

THE DIAGNOSIS OF HEPATIC DISEASES

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ANAMNESIS AND PHYSICAL FINDINGS IN HEPATIC DISEASES

The history and physical examination obtained on an animal with hepatic disease is often vague and non-specific. Because of the wide diversity of metabolic functions the liver performs and its great reserve capacity and regenerative ability, clinical signs rarely are evident until the disease is far advanced. Even when signs do exist, the clinician must maintain a high index of suspicion for hepatic disease because of the tendency for similar signs to be initially associated with many other diseases. The history is often one of intermittent abdominal disorders, anorexia, vomiting, and diarrhea or constipation. The owners may notice progressive depression and lethargy or the tendency for the abdomen to enlarge (ascites?). Observant owners may note the presence of acholic feces associated with complete bile duct obstruction or the "orange" urine associated with hyperbilirubinuria. The development of jaundice in the sclera and oral mucus membranes is rarely observed by the client, but may be the sole complaint on admission. The development of petechiation, hemorrhagic enteritis or hematuria enteritis or hematuria associated with hemorrhagic diatheses is an infrequent occurrence in hepatic diseases. The signs of hepatic encephalopathy such as dementia, circling, head pressing, aggression, transient blindness etc. can also be misinterpreted as signs of primary CNS disease. Polydipsia and polyuria are commonly present in dogs with hepatic failure and may be the only historical complaint of significance.

The physical examination of patients with hepatic disease is often no more rewarding than the history. The presence of jaundice, a palpably enlarged liver (symmetrical/asymmetrical, lumpy?) and ascites make the diagnosis uncomplicated, but rarely are all these findings present. Acholic feces and hepatomegaly are the only two definitive signs of hepatic abnormalities, since jaundice may be prehepatic in origin. The presence of normally pigmented stools in a constipated animal does not indicate the current status of bile flow. A fresh fecal sample should always be evaluated for the presence of normal bile pigmentation. Acholic feces tend to be fatty and clay or slate-colored from a lack of stercobilin. Jaundice, in the absence of anemia, is good evidence for hepatocellular or posthepatic biliary disease. Pain on palpation of the liver results from tension on Glisson's capsule and is indicative of acute disease processes (congestion or inflammation), as chronic hepatic disorders are rarely painful. The liver should be palpated, if possible, for size, shape, and location. The normal liver is difficult to palpate in the dog and cat. The borders are sharp and the liver substance is firm. Hepatomegaly is reliable evidence for some degree of injury.

The most common causes or processes associated with hepatomegaly are severe congestion and edema, bile engorgement, diffuse inflammation, nodular hyperplasia, infiltrative diseases (lipidosis or amyloidosis), and primary or secondary neoplasias. A decrease in hepatic size may exist in acute or subacute

necrosis, hepatic atrophy and cirrhosis. Ascites, although not a specific sign of hepatic disease, should make one suspicious of its existence. Palpation in the presence of ascites is often difficult, if not impossible, until the fluid is removed.

LABORATORY EVALUATION OF LIVER DISEASE

Well over 100 tests are currently available for the evaluation of hepatic disease. This large number of tests is better correlated with the large number of metabolic activities the liver is involved in rather than their usefulness as diagnostic aids. It is important for the clinician to select a few specific tests that fully evaluate the major anatomicophysiological divisions of the liver, and understand their significance, rather than run a barrage of randomly selected evaluations.

Whether the liver performs its functions well is determined largely by the integrity and vitality of its enzyme systems. Alterations in diagnostic tests are usually associated with cell damage, resulting either in an inability of the liver to perform a function, or the release of intracellular components that are measured in the blood.

INDICATIONS AND LIMITATIONS OF DIAGNOSTIC TESTS

The primary clinical indications for evaluating hepatic diagnostic tests are (1) to establish the diagnosis of primary and secondary hepatic diseases in jaundiced and nonjaundiced animals, (2) to differentiate between the various causes of jaundice, (3) to provide information necessary to establish a valid prognosis, and (4) to evaluate the effects of therapy in hepatic disease. Many animals with liver disease are only identified after obtaining laboratory data.

The limitations of liver function tests must be taken into consideration when interpreting laboratory results. No single test has been developed that will assess the liver's total functional status. Biochemical functions of the liver do not become equally impaired in every disease, as varying degrees of reserve function exists for each. Often, some functions are severely impaired (bile secretion) while others are totally unaffected, i.e., albumin synthesis. The sequence of loss and return of biochemical functions will vary from one disease to another. Because of the liver's great reserve and regenerative capacity, abnormal function tests may not appear until disease is quite far advanced. Most routine tests have great localizing value (sensitivity for liver disease) but limited specificity (will rarely discriminate between liver diseases). Tests should be considered as screening tests i.e. tests which are highly sensitive and localize a disease process to the liver (alanine transaminase [ALT], aspartate transaminase [AST], alkaline phosphatase [ALP], and gamma glutamyltransferase [GGT]), or as hepatic function tests which assess the liver's ability to perform metabolic work (albumin [ALB], total bilirubin [TB], serum bile acids [SBA], blood ammonia, bromosulfophthalein [BSP], glucose, conglutation profiles, uric acid [UA], cholesterol, blood urea nitrogen [BUN]).

Even though the presence of hepatic disease may be established, it is impossible to determine a morphological or etiological diagnosis from enzyme or function tests alone. This is because many different diseases with widely varied histological pictures produce similar biochemical results. Because of the potentially rapid changes in the status of liver screening and function tests, diagnosis and prognosis must be made on serial evaluations of specific functions rather than on single, isolated diagnostic panels.

The proper interpretation of laboratory data is one of the greatest challenges in internal medicine. The decision on what is "normal" for a given species in a given lab is critical. The "normals" discussed in this section are those the author is familiar with and may be quite different in other laboratories. Always establish accurate normal ranges for the species in question.

SERUM ENZYMES

Numerous serum enzyme determinations are available for evaluation of hepatocellular and biliary tract integrity. They are very valuable for as screening tests to identify the presence of hepatic disease as well as monitor patients over time. Serum enzymes are elevated in hepatic disease for several reasons. Necrosis of cells or increased membrane permeability is responsible for elevations of serum alanine aminotransferase (ALT), serum aspartate aminotransferase (AST), and arginase. Retention and increased production cause increased levels of serum alkaline phosphatase (SAP) and gamma glutamyltranspeptidase (GGT) occur in association with biliary tract disease.

Alanine Aminotransferase: The serum alanine aminotransferase enzyme is liver specific in the dog and cat and should be the enzyme used for the detection of hepatocellular necrosis and inflammation. ALT enzymes are localized to the cytoplasm of hepatocytes and are easily lost to the interstitial spaces and venous system with minor cellular injury. The intracellular concentrations of ALT and AST are 10,000 times that of extracellular fluid. In addition to leakage from cells associated with injury, ALT concentrations may increase in the serum secondary to enzyme induction by drugs (glucocorticoids anticonvulsants). Certain chemical agents are known to dramatically increase the basal intracellular concentration of this enzyme. This can result in mild increases in the "normal" serum concentration and with hepatic injury, very high serum concentrations will be produced by mild lesions. The half-life of both ALT and AST is reported variably as four hours to 4 days, thus if injury to the liver is transient, serum concentrations should return to normal fairly rapidly. Since ALT concentrations may remain elevated for up to 3 weeks following acute experimental toxic hepatitis in dogs, it has been proposed that the continued serum activity may reflect the involvement of this enzyme system in protein synthesis associated with hepatic regeneration and not progressive injury. Elevations occur after leakage from either necrotic cells or from cells surrounding the necrotic area that are stimulated to produce abnormal amounts of

this enzyme. Elevations in ALT in dogs are seen with infectious canine hepatitis, cholestasis, neoplasia, lipidosis, suppurative necrosis, anemias and any other condition resulting in active hepatocellular necrosis or inflammation. Normal values for ALT in the dog and cat vary between laboratories, but are generally less than 60 IU. In acute diseases, the magnitude of rise generally correlates with the severity of injury. Very high values do not necessarily carry a poor prognosis as large numbers of viable hepatocytes are necessary to produce and release massive quantities of the enzyme. In some chronic liver diseases (cirrhosis), the magnitude of rise in ALT does not correlate with the severity of illness, and, in fact values may be normal. In these situations, little active inflammation is taking place and the hepatic mass is sufficiently reduced so that very high levels are no longer physiologically possible. In acute severe disease in dogs, ALT may be increased 100 fold. In extrahepatic biliary obstruction, ALT values commonly are 20 to 70 times normal. Following enzyme induction with anticonvulsants, values may be 4 to 50X normal, while with glucocorticoids they typically are 2 to 10X normal. Dogs with hepatocellular carcinoma often have values for ALT that range from 3 to 35X normal. In cats ALT concentrations associated with acute necrosis or cholangiohepatitis range from 2 to 45 times normal. In lipidosis, values are 2 to 10 times normal. With severe anemia or sepsis or Felv associated diseases values range from 2 to 5 times normal. In cases of extra hepatic obstruction, ALT values in cats typically range from 5 to 45 times the upper normal range.

It is important to remember that although ALT is highly sensitive and specific for the presence of hepatocyte injury in dogs and cats, elevations are NOT specific for any particular disease.

Aspartate transaminase - This enzyme is not liver specific as it is present in high concentrations in extrahepatic tissues, notably skeletal muscle, kidney and brain tissue. The T 1/2 is 5 hrs in dogs and 77 minutes in the cat. Increased AST has a similar diagnostic value to ALT in dogs, however, if AST concentrations are increased and ALT concentrations are normal, some extrahepatic source (muscle?) is likely. In cats, however, AST may be a more sensitive indicator of hepatocellular injury than ALT. AST values are typically more elevated than ALT values in cats with hepatic necrosis, cholangiohepatitis, myeloproliferative disease, lymphosarcoma and chronic bile duct obstruction. The magnitude of rise in AST in severe acute hepatic injury in dogs is 10-30X and in cats it is 2-50X. With extrahepatic obstruction in dogs, AST values are often increased 25X and in cats 20X.

Serum Alkaline Phosphatase: Serum alkaline phosphatase (SAP) determinations are a very important part of any liver disease workup. Increases in SAP primarily occur in cholestatic liver diseases on dogs and cats or are a result of drug induction in dogs, particularly in association with glucocorticoid use.

Since there are dramatic differences between the cat and dog in terms of SAP interpretation and metabolism, they will be considered separately.

ALP is the most sensitive of the hepatic enzymes in dogs, but has low specificity. The serum concentration of alkaline phosphatase in the dog reflects the total activity of several isoenzymes derived from a number of different tissues. To date, 4 serum isoenzymes have been identified by cellulose acetate electrophoresis. These are from liver, bone, a glucocorticoid induced isoenzyme, and a fourth isoenzyme from non-hepatic neoplasms. Although other isoenzymes of alkaline phosphatase exist within placental, renal and intestinal tissue, their half-lives are so short in the circulation (less than 6 minutes) that significant serum concentrations do not develop. The half-lives of both the hepatic and steroid isoenzymes is similar i.e. 66 hours for the former and 70 hours for the latter. Normal SAP is primarily of hepatic origin with the bone isoenzyme of minor importance. Once within the circulation, alkaline phosphatase is not cleared by the liver but is catabolized like other serum proteins.

Many theories concerning the mechanism of SAP increases in hepatic disease have been proposed, but present evidence favors a modification of the regurgitation concept. Experimental work has confirmed that the rise in SAP associated with liver disease is secondary to varying degrees of intra or extrahepatic cholestasis. Cholestasis induces massive increases in the synthesis of alkaline phosphatase by both bile duct epithelial cells and hepatocytes. This massive increase in production in a dog who is unable to excrete the enzyme through blocked bile canaliculi or ducts leads to regurgitation of alkaline phosphatase into the circulation.

Pathologic increases in SAP occur in association with a variety of hepatic disorders, in association with increased endogenous or exogenous glucocorticoids, in certain diseases of bone, occasionally with nonhepatic or osseous neoplasms and following enzyme induction by number of therapeutic agents. Hepatic disorders are the most frequent cause for SAP elevations in the dog. Any hepatic disease that has a degree of cholestasis will be associated with variable increases in SAP concentration. Complete extrahepatic obstructive biliary tract diseases tend to produce the greatest rise, often being 10 to 15 times normal in 2 to 4 days. This may increase to 100X in 2-3 weeks. Severe intrahepatic cholestasis will also result in massive increases in SAP (100X). Diseases that are primarily centrolobular in location cause very mild increases in SAP, while those at the peripheral of the lobule, since they cause much more severe impairment to bile flow, often have very elevated concentrations. With early extrahepatic obstruction, the SAP and bilirubin are markedly increased while ALT concentrations will be mildly elevated. With time, however, obstructive jaundice induces a cholangitis and pericholangitis that results in significant alterations in S-ALT concentrations in addition to massively increased SAP concentrations. Acute hepatocellular necrosis often causes a 2 to 5X increase in SAP.

The identification of a steroid induced isoenzyme of SAP has been of great diagnostic help to veterinarians. The dog is uniquely sensitive to the effects of glucocorticoids as they relate to the hepatic synthesis of a steroid specific isoenzyme. Most dogs, but not all (~85-90%), will have increases in SAP following the administration of glucocorticoids or ACTH. In addition, dogs with pituitary dependent hyperadrenocorticism or functional adrenal cortical tumors frequently develop massive increases in SAP. The magnitude of rise in SAP associated with steroids may be 100 times normal, which is generally higher than that seen in most hepatobiliary disorders. The initial rise in SAP following steroid administration is quite rapid, often within a few days. The magnitude of rise appears to be dependent on the dose, type of steroid, duration of administration and individual patient response. Since hepatomegaly and increased SAP may be identified in many early Cushingoid dogs without other evidence for this disease, clinicians may elect to obtain SAP isoenzyme analyses, hepatic biopsy or ACTH stimulation and low dose dexamethasone test data to identify the cause of hepatic pathology. Steroid hepatopathy is associated with mild increases in ALT (2-5X normal), massive increases (usually) in AP, and marked hepatomegaly.

Abnormalities involving bone synthesis of SAP are uncommon. Situations reported to produce mild increases in SAP of osseous origin include young growing puppies, primary and secondary hyperparathyroidism, bone neoplasia, rickets, osteomalacia and osteoporosis. Although increases in SAP may occur in these conditions, it usually remains normal. When a rise is detected, its magnitude is less than 5 times normal. Bone neoplasia may be associated with 2 to 3 fold increases in SAP.

Several commonly used therapeutic agents have been shown to result in enzyme induction of the hepatic SAP isoenzyme. The most important include primidone, phenobarbital and phenytoin, all commonly used anticonvulsants. The magnitude of rise for phenobarbital is 30-40 fold, that for primidone and phenytoin is 2 to 12 fold. Primidone may also cause a parallel increase in S-ALT concentrations. As with steroids, clinicians must be aware that drugs they use to treat non-hepatic diseases may induce biochemical and/or morphologic changes in the liver.

Recent experimental and clinical work in the cat support the value of SAP as a diagnostic aid in this species. ALP is less sensitive as a diagnostic test in cats, but has higher specificity. It also does not increase in serum to concentrations seen in dogs. In general, although SAP does increase in serum of cats with hepatobiliary disorders, the frequency and magnitude of such pathological increases is low in spontaneous feline hepatic diseases. The reasons for this are several. Feline hepatic tissue contains only 1/3 the concentration of SAP per gram as the dog. The serum half-life of feline SAP is considerably shorter than dogs, 6 hours versus 66 hours, thus it is catabolized so rapidly that it takes major cholestatic disease for its serum activity to rise to a diagnostically useful concentration. The half-lives of other tissue enzymes (intestines and placenta) are so short (2 minutes)

that the only serum isoenzyme identified is that of the liver. Total bile duct ligation in cats produces 2 fold increase in 48 hrs and 9X increases in 2 to 3 weeks. Ligation of one hepatic lobe bile duct causes a 4 fold rise in activity. In conclusion, although SAP will rise in feline hepatobiliary disorders, only those with the most severe cholestatic diseases have diagnostically useful increases in this enzyme. Diseases we have associated with increased SAP include hepatic lymphosarcoma, chronic pancreatitis, cholangiohepatitis and severe lipidosis. Most feline hepatic disorders we have evaluated in our hospital have not had significant increases in SAP. Cats do not have increased SAP in association with glucocorticoid administration.

Gamma-Glutamyltranspeptidase-Another enzyme that is associated with cholestasis in dogs and cats is gamma-glutamyltranspeptidase (GGT). Gamma-glutamyltranspeptidase-GGT exists in high concentrations within the biliary tract and increases are common in obstructive biliary tract disease in dogs and cats. This enzyme has been evaluated in the dog and cat for diagnostic usefulness. The conclusions reached are that GGT has diagnostic implications similar to SAP but no increased usefulness in dogs. This may be because canine liver contains less of this enzyme per gram of tissue than in other species. The magnitude of rise in bile duct ligated dogs is 10-100X and in cats it will increase 2-16 fold. Acute inflammation is associated with mild increases in GGT in dogs and cats (0-3X). Drug induced changes in dogs are 2-3 fold increased over normal.

In cats, GGT is more sensitive and has greater magnitude of rise than SAP for most feline liver diseases. Combining results of GGT with SAP has differential diagnostic value in cirrhosis, extrahepatic obstruction and intrahepatic cholestasis, GGT values usually increase more than SAP. In hepatic lipidosis, SAP concentrations generally have a greater fold increase than GGT.

Selecting several laboratory tests in the initial evaluation of patients suspected to have liver disease is more likely to lead to a correct diagnosis than using single enzyme evaluations. The two most widely available and used tests are ALT and SAP. The combination of ALT and SAP as a screening test for diverse hepatic disorders should detect between 80 and 100% of all diagnosed cases of lipidosis, malignant neoplasia, hepatoma, cirrhosis and hepatitis. Evaluation of both tests together will produce significantly increased diagnostic sensitivity.

HEPATIC FUNCTION TESTS

Multiple tests of hepatic function exist for use in dogs and cats with hepatic disease. In contrast to enzyme tests which have high sensitivity and are very useful as screening tests to detect liver disease, function tests give information relative to the ability of the diseased liver to maintain its normal metabolic work load. Hepatic function tests, like hepatic enzymes, have little specificity in regards to discriminating between various hepatic diseases. They are useful in assessing overall hepatic biochemical health, give an indication relative to chronicity of disease and aid in establishing a rational prognosis.

TESTS OF HEPATIC EXCRETORY AND SECRETORY FUNCTION

Van den Bergh Reaction - A number of valuable and relatively simple laboratory determinations are available for the assessment of the excretory or secretory capabilities of the liver. The estimation of serum concentrations of the total, free, and conjugated bilirubin and urinary and fecal bile pigments are used primarily in the differential diagnosis of jaundice. The history and physical examination of the patient, including examination of urine and fecal color, will usually serve to establish whether jaundice is prehepatic (hemolytic), hepatic or posthepatic (obstructive) in origin. The use of laboratory evaluations confirms the clinical findings and establishes base line data for future use in evaluating therapy or prognosis.

The Van den Bergh test evaluates the amount of conjugated and total bilirubin in the serum and is used primarily to separate cases of hyperbilirubinemia into conjugated or unconjugated forms. Normal total serum bilirubin is less than 0.6 mg/gl in the dog and 0.2 mg/dl in the cat, the bulk of it being unconjugated. Prehepatic or hemolytic cause for jaundice have several typical findings. The animal should be moderately to severely anemic. Hepatic enzyme and function tests (except total bilirubin) should be normal in the early stages of disease. With time, however, hepatic enzymes are often mildly to markedly elevated (2 to 10X increase) if severe hemolytic anemia exists. The majority of the serum bilirubin is initially primarily free or unconjugated and total bilirubin values rarely exceed 10 mg/dl, with 2 to 5 mg/dl total serum bilirubin values being more typical of hemolytic disease. If hepatic hypoxia develops secondary to severe anemia, the hyperbilirubinemia often has a preponderance of the conjugated product present. This will occur within a few days following the onset of hyperbilirubinemia. The feces are darker brown or green and early in hemolytic cases, urine bilirubin is negative or trace positive. Bilirubinuria (conjugated only) also becomes marked within a few days following the onset of hemolytic jaundice due to secondary hepatic-injury or increased renal metabolism and excretion of free bilirubin. Urine urobilinogen concentration is high.

The Van den Bergh reaction is of little or no value in differentiating hepatocellular from posthepatic jaundice. In hepatocellular jaundice, no anemia is present or if present, it is mild (PCV = high 20's or 30's). Bilirubinuria is marked, conjugated hyperbilirubinemia is present (> 50% conjugated) but total bilirubin varies from 1 to 30 mg/dl. Hepatic biochemical profiles are significantly abnormal but values of individual tests are highly variable. The diseased liver cells still maintain reasonable conjugating but intrahepatic cholestasis causes regurgitation of the conjugated bilirubin into the blood.

Posthepatic jaundice is caused by partial or complete mechanical blockage of the common bile duct. Severe hyperbilirubinemia exists with the majority (60to 90%) being conjugated. Bilirubinemia is marked. Urine urobilinogen will be negative in complete obstructions. Coagulation profiles may be abnormal due to decreased vitamin K absorption. The stools may

be acholic (gray, pale and fatty). Anemia is absent unless coagulopathies are present. Many dogs and cats with posthepatic jaundice appear relatively healthy when compared to the depth of their jaundice. Posthepatic jaundice should be suspected in any dog or cat that feel relatively well but has severe icterus.

Regardless of the cause for icterus, it is important to determine the levels of serum bilirubin and the concentrations of conjugated to unconjugated product early in the disease, as the ratio of the conjugated to unconjugated product changes with time, making valid interpretations later in the disease more difficult.

Urine Bile Pigments-The two urine bile pigments of greatest clinical significance are urine bilirubin and urine urobilinogen. Bilirubin is seen in the urine 20 to 60% of normal dogs due to the low renal threshold and will be present in any febrile condition. Male dogs excrete more than females. The routine test to detect bilirubinuria is the tablet diazo reaction (Icto test, Ames Co.,) Pathological bilirubinuria in dogs is indicated by a 2+ to 3+ Icto test reaction at a urine specific gravity of 1.035 or less. In cats, bilirubinuria is always significant! Any hepatocellular or posthepatic liver disease will produce elevated levels of urine bilirubin and bilirubinuria may be the first indication of disease.

Urine urobilinogen results from the enterohepatic circulation of reduced bilirubin and its subsequent renal clearance. The presence of urine urobilinogen is evidence that the bile duct is at least partially patent, while its absence, unless on repeated urinalyses, carries little significance. If repeated negative urine urobilinogen determinations are recorded, an obstructed biliary system is most likely present.

Factors which may alter urine urobilinogen and result in false negative results are (1) dilution in urine to levels too low to detect by commercial reagents, (2) use of intestinal antibiotics that suppress intestinal bacteria responsible for formation of urobilinogen, (3) intermittent bile secretion into the bowel in fasting animals could result in sporadic urobilinogen production and absorption, and (4) reduced absorption of urobilinogen from the bowel in cases of malabsorption and diarrhea, and most importantly, conversion of urine urobilinogen to urobilin by exposure of samples to sunlight. Urine urobilinogen, if exposed to sunlight, is oxidized to a colored pigment, urobilin. It will not be detected in normal reactions for urobilinogen and must be tested for separately. Urine samples to be tested for urobilinogen should be kept in opaque containers or protected from sunlight if analysis is delayed for several hours.

Decreased urine urobilinogen is most often seen in cases of obstructive jaundice, a relatively rare entity in veterinary medicine, or secondary to the factors listed above. Increased urine urobilinogen levels occur in hemolytic states from the general increase in all bile pigments, in hepatitis, when reabsorbed intestinal urobilinogen is not efficiently ex-excreted

by the liver, and in cirrhosis with impaired hepatocellular function but minimally involved biliary excretory pathways.

SERUM BILE ACIDS (SBA)

Serum bile acid assays have supplanted BSP and ICG determinations as one of the most sensitive hepatic function tests available for routine use in clinical practice. They are very sensitive in anicteric animals with mild liver disease. They are more sensitive than total bilirubin since the SBA pool is larger than that for bilirubin, and SBA undergo more extensive enterohepatic circulation. SBA are superior to BSP as a function test and since SBA and bilirubin are removed by separate mechanisms, they are useful in icteric animals. They have been shown to be equal to blood ammonia concentrations in the diagnosis of portal systemic shunts in dogs and cats. They also provide information on the seriousness of a disease process, and may support the hypothesis that portal systemic encephalopathy is present when other tests are equivocal. They may be useful in the differential diagnosis of icterus as they are normal in hemolytic jaundice unless hypoxic injury to the liver has occurred. They are gaining wide acceptance as a useful adjunctive diagnostic test once screening tests determine that liver disease is present.

Bile acids are a normal component of bile. They are synthesized from cholesterol and are a normal elimination route for cholesterol. Within the liver they are conjugated, primarily with the amino acid taurine, in dogs and cats. The primary bile acids in dogs and cats are cholic and chenodeoxycholic acid. Once synthesized, they are secreted into bile canaliculi and stored in the gall bladder. During a meal, humoral and hormonal stimuli cause gall bladder contraction and release of bile acids into the duodenum. Two hours following a meal, a consistent increase in serum bile acids occurs in normal animals. Factors promoting gall bladder contraction include those active in the interdigestive and digestive periods. Motilin, a GI hormone is thought to affect gall bladder contraction during the interdigestive period and acid, fatty acids, amino acids and cholecystikinin (CCK) all play a role in stimulating the gall bladder to contract following a meal. Bile acids are important in the digestion of dietary lipids. They aid in the micellarization of triglycerides which enhances the action of pancreatic lipase. Within the intestine primary bile acids may be modified to the secondary bile acids; cholic acid to deoxycholic acid and chenodeoxycholic acid to lithocholic acid. The majority of intestinal bile acids are actively reabsorbed in the ileum, enter the portal vein and are reextracted by the liver to again be stored in the gall bladder. Daily fecal losses amount to only 2 to 5% of the total bile acid pool. Thus, hepatic synthesis of bile acids requires minimal hepatic function on a day to day basis due to the efficient recapturing of circulating bile acids. In fact decreased synthesis is not thought to be important in assessing serum bile acid concentrations in dogs and cats, since so little demand is placed

on the liver to replace the small daily fecal losses. During meals, the total bile acid pool recirculates 2 to 5 times. After a 12 hour fast, few bile acids remain in the circulation, having been efficiently removed from the blood and stored in the gall bladder. First pass extraction by the normal liver approximates 70 to 90%. This is true regardless of the quantity of bile acids in the portal blood. Since a fixed percent normally escapes hepatic extraction, mild increases occur for 4 to 6 hours following a meal in healthy dogs and cats. Portal vein bile acid concentrations are 6 times peripheral vein concentrations during fasting in dogs. A number of hepatic processes are involved in maintaining normal values for fasting or post-prandial bile acid concentrations. These include: intestinal absorption, portal delivery, hepatic extraction, hepatic conjugation and hepatocyte secretion into the bile must all function well. Most hepatic diseases associated with alterations in serum bile acids have impaired hepatic perfusion or promote regurgitation of bile acids into serum.

Bile acids are useful adjuncts to the diagnostic work up of patients with liver disease. They should not be used as screening tests but add significantly to the functional assessment of patients with liver disease once it has been identified. Both 12 hour fasting and two hour postprandial samples are usually obtained. Recent data on cats suggests that a 5 hour fast may be reliable in cats. In patients that have been anorectic for several days, fasting values may occasionally be normal. The failing liver still slowly removes bile acids, even though its functional capacity to do so is severely impaired. By feeding the animal, a large endogenous load of bile acids enters the intestines, are absorbed, and must be removed by the diseased liver. Normal fasting and post-prandial values for dogs are <5 and <15 micromoles/L, and for cats are <3 and <12 micromoles/L, respectively. Factors which may decrease SBA concentrations include small intestinal diseases that impair distal ileal absorption, rapid small bowel transit times or mucosal disease, and inability of the liver to synthesize sufficient bile acids to normalize serum levels. In spite of these concerns about falsely depressed serum concentrations, clinical evaluation of dogs with small intestinal diseases indicated they had normal fasting SBA concentrations. These dogs had normal liver function, however.

In the evaluation of patients with known liver disease fasting SBA values are usually obtained initially, with post-prandial values being used if fasting values are normal and an indication about the functional integrity of the liver is wanted. In addition, since occasional animals with serious liver disease will have normal fasting values, the post-prandial value confirms that the disease is, or is not, causing functional damage. In comparison testing of fasting SBA with other routine liver screening tests, they were determined to improve the diagnostic performance of all the others (ALT, AST, ALP, GGT, TB, ALB, urea). Fasting SBA of >30 had >90% specificity that significant liver disease was present on biopsy. If values exceeded 50, the specificity was 100%. In cats, specificity of fasting SBA was

>90% when values were >5 and 100% if >20 micromoles/L. Liver biopsy should be considered when fasting SBA values exceed 30 in dogs and 20 in cats. The best combination of tests to increase diagnostic sensitivity occurs by combining SBA with ALT, and TB for both dogs and cats. It is important to remember that SBA are not specific for any given disease, rather they detect significant abnormalities in hepatic function at early stages and predict whether hepatic lesions will be found, but do not give indications about the cause for the elevations.

Post-prandial SBA values occasionally do not rise above fasting values and sometimes actually decline 2 hours after a meal. A number of reasons for this phenomenon exists. Meal size appears to be important in stimulating gall bladder contraction. Frequently, 2 to 3 tablespoons of a canned, high fat diet are used as the test meal, while in developing the test, normal meals were fed to dogs and cats. Patients with hepatic failure may not readily consume a normal meal at one time. We usually use prescription diet p.d for dogs and c/d for cats, if signs of encephalopathy are absent. If the patient is protein intolerant, a reduced protein diet may be used and supplemented with 1 to 3 tablespoons of corn oil. It has been recently demonstrated that 0.5 tablespoons/kg body weight of c/d or 3 tablespoons c/d per cat was sufficient to consistently induce a significant rise in post-prandial SBA concentrations in normal cats. Other explanations for failure of post-prandial values to increase include: delayed gastric emptying, variations in how dogs and cats normally respond to food stimulated gall bladder contraction, slow delivery of bile acids to the ileum, mucosal diseases which impair absorption, and spontaneous emptying of the gallbladder during the fasting period. In animals with complete bile duct obstruction, SBA values would also not be expected to go up significantly following a meal since the gallbladder could not empty.

ORGANIC DYE EXCRETION

Uptake and excretion of a number of organic dyes have been used as a sensitive index of hepatic function for many years. The purpose of utilizing such clearance tests is to evaluate the functional capacity of the liver. Such tests assess the ability of the liver to remove a given volume of a substance per unit of time. These tests are some of the most reliable and sensitive indicators of hepatic function available. They are useful for assessing liver function when hepatic enzyme tests are normal but hepatic disease is still suspected. Of these dyes, sulfobromophthalein (BSP) and indocyanine green (ICG) have the greatest clinical application, at present. Both are primarily excreted from the body via the bile after uptake and conjugation by the liver. Because of the hepatic metabolic pathways involved in their excretion, they are sensitive indicators of hepatic disease. In addition to normal hepatic biochemical integrity, the excretion of these dyes requires a normal hepatic blood flow for proper elimination. Thus, elevated BSP and ICG retention will occur in conditions altering hepatic blood flow, such as congestive heart failure or other diseases inducing chronic

passive congestion. Unfortunately, due to rare anaphylactic reactions to BSP in man, this test is no longer used in human medicine. This has made the dye difficult to obtain for veterinarians. It can still be purchased through chemical supply houses but its availability is limited and few laboratories routinely perform this test at the present time. It has largely been replaced by measurement of fasting and post-prandial serum bile acid concentrations.

Measurement of BSP is the simpler and more economical of the two dye clearance tests. A standard dose of 5 mg/kg is injected intravenously and a pre-injection heparinized blood sample taken. Thirty minutes later, a second blood sample is taken from an alternate vein. Normal dogs have less than 5% retention of the dye after 30 minutes, although values up to 10% retention have been found in dogs with no histological evidence of hepatic disease. Delayed BSP excretion is seen in such diseases as infectious canine hepatitis, fibrosis, lipidosis, diabetes mellitus, leptospirosis, portal vascular anomalies and carbontetrachloride toxicosis. Normals should be determined for your clinical situation as different laboratories have variable techniques for interpreting the percentage dye retention. Determinations of BSP retention in jaundiced animals is not recommended, as bilirubin competes with BSP for uptake and delays in excretion will be difficult, if not impossible, to interpret accurately. It has been reported that total bilirubin concentrations of less than 5 mg/dl will not interfere with BSP uptake by the liver. The results of BSP testing may be significantly altered in hypoalbuminemic patients. BSP is bound to a great degree to albumin in the blood. Only the small fraction of unbound or free BSP is actively cleared by the liver. In hypoalbuminemic states more of the injected BSP is unbound and available for excretion in any given time period. Therefore, it has been proposed that BSP retention may be falsely lowered in hypoalbuminemic patients with hepatic disease. This has not been substantiated in clinical patients, however. Phenobarbital has also been reported to significantly increase the rate of removal of BSP from the circulation.

Indocyanine green is also a sensitive clearance test for canine liver function, although it requires more sophisticated equipment and is somewhat more expensive to use. ICG, after intravenous administration, has an exponential disappearance from the blood during the first 15 minutes. By taking 3 timed blood samples during that interval, the half time for ICG clearance can be determined. The advantages of ICG over BSP are that in addition to measuring dye clearance it can be used to estimate hepatic blood flow and plasma volume. The range for the fractional clearance of ICG is 5.5 to 9.8% per minute. For most clinical situations, the BSP retention test is as satisfactory as ICG determinations.

PROTEIN SYNTHESIS

Tests to evaluate the integrity of hepatic protein synthetic capabilities are useful in evaluating animals with hepatic disease. All the plasma albumin, fibrinogen, and prothrombin

synthesis occurs in the liver. With impairment of hepatocellular function, synthesis of plasma proteins is depressed and levels fall. Since many other conditions will depress plasma protein levels, hypoalbuminemia is not specific for hepatic damage and must be interpreted in conjunction with other hepatic diagnostic tests and ruling out the other causes for hypoalbuminemia (anorexia, GI loss, glomerular loss).

Normal total plasma protein levels in the dog and cat are between 6.0 and 7.5 gm/dl. The normal half-life of plasma albumin is approximately 9 days, thus, levels will decrease slowly even if synthesis is totally interrupted. In addition, hepatic albumin synthesis is normally occurring at 1/3 maximal capacity, during disease states, increased production can compensate for partial loss of function for variable time periods. A separation of serum protein into albumin and globulin fractions is absolutely necessary if proper interpretation of total serum protein values is to be made. Serum albumin levels can become quite depressed with longstanding hepatic diseases (<1 to 2 gm/dl), but because gammaglobulin levels are often elevated, total serum protein determinations may be near normal. The presence of hypoalbuminemia supports a diagnosis of chronic hepatic disease. In animals with hypoalbuminemia, a guarded to poor prognosis is generally warranted, irrespective of the cause for the liver disease. The one exception is animals with surgically correctable portal vascular anomalies.

Serum gamma globulins are often elevated in hepatic disorders (hepatitis, diffuse fibrosis), although the mechanisms for this elevation are poorly understood. The practice of using albumin-globulin ratios alone should be discontinued in deference to evaluating the absolute values for albumin and globulin concentrations.

Procoagulant Synthesis-The liver is capable of synthesizing all the coagulation factors except factor VIII and calcium. Abnormalities in prothrombin time (PT), activated clotting time (ACT), activated partial thromboplastin time (APTT), and thrombin time (TT) may all occur. In spite of these abnormalities being detectable on coagulation profiles, overt bleeding due to factor deficiencies is rare. Other factors often precipitate a bleeding episode such as GI ulcers, surgery or trauma. In general, significant coagulopathies are most likely to develop in animals with severe parenchymal failure or extrahepatic bile duct obstruction. The PT and PTT are most useful in differentiating these two conditions. If the animal has complete bile duct obstruction, providing 5 to 15 mg of vitamin K-1 should result in a 30% or greater shortening of coagulation parameters in 12 to 24 hours. In parenchymal failure, inability to synthesize vitamin K is the reason for the coagulopathy and it will not be corrected by parenteral vitamin-K.

AMMONIA METABOLISM-

Ammonium tolerance tests have been well evaluated in clinical and experimental hepatic disorders in dogs and cats in recent years. The test involves the oral administration of an

ammonium salt (ammonium chloride) and measuring the blood NH_3 concentration 30 minutes later. The test is particularly useful for assessing the integrity of the hepatic portal system. The orally administered drug enters the small bowel, the ammonium is converted to ammonia by the alkaline intestinal contents and absorbed. If significant portal vascular shunting, hepatocellular failure or deficiencies of urea cycle enzymes exist, blood ammonia concentrations will be significantly elevated. The procedure involves orally or rectally administering NH_4Cl at a dosage of 100 mg/kg up to a maximum of 3 gms. The animal should be fasted for 12 hours and the ammonium salt administered diluted in 20 to 50 ml of water to prevent vomiting. It may be given by syringe or stomach tube. This procedure has been well tolerated by many dogs and cats with severe hepatic failure except for occasional vomiting or nausea. Rarely, this test may precipitate a bout of hepatic encephalopathy. Samples of heparinized blood are drawn at zero time and 30 minutes following the drug. Normal values for the dog are between 60 and 120 $\mu\text{g}/\text{dl}$ on fasting samples and less than 200 $\mu\text{g}/\text{dl}$ at 30 minutes and generally there is less than a 2 fold increase. Although many animals with signs of hepatic encephalopathy have raised fasting blood ammonia concentrations, a significant number of dogs with severe hepatic failure will be normal, so that resting concentrations alone may not be diagnostic. We, therefore, routinely perform ammonia challenge tests unless the animal is showing obvious signs of encephalopathy. Significant increases are most often seen in portal vascular anomalies or chronic cirrhotics. We have found blood NH_3 concentrations to be particularly useful in dogs with congenital vascular anomalies while the BSP test has had greater reliability than NH_3 in a limited number of chronic fibrotic liver diseases. More clinical data is needed on cases where both tests have been run simultaneously in order to determine if either one has greater diagnostic accuracy in selected hepatic disorders. Ammonia tolerance testing has no greater sensitivity than pre and post-prandial serum bile acid values and is much more sensitive to errors in handling. Long venous occlusion during collection may also falsely elevate blood ammonia concentrations. Heparinized blood must be placed on ice and should be analyzed within 30 minutes of collection. Frozen plasma has unpredictable increases or decreases when compared to results run immediately from dogs. In cats frozen plasma values for NH_3 are stable for 48 hrs.

Low Blood Urea Nitrogen-Impaired urea synthesis from ammonia may occur as a late complication of liver disease due to a severely decreased functional mass (70%), or more commonly, secondary to acquired or congenital portal systemic shunting. BUN concentrations below normal have very low sensitivity and specificity for hepatic disease. Low values may be a result of anorexia, low protein diets, diuresis, and polydipsia. They may also be increased by many variables negating their value in most animals with hepatic failure.

BLOOD GLUCOSE

The liver plays a major role in normal carbohydrate metabolism. During fasting, it is responsible for maintaining blood glucose values via glycogenolysis and gluconeogenesis. The ability of most animals to maintain normal blood glucose values in the presence of severe hepatic disease is remarkable. Hypoglycemia is uncommonly documented. It is more likely to occur and be clinically significant in dogs with fulminant hepatic failure, congenital portal vascular anomalies or cirrhosis.

CHOLESTEROL METABOLISM

Measurement of serum cholesterol and cholesterol esters have been used as supportive evidence for the presence of hepatic disease and may be of limited value in prognosis. The liver is the main source of endogenous cholesterol production. Large amounts are excreted in the bile and partially converted to bile acids and their salts. The liver is also responsible for the normal esterification of cholesterol, esterified cholesterol comprising 60-80% of the total serum levels. Total serum cholesterol levels are often elevated in obstructive biliary disease. The elevations are due, in part, to regurgitation, but are also a result of increased bile salt release to plasma, which holds cholesterol and other lipids in solution, preventing their tissue uptake.

With hepatocellular disease or partial systemic shunting, both total and esterified fractions tend to fall as the disease progresses. Progressively decreasing total and esterified cholesterol values are considered a poor prognostic sign in man. Normal total cholesterol values range from 125 to 250 mg/dl and are affected by such variables as diet, exercise, and metabolic diseases (diabetes mellitus, Cushing's Syndrome, hypothyroidism, and nephrotic syndrome).

URIC ACID

Uric acid levels in serum were used at one time as an indication of hepatocellular damage since, in the dog, uric acid is converted in the liver to allantoin for excretion. With liver cell damage, uric acid will increase (> 0.6 mg/dl). Since the advent of newer and more sensitive tests (bile acids, SAP, ALT), the use of uric acid as a diagnostic test has little value. Increase of urine uric acid concentrations may predispose dogs and cats to the formation of ammonium urate or uric acid urocystoliths. In addition, the presence of ammonium urate ("biurate") crystals in urine is seen in many dogs with congenital portal systemic shunts.

RADIOGRAPHY

Plain and contrast radiographs and hepatobiliary ultrasonography can be used to evaluate the liver in suspected cases of hepatic disease. If ascites is present, it must be removed prior to survey radiographic evaluation for consistently reliable results to be obtained. For ultrasonography, however, fluid often aids in the procedure. Survey films enable the size,

shape and position of the liver to be ascertained. Normal radiographic liver size has not been precisely determined, partly because of the great variability between dogs and cats, and the fact that it changes position with respirations, positioning, obesity and disease in adjacent organs. Liver size is best evaluated in the lateral projection as often the liver shadow on the ventro-dorsal view is obscured by adjacent organs. In lateral recumbency the diaphragmatic crura are very mobile. The dependent crus sags forward more than the upper one causing the liver to look larger in right lateral recumbency. Respiratory movements also cause the liver to shift its position significantly. Upon inspiration, the liver moves caudally and on expiration, cranially. The normal caudal liver margin on the lateral projection thus may shift approximately one centimeter cranial or caudal to the costal arch in dogs. Hepatomegaly is not difficult to interpret. Reduced hepatic size, however, is often not fully appreciated radiographically. Microhepatica most often is seen with congenital vascular anomalies that produce hepatic atrophy, or in chronic, fibrotic liver disease with scar tissue contracture and cellular collapse. Small livers can only be assessed by the position of adjacent organs or by pneumoperitoneography or ultrasonography. When the liver is reduced in size adjacent abdominal viscera shift cranially, i.e. stomach, pylorus, spleen and duodenum. The stomach angle, rather than being vertical or angled slightly cranial to caudal will have the pylorus shifted cranially while the cardia remains fixed in its normal position. Putting a small quantity of gas (air) into the stomach with a stomach tube is an easy method of assessing the position of the caudal liver margin relative to the stomach.

Contrast angiography of the liver has received intense investigation as clinicians have become increasingly aware of the importance of congenital vascular anomalies as a clinical entity. Contrast angiography is at present the only dependable method of determining if a vascular shunt exists and if it is surgically correctable. In addition to identifying congenital vascular anomalies, angiography can determine if portal hypertension is due to intra or extrahepatic portal vein obstruction and aid in the diagnosis of hepatic and pancreatic neoplasia. Four radiographic techniques have been used to evaluate hepatic blood supply, cranial mesenteric arteriography, transabdominal splenoportography, operative mesenteric portography and coeliac arteriography. Splenoportography and mesenteric portography are the easiest techniques to use and do not require sophisticated radiographic equipment to perform. Although the technique for both oral and intravenous cholangiography have been well worked out in dogs and cats, they have limited clinical usefulness because of the infrequency of obstructive biliary tract disease.

Hepatic ultrasonography is very useful in the evaluation of animals with hepatic disease. It is particularly beneficial in animals with ascites. In addition to size, shape and position of the liver, its echotexture (density) can be assessed, and evaluations of the biliary and portal venous systems can be made. Extrahepatic biliary obstruction can be diagnosed via

ultrasonography. Hepatic neoplasia may also be presumptively diagnosed via ultrasonography. In some cases, ultrasound guided biopsy of focal hepatic lesions can be done. Biopsy of small livers can also be aided by ultrasonography.

BIOPSY

Liver biopsy is technically not a function test but it remains as one of the most important diagnostic aids available for clinical evaluation of patients with hepatic disease. In many cases, even after a careful clinical and laboratory evaluation, a specific etiologic or morphologic diagnosis is lacking and biopsy is the only method of obtaining such information. Liver biopsy is not an innocuous procedure and should only be considered when the potential benefit to the patient outweighs the inherent risks of the technique. Biopsy of the liver is indicated primarily when a specific diagnosis is lacking, as an aid to determine or evaluate therapy, and to establish a prognosis. Successful results of liver biopsy are most often obtained when diffuse hepatic diseases are involved (lipidosis, amyloidosis, diffuse neoplasia, glycogen storage diseases toxicities), or when diffuse necrosis and fibrosis exist, rather than with focal lesions. This is so because the size of the sample is small and the sample is most easily obtained by a blind technique.

Prior to performing liver biopsy, the clinician must be familiar with the techniques available and be prepared to control any of the potential (but rare) complications following biopsy (hemorrhage, bile peritonitis, sepsis, etc). As prebiopsy considerations, a complete clinical and laboratory evaluation must be performed and an activated clotting time determined. Abdominal ultrasonography can be very helpful prior to and during liver biopsy. Impression smears and cultures of the biopsy material should be taken prior to fixation in formalin and may yield useful diagnostic information. Special stains for determining if fat or glycogen is present should be available.

Potential post-biopsy complications are bile peritonitis, following inadvertent penetration of the gall bladder or an engorged bile duct under pressure, hemorrhage, injury to other organs and pneumothorax. Patients should be kept quiet for 24 to 48 hours after biopsy and the clinician should avoid any deep or excessive palpation. Any post-biopsy hemorrhage can usually be controlled by fresh, whole blood transfusions.

Needle biopsy is the most efficient in terms of time and cost of the various procedures available for obtaining hepatic tissue. The needles used most often are the Tru-Cut, Menghini or modified Vim-Silverman. The choice of needle or surgical approach depends more on clinician preferences than scientific fact. Aspiration needles, like the Menghini, have been considered to be less reliable for obtaining tissue from fibrotic livers and also generally obtain smaller samples. A recent study comparing the Menghini needle against the Tru-Cut indicates that except for greater fragmentation of samples, both needles obtained similar volumes of hepatic tissue and neither had better success in fibrotic livers.

Two recent reports summarized the results of a large number of liver biopsies obtained by the transthoracic approach, or a blind percutaneous transabdominal approach using a Menghini needle. Of these 182 total cases, only one mortality associated with the procedure was recorded. Significant complications were observed in 11/182 (6.0%), these included gall bladder puncture, (6), 2 required surgery to correct, 4 sealed spontaneously; biopsy of adjacent organs (3 cases, no complications); hemoperitoneum, 1 case, resolved with cage rest; and 1 case of transfer of ascites into the thorax through a diaphragmatic puncture which was controlled using thoracentesis and diuretics. The histopathological results from these two studies are difficult to compare because of the variability between pathologists reading hepatic biopsies. The ten most often made diagnoses were (1) steroid hepatopathy, a diagnosis reported only by one group (18.1%, 33/182, this includes both Cushing's disease and exogenous steroid administration; (2) normal liver tissue (15.9%, 29/182); (3) neoplasia (14.8%, 27/182); (4) inflammatory hepatitis, includes chronic active hepatitis which was diagnosed by only one group (27/182); (5) acute necrosis (11.5%, 75/182); (6) lipidosis (9.3%, 17/182); (7) no tissue obtained (6.0%, 10/182); (8) cholestasis (4.3%, 8/182); (9) cholangitis (2.2%, 4/182); and (10) cirrhosis (1.1%, 2/182). Twenty-five of these 182 cases eventually had necropsies and necropsy diagnoses correlated with the previous needle biopsies in 80%. It is obvious, that the biopsy information obtained in these cases significantly affected the prognosis and therapy of many of these animals, and this type of information was available by no other diagnostic means. Because needle biopsy of the liver is so easy to learn and become proficient at, veterinarians should strive to master this technique and have it become a routine part of hepatic disease workups.