 nm wavelength] is believed to be essential for normal calcium metabolism in reptiles, especially basking species. Female reptiles exhibit features of calcium metabolism similar to those of birds during egg production. During egg development, female reptiles exhibit hypercalcemia in response to estrogen and reproductive activity. The increase in the total plasma calcium is associated with an increase in protein-bound calcium during follicular development prior to ovulation and may increase two to four-fold. The total blood calcium concentration of a laying hen ranges between 20 and 30 mg/dl. The normal plasma concentration of calcium for most birds and reptiles ranges between 8 and 11 mg/dl. The normal plasma calcium concentration varies with species and physiologic status of the reptile and most likely other ectotherms. For example, some species of tortoises have low blood calcium concentrations [i.e. less than 8 mg/dl]. Hypocalcemia can occur with dietary calcium and vitamin D₃ deficiencies, excessive dietary phosphorus, alkalois, hypoalbuminemia, or hypoparathyroidism. Secondary nutritional hyperparathyroidism is a common disorder of herbivorous reptiles. Herbivorous diets are often deficient in calcium and contain excessive amounts of phosphorus. Also, dietary deficiency in vitamin D₃ or lack of proper exposure to ultraviolet light will predispose reptiles to hypocalcemia. Carnivorous birds and reptiles fed all meat calcium-deficient diets will also develop hypocalcemia associated with nutritional imbalances in calcium and phosphorus. Secondary renal hyperparathyroidism may also result in hypocalcemia in these animals. Hypercalcemia in birds and reptiles occurs with excessive dietary or parenteral vitamin D₃ and calcium. This is typically an idrogenic condition associated with oversupplementation of calcium and vitamin D₃. Other differentials for hypercalcemia include primary hyperparathyroidism, pseudohyperparathyroidism, and osteolytic bone disease; however, these disorders are rarely reported in lower vertebrates.

Normal plasma phosphorus concentration for most reptiles ranges between 1 and 5 mg/dl, whereas most birds range between 5 and 7 mg/dl. Hypophosphatemia may result from starvation or nutritional deficiency of phosphorus. Disorders resulting in hyperphosphatemia include excessive dietary phosphorus, hypervitaminosis D₃, and renal disease. Rare causes of hyperphosphatemia include severe tissue trauma and osteolytic bone disease. A factitious hyperphosphatemia can occur when the serum or plasma is not promptly separated from the clot allowing phosphorus to be released from erythrocytes.

Laboratory Evaluation of the Liver
Liver enzymes in birds and reptiles appear to be similar to those of mammals. The LD and AST activities are high in liver tissue of birds and reptiles and perhaps other ectotherms. Although few critical studies have been applied to the biochemical testing of the blood of ectotherms to evaluate hepatic disease, increases in these enzyme activities in plasma may suggest hepatocellular disease. Plasma AST is not considered to be organ-specific because its activity can be found in many tissues. In general, normal plasma AST activity for birds and reptiles is less than 275 IU/L. Increased plasma AST activity suggests hepatic or muscle injury. However, generalized diseases, such as septicemia or toxemia may damage these tissues resulting in increased plasma AST activity. Plasma LD is considered to have a wide tissue distribution in birds and reptiles. Therefore, increases in plasma LD [activity greater than 1,000 IU/L] may be associated with damage to the liver, skeletal muscle, or cardiac muscle. Hemolysis may also result in increased plasma LD activity. Like AST, plasma ALT is not considered to be organ specific in lower vertebrates. Normal plasma ALT activity for reptiles is usually less than 20 IU/L and less than 50 IU/L in birds. Although ALT activity occurs in the liver of these animals, increases in plasma ALT activity may not be as reliable in the detection of hepatocellular disease as increases in plasma AST or LD activity. Plasma ALT activity may be more useful for the detection of liver disease in carnivorous birds than the non-carnivores. Alkaline phosphatase [AP] is also widely distributed in the body of birds and reptiles and plasma activity of this enzyme is not considered to be organ-specific. In birds, the plasma AP activity primarily results from osteoblastic activity and does not appear to be useful in the detection of hepatobiliary disease. There is little information concerning the interpretation of increased plasma AP activity in reptiles; however, increased activity may also reflect an increase in osteoblastic activity.

Biliverdin, a green bile pigment, is generally considered to be the primary end product of hemoglobin catabolism in birds and reptiles. Green plasma results from the accumulation of biliverdin in the blood and is usually a pathological finding suggestive of hepatobiliary disease in these animals. However, a nonpathologic accumulation of biliverdin in the blood of some species of reptiles and perhaps other ectotherms that are rarely presented for clinical evaluation can occur [7]. The physiological advantage of this is not known. Biliverdin appears to be less toxic to tissues compared to bilirubin and the normal biliverdin concentration in the plasma of some species of lizards can be greater than 1000 umol/L [7]. Bile acid determination is a sensitive test for liver function in some species of birds, but has been poorly studied in other lower
Laboratory Evaluation of Plasma and Serum Proteins

The normal plasma total protein concentration of lower vertebrates is generally lower than that of mammals. Normal reptiles generally range between 3 and 7 gm/dl, whereas normal birds generally range between 2.5 and 4.5 g/dl. Female birds and reptiles demonstrate marked increases in plasma total protein concentration during active folliculogenesis. This estrogen-induced hyperproteinemia is associated with an increase in proteins, primarily globulins, necessary for yolk production. The plasma total protein concentration returns to normal following ovulation.

The biuret method is the most accurate method for determination of plasma or serum total protein concentration, although the refractometer method is commonly used to rapidly estimate plasma protein concentration in avian and reptilian blood. Protein electrophoresis provides an accurate assessment of serum or plasma albumin concentration in avian and reptilian blood. Absolute concentrations of the various plasma proteins are obtained by determination of the total protein using the biuret method in conjunction with electrophoretic separation of the proteins.

Hyperproteinemia occurs with dehydration or hyperglobulinemia associated with chronic inflammatory diseases. The alpha, beta, and gamma globulins may increase with infectious diseases. Hypoproteinemia is commonly associated with chronic malnutrition. However, other causes, such as malabsorption, maldigestion, protein-losing enteropathies, severe blood loss, and chronic hepatic or renal disease should also be considered.

Laboratory Evaluation of Glucose Metabolism

Normal blood glucose concentration of most reptiles ranges between 60 and 100 mg/dl, but is subject to marked physiologic variation. Normal blood glucose concentration of most birds ranges between 200 and 500 mg/dl. The blood glucose concentration of normal reptiles and perhaps other ectotherms varies with species, nutritional status, and environmental conditions. For example, an increase in temperature results in hypoglycemia in turtles, but hyperglycemia in alligators. Normal oral glucose tolerance curves in reptiles differ among the species and with temperature. Reptiles have pancreatic beta and alpha cell types as a source of insulin and glucagon, respectively, similar to mammals and other vertebrates [13]. The action of these hormones is affected by temperature.

Common causes of hypoglycemia include starvation and malnutrition, severe hepatobiliary disease, and septicemia. Hyperglycemia is often a result of iatrogenic delivery of excessive glucose. A persistent, marked hyperglycemia and glucosuria is suggestive of diabetes mellitus, a rarely reported disorder of reptiles, but sometimes reported in some species of birds. Hyperglycemia may also occur with glucocorticosteroid excess.

Laboratory Detection of Muscle Injury

Creatinine kinase is considered to be a muscle-specific enzyme and is used to test for muscle cell damage. Increases in plasma CK activity can result from muscle cell injury or exertion. Elevations in plasma CK are frequently observed in birds and reptiles that are struggling to resist restraint during blood collection or are exhibiting seizure activity. Increased plasma CK activity resulting from muscle cell damage occurs with traumatic injury, intramuscular injections of irritating drugs or fluids, and systemic infections that affect skeletal or cardiac muscle. Muscle injury will also result in mild to moderate increases in plasma AST and LD activities. However, these enzymes are not organ-specific for muscle and could increase with hepatobiliary disease. When plasma CK activity is not increased in the face of increased AST and LD activity, hepatobiliary disease should be suspected. Damage to both liver and skeletal muscle can occur simultaneously such as occurs with trauma and septicemia, which would result in elevations of plasma AST, LD, and CK.
References


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