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 practicing 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.

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.

INTRODUCTION

Hemograms consist of both quantitative data (total cell counts, differential cell counts, red cell indices, etc.) and qualitative data (blood film morphology). Proper interpretation depends on the integration of both.

Proper interpretation also depends upon the development of a systematic approach for both quantitative and qualitative data; we recommend evaluation of white cells first, followed by red cells, and then platelets.

For all cell compartments, interpretation can be guided by asking and answering a series of well-designed questions.

WHITE CELLS (Table 1)

Overview

- Quantitative data includes total white cell count and differential count
- Qualitative data is white cell morphology
- Key questions include:
  - Is there evidence of inflammation?
  - Is there evidence of stress?
  - Is there evidence of tissue necrosis?
  - Is there evidence of systemic hypersensitivity?
  - If inflammatory, can the response be classified as acute, chronic, or overwhelming?
  - Is there evidence of systemic toxemia?
Is there evidence of inflammation?

- Persistent eosinophilia, monocytosis, and a neutrophilic left shift (increased numbers of immature neutrophils), alone or in combination, suggest inflammation.
- Total white cell count merely reflects balance between marrow production and tissue utilization; in inflammation, total white cell counts may be low, normal, or high.
- Absolute neutrophilias of greater than 25,000/µl are also suggestive of inflammation.

Is there evidence of stress (high circulating levels of glucocorticoids)?

- Stress typically results in mild lymphopenia (lymphocyte counts between 750/µl and 1500/µl).
- Eosinopenia, mild neutrophilia, and mild monocytosis may also be present but are less consistent and nonspecific.

Is there evidence of tissue necrosis?

- Monocytosis indicates tissue necrosis and demand for phagocytosis. Monocytosis can occur with acute or chronic inflammation or necrosis.

Is there evidence of systemic hypersensitivity?

- Persistent eosinophilia and/or basophilia is an indicator of systemic hypersensitivity.
- Causes include:
  - Parasitic diseases with a systemic component, eg., heartworms, flea bite dermatitis
  - Allergic tracheobronchitis in dogs (pulmonary infiltrates with eosinophils)
- Feline asthma
- Allergic gastroenteritis
- Systemic mastocytosis
- Disseminated eosinophilic granuloma complex in cats
- Parasitic disease confined to the intestinal tract (e.g., whip worms) does not cause eosinophilia!!

Can the inflammatory response be classified as acute, chronic, or overwhelming?

- In many cases, inflammatory leukograms cannot be further classified.
- In other cases, the differential cell count is typical of acute, chronic, or overwhelming inflammation.
- These typical patterns reflect changes in leukocyte kinetics, or the balance between white cell production in the marrow and white cell utilization in the tissues. These changes are controlled by chemotactic factors and cytokines.
- The typical acute inflammatory leukogram is characterized by neutrophilia with increased band cells (a regenerative left shift), lymphopenia, and variable monocytosis.
  - Neutrophilia reflects a large bone marrow storage pool in dogs and cats and the movement of larger numbers of neutrophils from marrow into blood than are moving from blood into tissues.
  - The left shift suggests depletion of the marrow storage pool of neutrophils with the subsequent recruitment of younger cells into circulation.
  - The lymphopenia reflects stress, a common accompaniment of acute inflammatory processes.
- When present, the monocytosis reflects demand for phagocytosis/tissue necrosis.

➢ There are two patterns typical of chronic inflammation:

- Marked leukocytosis (50,000-120,000/μl) with marked neutrophilia and left shift, neutrophil toxicity, and monocytosis.
  ♦ Most commonly seen with severe focal suppurative lesions
  ♦ Usually accompanied by the anemia of inflammatory disease and hyperglobulinemia

- Normal to slightly elevated white cell count characterized by normal to slightly elevated neutrophil counts, no left shift, normal lymphocyte counts, and monocytosis.
  ♦ The normal to slightly elevated neutrophil count reflects a new balance between marrow production and tissue demand. This balance results from expanded production of neutrophils by the bone marrow in response to cytokines (growth factors) released at the tissue site of injury.
  ♦ The lack of left shift reflects the fact that marrow production of neutrophils has expanded to meet increased tissue demand.
  ♦ The normal lymphocyte count reflects the counterbalancing effects of stress and antigenic stimulation on lymphocyte numbers.
  ♦ Monocytosis reflects demand for phagocytosis/tissue necrosis.

➢ The typical overwhelming inflammatory response is characterized by reduced neutrophil numbers, a left shift, lymphopenia, and variable monocytosis.

- Reduced numbers of neutrophils suggest inability of marrow production to keep pace with tissue demand.

- Left shift indicates depletion of marrow neutrophil storage pools.
- Lymphopenia reflects stress.

- When present, monocytosis indicates tissue necrosis/demand for phagocytosis.

Is there evidence of systemic toxemia?

- The presence of toxic neutrophils on the blood film indicates systemic toxemia (see neutrophil morphology).
- Systemic toxemia is most commonly associated with bacterial infections.
- However, other causes, such as extensive tissue necrosis, must also be considered.

**RED CELLS**

**Overview**

- Quantitative data includes red cell count, hemoglobin, hematocrit, red cell indices (MCV, MCHC), and total protein.
  - Red cell count, hemoglobin, and hematocrit are all measures of red cell mass.
  - Total protein provides information about state of hydration. Elevations most commonly result from dehydration which can also falsely elevate indicators of red cell mass.
- Qualitative data is red cell morphology determined from the blood film.
- Key questions include:
  - Is red cell mass increased (polycythemia), decreased (anemia), or normal?
  - If decreased, is anemia regenerative or nonregenerative?
  - If regenerative, is the mechanism blood loss or hemolysis?
- If nonregenerative, can the mechanism be determined without bone marrow evaluation?
- If red cell mass is increased, is the polycythemia relative or absolute?
- If polycythemia is absolute, is it primary or secondary?

Is red cell mass increased, decreased, or normal?

- Answered by evaluating the indicators of red cell mass.

If decreased, is the anemia regenerative or nonregenerative (Figure 1)?

- Evaluating the blood film is the critical first step in recognizing regenerative anemias. Increased numbers of polychromatophilic erythrocytes on the blood film suggests red cell regeneration.
- Regeneration is confirmed by doing absolute reticulocyte counts. In dogs and cats, absolute reticulocyte counts of greater than 80,000/μl indicate regeneration.

If regenerative, is the mechanism blood loss or hemolysis?

- History, signs, and physical exam are key to differentiation. Most causes of blood loss will be recognized in this way.
- Hemoglobinemia or hemoglobinuria indicates hemolysis.
- Very high reticulocyte counts (>200,000/μl) are highly suggestive of hemolysis.
- Where hemolysis is suspected, red cell morphology should be scrutinized for abnormal red cells which are characteristic of certain hemolytic disorders. These include:
  - Spherocytes
- Heinz bodies
- Schistocytes
- Etiologic agents (*Haemobartonella, Babesia*)
- Ghost cells
- Eccentrocytes

If nonregenerative, can the mechanism be determined without bone marrow evaluation?

- The anemia of inflammatory disease is the most common anemia of dogs and cats and can be presumptively diagnosed from the hemogram. Characteristics include:
  - Mild to moderate normocytic normochromic anemia
  - An inflammatory leukogram

- Iron deficiency causes a characteristic microcytic hypochromic nonregenerative anemia which can be presumptively diagnosed from hemogram data and blood films.

- Megaloblastic anemias (nuclear maturation defect anemias) often have occasional giant red cells (macrocytes) in circulation. Megaloblasts may also be present on blood films. Marrow confirmation is required.

- Myelofibrosis of the bone marrow causes nonregenerative anemia with the following characteristics:
  - Poikilocytosis with dacryocytes and ovalocytes
  - Leukopenia
  - Variable platelet response. Marrow histopathologic confirmation is required.

- Nonregenerative anemias characterized by large numbers (>10/100 WBC counted) of nucleated red cells on blood films in the absence of polychromasia (an inappropriate
nucleated red cell response) indicates bone marrow stromal damage. Causes are most likely:

- Lead poisoning in dogs
- FeLV infection in cats

➢ All other nonregenerative anemias have nonspecific hemogram findings and can only be further assessed via bone marrow evaluation.

If red cell mass is increased, is polycythemia relative or absolute?

➢ Relative polycythemia (due to dehydration) is the most common form. It is characterized by:
  - Increased red cell mass
  - Increased total protein
  - Serum chemical indicators of dehydration

➢ When relative polycythemia is ruled out, the remaining cases are absolute polycythemias.

If absolute, is polycythemia secondary or primary?

➢ Secondary polycythemia is associated with (caused by) a number of other diseases including:
  - Cardiovascular disease
  - Pulmonary disease
  - Renal disease
  - Renal neoplasms (primary or metastatic)
  - Cushing’s disease
In the absence of such an underlying cause, polycythemia is considered to be primary (due to the myeloproliferative disorder polycythemia vera). Polycythemia vera is characterized by:

- Normal tissue oxygenation (normal arterial blood gas)
- Normal erythropoietin levels

PLATELETS

Overview

- The quantitative platelet test is platelet number.
- The qualitative platelet test is platelet morphology.
- Key questions include:
  - If there is increased platelet count (thrombocytosis), is it reactive or primary?
  - If there is a decreased platelet count (thrombocytopenia), can the mechanism be determined?
  - If the platelet count is normal but there is evidence of bleeding, could it be the result of dysfunctional platelets (thrombocytopathy)?
  - Is platelet morphology abnormal?

If there is thrombocytosis, is it reactive or primary?

- Reactive thrombocytosis is seen secondarily with:
  - Splenectomy
  - Excitement
  - Exercise
- Fractures
- High circulating glucocorticoid levels
- Post-blood loss (24 hours or more)
- Myelofibrosis
- Iron deficiency anemia

- Primary thrombocytosis is seen as a distinctive form of platelet leukemia or in association with other myeloproliferative disorders.

If there is a thrombocytopenia, can the mechanism be determined?

- Consumptive thrombocytopenias are associated with inflammation, DIC, and infectious diseases such as Ehrlichiosis and other tick-borne diseases. Features include:
  - Inflammatory leukogram
  - Mild to moderate thrombocytopenia (platelet counts generally greater than 50,000)
  - Schistocytes (in dogs)
  - Normal numbers of marrow precursors

- Sequestration thrombocytopenias are associated with hepatosplenomegaly

- Hypoproliferative thrombocytopenias are associated with reduced numbers of marrow precursors.

- Destructive thrombocytopenias are immune-mediated. They may occur alone or in combination with immune mediated hemolytic disease. Common features include:
  - Marked thrombocytopenia (<50,000/μl)
  - Normal to increased numbers of marrow precursors
If platelet count is normal but there is evidence of bleeding, could it be the result of thrombocytopathy?

- First rule out other causes of bleeding
  - Trauma
  - Coagulation defects (activated partial thromboplastin time, APTT, and prothrombin time, PT, are normal)
  - DIC – fibrinogen and fibrin split products are also within normal
- Run buccal mucosal bleeding time (BMBT)
  - If prolonged, consider thrombocytopathy as a possible (likely) cause

Is platelet morphology abnormal?

- The presence of significant numbers of small platelets (microplatelets) suggests the early phase of a possible immune-mediated thrombocytopenia
- Platelet anisocytosis characterized by significant numbers of enlarged platelets (macroplatelets) suggest increased marrow production of platelets. Commonly seen in responsive thrombocytopenias and regenerative anemias.
- Poorly granulated platelets with a few large granules suggest developmental abnormalities. Evaluate bone marrow.
- Excessively large granules in platelets should be examined as potential inclusions such as those associated with *Ehrlichia platys*. 
<table>
<thead>
<tr>
<th></th>
<th>WBC</th>
<th>Seg</th>
<th>Band</th>
<th>Lymph</th>
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<td>Increased</td>
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<td>Increased or no change</td>
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<td>Increased in dogs; increased or no change in cats</td>
<td>No change</td>
<td>No change in dogs; increased in cats</td>
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<td>Decreased</td>
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Decreased HCT
Consider State of Hydration
Reticulocyte Count – Dogs/Cats

Regenerative
>80,000 Reticulocytes/µl for Dogs
>60,000 Reticulocytes/µl for Cats

Nonregenerative
<80,000 Reticulocytes/µl for Dogs
<60,000 Reticulocytes/µl for Cats

Bone Marrow Evaluation

Blood Loss Anemia
External hemorrhage
Internal hemorrhage
Parasites (fleas, hookworms)
Takes 1-3 days to see HCT decrease

Hemolytic Anemia
Immune-mediated hemolytic anemia with spherocytosis
Heinz body hemolytic anemia from ingesting onions, acetaminophen, etc.
Fragmentation

Blood Marrow Hypoplasia
Anemia of inflammation
Anemia of chronic renal disease
Myelophthisis – bone marrow disease
Toxicosis – chemotherapy

Bone Marrow Hyperplasia
with ineffective erythropoiesis

Nuclear Maturation Defect
FeLV
Drug toxicity

Cytoplasmic Maturation Defect
Lead toxicity
Iron deficiency including chronic blood loss

Figure 1. Interpretive Approach to the Evaluation of Anemia
The liver performs a wide variety of different and seemingly unrelated functions. For example, it plays a central role in plasma protein synthesis, carbohydrate metabolism, lipid metabolism, and detoxification of both endogenous and exogenous substances. In addition, the liver is the site of bilirubin metabolism and bile synthesis, as well as synthesis of most circulating coagulation factors. The Kupffer cells of the hepatic sinusoids form one of the major elements of the monocyte-macrophage continuum (mononuclear phagocyte system).

The diversity of hepatic function suggests that a chemistry organ panel assessing the liver must also be diverse. The screening panel includes tests of primary importance as well as a group of additional tests to be more closely evaluated when abnormalities are present in any of the screens.

**Primary Hepatic Panel**

*Serum Alanine Aminotransferase (ALT)*

Serum alanine aminotransferase (ALT) is probably the most accurate indicator of liver disease in small animal medicine. However, it is important to realize that ALT is *not* a liver function test but rather an indicator of hepatocyte injury. ALT is a liver-specific enzyme present in high concentrations within the cytoplasm of hepatic parenchymal cells. As such, serum ALT activity is obviously increased with necrosis. However, a common response to non-lethal
hepatocellular injury involves membrane blebbing with subsequent release of cytoplasmic-rich vesicles such that increased ALT activity is seen in the serum. Therefore, in a general way, the degree of elevation correlates not with the severity of hepatocellular damage but rather with the number of hepatocytes involved. In other words, diffuse fatty change may result in more extreme ALT activity elevations than focal hepatic necrosis.

As with other serum enzymes, interpretation of ALT values is largely dependent upon circulation dynamics. Serum alanine aminotransferase activity reaches maximal elevation approximately 48 hours after acute injury. The half-life of ALT is approximately 2 to 4 days in the dog and approximately 6 hours in the cat. Consequently, elevations of ALT activity following single episodes of hepatocellular damage will be transient; continuous and persistent elevations imply ongoing hepatocellular damage.

Serum Alkaline Phosphatase (ALP)

Serum alkaline phosphatase (ALP) is a membrane-bound enzyme produced at the bile canalicular surface of hepatocytes. Increased ALP production is induced whenever cholestasis occurs with resultant elevation in circulating enzyme activities. Thus, ALP is not an indicator of hepatocellular leakage, as is ALT; instead ALP is used as an indicator of either intrahepatic or extrahepatic biliary obstruction.

Unfortunately, ALP is not liver-specific; the enzyme is also found in bone, placenta, intestine, kidney and leukocytes. In addition, both exogenous steroid administration and endogenous adrenal glucocorticoid production can induce the production of a second isozyme of ALP in the dog (but not in the cat). Furthermore, drugs such as primidone and phenobarbital can
directly induce ALP production. In general, in dogs 2- to 3-fold elevations of ALP activity are regarded as non-specific and may be the result of liver disease, bone disease, or drug/exogenous steroid administration. Also in dogs, 4-fold elevations or greater are virtually always the result of cholestasis or induction of the corticosteroid isozyme of alkaline phosphatase.

Interpretation of serum ALP activity in cats is quite different. First, normal ALP activity in the liver of cats is much lower than in dogs. In addition, the circulating half-life of ALP in cats is significantly shorter than that of dogs. As a consequence, any elevation in ALP activity in cats is regarded as suggestive of cholestasis.

ALP elevations secondary to cholestasis may occur with or without concurrent elevations of ALT. Many acute conditions causing hepatocellular injury and ALT release also cause hepatocellular swelling and intrahepatic cholestasis. In contrast, many more chronic hepatic disorders are characterized by periportal fibrosis with resultant cholestasis and elevated ALP levels but little active hepatocellular degeneration.

*Serum Gamma Glutamyl Transferase (GGT)*

Serum gamma glutamyl transferase (GGT) is a second membrane bound enzyme associated with bile duct epithelium commonly included in the primary hepatic diagnostic panel. Both ALP and GGT are indicators of cholestasis. It has been suggested that GGT may be more useful than ALP because GGT activity elevations are not directly induced to a significant magnitude by glucocorticoids and drugs such as primidone. However, in most cases this distinction is academic; most drugs which directly induce ALP also cause hepatocellular swelling which secondarily causes intrahepatic cholestasis and elevated GGT activity.
Measuring both GGT and ALP activities is probably most useful in cats where elevations in ALP are often more subtle. Elevations of both enzymes simultaneously provides supportive evidence that cholestasis is present. In cats, a relatively greater increase in ALP than GGT is suggestive of hepatic lipidosis.

*Total Protein (TP) and Albumin*

The majority of the plasma proteins are produced in the liver and severe liver disease may be a cause of hypoproteinemia due to decreased production. Due to the relatively long half-lives of plasma proteins (7-10 days), such alterations are usually seen only in chronic liver disease. Hypoproteinemia of this type is usually predominantly the result of hypoalbuminemia.

If only total protein (TP) is measured (and not albumin), the hypoproteinemia of liver disease may be missed. This is because hepatic disease is sometimes accompanied by hypergammaglobulinemia (gamma globulins are produced by cells of the immune system rather than hepatocytes), which may keep TP levels in the normal range. Hypergammaglobulinemia can develop in chronic liver disease because there are increased levels of circulating foreign proteins which have not been removed by the liver; this results in systemic antigenic stimulation.

*Secondary Hepatic Panel*

*Blood Urea Nitrogen (BUN)*

In the liver, ammonia is metabolized to urea, the principal nitrogenous waste product of mammalian systems. The blood carries urea to the kidneys, where it is excreted as a part of the
glomerular filtrate. In cases of reduced hepatic blood flow (congenital or acquired portosystemic shunts) and possibly with reduced functional hepatic mass, urea production from ammonia may be markedly reduced with a resultant decrease in circulating blood urea nitrogen (BUN) levels. It should be emphasized that a decreased BUN is not specific for liver disease of this nature; on the contrary, a common cause of decreased BUN is diuresis. Establishing liver disease as a cause of decreased BUN is best accomplished by demonstrating a concomitant elevation in circulating ammonia, by measuring pre and postprandial ammonia levels, or by measuring pre and postprandial bile acid levels. Both serum ammonia and serum bile acids are special tests not usually included in the large chemistry profile and therefore beyond the scope of this text.

*Serum Bilirubin, Urine Bilirubin*

When senescent RBCs are phagocytized and degraded by macrophages, the hemoglobin they contain is converted to heme and globin. The protein moiety, globin, is degraded to its amino acid constituents and recycled. The tetrapyrrole ring, heme, is enzymatically cleaved with release of iron and, following further degradation, is converted to free (unconjugated) bilirubin. Unconjugated bilirubin is complexed to albumin and circulated to the liver where it is conjugated with glucuronic acid and excreted in bile as bilirubin diglucuronide.

Sera from normal individuals contain a small amount of both conjugated and unconjugated bilirubin. Increases in total circulating bilirubin may result from prehepatic, intrahepatic or posthepatic causes. Prehepatic elevations are the result of hemolysis; increased breakdown of RBCs leads to increased levels of circulating bilirubin. As might be expected in the acute phase of hemolysis, the majority (more than 75%) of the elevations in bilirubin are
usually the result of elevation in unconjugated (indirect) bilirubin. Elevations due to intrahepatic cholestasis are usually the result of increases in both conjugated (direct) and unconjugated bilirubin. Elevations resulting from posthepatic cholestasis usually feature predominant (75%) elevations in conjugated bilirubin acutely, although levels of unconjugated bilirubin may also be increased. However, the reader is cautioned that these patterns of elevation are suggested as general guidelines and become increasingly less reliable as disease processes progress.

Circulating conjugated bilirubin passes the glomerulus with the glomerular filtrate and is excreted in the urine. Therefore elevated urine bilirubin levels may also be used as an indicator of hepatic disease with cholestasis particularly in the dog, which normally has a low normal renal threshold for bilirubin. In the dog, normal urine contains only small amounts of bilirubin when evaluated by standard reagent dip strip methods; an increased amount is therefore a significant finding. However, occasional increased urine bilirubin with no evidence of liver disease is seen in some dogs. The cause of this phenomenon is uncertain. In other cases, bilirubinuria may precede bilirubinemia in the progression of liver disease. Since only conjugated bilirubin passes the glomerulus, urine bilirubin levels do not usually reflect presence of prehepatic bilirubinemia. The normal cat has a high renal threshold for bilirubin; the reagent dip strip test is almost always negative even when serum bilirubin levels are significantly elevated. Positive urine bilirubin tests in cats are only obtained in the most severe cases of liver disease, usually after clinical icterus is apparent.

Urine bilirubin and serum bilirubin are included only as secondary liver screening tests because they are less sensitive indicators of cholestasis than ALP or GGT. As a general rule in dogs, ALP and GGT elevate earlier than urine bilirubin levels, which in turn can be detected earlier than elevations in serum bilirubin levels.
**Delta Bilirubin**

Delta bilirubin is conjugated bilirubin that has been bound to albumin. In previously used diazo reagent methods, all conjugated bilirubin, whether protein-bound (delta) or not, was measured as direct or conjugated bilirubin. Some newer assays are specific for non-protein-bound conjugated bilirubin, the fraction that most closely parallels active cholestasis. The delta bilirubin fraction is then calculated by subtracting unconjugated and conjugated bilirubin values from total bilirubin.

Delta bilirubin is not readily excreted and therefore has nearly the same circulating half-life as albumin. In contrast, both conjugated and unconjugated bilirubin are readily cleared. Consequently, when liver disease resolves, delta bilirubin persists while conjugated and unconjugated fractions are rapidly excreted. In people with liver disease, if total bilirubin is elevated and the major form is delta bilirubin, the prognosis is favorable. Although less is known with regard to animals, there is some evidence to suggest that the same is true in dogs.

**Cholesterol and Triglycerides**

Because the liver is central to lipid metabolism, hepatic disease greatly influences circulating lipid levels. It is well established that serum cholesterol and triglycerides are often elevated in liver diseases in both man and animals. However, these tests are listed only as components of the secondary liver screen because they are far from specific for hepatic disease. Elevations occur in a large number of diseases such as pancreatitis, diabetes mellitus,
hypothyroidism, etc. These two tests are therefore considered a part of several organ system panels. In contrast, very few conditions result in decreased serum cholesterol. The primary differential for hypocholesterolemia is reduced synthesis secondary to hepatic insufficiency.

*Glucose*

Chronic severe liver disease can cause hypoglycemia or hyperglycemia. This is a reflection of reduced glycogen storage capacity and reduced functional hepatic mass. The presence of hypoglycemia in cases of obvious liver disease is therefore a poor prognostic sign. Hyperglycemia in liver disease is a postprandial event also due to reduced functional hepatic mass where there is no longer a place for glucose storage.

*Reprinted with permission from Biochemical Profiling in the Dog and Cat, A.H. Rebar, G.D. Boon, J.A. Christian,*

URINARY SYSTEM INTRODUCTION

The kidney, like the liver, performs a variety of functions of major importance to the maintenance of normal 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) 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

Urine 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 catheterized 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 and 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.