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A CASE ORIENTED APPROACH TO URINARY SYSTEM LABORATORY PROFILING IN DOGS AND CATS

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Biochemical profiling may be defined as the use of multiple blood chemistry determinations to assess the health status of various organ systems simultaneously. Biochemical profiling rapidly has become a major diagnostic aid for the practising veterinarian for several reasons. First, a more educated clientele has come to expect increased diagnostic sophistication. Secondly, the advent of high-volume clinical pathology laboratories has resulted in low prices that make profiling in veterinary practice feasible and convenient. In addition, improved technology has resulted in the development of procedures that can be used to obtain accurate analyses on microsamples of serum. Such procedures offer obvious advantages to veterinarians, who in the past were hindered by requirements for large sample size.

Limitations

Although biochemical profiling offers exciting potential, it is not a panacea. Since standard chemical screens provide 12 to 30 test results, interpretation of data may be extremely complex. Interpretation is often clouded by the fact that perfectly normal animals may have, indeed, are expected to have, an occasional abnormal test result. It is estimated that in a panel of 12 chemistry tests, approximately 46% of all normal subjects will have at least one abnormal test result. Such abnormalities do not reflect inaccuracies in laboratory test procedures but rather the way in which reference (or normal) values are determined. In order to establish the ‘normal range’ for a given test, the procedure is performed on samples from a large population of clinically normal individuals. A mean and a standard deviation are determined. The reference values are then defined as those values falling within two standard deviations above and below the mean. Since two standard deviations above and below the mean only include 95% of all determined values, 5% of the values obtained from a normal population are by this definition abnormal.

It is important to realize that determination of reference values in the manner described above assumes a Gaussian or bell-shaped distribution for measured values. Additional problems with the establishment of reference values can be expected if Gaussian distributions are not present. In these instances, population distributions must be normalized to a bell-shaped distribution before reference values are established.

Just as healthy individuals may have occasional abnormal test results, so can individuals with severe organ disease have test results that are within the reference intervals. For example, elevated serum alanine aminotransferase (ALT) levels long have been considered important indicators of liver disease in dogs. However, ALT levels will only be elevated under specific circumstances. ALT is an enzyme normally found in the cytosol of hepatocytes. Consequently, serum levels will only be elevated in conditions where there is increased permeability of plasma membranes. In more chronic liver disease, plasma membrane permeability is often normal. Additionally, ALT levels reflect the number of hepatocytes with leaky membranes; therefore, marked elevations are more commonly seen in diffuse than in localized liver disease. ALT levels also will vary with the stage of the disease when the sample is collected. ALT has a circulatory half-life of two to four days; therefore, a two-fold elevation in ALT due to acute liver necrosis may be expected to have returned to the normal range within two days.

The clinician must also be aware that abnormalities in one organ system may cause abnormalities in chemistry test results that are used primarily to indicate disease in a different organ system. For example, elevated serum amylase levels are used primarily as indicators of pancreatic disease. However, amylase normally is excreted by the kidney as a part of glomerular filtrate. Consequently, anything that reduces glomerular filtration may result in elevated serum amylase levels.

Hopefully, the preceding paragraphs have succeeded in illustrating some of the more important difficulties encountered in the interpretation of clinical chemistry data. It is apparent that a single test should never be used to assess the total health status of an organ. It is equally apparent that one must understand the factors affecting a given test result, such as the causes of elevations, circulating half-lives, and routes of excretion. Then too, interactions between different organ systems and their effects upon test results must be considered. In the final analysis, it is apparent that only through systematic assessment of chemistry data can misinterpretation and confusion be avoided. A final point to consider is that chemistry profiling should not be undertaken without simultaneous evaluation of a complete blood count (CBC) and urinalysis.

Urinary system

Introduction

The kidney, like the liver, performs a variety of functions of major importance to the maintenance of normal
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homeostasis. It is involved in the excretion of wastes and the regulation of acid-base balance, electrolyte balance, and state of hydration.

The performance of these functions depends upon both normal glomerular filtration and normal renal tubular integrity. The primary renal panel assesses both. It is important to note that urinalysis, although not a part of our large chemistry profile, is an essential part of the primary renal panel. The secondary renal panel is primarily designed to evaluate changes that may occur secondary to renal disease.

**Primary renal panel**

**Blood urea nitrogen (BUN)**

Urea is a nitrogenous waste that is excreted by the kidney via glomerular filtration. Blood urea nitrogen (BUN) level is primarily used as an indicator of glomerular filtration rate. Azotemia (elevations in BUN) may be prerenal due to reduced renal perfusion, renal due to primary kidney disease, or postrenal due to ureter, bladder or urethral obstruction or rupture.

BUN should only be interpreted in light of urine specific gravity (see urinalysis handout). If BUN is elevated and urine specific gravity indicates that the renal tubules are concentrating, then the azotemia is most likely prerenal. If BUN is elevated but urine specific gravity is isosthenuric (between 1.008 and 1.017; the concentration of plasma), then primary renal disease is suspected. Despite the value of BUN as a test of renal function, it is not a terribly sensitive or specific test. In primary renal disease, approximately 3/4 of both kidneys must be non-functional before BUN will elevate. Also, circulating levels of urea nitrogen are influenced by many other factors. To better understand how to interpret BUN values, it is first necessary to understand how urea is produced.

**Creatinine**

Creatinine, a by-product of muscle metabolism, is excreted exclusively by glomerular filtration. Therefore, serum creatinine levels, like BUN levels, are used as estimates of glomerular filtration rate. Interpretations of elevated serum creatinine and elevated BUN are nearly identical; however, creatinine is less influenced by nonrenal factors than is BUN. For this reason, some authors have suggested that sequential serum creatinine determinations may be used for prognostic purposes. When factors such as diet and hydration are constant, patients with renal disease and sequentially elevating serum creatinine levels have a much more guarded prognosis than patients with diagnosed renal disease and decreasing serum creatinine levels.

**Urinalysis**

The urinalysis, like the CBC, is not part of the large chemistry profile. However, in our laboratory whenever the large profile is requested, the urinalysis is also done for several reasons. First, like the CBC, the urinalysis provides valuable information concerning general health status and state of hydration. Second, renal parameters in the large chemistry profile - BUN, and creatinine - cannot be interpreted without accompanying urinalysis data.

Urinalysis has three components: physical examination, chemical examination, and urine sediment examination. Physical examination includes evaluation of color, turbidity, and specific gravity. Chemical evaluation includes semiquantitative evaluation of urine protein, ketones, glucose, bilirubin, urobilinogen and occult blood. Urine pH is also determined. Urine sediment examination is the microscopic evaluation of the formed elements to the urine casts, crystals and cells.

**Physical Examination**

**Color**

Normal urine is yellow to amber. In general, the more dilute the urine, the less intense the color. Numerous abnormalities result in color changes. Frank hemorrhage will color urine red. Hemoglobinuria or myoglobinuria gives urine a deep red-brown discoloration. Bilirubin gives urine an orange-brownish cast. Drug therapy may also alter urine color.

**Turbidity**

Normal feline and canine urines are clear; increased turbidity is generally a reflection of increased particulate matter in the urine. Such particles will be identified during the microscopic examination of the sediment.

**Specific Gravity (Sp. Gr.)**

Specific gravity is used to estimate the ability of the renal tubules to concentrate or dilute the urine; therefore, it is a true renal function test. There is no 'normal' value for urine specific gravity. Urine may have a specific gravity between 1.001 and 1.060 in the dog (up to 1.080 in the cat). The normal specific gravity of plasma is between 1.008 and 1.012; when urine specific gravity is in this range the kidney has done neither concentrating nor diluting work. Urine with a specific gravity of 1.008-1.012 is therefore said to have a specific gravity in the fixed or isosthenuric range. (In practice, most authors extend the fixed range up to 1.017). Urine specific gravity of greater than 1.025 implies renal tubular concentration; specific gravities below 1.008 indicate dilution.

Normal animals may have urine specific gravities in the dilute, isosthenuric, or concentrated range, depending upon the state of hydration. Animals which are diuresing are expected to have urine specific gravities in the fixed or dilute range. In contrast, dehydrated animals are expected to concentrate urine.

As stated earlier, the interpretation of azotemia is largely dependent upon urine specific gravity. Prerenal azotemia is the result of reduced renal perfusion seen with conditions such as dehydration and shock, and the elevated BUN and creatinine should be accompanied by a high urine specific gravity. In contrast, primary (renal)
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Azotemia is usually associated with inability of the tubules either to concentrate or to dilute; therefore, the marked evaluation in BUN is generally accompanied by a specific gravity in the isosthenuric range. A fixed specific gravity with azotemia or dehydration indicates that at least two-thirds of the tubules are nonfunctional. Certain caution must be exercised in the interpretation of urine specific gravity. Because even normal animals have occasional urine samples with specific gravities in the fixed range, the significance of a single demonstration of isosthenuria must be questioned. If the animal is in a normal state of hydration and not azotemic, no statement can be made regarding renal function and further evaluation is necessary.

Chemical Examination

Urine Protein

Urinary protein levels are most conveniently determined with a dipstick. Like most renal parameters, urine protein levels must be evaluated in light of urine specific gravity. A 1-2+ proteinuria is far more significant in a dilute urine sample than in a concentrated one. There are many causes of proteinuria and in most cases differentiation depends upon other reagent strip or sediment findings. Hemorrhage or inflammation in the urinary tract may cause proteinuria and will be recognized on the basis of many cells in the sediment. Myoglobinuria or hemoglobinuria, detected as occult blood, also may be a cause of proteinuria. If the preceding causes of proteinuria are lacking, then possible proteinuria due to glomerular leakage must be considered. However, it must be remembered that conditions such as shock or fever may cause a mild nonspecific proteinuria.

Ketones

The presence of ketones in the urine may also be readily established with reagent strips. Ketone bodies are found in the urine when fat metabolism has replaced carbohydrate metabolism as the principal energy-producing pathway. This occurs in a wide variety of conditions, including starvation and diabetes mellitus. Ketonuria is usually associated with a metabolic acidosis.

Glucose

In normal animals, circulating glucose is filtered into the glomerular filtrate and then reabsorbed into general circulation by the renal tubules. Glycosuria is seen in association with hyperglycemia when the tubular reabsorption maximum (180 mg/dl) of the kidney has been exceeded, occasionally in renal disease as a nonspecific finding and, rarely, in congenital renal glycosuria where blood glucose levels are normal but the renal tubules has reduced reabsorptive capabilities. The most common clinical condition with glycosuria is therefore diabetes mellitus. The glucose in the urine in this condition also predisposes to bacterial cystitis; if urine is allowed to stand after collection, glycosuria may not be detected because of bacterial metabolism. False positives may be obtained in cats with hematuria. False negatives may be seen with the reagent strip in animals excreting ascorbic acid in their urine as occurs in diabetes mellitus.

Urobilinogen

Urobilinogen is produced in the intestine by bacterial reduction of bilirubin. Approximately 10% of that produced is recirculated by portal circulation to the liver and via the bile back into the intestine. Ten percent of that recirculated to the liver reaches general circulation, becomes a part of the glomerular filtrate, and is excreted in the urine. Because urobilinogen is produced only from bilirubin that has entered the intestinal tract, the presence of urinary urobilinogen is considered an indication that the bile duct is at least partially patent. Similarly, in theory the absence of urinary urobilinogen should indicate bile duct obstruction. Unfortunately, the test is of low sensitivity and urobilinogen is converted to an inert form almost immediately upon standing. This test is therefore of little interpretive value.

Occult Blood

This test is for the presence of myoglobin or hemoglobin and may be positive in the face of hematuria, hemoglobinuria, or myoglobinuria. Myoglobinuria is seen with muscle disease, hemoglobinuria may be seen with overwhelming hemolysis, and hematuria is seen with hemorrhage anywhere in the urogenital tract. Hematuria is established by the presence of red cells in the urine sediment. Myoglobin may be distinguished from hemoglobin with the ammonium chloride precipitation test.

Urine pH

Normally, the urine pH of carnivores is acidic (less than 7.0). In cystitis pH may be alkaline because of the presence of urea-splitting bacteria. Urine which has been allowed to stand before testing may also be alkaline because of bacterial action.

Urine Sediment Examination

Cells

Three kinds of cells may be found in the urine sediment: white blood cells, red blood cells, and epithelial cells. In voided urine 4-5 red cells/high power field (HPF), 5-8 leukocytes/HPF and occasional epithelial cells/HPF fall within normal limits. Slightly higher numbers may be seen in cathereterized samples. Increased numbers of red blood cells indicate hemorrhage in the urogenital tract, while increased numbers of leukocytes indicate inflammation anywhere in the urogenital tract. Increased numbers of epithelial cells in the sediment are more difficult to interpret. Three types of epithelial cells are found in urine: squamous epithelium from the vagina or prepuce, transitional cells from the lower urinary tract, and the smaller renal epithelial cells. In many cases
epithelial cell type is difficult to establish. In general, increased numbers of epithelial cells in the sediment are associated with inflammation, degeneration or neoplasia of the urogenital tract. Cytologic evaluation of an air-dried stained sediment smear is recommended in cases where increased numbers of epithelial cells are seen.

**Crystals**

Urine of normal dogs and cats contains triple phosphate crystals and usually accumulations of amorphous phosphates. Crystals of pathologic significance in dogs and cats include ammonium biurate and tyrosine crystals (associated with liver disease), oxalate crystals (associated with ethylene glycol toxicosis), and cystine crystals (associated with an inherited metabolic defect). The morphology of these crystals has been discussed elsewhere.

**Casts**

Casts are probably the most important finding in the urine sediment because they localize injury to the kidney. The presence of any casts in the urine is abnormal and usually implies some degree of renal damage. The morphology of casts is described and illustrated elsewhere; only the interpretive significance will be considered here.

Casts may be hyaline, cellular, granular, or waxy. Hyaline casts are composed of mucoprotein and are seen with mild renal injury and glomerular leakage. Febrile animals with normal kidneys may have occasional hyaline casts in the urine. Cellular casts may be red cell, white cell, or epithelial cell in composition. Red cell casts indicate renal hemorrhage or inflammation, white cell casts indicate renal inflammation, and epithelial cell casts indicate acute tubular degeneration.

Granular casts are simply older epithelial cell casts in which the epithelial cells have degenerated to the point that they can no longer be identified as individual cells. Granular casts are of two forms: coarsely granular (early stage) and finely granular (late stage). Both forms are interpreted as evidence of tubular degeneration. With time the finely granular cast is further modified to form a fairly homogeneous cast - the waxy cast. Waxy casts indicate chronic tubular degeneration and must be distinguished from hyaline casts.

It is possible to see epithelial cell, granular, and waxy casts simultaneously in the urine sediment of an animal with ongoing tubular degeneration.

**Bacteria**

Bacteria in urine are only significant in aseptically collected bladder samples which are immediately evaluated. Greater than 100,000/ml indicates bacterial infection of the urogenital tract. Immediacy is important because bacteria multiply readily in standing urine samples.