Clinical Chemistry of Companion Avian Species: A Review

Kendal E. Harr

Birds have evolved alternate physiologic strategies to contend with dehydration, starvation, malnutrition, and reproduction. Basic anatomic and functional differences between birds and mammals impact clinical chemistry values and their evaluation. Interpretation of the results of standard biochemical analyses, including BUN, alanine aminotransferase, aspartate aminotransferase, creatine kinase, gamma glutamyltransferase, bilirubin, ammonia, alkaline phosphatase, cholesterol, bile acids, glucose, albumin, globulins, calcium, phosphorus, prealbumin (transthyretin), fibrinogen, iron, and ferritin, is reviewed and discussed in relation to these physiological differences. The use and interpretation of alternative analytes appropriate for avian species, such as uric acid, biliverdin, glutamate dehydrogenase, and galactose clearance, also are reviewed. Normal avian urine and appropriate use of urinalysis, an integral part of laboratory diagnosis in mammalian species that frequently is omitted from avian diagnostic protocols, is discussed. (*Vet Clin Pathol.* 2002;31:140-151)

Key Words: Avian, chemistry, electrolytes, enzymes, metabolites, proteins, sample collection

Introduction

In the past 20 years, the number of veterinarians practicing companion avian medicine has increased exponentially in response to the increased numbers of pet birds owned in the United States. Bird owners are looking for veterinarians that can provide quality medical attention. Plasma biochemistry along with hematology is the cornerstone of medical diagnosis of disease. Veterinarians therefore have an increasing need for accurate and useful biochemical analyses for avian and exotic animal species. Veterinary laboratories and clinical pathologists must meet these expectations and develop the techniques and services needed for avian biochemical analysis and interpretation.

Biochemical reference intervals for common species of psittacines (parrots), passerines (canaries and finches), and galliformes (turkeys and chicken) have been established by specialized laboratories, eg, California Avian Laboratory, Citrus Heights, Calif. Reference intervals should be developed by each laboratory analyzing avian samples, but are still lacking in some university and private laboratories. Although a great deal now is known regarding avian medicine, research into the diagnostic application, sensitivity, specificity, and positive and negative predictive values of biochemical analytes still is needed.

Physiological and anatomical differences sometimes require the use of different analytes in birds compared with mammals (eg, uric acid versus BUN). Therefore, basic knowledge of avian physiology is vital to appropriate interpretation of blood chemistry values. This article is organized by organ system, with a review of applicable chemical methodology and basic avian anatomic and physiological differences. This literature review will likely lead to more questions than answers, but hopefully, in the next 20 years, we will be able to answer those questions.

Sample Collection

Collection of blood volume equivalent to 1% of a bird's body weight is usually not associated with any adverse effect. Illness, such as anemia or dehydration, should be considered when determining appropriate sample volume. Adequate blood volume for laboratory testing can usually be obtained as long as the animal weighs more

From Florida Veterinary Pathology, Inc, 400 NW 122nd Street, Gainesville, FL 32607 (harrk@mail.vetmed.ufl.edu).

than 200 g, as in many common avian species. However, some of the smaller species, eg, the average 30-g budgerigar, may not yield enough plasma for complete biochemical analysis. 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.

In the United States, many exotic animal practitioners perform venipuncture using a needle that has been heparinized 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 heparin. Reducing the amount of heparin in the sample is desirable when using automated hematology analyzers, such as the Cell Dyn 3500 (Abbott Laboratories, Abbott Park, Ill) because of a decreased coefficient of variation of the WBC count[1] as well as decreasing potential dilution and assay artifact in a biochemical sample. If most of the heparin is expelled from the needle hub, 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.[2-4] Samples for biochemical analyses should be placed into lithium heparin Microtainer or Vacutainer tubes (Becton Dickinson, Franklin Lakes, NJ, USA) filled to the appropriate volume to avoid variable clotting time and gelling of serum.

Avian plasma samples frequently are yellow due to carotenoid pigments, not bilirubin.[5] Pink or red plasma is usually indicative of hemolysis. When working with smaller species of birds, tuberculin or insulin syringes are frequently used, however, not all of these syringes have detachable needles. Ejecting blood through a 25-gauge or smaller needle may cause moderate to marked hemolysis that will invalidate many biochemical analyses.[6] Attached needles can easily be cut from the syringe using a pair of large veterinary nail clippers or scissors. Insulin syringes also are available with detachable needles.

The collection and handling of avian blood are important for obtaining accurate results. Discussion with the practitioner about appropriate materials, venipuncture technique, and sample handling can significantly improve the quality of the sample and therefore the result.

Genitourinary Tract

Renal function and osmoregulation

There are marked differences in renal function and osmoregulation among mammalian, avian, and reptilian species. The avian kidney lacks a renal pelvis and distinct demarcation of cortical and medullary regions on gross examination.[7,8] There are 2 distinct types of nephrons in birds: the mammalian-type nephron and the "reptilian nephron". The reptilian nephron has a less convoluted glomerulus and lacks a loop of Henle. Mammalian-type nephrons are located more deeply than reptilian types. Urine flows from the distal tubules to collecting ducts to the ureter that empties into the urodeum of the cloaca. Although individual nephrons in avian species have low glomerular filtration rates (GFRs), there are more nephrons per kidney in birds than in mammals, such that overall GFR is similar.

Studying blood flow to the avian kidney is difficult due to the complexity and inaccessibility of the renal vasculature.[7] Therefore, a reduced avian kidney model with a single arterial supply and accessible renal vein has been used. This model has limitations, however, in its ability to accurately assess blood flow and clearance. Up to 50% of renal blood flow comes from the ischiac and external iliac veins that comprise the portal system.[9] The percentage of portal circulation filtered by the kidney varies with species, stress, and temperature. Portal circulation is of significant clinical importance when injections or intravenous fluid are administered in the leg or lumbar region, because medication will be transported directly to the kidneys and filtered, magnifying any nephrotoxic effects and preventing adequate medication from reaching the site needing treatment.

The avian kidney filters a large volume of fluid (approximately 11 times the entire body water each day for a 100-g bird) and then reclaims most filtered water by tubular reabsorption.[8] The GFR decreases with dehydration, and with injection of arginine vasotocin, the avian analog of mammalian antidiuretic hormone.[10,11] Avian kidneys shunt blood from reptilian- to mammalian-type nephrons when GFR is decreased, increasing a bird's ability to concentrate urine.[12] The ability of the avian kidney to concentrate urine appears to vary inversely with size, ie, smaller birds have a greater ability to concentrate urine.[13]

Osmoregulation in birds is accomplished by contributions from the kidneys, intestinal tract, salt glands, and, to some extent, the skin and respiratory tract.[8,14] 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.[10] This affects the specific gravity, electrolyte concentrations, and bacterial contamination of urine.

Uric acid is the major nitrogenous waste product of birds. It is hypothesized that this evolved due to oviparity.[15] 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 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.[7,8,16] This percentage can be markedly altered in pathologic conditions.

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.[16] Raptors and other carnivores have higher reference values for uric acid, and marked increases in plasma uric acid concentration may be observed postprandially.[17-19] Therefore, sampling of carnivorous birds should be performed after a 24-hour fast. Fasting also will decrease the likelihood of lipemia, which frequently is observed in postprandial samples.

Both BUN and creatinine levels are normally low in birds and may be below the minimum detectable limit of the assays in the laboratory (Table 1). Prerenal azotemia may be observed in dehydrated birds.[20,21] In one study in penguins it was shown that BUN was not elevated postprandially, as was uric acid.[22] In another study in peregrine falcons (*Falco peregrinus*) both urea and uric acid increased above normal reference intervals in postprandial samples within 8 hours.[23] Azotemia also has been documented in birds with renal disease.[15] It may be useful to evaluate BUN and uric acid together to differentiate among dehydration, postprandial effects, and renal pathology. Differentiating pathologic conditions from prerenal azotemia also should be done using history, physical exam, and the results of other chemical tests and diagnostic techniques.[24]

Ammonia may comprise up to 25% of the total nitrogen in urine. Blood ammonia concentration has not been evaluated for diagnostic relevance in companion avian species. Acid-base alterations may result in increased ammonia concentration in birds.[25]

Urinalysis

Urinalysis is indicated when there is azotemia, uratemia, polyuria/polydipsia, abnormal urates, or genitourinary masses. Birds with renal pathology frequently will have polyuria, resulting in a urine sample of adequate volume for analysis. Avian urine is generally collected from a voided sample 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 and minimize fecal contamination. Ureteral catheterization has been performed but requires anesthesia and is difficult.[26]

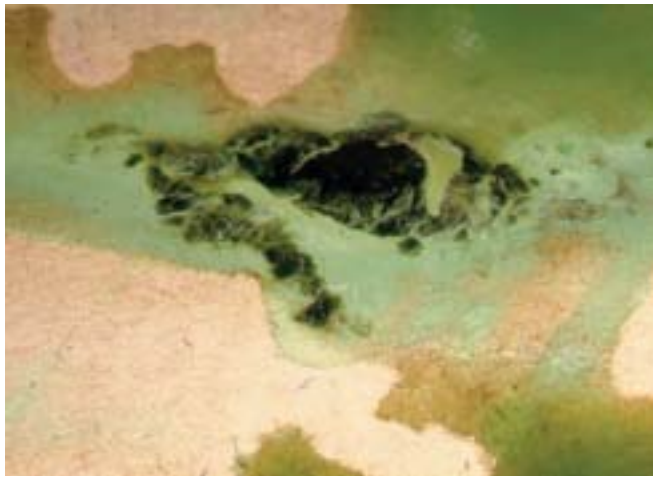

Figure 1. Biliverdinuria in excrement from an Amazon parrot. Note the green coloration of the urates, which normally are white.

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.[14,27] Specific gravity in most clinically normal birds has been reported as 1.005-1.020, and avian urine is usually acidic.[19] Normal urine sediment is composed of uric acid precipitates and crystals, sloughed squamous epithelial cells, <3 WBC/×40 field, <3 RBC/×40 field, and low quantities of predominantly gram-positive bacteria. Bacteria in otherwise normal urine samples are attributable to fecal contamination.

The majority of uric acid in avian urine exists as a white to light yellow colloidal suspension (urates) made up of small spherical conglomerates that range in diameter from 0.5 to 15 µm.[19] 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 tends to be 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 detectable protein on a urine dipstick.[28] Needle-shaped uric acid crystals also may be observed in normal avian urine.[19,29] Uric acid crystals polarize and can be tested chemically using the murexide test.[15] For this 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. Uric acid crystals can be dissolved by adding several drops of sodium hydroxide to a urine sample.[30] This can facilitate the identification of casts, bacteria, and cells.

Biliverdinuria is not a normal finding and is most

commonly caused by liver compromise resulting in bile stasis due to inflammation, infection, neoplasia, toxic insult, or lipidosis (Figure 1).[31] It also may be seen in birds with hemolytic anemia.[30] Biliverdinuria associated with nephrosis has been described histologically.[32] Prior to diagnosing biliverdinuria, one should evaluate whether fecal contamination is present, by measuring urobilinogen with a urine dipstick. A positive urobilinogen result supports positive fecal contamination.

Hemoglobinuria has been documented in heavy-metal poisoning, specifically lead toxicosis, in Amazon and African grey parrots secondary to intravascular hemolysis.[33] Ketonuria is not observed in normal birds.[34] Ketones have been found in the urine of migratory birds, but otherwise support a diagnosis of diabetes mellitus.[34-36]

Even in free catch samples, culture and sensitivity are indicated when bacterial infection is suspected based on clinical presentation or urinalysis results, eg, pyuria. Renal biopsy and culture can also be performed if inflammation or infection is believed to involve the kidney. Pathologic disorders of the avian genitourinary system have been well-reviewed by Phalen.[32]

Liver, Muscle, and Bone

Enzymes

Liver disease is common in avian species,[31] however, interpretation of results for standard hepatic analytes used in dogs and cats must be modified due to physiological differences in birds.

Aspartate aminotransferase (AST) is not specific for hepatocellular damage but is highly sensitive in detecting liver damage caused by ethylene glycol in pigeons.[15] Plasma AST activity returned to normal reference values within 100 hours after doxycycline-induced muscle trauma in pigeons. AST activity currently is considered to be a very sensitive but nonspecific indicator of hepatocellular disease in other avian species as well, and is frequently used with the muscle-specific enzyme, creatine kinase (CK) to differentiate between liver and muscle damage.[37,38]

As in horses and ruminants, alanine aminotransferase (ALT) is found in hepatocyte cytosol as well as in muscle and other tissues of birds.[39] Intramuscular injection of doxycycline in pigeons caused plasma ALT levels to increase above reference values for more than 200 hours.[15] Becase of the effect of injections, ALT has poor specificity for liver disease, and the clinical relevance of an increased ALT value is decreased. For this reason, ALT frequently is omitted from avian clinical chemistry panels.

Glutamate dehydrogenase (GDH) is found in hepatocyte mitochondria and is considered the most specific indicator of hepatocellular damage in birds.[39] There also is high GDH activity in renal tissue; however, most of the enzyme is excreted directly into urine and never reaches the blood.[40] Plasma GDH activity is only increased when there is hepatic necrosis, and therefore it has low sensitivity.[15]

Lactate dehydrogenase (LDH) isoenzymes are found in most avian tissues such that increased plasma LDH activity has low specificity for liver disease.[39] Currently, LDH isoenzymes are not commonly measured for the clinical evaluation of birds. Contrary to prior statements in the literature, LDH has not proven to be useful in assessing fitness in raptors.[41,42]

Gamma glutamyltransferase (GGT) is probably specific to biliary and renal epithelium in birds, similar to dogs and cats.[43,44] Although GGT is considered "insensitive and inconsistent in the diagnosis of liver disease"[45] in birds, Lumeij found increased plasma GGT activity in the majority of pigeons with experimentally-induced liver disease.[15] Marked increases in GGT activity in birds with bile duct carcinoma also have been reported.[46]

Although reference intervals have not been established, GGT values of 0-10 U/L are considered "normal" at the Schubot Exotic Bird Health Center (College Station, Texas, USA). GGT values appear to be slightly higher in older Amazon parrots, which may have up to 16 U/L without other evidence of liver disease (Phalen DN, personal communication). These GGT values are higher than the reference intervals for GGT reported by Lumeij[15] of < 3 or 4 U/L in most species except Amazon parrots, which had a high normal value of 10 U/L. Differences in methodologies for measuring GGT may account for differences in reference values.

There are numerous reports of birds with bile duct carcinoma or cholangiocarcinoma, in which no concurrent increase in GGT activity was reported.[47-50] This is likely because GGT was not measured, since GGT activity would be expected to increase in these hepatic diseases. GGT activity is more likely to be increased in cholestatic conditions and biliary epithelial disorders in birds as it is in mammals; GGT is not sensitive to hepatocellular damage alone. The clinical utility of GGT in the diagnosis of biliary conditions in birds has not been adequately evaluated.

Very low levels of alkaline phosphatase (ALP) activity have been found in the liver of avian species studied.[39] Marked increases in plasma ALP activity appear to be specific for osteoblastic activity and bony change associated with growth, trauma, repair, osteomyelitis, neoplasia, nutritional secondary hyperparathyroidism, and egg-shell deposition.[39]

Table 1. Reference values for clinical chemistry parameters in selected companion avian species.*

	Units	*Psittacus* sp. (African grey)	*Amazona* sp. (Amazon Parrot)	*Melapsittacus* undulatus (budgerigar)	*Ara* sp. (macaws)	*Nymphicus hollandicus* (cockatiel)	*Cacatua* sp. (cockatoo)	*Anas* sp. (duck)	*Columba livia* (pigeon)	*Serinus* sp. (canary)
n		5571	8375	1542	5338	9267	5928	73	>50	300
Albumin	g/dL	0.2-2.4 (1.7)	0.3-2.4 (1.8)	0.9-1.2 (1.1)	0.3-2.4 (1.7)	0.8-1.8 (1.5)	1.2-2.4 (1.8)	1.7-2.2 (2.0)	1.3-2.2 (1.75)	—†
	g/L	2-24 (17)	3-24 (18)	9-12 (11)	3-24 (17)	8-18 (15)	12-24 (18)	17-22 (20)	13-22 (17.5)	—
A/G ratio	—	0.42-3.00 (1.20)	0.69-1.75 (1.10)	1.00-1.75 (1.31)	0.50-1.40 (0.93)	1.00-2.29 (1.50)	1.00-2.36 (1.37)	1.00-1.80 (1.18)	1.50-3.60	—
ALP	U/L	12-92 (34)	8-100 (52)	24-96 (68)	12-100 (50)	12-100 (51)	24-104 (59)	—	—	—
Amylase	U/L	415-626 (511)	184-478 (330)	302-560 (437)	239-564 (421)	113-870 (361)	228-876 (558)	—	—	—
AST	U/L	110-340 (174)	150-344 (221)	156-375 (262)	65-168 (122)	128-396 (245)	140-360 (204)	12-73 (34)	45-123 (58.6)	132-351 (224)
Bile acids	µmol/L	12-96 (56)	33-154 (89)	32-117 (81)	7-100 (49)	44-108 (76)	34-112 (70)	22-82 (55)	22-60	—
BUN	mg/dL	2.0-6.7	2.5-27.6	—	0.8 –9.2	—	2.2-5.9	—	1.1-2.0	—
	mmol/L	1.43-4.78	1.79-9.20	—	0.60-6.60	—	1.60-4.20	—	0.78-1.43	—
Calcium	mg/dL	8.0-14.0 (9.1)	8.0-13.9 (9.8)	8.0-11.2 (9.3)	8.4-11.9 (9.7)	8.2-10.9 (9.2)	8.2-11.5 (9.4)	8.7-12.7 (10.0)	7.6-10.4 (9.2)	—
	mmol/L	2.0-3.5 (2.3)	2.0-3.5 (2.5)	2.0-2.8 (2.3)	2.1-3.0 (2.4)	2.1-2.7 (2.3)	2.1-2.9 (2.4)	2.2-3.2 (2.5)	1.9-2.6 (2.3)	—
Cholesterol	mg/dL	100-250 (193)	148-228 (191)	120-230 (181)	96-264 (168)	90-200 (152)	96-212 (166)	104-244 (170)	—	—
	mmol/L	2.6-6.5 (5.0)	3.8-5.9 (4.9)	3.1-6.0 (4.7)	2.5-6.8 (4.3)	2.3-5.2 (3.9)	2.5-5.5 (4.3)	2.7-6.3 (4.4)	—	—
CK	U/L	140-411 (303)	117-425 (257)	117-368 (235)	88-361 (215)	160-420 (269)	147-418 (266)	165-378 (266)	110-480 (203)	—
Creatinine	mg/dL	0.26-0.45	0.21-0.37	0.10-0.40	0.40-2.00 (0.73)	—	0.30-1.90 (0.77)	—	0.26-0.40 (0.32)	—
	µmol/L	23-40	19-33	8.8-35.4	35.4-247.5 (64.5)	—	26.5-167.9 (68.1)	—	23-36 (28)	—
Fibrinogen	mg/dL	100-280	100-340	—	100-330	—	—	—	—	—
	g/L	1.0-2.8	1.0-3.4	—	1.0-3.3	—	—	—	—	—
Globulins	g/dL	1.2-3.6 (1.9)	1.6-3.7 (2.3)	0.7-1.5 (1.1)	2.1-3.8 (2.6)	2.1-3.8 (2.8)	2.0-3.4 (2.5)	3.5-6.0 (4.6)	—	—
	g/L	12-36 (19)	16-37 (23)	07-15 (11)	21-38 (26)	21-38 (28)	20-34 (25)	35-60 (46)	—	—
Glucose	mg/dL	256-360 (281)	246-378 (287)	216-456 (330)	210-360 (277)	228-440 (326)	206-418 (268)	127-319 (207)	232-369 (299)	160-360 (297)
	mmol/L	14.2-20.0 (15.6)	13.7-20.0 (15.9)	12.0-25.3 (18.3)	11.7-20.0 (15.4)	12.7-24.4 (18.1)	11.4-23.2 (14.9)	7.1-17.7 (11.5)	12.9-20.5 (16.6)	8.9-20.0 (16.5)
LDH	U/L	154-378 (248)	160-368 (230)	156-384 (252)	70-220 (135)	122-378 (255)	208-414 (303)	120-246 (194)	30-205 (57)	—
Total protein	g/dL	2.7-4.4 (3.5)	2.6-4.5 (3.7)	2.1-4.3 (2.8)	2.4-4.4 (3.4)	2.1-4.8 (2.9)	2.6-4.8 (3.6)	3.5-5.5 (4.5)	2.1-3.5 (2.7)	—
	g/L	27-44 (35)	26-45 (37)	21-43 (28)	24-44 (34)	21-48 (29)	26-48 (36)	35-55 (45)	21-35 (27)	—
Uric acid	mg/dL	2.0-11.0 (5.55)	2.2-10.0 (5.1)	4.8-13.0 (8.6)	1.8-12.0 (5.6)	3.4-11.0 (7.1)	3.8-11.0 (6.7)	2.0-12.0 (6.4)	2.5-12.6 (6.3)	4.1-13.0 (8.7)
	µmol/L	118-648 (330)	132-618 (303)	286-765 (512)	107-701 (331)	202-648 (419)	226-654 (398)	119-701 (380)	150-765 (375)	246-750 (516)

*Data are minimum-maximum for adults of both sexes, as obtained from Lumeij[15] (pigeon data, BUN and creatinine data), Hawkey and Hart[87] (fibrinogen data), and Fudge AM, *Laboratory Medicine: Avian and Exotic Pets*[79] (all remaining data; reprinted with permission). When available, mean values are shown in parentheses. See text for abbreviations.

†— Not determined.

Metabolites

Most birds have very little biliverdin reductase and therefore do not produce bilirubin in measurable quantities. Biliverdin (the tetrapyrrole dehydrobilirubin) can be measured in research laboratories by high-performance liquid chromatography[51] but currently it is not measured in clinical laboratories. Further investigation into the potential clinical importance of this analyte is warranted.

Plasma ammonia concentration has not been clinically evaluated in healthy or ill birds. Some clinicians have observed up to a fourfold increase in blood ammonia values in birds with liver failure (Phalen DN, personal communication). Further study of ammonia as an indicator of hepatic insufficiency is warranted.

Cholesterol metabolism in companion avian species is similar to that of mammals, but there are differences in the clinical presentation of birds with abnormal cholesterol values. In oviparous species, such as birds, a marked increase in plasma cholesterol concentration can be seen during vitellogenesis and egg formation.[52] Cholesterol values may be increased before the egg(s) can be visualized on radiographs.[53]

The primary bile acids (BAs) in human beings, dogs, and cats are cholic and chenodeoxycholic acid.[54] In granivorous birds, chenodeoxycholic acid is the predominant bile acid, followed by cholyltaurine in chickens and phocaecholyltaurine in ducks.[55] Over 90% of bile salts are reabsorbed in the jejunum and ileum, predominantly as glycocholate and taurocholate.[56] In most avian species studied, postprandial BA levels are higher than preprandial levels.[15,57] One report states that preprandial and postprandial bile acid concentrations are variable.[58]

The pre- and postprandial sampling recommendation for BA determination in dogs and cats would likely be ideal for birds, as well. However, the crop, an esophageal diverticulum for food storage, has varying emptying times in different species, and crop stasis is common in sick birds, such that standardization of postprandial sampling is difficult.[57] A fasted sample is preferred to eliminate random postprandial increases in BA concentration. Many birds can be fasted overnight (for 12 hours); however, fasting should be done with caution in debilitated birds and smaller species. Raptors can be fasted for up to 24 hours. Pigeons, ostriches and some parrots lack a gall bladder, such that fasting is not applicable when determining BA levels.[56]

Although BA reference intervals have been established using the enzymatic method (Table 1),[59,60] neither the enzymatic method nor any of the radioimmunoassays (RIAs) have been validated for avian species. The RIAs measure nonsulfated conjugated bile acids.[57] There is variability in results obtained in birds using human RIA test kits, likely because different antibodies bind different amino acid conjugates in avian BAs. Additionally, the predominant BAs in human beings differ from those in different bird species. Consequently, human-based kits yield values that may or may not be representative of total BAs in avian species. Furthermore, many kits are linear to only 50 µmol/L, and because avian BA concentrations tend to be higher, dilutions frequently are needed. The enzymatic BA method, validated for canine, feline, and human samples, measures the 3-α-hydroxyl group present in most BAs. This test would be expected to best approximate total BA concentration in most avian species. Unfortunately, the enzymatic method relies on spectrophotometry, which is markedly affected by hemolysis and lipemia, thereby requiring careful sample handling and quality control.[6] Automated spectrophotometric chemistry analyzers made by Abbott Laboratories (Abbott Spectrum Series II) and Boehringer Mannheim (Hitachi 911, Indianapolis, Ind, USA) have been used to measure avian BAs.[59] Using the enzymatic method, BA values >100 µmol/L are considered abnormal and >75 µmol/L are suspect for hepatic insufficiency.[15,57] Amazon parrots normally have slightly higher BA concentrations than do other companion avian species (Table 1). Of course, reference intervals for individual species should be generated in each laboratory, and validation is necessary.

Galactose clearance and galactose single-point concentrations (GEC-SA) were evaluated during a prospective study on the effects of partial hepatectomy in cockatoos.[37] In the study, both galactose clearance and GEC-SA appeared to be more sensitive indicators of hepatic insufficiency than plasma enzyme activities or BA levels, and were able to detect an 18% loss of hepatic mass. The authors concluded that GEC-SA has the potential to be a simple, sensitive method of screening birds for decreased hepatic function.

Evaluation of liver function using standard analytes, such as albumin and BUN, has not been performed in companion avian species. The accuracy of albumin quantitation using the bromcresol green method (BCG) is questionable and will be discussed later in this article. BUN often is not included on standard avian panels; nor has it been evaluated in prospective studies as an indicator of hepatic function. Further evaluation of these two potentially useful analytes is warranted.

Lactate is produced during anaerobic metabolism in muscle, and is metabolized in the liver. Comparisons of pre- and post-training lactic acid levels in healthy conditioned and unconditioned raptors have been used to determine fitness and releaseability, and establish flight-training protocols.[42] In that study, blood lactate levels peaked at 120mg/dL 2 min postexercise and gradually decreased to preflight levels (50-80mg/dL) or lower

within 10 min in well-conditioned raptors. Unconditioned raptors exhibited peak (2 min) blood lactate levels over 200 mg/dL.

Glucose

As in mammals, glucose metabolism in birds is modulated by insulin and glucagon. However, healthy birds maintain blood glucose concentrations of >150 mg/dL (Table 1), with levels up to 800 mg/dL in hummingbirds.[61] The glucose concentration in clinically normal birds may fall above the linear range of some hand-held glucometers, such as the One Touch Ultra (Life Scan, Milpitas, Calif, USA), which uses a glucose oxidase biosensor and has an upper linear limit of detection of 600 mg/dL. Liquid chemistry analyzers, such as the Hitachi 911 (Boehringer Mannheim), measure NAD generated from hexokinase and glucose-6-phosphate dehydrogenase (Infinity, Sigma Diagnostics, St. Louis, Mo), and assays are linear to 1000 mg/dL or up to 2000 mg/dL with automatic dilution. The hexokinase method, therefore, is preferred in avian species with high glucose values, including smaller species of birds, and birds with disorders associated with hyperglycemia.

There are species differences in the way in which birds regulate blood glucose. The insulin content of the pancreas of a granivorous species is about 1/6 that of the mammalian pancreas, while glucagon content is about 2 to 5 times greater.[62] Pancreatectomy induces hypoglycemic crisis in granivorous birds but produces diabetes mellitus in carnivorous birds.[15] This 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 blood insulin concentration in comparison with normal birds[35] and a positive response to insulin therapy.[63] It is therefore possible that either glucagonemia or hypoinsulinemia are responsible for diabetes in psittacines and other species.

Hyperglycemia is induced in birds by high levels of endogenous or exogenous glucocorticoids.[64] Although a positive urine glucose test is an indicator of hyperglycemia, stress hyperglycemia may cause up to 3+ glucosuria on a urine dipstick. Since avian urine/urates are retropulsed into the colorectum, false-positive glucosuria and proteinuria may occur due to fecal contamination, especially in frugivorous birds. Results of clinical chemistry tests, including repeated blood glucose, glucagon, and insulin concentrations (in comparison with a control bird or established reference interval), urinalysis, and clinical signs all should be considered prior to making a diagnosis of diabetes mellitus.

Carnivorous birds can maintain fasting blood glucose levels much longer than granivorous species.[36] Smaller granivorous species may become hypoglycemic after a 12-hour fast, especially if debilitated. Hypoglycemic seizures also have been reported in raptors when blood glucose concentration fell below 80 mg/dL.[36] Low glucose levels were attributed to flight training after restricted food intake.

Maldigestion and malabsorption may occur in both granivorous and carnivorous avian species. Diagnosis is usually made by gross examination of feces with undigested seed or other food.[30] A carbohydrate absorption test, such as xylose absorption, can be performed in birds in a manner similar to that used for mammals.[62,65] Evaluation of different sugars and validation of testing protocols is warranted in birds to aid in the diagnosis of gastrointestinal disease.

Electrolytes

There has been extensive study on the intestinal absorption of electrolytes and intestinal calcium transport in chickens. See *Sturkie's Avian Physiology* for a thorough review of the literature.[56] The predominant intracellular and extracellular anions and cations in birds are similar to those in mammals.

Plasma potassium concentration decreases by approximately 60% within 2 hours after sampling in pigeon blood and to a lesser extent (30% in 2 hours) in chickens.[15] Potassium concentration increases in macaw blood samples by approximately 30% within 4 hours.[66] The degree of artifactual change in potassium values appears to be species-specific, and warrants immediate separation of plasma from RBCs in all avian samples.

Renal disease in birds has been associated with hyperphosphatemia and hyperkalemia,[15] however, hypophosphatemia and hypokalemia also have been reported.[67] It is possible that the pattern of change in these values follows that of mammals with acute and chronic renal disease.

Avian total calcium values can be much higher under normal physiological circumstances than would be tolerated by a mammal. Dramatic increases in plasma total calcium concentration are seen in reproductive, oviparous females due to estrogen-induced transport of calcium-bound yolk proteins to the ovary.[68] Therefore, sex- and possibly season-specific reference values are required for accurate clinical evaluation of calcium values, although few have been published. Reproductive pathology such as egg binding and egg yolk coelomitis also can result in marked total hypercalcemia.[45] Marked hypocalcemia may be caused by malnutrition or reproductive abnormalities such as chronic egg laying.

The relationship between plasma total calcium concentration and total protein and albumin concentrations

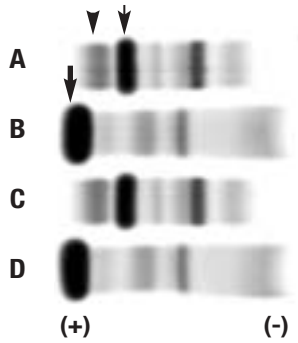

Figure 2. Human serum (**B, D**) and cockatiel plasma (**A, C**) gel electrophoresis. Duplicate samples were electrophoresed on the same agarose gel. Note that human albumin (thick arrow) migrated farther towards the cathode than cockatiel albumin (thin arrow) and prealbumin (transthyretin; arrowhead). This difference is believed to be due to variations in conformation and surface-charge distribution of albumin.

has been evaluated in several bird species.[69-72] No significant relationship was found between protein or albumin concentration and calcium concentration in Amazon parrots (*Amazona* sp).[70] A significant correlation was noted between albumin and calcium concentrations in African gray parrots.[70] The correlation formula, adjusted Ca (mmol/L) = Ca (mmol/L) – 0.015 × albumin (g/L) + 0.4, was obtained. A significant correlation also was found between total calcium and total protein concentrations in ostriches.[73] The correlation formula, adjusted Ca (mmol/L) = Ca (mmol/L) – 0.09 × total protein (g/L) + 4.4 was derived. Peregrine falcons (*Falco peregrinus*) also appear to have a linear relationship between both albumin and total protein concentrations and total calcium concentration.[71] Species differences in protein-calcium correlations indicate that 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.

There has been minimal study of ionized calcium and its relationship to disease in companion avian species. It is likely that ionized calcium determination would help to differentiate pathologic from physiologic increases in total calcium concentration. Some companion avian veterinarians use ionized calcium in birds for this purpose and consider ionized calcium values of 1.0-1.3 mmol/L to be normal (P. Gibbons, personal communication), although reference values have not been determined. A report in chickens found free calcium concentrations in normal laying hens to be between 1.3 and 1.6 mmol/L, with moderate variation during the ovulatory cycle.[74] Ionized calcium concentration is highly conserved within a species and species-specific values should be generated within the laboratory.

Proteins

Reference intervals for avian total protein concentration are substantially lower than those for mammalian species (Table 1). There have been several articles comparing refractometer versus biuret analysis of total protein in birds.[75-78] The total protein value determined using a refractometer is frequently inaccurate in companion avian species due to interference by high concentrations of other refractive compounds in plasma, such as chromagens, lipids, and glucose.[76,77] Studies in chickens, turkeys, and ducks have shown good correlation between protein concentrations obtained by refractometer (AO Goldberg Refractometer, American Optical Corporation, Buffalo, NY) and biuret methods. However, these species tend to have lower blood glucose values than most psittacines and smaller birds.[79] Correlation studies in avian species with high blood glucose levels, such as pigeons, have shown marked discrepancies between refractometer and biuret methods using a different brand of refractometer (Atago Corporation, Atago Ltd, Tokyo, Japan), which may have contributed to the difference in results.[76,77] There is marked variation in normal blood glucose levels in avian species. As previously noted, a hummingbird normally has a blood glucose concentration of 800 mg/dL. The biuret method is most accurate for quantifying total protein concentrations in the clinical laboratory, where samples from many different species may be evaluated.

The BCG method for albumin determination has not been validated in birds. Significant discrepancies have been shown between results obtained using BCG and gel electrophoresis.[80] This disparity is caused, in part, by use of human albumin standards and controls, which have different binding affinity for the dye than does avian albumin. Gel electrophoresis is the recommended method of albumin determination in avian species at this time.[15,81] Reference intervals for each species of bird should be established in each laboratory.

Plasma gel electrophoresis can be used to accurately determine albumin concentration and globulin distribution. Electrophoresis is useful for staging acute and chronic inflammatory conditions and for monitoring therapeutic response in birds, which frequently show few overt clinical signs.[81] Plasma proteins identified in the classic banding pattern of avian species include transthyretin (prealbumin fraction), albumin, α-1-antitrypsin (α-1-globulin fraction), α-2-macroglobulin (α-2-globulin fraction), fibrinogen, β-lipoprotein, transferrin, complement, and vitellogenin (β-globulin fraction), and immunoglobulins and complement degradation products (γ-globulin fraction).[81,82]

Transthyretin has replaced prealbumin in the human medical vocabulary. This protein binds thyroid

hormones and retinol with varying affinities for different thyroid hormones across mammalian, avian, and reptilian species. Chang et al[82] have isolated and sequenced transthyretin in 9 vertebrate species, including human, emu, chicken, ostrich, and pigeon. Transthyretin has greater than 98% homology and has a very similar banding pattern across species.[82,83]

There are species differences in albumin migration. Cockatiel albumin migrates to a position equivalent to chicken α-globulins, while the migration of cockatiel prealbumin is similar to that of chicken albumin.[84] Different migration patterns are attributed to variable conformation and surface charge distribution of albumin molecules (Figure 2). A difference in surface charge and conformation also may explain differential binding of BCG, and the variable results obtained for albumin determination using a human standard.[80] Decreased albumin concentration has been observed in birds with maldigestion, malabsorption, and protein-losing enteropathy.[85,86] Other differential diagnoses for hypoalbuminemia include protein-losing nephropathy and liver failure.

Fibrinogen concentration was increased above the reference interval in 77% of 89 birds, representing 20 species, with confirmed bacterial infection.[87] Fibrinogen concentration is being quantified and used to assess inflammation in raptors,[41] although data on specificity and sensitivity are lacking. Currently, total plasma protein/fibrinogen ratios in raptors are interpreted as follows: <1.5 = infection/inflammation, >5 = dehydration.

Albumin/globulin (A/G) ratio may be decreased in inflammation, protein-losing nephropathy, and liver failure.[45] Females of oviparous species may have a physiological decrease in A/G ratio concurrent with an estrogen-induced hyperproteinemia composed of proteins involved in egg formation.[15,68] The majority of yolk proteins and chalazae band in the globulin region and cause a marked increase in globulin fractions. Albumin concentration may be mildly increased during egg formation. Egg formation therefore results in a decreased A/G ratio that is not indicative of disease.

Iron

Hemochromatosis in human beings is an inherited, pathologic accumulation of iron in tissues due to aberrant intestinal iron absorption caused by the gene *HHC.* Screening for hemochromatosis includes measurement of transferrin saturation, serum iron concentration, serum ferritin concentration, and, frequently, genetic testing. The diagnosis is confirmed by evaluating hepatic iron levels.[88]

A genetic predilection for hemochromatosis is less certain in birds, although the disorder appears to be more common in ramphastids (toucans), sturnids (birds of paradise), some passeriforms (mynahs), and quetzals.[89] Death due to iron overload also has been reported in Psittaciformes (parrots) and Cracidae (curassow).[90,91] A study involving 46 toucans showed no difference in prevalence or severity between sexes or between wild-caught and captive-bred toucans.[89] These results argue against an underlying genetic defect. It has been hypothesized that affected species of birds absorb iron from intestinal contents to a greater extent than do other birds and that increased quantities of bioavailable iron may be present in some protein sources.[92]

Serum chemistry results in birds with hemochromatosis may include increased liver enzyme activities, usually AST, which is believed to be due to iron-induced hepatocellular damage.[15] A few anecdotal reports describe increased serum iron concentration in sick versus control birds.[15] Other studies have 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.[89,93] However, the reference values used in Worell's study[89] were considerably higher than those used in domestic mammals and human beings. Additionally, some birds with possible inflammation, ie, leukocytosis, heterophilia, and monocytosis, were included in the study.

Although serum ferritin concentration correlates significantly with nonheme 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 ELISAs and RIAs.[94,95] 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.

Summary

There is a great deal of research and literature about the clinical chemistry of companion avian species. However, it does not compare with the wealth of knowledge we have on human beings and other companion animals. Additionally, many targeted, single-variable experiments have yet to be incorporated into the practice of avian medicine. The current use of avian "minipanels" limits the data available for interpretation by avian practitioners and prevents comprehensive retrospective study by researchers. Larger, more accurate databases of avian clinical chemistry values are needed to improve the quality of avian internal medicine. ◊

Acknowledgment

The author acknowledges and thanks Drs. April Romagnano and Darryl Heard for reviewing and editing the manuscript.

References

1. Vatn S, Framstad T, Torsteinbø WO. Hematologic evaluation of normal and anemic lambs with the Technicon H*1 using EDTA or heparin as anticoagulants. *Vet Clin Pathol.* 2000;29:62-68.
2. Bonvicini P, Ceriotti G, Plebani M, Volpe G. Heparin interferes with albumin determination by dye-binding methods. *Clin Chem.* 1979;25:1459-1460.
3. Finotti P, Pagetta A. Heparin-induced structural modifications and oxidative cleavage of human serum albumin in the absence and presence of glucose—implications for transcapillary leakage of albumin in hyperglycaemia. *Eur J Biochem.* 1997;247:1000-1008.
4. Perry BW, Doumas BT. Effect of heparin on albumin determination by use of bromcresol green and bromcresol purple. *Clin Chem.* 1979;25:1520-1522.
5. Campbell TW. *Avian Hematology and Cytology.* 2nd ed. Ames, Ia: Iowa State University Press; 1995.
6. Meyer DJ, Harvey JW. *Veterinary Laboratory Medicine: Interpretation and Diagnosis.* 2nd ed. Philadelphia, Pa: WB Saunders; 1992.
7. Wideman RF, Jr, Gregg CM. Model for evaluating avian renal hemodynamics and glomerular filtration rate autoregulation. *Am J Physiol.* 1988;254:R925-R932.
8. Goldstein DL, Skadhauge E. Renal and extrarenal regulation of body fluid composition. In: Whittow GC, ed. *Sturkie's Avian Physiology.* San Diego, Calif: Academic Press; 2000:265-298.
9. Shideman JR, Evans RL, Bierer DW, Quebbemann AJ. Renal venous portal contribution to PAH and uric acid clearance in the chicken. *Am J Physiol.* 1981;240:F46-F53.
10. Goldstein DL, Braun EJ. Contributions of the kidneys and intestines to water conservation, and plasma levels of antidiuretic hormone, during dehydration in house sparrows (*Passer domesticus*). *J Comp Physiol*. 1988;158B:353-361.
11. Arnason SS, Rice GE, Chadwick A, Skadhauge E. Plasma levels of arginine vasotocin, prolactin, aldosterone and corticosterone during prolonged dehydration in the domestic fowl: effect of dietary NaCl. *J Comp Physiol.* 1986;156B:383-397.
12. Braun EJ. Comparative aspects of the urinary concentrating process. *Ren Physiol.* 1985;8:249-260.
13. Goldstein DL, Braun EJ. Structure and concentrating ability in the avian kidney. *Am J Physiol.* 1989;256:R501-R509.
14. Goldstein DL, Braun EJ. Lower intestinal modification of ureteral urine in hydrated house sparrows. *Am J Physiol.* 1986; 250:R89-R95.
15. Lumeij JT. Avian clinical biochemistry. In: Kaneko JJ, Harvey JW, Bruss ML, eds. *Clinical Biochemistry of Domestic Animals.* San Diego, Calif: Academic Press; 1997:857-884.
16. Shideman JR, Zmuda MJ, Quebbemann AJ. The acute effects of furosemide, ethacrynic acid and chlorothiazide on the renal tubular handling of uric acid in the chicken. *J Pharmacol Exp Ther.* 1981;216:441-446.
17. Braun EJ. Glomerular filtration in birds—its control. *Fed Proc.* 1982;41:2377-2381.
18. Dantzler WH, Braun EJ. Comparative nephron function in reptiles, birds, and mammals. *Am J Physiol.* 1980;239:R197-R213.
19. Braun EJ. Comparative renal function in birds, reptiles, and mammals. *Sem Avian Exotic Pet Med.* 1998;7:62-71.
20. Lumeij JT. Plasma urea, creatinine, and uric acid concentrations in response to dehydration in racing pigeons (*Columba livia domestica*). *Avian Pathol.* 1987;16:377-382.
21. Lumeij JT. The diagnostic value of plasma proteins and nonprotein nitrogen substances in birds. *Vet Q.* 1987;9:262-268.
22. Kolmstetter CM, Ramsay ER. Effects of feeding on plasma uric acid and urea concentrations in blackfooted penguins (*Spheniscus demersus*). *J Avian Med Surg.* 2000;14:177-179.
23. Lumeij JT, Remple JD. Plasma urea, creatinine and uric acid concentrations in relation to feeding in peregrine falcons (*Falco peregrinus*). *Avian Pathol.* 2002;20:79-83.
24. James SB, Raphael BL, Clippinger T. Diagnosis and treatment of hepatic lipidosis in a barred owl (*Strix varia*). *J Avian Med Surg.* 2002;14:268-272.
25. Long S, Skadhauge E. Renal acid excretion in the domestic fowl. *J Exp Biol.* 1983;104:51-58.
26. Wideman RF, Jr., Braun EJ. Ureteral urine collection from anesthetized domestic fowl. *Lab Anim Sci.* 1982;32:298-301.
27. Casotti G, Braun EJ. Renal anatomy in sparrows from different environments. *J Morphol.* 2000;243:283-291.
28. Janes DN, Braun EJ. Urinary protein excretion in red jungle fowl (*Gallus gallus*). *Comp Biochem Physiol.* 1997;118A:1273-1275.
29. Long S, Skadhauge E. The role of urinary precipitates in the excretion of electrolytes and urate in the domestic fowl. *J Exp Biol.* 1983;104:41-50.
30. Harrison GJ, Ritchie BW. Making distinctions in the physical examination. In: Ritchie BW, Harrison GJ, Harrison LR, eds. *Avian Medicine: Principles and Application.* Lake Worth, Fla: Wingers Publishing, Inc; 1994:144-175.
31. Lumeij JT. Hepatology. In: Ritchie BW, Harrison GJ, Harrison LR, eds. *Avian Medicine: Principles and Application.* Lake Worth, Fla: Wingers Publishing, Inc; 1994:522-537.
32. Phalen DN. The avian urinary system: form, function, diseases. *Proc Assoc Avian Vet.* Lake Worth, Fla: Assoc Avian Vet Publications; 1990:44-57.
33. Dumonceaux G, Harrison GJ. Toxins. In: Ritchie BW, Harrison GJ, Harrison LR, eds. *Avian Medicine: Principles and Application.* Lake Worth, Fla: Wingers Publishing, Inc; 1994:1030-1052.
34. Rosskopf WJ, Woerpel RW. Kidney disease in a military macaw, a yellow-naped Amazon parrot, and an unbrella cockatoo. *Proc Assoc Avian Vet.* Lake Worth, Fla: Assoc Avian Vet Publications; 1988:207-218.
35. Candeletta SC, Homer BL, Garner MM, Isaza R. Diabetes mellitus associated with chronic lymphocytic pancreatitis in an African Grey Parrot (*Psittacus erithacus erithacus*). *J Assoc Avian Vet.* 1993;7:39-43.
36. Wallner-Pendleton EA, Rogers D, Epple A. Diabetes mellitus in a red-tailed hawk (*Buteo jamaicensis*). *Avian Pathol.* 1993; 22:631-635.
37. Jaensch MJ, Cullen L, Raidal SR. Assessment of liver function in galahs/cockatoos (*Eolophus roseicapillus*) after partial hepatectomy: a comparison of plasma enzyme concentrations, serum bile acid levels, and galactose clearance tests. *J Avian Med Surg.* 2000;14:164-171.

38. Dabbert CB, Powell KC. Serum enzymes as indicators of capture myopathy in mallards (*Anas platyrhynchos*). *J Wildl Dis.* 1993;29:304-309.

39. Lumeij JT, Westerhof I. Blood chemistry for the diagnosis of hepatobiliary disease in birds. A review. *Vet Q.* 1987;9:255-261.

40. Battison AL, Buczkowski S, Archer FJ. The potential use of plasma glutamate dehydrogenase activity for the evaluation of hepatic disease in the cockatiel (*Nymphicus hollandicus*). *Vet Clin Pathol.* 1996;25:43-47.

41. Joseph V. Raptor hematology and chemistry evaluation. *Vet Clin North Am Exotic Anim Pract.* 1999;2:689-99.

42. Chaplin SB, Mueller LR, Degernes LA. Physiological assessment of rehabilitated raptors prior to release. In: Redig PT, Cooper JE, Remple JD, Hunter DB, eds. *Raptor Biomedicine.* Minneapolis, Minn: University of Minnesota Press; 1993:167-173.

43. Kramer JW, Hoffmann WE. Clinical enzymology. In: Kaneko JJ, Harvey J, Bruss ML, eds. *Clinical Biochemistry of Domestic Animals.* San Diego, Calif: Academic Press; 1998:303-325.

44. Lumeij JT. *A Contribution to Clinical Investigative Methods for Birds, with Special Reference to the Racing Pigeon, Columba livia domestica.* Utrecht: Rijksuniversiteit Utrecht; 1987.

45. Jones MP. Avian clinical pathology. *Vet Clin North Am Exotic Anim Pract.* 1999;2:663-87.

46. Phalen DN, Homco L, Jaeger L. Investigations into the etiologic agent of internal papillomatosis of parrots and ultrasonographic and serum chemical changes in Amazon parrots with bile duct carcinomas. *Proc Assoc Avian Vet.* Lake Worth, Fla: Assoc Avian Vet Publications; 1997:53-56.

47. Munson L, Clyde VL, Orosz SE. Severe hepatic fibrosis and bile duct hyperplasia in four Amazon parrots. *J Avian Med Surg.* 1996;10:252-257.

48. Hillyer EV, Moroff S, Hoefer H, et al. Bile duct carcinoma in two out of ten Amazon parrots with cloacal papillomas. *J Assoc Avian Vet.* 1991;5:91-95.

49. Elangbam CS, Panciera RJ. Cholangiocarcinoma in a blue-fronted Amazon parrot (*Amazona estiva*). *Avian Dis.* 1988;32: 594-596.

50. Coleman CW. Bile duct carcinoma and cloacal prolapse in an orange-winged Amazon parrot (*Amazona amazonica*). *J Assoc Avian Vet.* 1991;5:87-89.

51. Itoh S, Imai T, Kondo M. Relationships between serum (ZZ)-bilirubin, its subfractions and biliverdin concentrations in infants at one-month check ups. *Ann Clin Biochem.* 2001;38:323-328.

52. Johnson AL. Reproduction in the female. In: Whittow GC, ed. *Sturkie's Avian Physiology.* San Diego, Calif: Academic Press; 2000:569-596.

53. Harr KE, Alleman AR, Dennis PM, Maxwell LK, Lock BA, Bennett RA et al. Morphologic and cytochemical characteristics of blood cells and hematologic and plasma biochemical reference ranges in green iguanas. *J Am Vet Med Assoc.* 2001;218:915-921.

54. Hornbuckle WE, Tennant BC. Gastrointestinal function. In: Kaneko JJ, Harvey JW, Bruss ML, eds. *Clinical Biochemistry of Domestic Animals.* San Diego, Calif: Academic Press; 1997:367-406.

55. Elkin RG, Wood KV, Hagey LR. Biliary bile acid profiles of domestic fowl as determined by high performance liquid chromatography and fast atom bombardment mass spectrometry. *Comp Biochem Physiol.* 1990;96B:157-161.

56. Denbow DM. Gastrointestinal anatomy and physiology. In: Whittow GC, ed. *Sturkie's Avian Physiology.* San Diego, Calif: Academic Press; 2000:299-326.

57. Hoeffer H. Bile acid testing in psittacine birds. *Sem Avian Exotic Pet Med.* 1994;3:33-37.

58. Flammer K. Serum bile acids in psittacine birds. *Proc Assoc Avian Vet.* Lake Worth, Fla: Assoc Avian Vet Publications; 1994; 9-12.

59. Battison AL, Buckzowski S, Archer FJ. Plasma bile acid concentration in the cockatiel. *Can Vet J.* 1996;37:233-234.

60. Lumeij JT, Remple JD, Remple CJ, Riddle KE. Plasma chemistry in peregrine falcons (*Falco peregrinus*): reference values and physiological variations of importance for interpretation. *Avian Pathol.* 1998;27:129-132.

61. Diamond JM, Karasov WH, Phan D, Carpenter FL. Digestive physiology is a determinant of foraging bout frequency in hummingbirds. *Nature.* 1986;320:62-63.

62. Hazelwood RL. Pancreas. In: Whittow GC, ed. *Sturkie's Avian Physiology.* San Diego, Calif: Academic Press; 2000:539-556.

63. Altman RB, Kirmayer AH. Diabetes mellitus in the avian species. *J Assoc Avian Vet.* 1977;12:531-537.

64. Hazelwood RL. Carbohydrate metabolism. In: Sturkie PD, ed. *Avian Physiology.* San Diego, Calif: Academic Press; 1986:303-325.

65. Riesenfeld G, Sklan D, Barr A, et al. Glucose absorption and starch digestion in the intestine of the chicken. *J Nutr.* 1980;110: 117-121.

66. Harr KE, Raskin RE, McKinsey JE. Hematologic and biochemical changes caused by commonly used anticoagulants on macaw (*Anodorhynchus* sp.) blood over time [abstract]. *Vet Clin Pathol.* 2002;31:157.

67. Laverty G, Dantzler WH. Micropuncture study of urate transport by superficial nephrons in avian (*Sturnus vulgaris*) kidney. *Pflugers Arch.* 1983;397:232-236.

68. Simkiss K. *Calcium in Reproductive Physiology: A Comparative Study of Vertebrates*. New York, NY: Reinhold Publishing Corp; 1967: 155-197.

69. Verstappen FA, Lumeij JT, Bronneberg RG. Plasma chemistry reference values in ostriches. *J Wildl Dis.* 2002;38:154-159.

70. Lumeij JT. Relation of plasma calcium to total protein and albumin in African grey (*Psittacus erithacus*) and Amazon (*Amazon* spp) parrots. *Avian Pathol.* 1990;19:661-667.

71. Lumeij JT, Remple JD, Riddle KE. Relationship of plasma total protein and albumin to total calcium in peregrine falcons (*Falco peregrinus*). *Avian Pathol.* 1993;22:183-188.

72. Bailey TA, Wernery U, Howlett J, Naldo J, Samour JH. Age-related plasma chemistry findings in the buff-crested bustard (*Eupodotis ruficrista gindiana*). *Zentralbl Veterinarmed.* 1998;45B: 635-640.

73. Verstappen FA, Lumeij JT, Bronneberg RG. Plasma chemistry reference values in ostriches. *J Wildl Dis.* 2002;38:154-159.

74. Kolling K, Hofmeier A, Merkenschlager M. Ionized calcium in the blood of domestic chickens: dependence on the laying cycle. *Zentralbl Veterinarmed.* 1992;39A:115-120.

75. Andreasen CB, Latimer KS, Kircher IM, Brown J. Determination of chicken and turkey plasma and serum protein concentrations by refractometry and the biuret method. *Avian Dis.* 1989;33:93-96.

76. Lumeij JT, Maclean B. Total protein determination in pigeon plasma and serum: comparison of refractometric methods with biuret method. *J Avian Med Surg.* 1996;10:150-152.

77. Lumeij JT, de Bruijne JJ. Evaluation of refractometric method for the determination of total protein in avian plasma or serum. *Avian Pathol.* 1985;14:441-444.

78. Morgan GW, Thaxton P, Eden FW. Estimation of protein content in the plasma of young chickens by a refractometric method. *Poult Sci.* 1975;54:1312-1314.

79. Fudge AM. Laboratory reference ranges for selected avian, mammalian, and reptilian species. In: Fudge AM, ed. *Laboratory Medicine: Avian and Exotic Pets.* Philadelphia, Pa: WB Saunders; 2000:376-400.

80. Spano JS, Whitesides JF, Pedersoli WM, Krista LM, Ravis WM. Comparative albumin determinations in ducks, chickens, and turkeys by electrophoretic and dye-binding methods. *Am J Vet Res.* 1988;49:325-326.

81. Cray C, Tatum L. Applications of protein electrophoresis in avian diagnostics. *J Avian Med Surg.* 1998;12:4-10.

82. Chang L, Munro SL, Richardson SJ, Schreiber G. Evolution of thyroid hormone binding by transthyretins in birds and mammals. *Eur J Biochem.* 1999;259:534-542.

83. Farer LS, Robbins J, Blumberg GS, et al. Thyroxine serum protein complexes in various animals. *Endocrinology.* 1962;70:686-696.

84. Archer FJ, Battison AL. Differences in electrophoresis patterns between plasma albumins of the cockatiel (*Nymphicus hollandicus*) and the chicken (*Gallus gallus domesticus*). *Avian Pathol.* 1997;26:865-870.

85. Wilson GH, Greenacre CB, Howerth EW, et al. Ascaridosis in a group of psittacine birds. *J Avian Med Surg.* 1999;13:32-39.

86. Stone EG, Redig PT. Preliminary evaluation of hetastarch for the management of hypoproteinemia and hypovolemia. *Proc Assoc Avian Vet.* Lake Worth, Fla: Assoc Avian Vet Publications; 1994:197-199.

87. Hawkey C, Hart MG. An analysis of the incidence of hyperfibrinogenemia in birds with bacterial infections. *Avian Pathol.* 1988;17:427-432.

88. Tolman KG, Rej R. Liver function. In: Burtis CA, Ashwood ER, editors. Tietz *Fundamentals of Clinical Chemistry.* Philadelphia, Pa: WB Saunders Co; 2001:747-770.

89. Worell AB. Further investigations in rhamphastids concerning hemochromatosis. *Proc Assoc Avian Vet.* Lake Worth, Fla: Assoc Avian Vet Publications; 1993:98-107.

90. Rosskopf WJ, Woerpel RW, Fudge AM, et al. Iron storage disease (hemochromatosis) in a citron-crested cockatoo and other psittacine species. *Proc Assoc Avian Vet.* Lake Worth, Fla: Assoc Avian Vet Publications; 1992:98-107.

91. Turner R. Iron storage disease (hemochromatosis) in the curassow. *Proc Assoc Avian Vet.* Lake Worth, Fla: Assoc Avian Vet Publications; 1994:265-267.

92. Gosselin SJ, Kramer LW. Pathophysiology of excessive iron storage in mynah birds. *J Am Vet Med Assoc.* 1983;183:1238-1240.

93. Dorrestein GM, Grinwis GM, Dominguez L, et al. An induced iron storage disease syndrome in doves and pigeons: a model for hemochromatosis in mynah birds? *Proc Assoc Avian Vet.* Lake Worth, Fla: Assoc Avian Vet Publications; 1992:108-112.

94. Smith JE. Iron metabolism and its disorders. In: Kaneko JJ, Harvey JW, Bruss ML, eds. *Clinical Biochemistry of Domestic Animals.* San Diego, Calif: Academic Press; 1997:223-239.

95. Gonzalez D. Comparative study of ferritins from dove (*Columba oena*) and chicken (*Gallus domesticus*) livers. *Comp Biochem Physiol.* 1983;76:568.