

Avian Kidney Disease

Part II: Diagnosis of Renal Disease

M. Scott Echols, DVM, Diplomate ABVP - Avian Practice

Director of Avian Medical and Surgical Services, Westgate Pet and Bird Hospital, 4534 Westgate Blvd, Suite 100, Austin, Texas 78745, USA.

Avian renal diseases are as diverse in type and diagnosis as is reported in better-studied mammals. For proper diagnosis, emphasis is given toward a thorough historical, physical and laboratory evaluation of each patient. Definitive diagnosis of renal disease is often limited to a renal biopsy and histopathologic evaluation. Even without a renal biopsy and with a proper work-up, a good presumptive diagnosis of avian renal disease can be made and guide the clinician to provide an appropriate treatment plan.

A Review of Diagnosis and Management of Avian Renal Disease

History and physical examination

A historical review of a bird's environment, diet, source, exposure to infectious agents and toxins, genetics and behavior becomes important for both diagnosis and management of avian renal disease. Environmental factors can include exposure to known aerosolized, ingested or topical toxins. Adverse conditions that might lead to dehydration or other stresses may also be identified. The diet should reflect what is appropriate for that species and the history should include any additional dietary supplementation or changes. Understanding the bird's origin, whether from a specific aviary, store, quarantine station, the wild, etc, may suggest the possibility of problems seen in other avian species from the same source. Known exposure to infectious agents (and again, toxins) is especially important as definitive diagnosis of bacterial, viral, parasitic, fungal and toxic agents is not always possible without cultures, special stains, electronmicroscopy, in situ DNA hybridization, PCR probes or other diagnostics. Genetic problems are poorly described in birds, but with intense in-breeding, development of mutations or conservation breeding efforts from an extremely limited gene pool, it is reasonable to assume that hereditary defects will become more common. Behavioral changes including depression, anorexia, anuria, oliguria, polyuria, polydipsia, feather picking over the synsacrum, self-mutilation, seizures and others may be associated with renal disease and should be noted in the history.

Most physical examination abnormalities associated with avian renal disease are non-specific, but there are some key findings that tend to warrant further investigation. It is highly likely that a bird with articular gout has had or currently has some form of renal disease. For this reason, consider renal biopsy in some birds with articular gout to help rule out, or specifically, identify kidney disease. Not all birds afflicted with articular gout, however, have renal disease. Unilateral leg lameness or paresis may accompany renal disease. This is particularly true if kidney disease causes inflammation or compression on the lumbar and/or sacral nerve plexus that is so intimately associated with the dorsal renal parenchyma. Birds with renal disease may also exhibit, dehydration, generalized weakness, regurgitation and decreased muscle mass with or without historical anorexia, all of which are nonspecific signs.

Diagnostic tests

Multiple diagnostic tests are available to help clinicians identify and define multiple disease processes in birds. As diagnostic technology improves, so will our ability to accurately diagnose diseases in birds. The tests listed below are ones that are most frequently discussed or used in diagnosing renal disease in birds. Many diagnostics such as fecal floatation, which help diagnose renal coccidiosis, are not discussed but should be included in a minimum database when evaluating sick birds. Some new, or unfamiliar, diagnostics are also introduced.

Considering all of the diagnostic tests available, the author has noticed a pattern of laboratory abnormalities that is often strongly correlated with many forms of renal disease in birds. This includes persistently elevated uric acid (at least two consecutive tests on a well hydrated and fasted bird), elevated creatinine phosphokinase (CPK), mild anemia and a relative heterophilia with or without a total heterophilia. Elevated CPK is a very non-specific indicator of multiple types of tissue damage and is not mentioned further. Using the currently available diagnostics, the actual type and degree of renal disease can only be confirmed with a kidney biopsy.

Complete blood count:

Some nonspecific CBC changes may be associated with avian renal disease. A marked (relative) heterophilia was noted in two chickens with urolithiasis, but no total white blood cell count was given. Heterophilia, monocytosis, lymphopenia and normocytic-normochromic anemia were noted in broiler chicks with various forms of histologically confirmed renal disease, but specific details were not given. In a different study in chickens, clinically affected birds with histologically identified nephritis had significant heterophilic leukocytosis when compared to 'normal' birds. The author has reported that many pet birds (geese, doves and various psittacine birds) with different forms of renal disease have demonstrated a mild to marked relative heterophilia with a normal total white blood count. These changes are nonspecific, however, and can be seen in healthy birds under stress alone.

Serum or plasma-based biochemistries:

Selected plasma biochemistries may provide several useful clues towards renal disease in avian patients. Although many serum and plasma-based tests may be 'abnormal' in birds with renal disease, only specific diagnostics are covered.

Uric acid

Plasma uric acid can be useful as a screening tool for advanced renal disease. With the exception of gastrointestinal uricolysis, uric acid and its salts (urate) are the end product of nitrogen metabolism in birds. Elevated uric acid has been correlated with histologically confirmed severe renal disease in chickens (tubular nephrosis and interstitial nephritis). In a separate study involving dehydrated chickens, increased serum uric acid was associated with histologic renal lesions. Broilers given oosporein (renal toxin), developed visceral and/or articular gout, swollen pale kidneys and had a 48% increase of uric acid over control birds. In a similar study with oosporein in turkey poults, intoxicated birds had dose-dependant increases in uric acid (over controls) ranging from 76% to 140%. Lumeij noted that fasting hyperuricaemia (> 16.7 mg/dl [$>1,000$ $\mu\text{mol/L}$]) in peregrine falcons (*Falco peregrinus*) indicates renal failure.

Uric acid is produced and secreted in the avian liver, kidney and pancreas. Although predominately produced in the liver, at least 17% of the uric acid found in chicken urine may be synthesized in the kidney. Specifically, nephrogenic uric acid synthesis may increase when plasma purine precursors are elevated. These findings suggest the avian kidney has an important role in the synthesis, in addition to elimination, of uric acid, especially when increased precursors are available. Precursors, including body proteins degraded because of poor nutritional status, have been suggested as a cause of elevated uric acid and should be considered in birds with hyperuricaemia.

An interesting secondary role of uric acid in birds is its antioxidant capability. In chickens, it has been clearly shown that plasma uric acid concentrations are inversely correlated with oxidative activity. Simoyi et al stated that 'uric acid constitutes one of the most important antioxidants in birds and is directly linked to their longevity'.

Uric acid is mainly cleared via tubular secretion and is largely independent of glomerular filtration, water resorption and urine flow rate. Blood uric acid levels are mildly affected by a bird's hydration status, but rather reflect the functional capacity of the renal proximal tubules. However, in a study with dehydrated chickens, uric acid levels increased after 24 to 48 hours of water restriction, but only in those birds allowed free access to food. Serum uric acid levels actually dropped within 24 hours in birds denied food and water. It has been estimated that renal function must be below 30% of its original capacity before hyperuricaemia develops. Suggested normal avian uric acid levels range from less than 1 to 10 mg/dl.

Hyperuricaemia is defined as 'any plasma uric acid concentration higher than the calculated limit of solubility of sodium urate in plasma'. In bird plasma, this theoretical limit of solubility of sodium urate is estimated to be 600 $\mu\text{mol/L}$.

Uric acid represents 80% or more of the nitrogen excreted by birds. Therefore, a significant increase in the proportion of nitrogen excreted as uric acid is not likely, even with increased dietary protein consumption. At least in chickens, hyperuricaemia is likely due to reduced renal tubular secretion of uric acid and not excessive production as can occur in humans. These findings imply that renal tubular diseases are likely responsible for hyperuricaemia and uric acid abnormalities may not be evident until very high protein diets are fed. Specifically in chickens, dysfunctional proximal convoluted tubules result in reduced urate secretion and can lead to hyperuricaemia if severe.

In chickens, the uric acid renal tubule transport system does not appear to become saturated until plasma uric acid levels exceed 60 mg/dl. Chickens genetically predisposed to hyperuricaemia and fed high (60%) protein diets develop an elevated steady state of plasma uric acid (10-60 mg/dl) in order to excrete their daily loads of this by-product. The increased basal plasma uric acid made the affected chickens susceptible to articular gout formation. One group suggested that these chickens genetically predisposed to gout had a defective uric acid transport mechanism at the peritubular membrane.

In birds of prey, uric acid production is directly related to the amount of protein consumed and transient rises are noted following high protein meals. Peregrine falcons (*Falco peregrinus*) and red-tailed hawks (*Buteo jamaicensis*) are reported to have a 'significant' post-prandial increase in plasma uric acid concentration (hyperuricaemia) for up to 12 hours after ingesting a natural meal. The 'significant' post-prandial uric acid increase noted in peregrine falcons was up to 32 mg/dl (reported as 1,881 $\mu\text{mol/L}$) between 3 and 8 hours after being fed. Lumeij stated that significant post-prandial increases in both urea and uric acid persist for up to 15 hours in peregrine falcons. It was not clear why these birds of prey did not develop gout lesions, but the authors recommended a 24-hour fast prior to evaluating serum uric acid in peregrine falcons. The authors further recommend that a 24-hour fast should be considered for all carnivorous avian species prior to blood uric acid testing. Almost identical findings of post-prandial hyperuricaemia were noted in blackfooted penguins (*Spheniscus demersus*) and represent another species that should be fasted before measuring uric acid levels.

Uric acid production following a high protein meal has been studied in various psittacine birds. In one study with African grey parrots (*Psittacus erithacus* sp.), plasma uric acid concentrations showed a positive correlation with dietary protein consumption. However, even though the fed protein level was as high as 30%, plasma uric acid levels remained within normal ranges. In cockatiels fed 11, 20, 35 and 70% protein for 11 months, serum uric acid increased linearly with dietary protein levels.

However, the serum uric acid level was significantly greater only in birds fed 70% protein diets. Because no histologic or gross renal lesions were found at necropsy, the authors concluded that the rise of uric acid was related to dietary protein concentration and not kidney damage. Angel and Ballam found that feeding diets containing 13.5, 18.2 and 24.6% protein for up to 24 weeks had no effect on serum uric acid levels in parakeets.

In consideration of the above-described causes of elevations in uric acid, this single biochemistry value can help identify significant renal disease. The author prefers to repeat (fasting) uric acid levels on well-hydrated birds before a suggestion of renal disease is made. In birds with suspect renal disease that have a single laboratory value of hyperuricemia, the author will often give a total of 100 ml/kg SQ, SID to BID of isotonic fluids for 2 days and then recheck the uric acid level. In the author's experience, birds with persistent hyperuricaemia after fluid therapy and/or fasting have some form of renal disease.

Urea

Unlike mammals, urea in birds is produced only in small amounts (by renal mitochondrial breakdown of arginine) and does not serve as the end product of protein metabolism. Plasma urea in birds is excreted by glomerular filtration, and unlike uric acid, blood urea concentrations are more significantly affected by the bird's hydration status. During normal hydration, filtered urea is 100% excreted but is 99% reabsorbed in the tubules during dehydration. Plasma urea has also been shown to significantly increase in peregrine falcons for up to 15 hours post meal.¹⁴³ In studied cockatiels, serum urea levels increased linearly with dietary protein levels (11, 20, 35 and 70%). Separate studies involving the domestic fowl and pigeons demonstrated decreased urea elimination and/or increased blood urea levels (6.5 to 15.3 fold increase in pigeons) in dehydrated birds. Pegram et al showed that plasma urea nitrogen 'increased in a dose-dependant fashion (in turkeys) at every level of dietary oosporein (nephrotoxin)'. It should also be considered that these intoxicated turkey poults were also showing signs of dehydration. Lumeij has proposed that plasma urea is the single most useful indicator of prerenal (dehydration) causes of kidney failure in birds.

The urea/creatinine and urea/uric acid ratio can be used to better define pre- and post-renal azotemia. Because reabsorption of urea is disproportionally higher than both creatinine and uric acid, these ratios should be high during dehydration and ureteral obstruction. The formulas for these ratios are listed below:

$$\frac{\text{urea}}{\text{creatinine}} = \frac{\text{urea (mmol/L)} \times 1,000}{\text{creatinine } (\mu\text{mol/L})}$$

$$\frac{\text{urea}}{\text{uric acid}} = \frac{\text{urea (mmol/L)} \times 1,000}{\text{uric acid } (\mu\text{mol/L})}$$

Creatinine

Birds produce little creatinine from its precursor, creatine. Creatinine is eliminated by tubular secretion but clearance is variable. Clinically, creatinine may be elevated in pet birds by feeding high protein diets. Lumeij showed that plasma creatinine will also increase significantly in dehydrated pigeons. The relationship between creatine and creatinine in birds with renal disease is poorly understood and differentiation does not appear to be useful clinically.

Proteins

Although hypoproteinaemia has been noted as 'associated with renal failure', few studies have evaluated serum protein levels in birds with renal disease. Biochemically determined low serum

protein has been noted in chickens with advance tubular nephrosis and interstitial nephritis. In two chicken flocks with spontaneously occurring urolithiasis, plasma protein level changes (method of determination not disclosed) were not significantly associated with renal disease. While affected birds developed articular and/or visceral gout, gross renal changes and death, broilers intoxicated with oosporein (fungal nephrotoxin) had, with the exception of one group, no significant changes in plasma protein (biuret method) over the normal (control) birds. A single group of broilers receiving a mid-range amount of oosporein had a statistically significant rise in plasma protein over controls. The cause for this single discrepancy was not determined. In a similar study using oosporein-intoxicated turkey poults, statistically significant decreased albumin:total protein was noted at all levels of intoxication over controls, but total protein remained unchanged and albumin was not significantly decreased until the highest levels of the toxin were given. These few studies show a couple important facts; there is limited information properly associating plasma proteins with renal disease and, differing species may have dissimilar plasma protein levels under similar disease conditions. Protein levels should be evaluated electrophoretically (in addition to the more common biochemical methods).

Plasma electrolytes:

The effect of renal disease on plasma electrolytes is poorly studied in birds. Hyperkalaemia and hyperphosphatemia have been loosely 'associated with renal failure', but studies are limited in birds. No significant associations between renal disease and plasma sodium, potassium, calcium, magnesium, chloride and phosphate levels were noted in birds from two chicken flocks with spontaneously occurring urolithiasis. Specific sample collection/storage was not discussed and the authors conceded that their handling of the samples might have affected the results. Dehydrated chickens allowed free access to food, developed significantly elevated serum sodium and phosphorous by 24 hours and after 24 hours, respectively, but maintained normal potassium levels. Histologically, these chickens had mild renal tubular dilatation. Turkey poults intoxicated with oosporein (nephrotoxin) developed significantly decreased plasma potassium and phosphorous and had no changes in sodium compared to controls. As the avian kidney is responsible for electrolyte regulation, it is reasonable to assume that electrolyte disorders can be present in birds with renal disease.

Microbiologic analysis:

Microbiologic assays may be useful in identifying infectious causes of avian renal disease. Bacteria may enter the renal system either hematogenously, ascending from the ureters and cloaca or as an extension of surrounding organ infection. The avian cecocolicomesenteric vein drains the mesentery of the hind-gut into the hepatic portal and/or the renal portal vein. It is conceivable that colitis may serve as a hematogenous source of infectious agents, toxins and inflammatory products to the kidney if blood flow draining the colon is diverted into the renal vasculature. For this reason, collection of a cloacal or fecal microbial culture is a rational portion of the supportive laboratory database in birds with suspected renal disease. Severe ulcerative colitis caused by *Salmonella* infection resulted in ascending bacterial nephritis in four African grey parrots.

Bacterial nephritis in birds is often a component of systemic infection and multiple organs may be involved. In one study, 50% of birds with systemic bacterial infections had kidney involvement suggesting that any bacterial septicemia can potentially result in nephritis. Identification of bacteria within renal tissue may be difficult as has been noted in dogs and swine with renal disease putatively associated with a bacterial aetiology. Blood cultures are an appropriate consideration if septicemia is suspected. Prior to blood collection, the skin over the venipuncture site is aseptically prepared by thorough cleaning with alcohol and organic iodine (as with surgical preparation). The jugular and basilic veins are described as appropriate blood collection sites in septicemic birds. Using aseptic techniques, renal biopsy specimens can also be sampled for microbial cultures. The cause of infectious nephritis in birds is not limited to bacteria and various culture methods may also be useful for identifying fungal, viral and parasitic organisms.

Urinalysis:

Biochemical and cytological sediment analysis of avian urine has been advocated as potentially useful in diagnosing avian renal disease. In birds, hematuria may be noted with renal disease but should be carefully differentiated from bleeding originating from the gastrointestinal and reproductive tracts. Haemoglobinuria, as noted in *Amazona spp.* parrots with lead intoxication and in other species with differing disorders, may or may not be related to renal disease. Toxic, neoplastic, bacterial and viral nephropathies may be more frequently seen associated with hematuria in birds. White blood cells were seen in 45% of urine sediment from pigeons with paratyphus, many of which had interstitial nephritis. Sediment analysis should be a part of an avian urinalysis and specific cellular urinary components have been discussed.

Several significant factors complicate interpreting avian urinalysis. First, urine is mixed with feces in the cloaca. The one possible exception is the ostrich, which appears to eliminate urinary waste separate from the feces. Second, in many species ureteral urine is refluxed orad into the lower intestines to the caeca where water, and sometimes electrolyte, reabsorption takes place. Additionally, diseases of the lower intestine may alter urine production and composition. Gastrointestinal bleeding, inflammation, normal and abnormal organisms, etc may end up in an 'urinalysis' harvested from a dropping giving the false impression that red and white blood cells and/or infectious agents, respectively, came from the urinary tract. In short, the 'urine' present in a dropping is not the same urine produced from the kidneys. Urinalysis results should be carefully interpreted.

Collection

True urine can be collected in birds with some difficulty. Once emptied of feces, specially designed cannulas can be inserted into the cloaca for collection of ureteral urine. One group used a foley catheter to 'occlude the rectum but not the ureters' and successfully collected ureteral urine in chickens. Casotti and Braun used small close-ended cannulas constructed from micropipette tips to collect ureteral urine from house (*Passer domesticus*) and song sparrows (*Melospiza melodia*). The opening of the close-ended cannula was placed over the 'ureteral orifices'. A similar design was used in house sparrows to make cloacal cannulas from PE-240 tubing with a hole cut near the sealed end. The sealed end prevented intestinal fluids from contaminating the urine once the cannula was in place. Under local anesthesia, Roberts sutured a 1.5 ml microcentrifuge tube into the cloaca of chickens to allow collection of ureteral urine. Forman and Wideman used cyano-acrylate to glue a cannula over the ureteral orifice of chickens. Several obvious drawbacks include restraint or sedation of the patient while urine is slowly produced and the cannulation itself may induce diuresis. Clearly there are numerous methods, with varying degrees of difficulty, used to collect ureteral urine.

Casts

Urinary casts represent cellular and/or acellular material sloughed from the inner lining of various renal tubules. This material is generally in the shape (or a 'cast') of the tubule from which it originated. Casts are sometimes noted on histologic sections. Protein and cellular casts were histologically noted in an Australian diamond firetail finch (*Steganopleura bella*) with *Cryptosporidium sp.* and multifocal amyloidosis. Narcisi et al noted 'albuminous' casts in renal tubules of pigeons infected with virulent *Trichomonas gallinae*. Hyaline casts were identified in kidney sections of birds experimentally infected with infectious bursal disease (Gumboro disease). Eosinophilic granular casts have been found within the renal tubules of turkeys afflicted with salt toxicosis. Phalen et al reported eosinophilic tubular casts, possibly containing myoglobin, in an ostrich with acute muscle necrosis and anuric renal failure. A rhea with haemoglobinuric nephrosis developed eosinophilic casts in the renal collecting tubules. Both hyaline and granular tubular casts were present in racing pigeons infected with avian paramyxovirus type 1. Granular, hyaline and albuminous casts were seen in the renal tubules of chickens experimentally infected with several pathogenic bacteria.

Identifying casts in urine is reported as 'highly significant', a 'sign of renal disease' and/or can be a

non-specific indicator of tubular renal disease in birds. With that said, the papers above describe histological sections with no discussion of casts in the urine. The author disagrees that urinary casts are 'highly significant' or a definite 'sign of renal disease' as there is little information correlating casts found in an urinalysis with any type of renal disease in birds. However, casts should be noted and may have correlation with some forms of avian renal disease. Mushi et al found epithelial casts in 2 out of 35 ostrich urine samples but no correlation was made with any renal parameters. Epithelial casts were noted in 20% of urine samples from *Salmonella typhimurium* infected pigeons. Although many birds did have histologically confirmed renal disease, no correlation was made between those pigeons with kidney lesions and those with urinary casts. The large variety of 'types' of casts reported also suggests that an inconsistent naming system exists within the current literature.

Urine chemistries and electrolytes

Standard mammalian dipsticks may be used but not all components are applicable to avian urine. 167 Chicken urine reportedly contains non-uric acid chromagen. Non-protein chromogens are known to interfere with refractometric and chemical measurement of plasma proteins and may also apply to avian urine sampling.

Few studies even mention test strips used in avian urinalyses. One study evaluated commercial urine dipsticks (Combur-9 stix [Boehringer Mannheim]) on normal urine of 35 ostriches. Because ostriches can eliminate urinary waste separate from feces, these values may not apply to most other birds. In the study, 31/35 (89%) and 35/35 (100%) of the urine samples were positive for nitrite and protein, respectively. The urine chemistry strips were negative for glucose, urobilinogen, bilirubin and ketones in all ostriches. No association with renal disease was made. Using the Combur-9 Stix strips, nitrite and protein were also positive in 90% (18/20) and 50% (10/20), respectively, of the ureteral urine samples from pigeons with paratyphus. The same strips identified blood in all samples, which correlated to red blood cells seen in only 45% of urine sediments. Urine strips may also detect undigested hemoglobin found in the excrement of the bird, especially carnivorous species with short digestion times, and give a positive result. Myoglobinuria may also cause positive reactions and can only be distinguished from hemoglobinuria by spectrophotometry. Finally, porphyrinuria, as seen with lead-poisoned Amazon parrots (*Amazona spp.*), may result in red-colored urine visually mimicking hemoglobinuria. Because of the inconsistent results and limited critical studies noted in the literature, difficulty in obtaining ureteral urine and clinical experience, the author feels that the currently available chemistry strips have limited value in an avian urinalysis.

Urine electrolytes and chemistries can be collected, but there is limited information on their interpretation. Lumeij suggested that because renal intracellular enzymes are likely voided in the urine, urinary chemistries might be useful in detecting kidney damage. Urine sodium and potassium were measured, and insignificantly changed, in house sparrows undergoing trials with the antidiuretic arginine vasotocin. One study noted that in normal and dehydrated starlings (*Sturnis vulgaris*), cloacal urine contained significantly higher concentrations of magnesium, phosphate, potassium and total osmolality than found in ureteral samples. This study supports the recommendation that ureteral samples must be collected to obtain a 'true' evaluation of avian urine, again making urinary chemistry evaluation impractical in a clinical setting.

One renal enzyme, N-acetyl- β -D-glucosaminidase (NAG), has been successfully evaluated in the urine of chickens as a marker for kidney damage. In mammals and chickens, NAG is a renal tubular enzyme. In humans, urinary NAG has been suggested for use as an early predictor of renal tubular damage and may be a good non-invasive indicator of disease progression. Elevated urinary (ureteral urine), but not plasma, NAG was noted at 40 days of excessive D₃ supplementation in chickens. Although the information is limited, further studies may show that NAG, and possibly other urinary enzymes, may become useful as early markers of renal disease in birds.

Osmolality and specific gravity

Avian urine is typically isosmotic because the predominant reptilian-type nephrons cannot concentrate urine beyond plasma osmolality. In normal birds, urine osmolality can be maximally increased to 2.0-2.5 times that of plasma osmolality. Even this number is high for some species as emus (*Dromaius navaehollandiae*) are reported to have maximal urine to plasma osmotic ratio of only 1.4 to 1.5. This is minimal in comparison to some mammals that can concentrate urine osmolality 25 to 30 times that of plasma.

There is limited information on urine specific gravity or osmolality in avian health or disease. The reported average (refractometrically determined) urine specific gravity of ostriches (*Struthio camelus*) is 1.020 with a range of 1.010 to 1.050. Gevaert et al noted consistent polyuria and hyposthenuria (60% had specific gravity below 1.007) in *Salmonella typhimurium* infected pigeons, many of which had interstitial nephritis. In a separate evaluation, urine osmolality significantly increased up to 3 times control levels in post-flight and dehydrated pigeons. The author has used urine specific gravity diagnostically as discussed under 'Water deprivation testing'.

Urine pH

Urine pH is highly variable in birds. The urine pH may be acid (down to 4.7) in egg laying female birds during calcium deposition. Once the egg is laid or calcium is no longer being deposited, urinary pH may climb to 8.0. Male birds have an approximate urine pH of 6.4. Hypoxia, as noted in diving ducks, may drop urine pH to 4.7. Normal ostriches have a urine pH range of 6.1 to 9.1, with a mean of 7.6.

Electrophoresis:

Plasma Protein Electrophoresis

Properly determined hypoalbuminemia (via plasma electrophoresis) is not reported in confirmed active cases of avian renal disease. However, it is possible that birds may develop low albumin/protein with some kidney disorders. Biochemically determined hypoalbuminemia has been noted in some active avian renal disease cases.

The literature states that as the currently available biochemical tests likely do not accurately report avian albumin levels, serum/plasma protein electrophoresis is necessary to properly quantitate blood proteins and should be performed if hypoalbuminemia is suspected. Decreased albumin and elevated betaglobulins and alpha₂ macroglobulin, as recorded with serum electrophoresis, have been reported with avian 'nephritis'. However, there are no controlled studies to support these above statements that correlate protein electrophoresis abnormalities with any renal pathology in birds.

With the above stated, one study showed that an analyzer using the biuret and bromocresol green dye-binding methodologies for total protein and albumin determination, respectively, had good agreement between whole blood and plasma samples. On the contrary, there was poor correlation between the results from the studied analyzer and samples evaluated via electrophoresis used at 2 major reference laboratories. Due to the discrepancies, the authors concluded that neither reference laboratory using electrophoresis served as the 'gold standard' for total protein and albumin determination.

These very limited studies suggest inconsistencies in the 'gold standard' method of serum/plasma total protein and albumin determination and questions the true value of these diagnostics in birds with renal disease. Regardless, the author feels that monitoring serum and/or plasma protein levels has diagnostic value in birds, even if not necessarily used in renal disease cases. The author recommends consistently using one of the common biochemical methods of protein determination and comparing those results to electrophoresis. The goal being to become familiar with test results from one or two diagnostic methods and correlating those results to (histologically) confirmed disease.

Urinary protein electrophoresis

In mammals, proteinuria is broken down into preglomerular, glomerular and postglomerular urinary protein loss. Preglomerular proteinuria occurs when large amounts of small molecular weight proteins (immunoglobulin fragments, hemoglobin and myoglobin) that readily pass through normal glomerular walls, are lost in the urine. Glomerular proteinuria occurs when diseased glomerular membranes allow large proteins (albumin, immunoglobulins, some coagulation proteins/antithrombin III) to pass. Post-glomerular proteinuria results from normal genital secretions, as well as urogenital infections, trauma and neoplasia. Although uncommon in mammals, defects resulting in proximal renal tubular protein resorption result in (postglomerular) tubular proteinuria.

Avian urine normally contains a large amount of protein (average of 5 mg/ml up to 15 mg/ml), especially when compared to that of mammals (< 0.09 mg/ml in dogs and humans). Amino acids are freely filtered at the glomerulus, but are normally almost completely reabsorbed by the renal tubules in birds. Because uric acid is poorly water soluble, very little avian ureteral urine is required to eliminate this protein waste. Instead, proteinuria is likely necessary to maintain the excreted uric acid-containing spheres in a colloidal suspension, preventing aggregation and renal tubular blockage. Within the proximal tubule, uric acid is bound to a protein to solubilize the waste product and prevent crystal formation. The reflux of urine into the cloaca may be a mechanism to recover some of the urinary protein as cloacally voided fluid contains very little protein compared to ureteral samples.

Serum albumin, among other proteins, is found in both the liquid urine and uric acid spheres in chickens. In the normal jungle fowl (*Gallus gallus*), the urinary proteins (averaged 2.01 mg/ml urine) identified closely matched the plasma proteins. This led to the conclusion that protein is passed through a glomerular filtration barrier differently than occurs with most mammals. There are however, differences in concentrations of plasma and urinary proteins suggesting differential filtration and/or absorption of some proteins by renal tubules.

Pathologic proteinuria is poorly described in birds. In one study, control chickens and those with experimentally induced autoimmune glomerulonephritis produced urinary protein (measured via 3% sulfosalicylic acid with a bovine serum albumin standard) at 5 mg/24 hours. Test birds developed no abnormal proteinuria but were considered moderately proteinuric after given IV colloidal carbon (3 to 8 times increase in proteinuria). Colloidal carbon induces proteinuria in other species, but the mechanism is not clear. As discussed in 'Types of Avian Renal Disease', birds may not be capable of developing pathologic proteinuria with glomerular disease as is recognized in mammals. However, it is possible that pathologic proteinuria develops more slowly in birds compared with mammals, and as a result, has not been frequently discussed or evaluated in clinical cases. If pathologic proteinuria is suspected, urine protein electrophoresis should be used to differentiate protein type and size. If performed, it would be beneficial to compare urinary protein levels from a 'sick' patient with samples from a 'healthy' member of the same species. Finally, ureteral urine should be collected to rule out any effects from protein absorption or from other proteins present in the lower intestine. In a normal clinical setting, these collection requirements and limited studies make meaningful urinary protein interpretation in birds impractical.

Imaging:

Radiography

Plain and contrast radiography, nuclear scintigraphy, ultrasound, magnetic resonance imaging and computed tomography can be used to 'image' the avian kidneys. The avian kidney lies in a fossae created by the ventral surface of the synsacrum. With bone dorsal and air sacs surrounding ventrally, imaging of the avian renal system is difficult with some techniques. Indirect methods such as positive contrast radiography of the alimentary tract, may be helpful in outlining renal masses.

A lateral view is the best method to radiographically view the kidneys. As viewed with a lateral radiograph, the absence of the normal dorsal diverticulum of the abdominal air sac (dorsal to the kidney and ventral to the synsacrum) may indicate renal enlargement. Improper positioning can artifactually change the appearance of this air filled diverticulum. Because the renal silhouettes are superimposed on a lateral view of the abdomen, an oblique view may also be used to distinguish each kidney. Renal density and gross size changes may indicate renal disease. Radiographically visible renomegaly was noted in a salmon-crested cockatoo with chronic interstitial nephritis and calcification as the result of hypervitaminosis D₃. Nephrocalcinosis was detected radiographically in ostriches and appeared as multiple radio-opacities throughout the renal parenchyma.

Ultrasound

Due to the presence of surrounding air sacs (ventrally) and bone (dorsally and laterally), ultrasonographic imaging of normal avian kidneys is difficult. In one study of 386 mixed bird species that underwent ultrasonographic evaluation of the urogenital tract, abnormalities, such as renal cysts (6), cancer (12) and inflammatory nephromegaly (11), were identified in only 29 patients. The authors concluded that sonographic imaging of the normal kidney was not possible. Some disease conditions that either obliterate the air sacs or result in fluid accumulation in the coelomic cavity may actually improve renal ultrasonographic imaging. In these abnormal situations, ultrasonography can serve as a non-invasive and safe means to evaluate coelomic structures such as the kidneys.

Intravenous excretory urography

Intravenous excretory urography has been described in birds as a method to gain information on kidney size, shape and function. Lumeij reports using organic iodine compounds (Urographine 76) given IV at 2 mg/kg in the basilic vein. The organic iodine can be visualized radiographically in the heart and pulmonary artery within 10 seconds and outlining the kidneys and ureters 20 to 50 seconds later. After 2 to 5 minutes, the cloaca will be outlined. This technique should not be used in birds with severe renal compromise.

The author feels that intravenous excretory urography may have some limited uses in a clinical setting as demonstrated in the case report below. Dennis and Bennett successfully used a water-soluble iodinated contrast agent (Renografin-76, Squibb Diagnostics, Princeton, NJ) to evaluate the ureters post-ureterotomy in a double-yellowheaded Amazon parrot (*Amazona ochrocephala*). The agent was dosed at 400 mg/kg and was given in the right medial metatarsal vein. Radiographic images were taken at 1, 2, 7 and 10 minutes post-injection. Ureter peristaltic movement and size were successfully evaluated using this technique.

Renal scintigraphy

Avian renal scintigraphy has also been described. The radioisotope 99mTc-dimercaptosuccinic acid (99mTc-DMSA) was used in an unspecified avian species. The kidney parenchyma took the 99mTc-DMSA well and activity in the cloaca indicated renal excretion. Although the information was limited, renal scintigraphy may prove to be a useful tool in diagnosing and evaluating avian renal disease.

Water deprivation testing:

Water deprivation testing is considered when attempting to rule out unknown causes of polyuria/polydipsia (PU/PD) including central and nephrogenic diabetes insipidus and psychogenic polydipsia. There are numerous causes of PU/PD in birds that must first be ruled out using a complete historical, physical and laboratory evaluation. Some of the many causes of PU/PD in birds include organic (liver, kidney, intestine, cardiac, etc), endocrine (diabetes mellitus) and metabolic (hypercalcaemia) diseases.

A water deprivation test is carefully performed using a simple cage. The bird is weighed and blood

and urine are collected. Evaluate the packed cell volume (PCV), total solids and osmolality of blood and specific gravity and osmolality of urine. In one report of an African grey parrot (*Psittacus erithacus erithacus*) undergoing a water deprivation test, the authors evaluated plasma sodium, potassium and osmolality in addition to the above listed urine parameters.

Place the avian patient in a cage with no food or water for the duration of the test. Evaluate both blood and urine parameters every 3 to 24 hours for 12 to 48 hours, depending on the species and physical condition of the bird. The reported African grey parrot was evaluated every 24 hours. As a normal response, some birds such as European starlings may become distressed within 24 hours of water deprivation, which should be considered when interpreting the results. On the other hand, pigeons deprived of water for 36 hours had little change in plasma osmolality, demonstrating the variable responses to dehydration in differing species. As a general rule, smaller birds should be evaluated more frequently.

The bird's behavior and laboratory results give a presumptive diagnosis. Birds with psychogenic polydipsia should tolerate this test well and develop more concentrated urine (increased osmolality and specific gravity) and an increase in PCV, total solids and plasma osmolality, all consistent with dehydration. This was the pattern seen in the African grey parrot and subsequent treatment with water restriction proved curative. These individual values should all be carefully interpreted as noted in a study of dehydrated starlings where the haematocrit remained unchanged (compared with hydrated birds) and was not a reliable indicator of hydration.

Birds with central (lack of production of arginine vasotocin [AVT]) or nephrogenic (inadequate response to AVT) diabetes insipidus should have different results than those with psychogenic causes. Birds with diabetes insipidus become dehydrated (as supported by plasma variables) but maintain dilute urine (low specific gravity and osmolality). Normal house sparrows given arginine vasotocin (0.4 ng/kg/min to 1.6 ng/kg/min) had a significant drop in urine flow rate (50.2% to 28.9% of normal, respectively) and increased urine osmolality (150.1% to 196% of normal, respectively). A similar response would be expected in other normal bird species.

A strain of chickens with hereditary diabetes insipidus has been described. These polyuric chickens produce low osmolality urine and maintain high circulating level of AVT. The vital functions of these chickens become impaired after 48 hours of water deprivation. When given AVT, additional to their high circulating levels, these birds have minimal response. Either the birds have improperly responding kidneys or the AVT was defective.

In the author's experience with 1 male canary winged parakeet (*Brotogeris versicolorus*) with suspected diabetes insipidus, the bird became panicked within 4 hours as he became rapidly dehydrated but maintained excessive production of dilute urine. The canary winged parakeet had normal plasma biochemistries, complete blood count, screening radiographs and renal biopsy (light microscopy) and had a history of severe PU/PD since weaning. A diagnosis beyond presumptive diabetes insipidus was not made since AVT levels were not evaluated.

Identifying uric acid crystals:

Gout results when uric acid precipitates out as a solid, chalky substance in joints (articular) or on tissue surfaces (visceral). Articular gout material may be recovered using fine needle aspiration. Uric acid crystals are easily confirmed using microscopy or the murexide test. Cytologically, 'gouty' material typically presents as uric acid crystals surrounded by a pyogranulomatous infiltrate, usually without organisms. The needle-shaped crystals are easy to identify on direct and stained smears. To perform the murexide test, place a small amount of the suspect material on a slide and mix with nitric acid. Use a flame to evaporate and/or dry the mixture. Once cool, add one drop of concentrated ammonia. If

urates are present, a mauve color will appear. Due to their water-soluble nature, urates will dissolve in formalin and therefore the crystalline form will not be seen on conventionally fixed tissue. However, urates can be seen in alcohol fixed tissue using Gomori's methenamine silver impregnation technique.

Evaluating glomerular filtration rate:

Glomerular filtration rate has been studied in chickens as a method to evaluate renal function. Glomerular filtration rate is considered the most reliable quantitative index of renal function and is an important tool for the diagnosis and management of kidney disease of mammals. Most methods of measuring glomerular filtration rate and effective renal plasma flow are difficult and time consuming. 199 As a result, determining glomerular filtration rate in birds is often limited to research situations.

In general, urine flow rate (UFR) is first calculated as the volume (of ureteral urine) collected per kilogram of body weight per minute. The urine to plasma concentration ratio of a (usually parenterally administered) marker substance, such as inulin, is multiplied by the urine flow rate. Glomerular filtration rate (milliliters per kilogram body weight per minute) can then be calculated by measuring the clearance of the marker substance. The basic formula is as follows:

$$\text{Glomerular filtration rate} = \frac{\text{UFR} \times \text{urine marker substance concentration (inulin)}}{\text{plasma marker substance concentration (inulin)}}$$

The single injection, double isotope method, utilizing 3H-inulin ([methoxy-3H]-inulin) and 14C-PAH (para-[glycyl-1-14C]-aminohippuric acid), has been shown to be a simple, reliable and rapid method for evaluating renal function in chickens. If needed, the specific procedures of evaluating glomerular filtration in birds can be reviewed in the literature.

Biopsy:

When history, physical examination and/or laboratory abnormalities support the presence of renal disease, consider biopsy. Currently, the only way to definitively diagnose avian renal disease and specific pathologic patterns is with a kidney biopsy and histopathologic evaluation. A renal biopsy is most frequently performed during endoscopic examination of the coelomic cavity and specifically, kidneys. Before a renal biopsy is performed, the cost: benefit of the surgical procedure versus conservative therapy must be considered as many birds have compromised health, especially if they have kidney disease.

Several methods of renal biopsy, primarily via endoscopy, and detailed accounts of avian kidney anatomy and physiology have been previously discussed. For the most part, renal tissues can be stored in 10% formalin for light microscopy. If available, additional tissue may be stored in glutaraldehyde (electron microscopy), culture media (organism recovery) and alcohol (visualizing uric acid crystals) or frozen (PCR studies).

Renal histologic lesions are rarely pathognomonic for a specific disease process. Many different diseases cause similar renal lesions. Additionally, different pathologists may make differing morphologic diagnoses on the same renal tissue. The author encourages veterinarians to work with a pathologist familiar with normal and abnormal avian histology. Oftentimes, it is the pathologist's interpretation of a renal biopsy combined with the attending veterinarian's case familiarity that enables both parties to make a definitive diagnosis or build a reasonable differential diagnoses list compatible with the kidney lesions noted. This approach has a key role in the formation of a viable therapeutic plan for the patient.