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The Diagnosis of Liver Disease in the Dog and Horse

Charles E. Cornelius, D.V.M., Ph.D.

The diagnosis and prognosis of liver disease is dependent upon an understanding of the biochemistry of hepatic function and its clinical and laboratory manifestations. A complete history and an exhaustive clinical examination are of prime importance to correlate with the indicated laboratory tests. Due to the rapid regenerative ability of the liver\(^1\), interpretations of all function tests must be viewed only in terms of short time intervals. The use of a few procedures which can be interpreted on the basis of past experience is most desirable.

Only few clinical signs are present in diseases of the liver; these may be: icterus, the amine-like odor\(^2\) of massive liver degeneration, a tender abdomen, digestive disturbances, clay-colored stools, a yellow-green urine from bilirubin-glucuronide regurgitation, and only rarely petechiae or massive hemorrhage from prothrombin deficiency. In animals with hidden liver disease, the diagnosis is entirely dependent upon either a biopsy for histopathological examination or liver function tests.

The presence of icterus in all domestic animals cannot be interpreted similarly. The enterohepatic circulation of bile pigments is presented in fig. 1. The heme liberated from the turnover of erythrocyte hemoglobin is reduced within the reticulo-endothelial cells of the body to first biliverdin and later to free bilirubin. The free-bilirubin (pre-hepatic) is subsequently conjugated with glucuronic acid within the hepatic cell and excreted into the bile. Urobilinogen results from the bacterial reduction of bilirubin-glucuronide in the small intestine. The major portion of the urobilinogen (also referred to as stercobilinogen) is excreted and imparts the normal color to the stool. A smaller part is reabsorbed into the portal blood where it enters the liver, only to be excreted again into the bile (enterohepatic circulation). Trace amounts, however, of the reabsorbed urobilinogen escape into the general circulation from the liver and are subsequently eliminated into the urine.

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Fig. 1. Enterohepatic circulation of bile pigments.

Elevations in the serum free bilirubin (Indirect Van den Bergh) may occur from (3):

1. Excess production of free bilirubin from hemolysis.
2. Decreased uptake of free bilirubin from hepato-cellular pathology. Elevations in the serum bilirubin-glucuronide (Direct Van den Bergh) may occur from (3):
   a. Intrahepatic obstruction of biliary canaliculi by hepatocellular pathology and regurgitation into the blood and lymph.
   b. Extrahepatic obstruction of the bile duct system and regurgitation into the blood and lymph.

Free serum bilirubin (pre-hepatic) will not pass into the urine as does the regurgitated bilirubin-glucuronide (post hepatic). The presence of bile pigment in the urine is, therefore, due to regurgitation following its normal cellular conjugation in the liver. This regurgitation may be from either lesions producing occlusion of the intrahepatic biliary canaliculi or extrahepatic bile duct closure. The presence of urobilinogen in the urine indicates that an open bile duct exists but its absence at one sampling need not indicate bile duct closure since diurnal variations are possible. In hepatocellular damage, there is a defect in the excretion of urobilinogen by the liver into the bile from the portal blood; this results in the escape of a greater percentage of the pigment into the general circulation and finally its excretion into the urine. In hemolytic anemias, larger amounts occur in the urine from both secondary hepatic insufficiency and the presence of a greater quantity of bile pigments within the enterohepatic circulation. The finding of a 3 or 4 plus urine urobilinogen reaction accompanying a 2 to 4 plus urine bilirubin-glucuronide reaction is quite suggestive of hepato-cellular damage. A 3 to 4 plus urinary bilirubin-glucuronide, and a consistently negative urobilinogen, is indicative of an extrahepatic bile duct occlusion or, rarely, a progressive chronic fibrosis. High levels of urinary urobilinogen accompanied by very little or no bilirubin-glucuronide would be compatible with a hemolytic crisis.

The clinical examination of the animal may indicate the type of icterus or liver lesion present. The anemia and icterus in an animal without any appreciable bilirubin-glucuronide in a normal urine would be of a hemolytic nature since the free bilirubin would not pass the renal filter. The presence of clinical icterus without anemia and with normal colored stools would indicate most likely a specific hepatic involvement. An icteric animal with a dark yellow-green urine and clay colored stools would typify a case of complete bile duct closure (extra-hepatic obstruction). The gross urine examination can be performed by vigorously shaking the urine sample and observing the yellow-green foam which is positive for regurgitated bile pigments (bilirubin-glucuronide). The gross fecal examination for the presence of the clay colored stools of obstructive icterus can be confused with the light fawn-colored fatty stools of chronic pancreatic fibrosis or possibly the stools following bone ingestion by canines. Without the presence of such clinical signals as icterus, bile-containing urine and clay-colored stools, hidden hepatoopathy may remain undiagnosed unless liver function tests are performed.
Indications for Liver Function Tests

1. Primary liver disorders such as infectious hepatitis, leptospirosis, suppurative hepatitis, diffuse hepatic fibrosis, acute toxic diffuse necrosis, hepatic hemangioma, hepatoma, and bile duct adenoma and carcinoma.
2. Secondary liver disorders such as severe infiltrative and degenerative lipoidosis accompanying hypothyroidism, diabetes mellitus, and pancreatic atrophy and fibrosis; also, chronic passive congestion in cardiac decompensation, secondary amyloidosis, and metastatic hepatic malignancy.
3. Differential diagnosis of icterus from hemolytic crisis, intrahepatic obstruction within the liver, and extrahepatic obstruction of the duct system.
4. Anemias of undetermined origin. Normocytic, normochromic anemias from hemorrhages in prothrombin deficiency or from chronic progressive diffuse hepatic fibrosis can occur.
5. Prognosis of hepatic disease and evaluation of therapy.

Classification of Liver Function Tests

A. Tests depending primarily on biliary excretion:
   1. Serum bilirubin (Van den Bergh test)
   2. Bile pigments in the urine
      a. Bilirubin conjugate of glucuronic acid
      b. Urobilinogen
   3. Bromsulphalein (BSP) dye excretion test
   4. Alkaline phosphatase

B. Tests dependent upon specific independent biochemical functions:
   1. Serum protein production (paper electrophoresis and chemical “salt-ing out” techniques)
   2. Prothrombin production (prothrombin time or coagulation time by capillary tube technique)
   3. Serum cholesterol and cholesterol esters
   4. Blood uric acid

C. Tests dependent upon the abnormal release of hepatic cellular enzymes into the blood:
   1. Serum glutamic oxaloacetic-transaminase
   2. Serum glutamic pyruvic-transaminase

Canine Liver Function Tests

The Van den Bergh test is interpreted similarly as in man except the renal threshold is much lower for the direct-reacting pigment. The direct reacting bilirubin-glucuronide passes rapidly upon its regurgitation within the liver into the blood and the urine. All direct reacting serum bilirubin levels are much lower than in other species and the measurement of this pigment in the urine is an extremely sensitive test for early hepatocellular changes. Care should be exercised in its interpretation in the dog, since in any febrile state, it may be present in the urine in a low concentration. The presence of a 2-3 plus reaction on commercial pill tests is considered of diagnostic significance. The bilirubin-glucuronide will be found in the urine for several days in experimental biliary obstruction before clinical icterus may become apparent. The measurement of bilirubin-glucuronide is easy for the practitioner to perform, and is the most specific test to show partial or complete obstructive icterus.

The urinary urobilinogen test, if positive, indicates an open bile duct exists. High levels will be found in both hepatic damage and hemolytic crisis. Changes in the specific gravity of urine in the maintenance of water balance will vary the urobilinogen concentration considerably.

The Bromsulphalein (BSP) test appears to approach the nearly perfect function test for latent liver damage in the dog. The hepatic blood flow, the complex enzymatic cellular uptake of BSP and its excretion, and the patency of the bile duct are all quantitatively assessed together. The use of 5 mg. per Kg. of body weight has generally been accepted(4), however, the use of 10 mg. BSP per Kg. would result in identical percentage retention values and allow for easier
clinical laboratory manipulations\(^3\). Using 5 mg. BSP per Kg., the weight of the dog in pounds is divided by 22, and the resultant quotient equals the mls. of 5 per cent BSP solution to be injected intravenously. Less than 5 per cent retention at 30 minutes is considered within normal limits. The great sensitivity of the test may result in a significant degree of retention in some functional disturbances as high fevers, shock and severe dehydration, which may be of a temporary nature. The BSP test is of little use in clinical icterus since any mechanical effects of biliary obstruction would naturally produce retention.

The determination of the serum glutamic pyruvic—transaminase (SGP-T) is the test of choice for the estimation of canine hepatic cell necrosis. When the hepatic cells undergo necrosis or changes in permeability to proteins, enzymes escape to the serum and can be measured spectrophotometrically\(^3\) or colorimetrically\(^6,7,8\). SGP-T data from our laboratory as correlated to conventional microscopic examinations has indicated the following: Normal values range between 10-40 Sigma-Frankel (S.F.) units; moderate liver necrosis 40-400 S.F. units; and over 400 S.F. units for severe liver necrosis. Following carbon tetra-chloride ingestion (0.25 ml. per pound), levels up to 8,000 S.F. units were found in dogs\(^3\). Serum glutamic oxaloacetic transaminase (SGO-T) levels are elevated in liver disease and in myocardial necrosis.

Alkaline phosphatase has been shown by Armstrong et al\(^9\) to increase in the blood of dogs in experimental obstructive icterus. Other workers found elevated values in hepatitis, hepatic fibrosis and amyloidosis; but levels were always consistently higher in extrahepatic obstructive icterus. Normal values for the dog range from 3 to 6 units per 100 ml. of serum (Bodansky method) with values above 10 in extrahepatic obstructive icterus. The determination of serum alkaline phosphatase levels is of questionable significance in the dog as a liver function test. Any clinical syndrome accompanied by secondary hyperparathyroidism may be confusing due to marked increases in the blood alkaline phosphatase levels.

Normal values for total serum proteins in dogs should range between 5 and 7 grams per 100 ml. While no alteration in the serum proteins is entirely specific for liver damage, the combination of a low albumin with a high gamma globulin is quite typical. The rapid fractionation methods of Wolfson et al\(^10\) or paper electrophoresis may be used. Since the albumin-globulin ratio in the dog and some other mammalian species is 0.7 or less, false positive reactions will occur in most all of the turbidimetric and flocculation tests used upon humans in which the albumin-globulin ratios are normally above 1. Apart from liver disease, low albumin values are found in nephritis, nephrosis, malnutrition, circulatory diseases, and a host of chronic diseases resulting in cachexia. Hyperglo-bulinemia may occur in such malignancies as lymphoscarcoma and plasmacytoma.

Total serum cholesterol levels are normally between 125-250 mg. per 100 ml. of which about 60-70 per cent is esterified. In hepatic disease in domestic animals, striking alterations in both the total and the esterified portion may occur. Impaired hepatic function causes an immediate fall in the cholesterol ester but need not change the total cholesterol ester. In extrahepatic biliary obstruction, the total cholesterol level climbs without altering the percentage of ester present. The cholesterol phenanthrene ring is normally metabolized from 2 carbon units and excreted in the bile as the conjugated cholic acids or bile salts. High blood cholesterol levels occur in other diseases in the dog such as hypothyroidism, diabetes mellitus and advanced nephrosis.

In addition, blood coagulation time by the capillary tube test and the blood uric acid level are commonly used to indicate hepatic damage. Coagulation times with the capillary tube of upwards of 2 minutes should be interpreted as prolonged, with normal values near 1 to 1.5 minutes\(^3\). Blood uric acid levels above 1.0
mg. per cent are suggestive of hepatic disease since the end product of purine metabolism in the dog is allantoin, whose conversion site from uric acid is within the hepatic parenchyma.

Case Reports

Case 1: A spayed bitch 9½ years of age was presented with symptoms including distended abdomen, slight icterus, and a temperature of 101.8°F. X-ray indicated a fluid line in the abdomen and a slight irregularity of the ventral border of the liver. The stools were of normal color but the urine was dark greenish-yellow indicating regurgitation of bilirubin-glucuronide.

Blood Studies:

R.B.C. 4.97 x 10⁶ per cu. mm M.C.V. 66.5
W.B.C. 6,450 per cu. mm M.C.H.C. 33.0
Hemoglobin 11.8 Gm% Icteric index 9 units
Sed. Rate 50/30 min.

Differential: 1.5% Morphology of the blood film:
Neut. 90.0%, Moderate rouleaux, frequent target cells
Lymph. 5.5%
Mono. 2.5%
Unclass. 0.5%

The ascitic fluid contained:
Specific gravity: 1.007 (transudate range)
Cell counts: R.B.C. 210 per cu. mm
W.B.C. 100 per cu. mm
Differential: Neut. 32%
Small mononuclears 36%
Large mononuclears & tissue cells 32%

Liver Function Tests:
Serum proteins: Albumin 1.5 Gm%
globulin ratio = 0.4
Total globulin 3.8 Gm%
Gamma globulin 1.2 Gm%

Bromsulphalein Test:
15 minute retention 75%
30 minute retention 51%

Urinalysis:
Bilirubin glucuronide 4 plus
Urobilinogen 3 plus

Fig. 2. Atrophied canine liver exhibiting diffuse fibrosis. BSP retention was 51 per cent at 30 minutes after injection. Note the absence of vascularity in the periphery of the organ.

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Conclusions: The physical findings indicated first that hepatic lesions were present producing ascites, icterus and intrahepatic obstruction of bile canaliculi. The severity of the hepatopathy was confirmed in the laboratory by a normocytic normochromic anemia, a serum albumin deficiency, and a high retention of BSP. The urine also indicated that a hepatogenous icterus was present.

Autopsy: A diffuse hepatic fibrosis was observed, the cause of which remained undetermined. See fig. 2.

Case 2: A 6 year old bitch was presented exhibiting pyometra and having a history of progressively lighter colored stools and darker colored urine during the last 6 months. X-ray studies indicated dense circumscribed areas in the anterior abdomen in the region of the liver. A tentative diagnosis of a chronic progressive hepatic lesion accompanying pyometra was made.

Blood Studies:

Hemoglobin 14 Gm%  Packed cell vol. 37%
W.B.C. 39,150 per cu. mm  Icteric Index 20%
Diff. Neut. 70%  Lymph. 1%
Mono. 27%  Eos. 2%

Urinalysis:
Specific gravity 1.020
pH 7.5
albumin trace

Liver Function Tests:
Bromsulphalein retention: 60% at 30 minutes
Van den Bergh: direct reacting (bilirubin-glucuronide) 6.0 mg%
Indirect reacting (free bilirubin) 2.3 mg%

Conclusions: All clinical findings suggested progressive liver lesions with or without complete obstruction at the time of examination. Laboratory findings confirmed the physical examination and established through the urobilinogen test that the bile duct was not occluded.

Autopsy: The liver contained fibrotic bands infiltrated with inflammatory cells (see fig. 3) and small liver abscesses which contained a coagulase positive *Micrococcus pyogenes*. In addition to the post-necrotic scarring and biliary hyperplasia, numerous giant cells, biliary casts and focal fatty changes were observed. In addition, a nephrosis and endometrial hyperplasia were present. A uterine culture established the presence of Proteus and Streptococci and cast doubt concerning a metastatic pathogenesis of the liver lesions following the pyometra.

Fig. 3. Section of a canine liver exhibiting post-necrotic scarring, cellular infiltration and bile duct hyperplasia. BSP retention at 30 minutes after injection was 60 per cent. X 100.
EQUINE LIVER FUNCTION TESTS

This field in the large domestic animals has been sadly neglected. The exact interpretation of the Van den Berg test has not been clarified at present in the horse but most clinicians use the conventional interpretation from other species. Normal bilirubin levels in equine plasma may normally occur up to 3 to 4 mg per cent but are most commonly found at near 1 mg. per cent, which is of an indirect-reacting chemical behavior with the diazo reagent. It is not uncommon to find clinically icteric horses with free bilirubin levels of 4 to 5 mg per cent with only 1 mg. per cent of a direct acting pigment. There is need for basic studies concerning bilirubin metabolism in the horse. Horses with colic and catarrhal duodenitis commonly have elevations in serum bilirubin levels from possible extrahepatic obstruction.

The Bromsulphalein clearance test has been developed recently for the horse. In this test, 1 gram of BSP is injected intravenously and the rate of BSP disappearance is measured from serum samples taken between 5 and 12 minutes after injection. The 2 BSP serum concentrations are plotted on semilog paper and the T₁/₂ (half-time) is calculated, that is, the time required for the serum concentration of BSP to be halved. See Fig. 4 for the method of T₁/₂ calculation. T₁/₂ for BSP clearance in normal control horses was 2.81 ± 0.5 minutes with a range of 2.0 to 3.7 minutes. The test is quite sensitive for liver insufficiency in the horse. Contraindications to its use are severe febrility and extensive dehydration, which lowers the portal blood flow and hence BSP uptake. The BSP clearance test has also been investigated in the cow and sheep.

At present, intensive studies are underway to clarify the Van den Bergh test in the horse. In addition, the serum transaminases (SGO-T and SGP-T) are being investigated to clarify their use as indicators of hepatic necrosis in horses, cattle and pigs. Since very little is known concerning the intermediary metabolism and compartmental distribution of the lipids, purines, and proteins in horses, the use of such tests to determine the serum protein, cholesterol and cholesterol ester, and alkaline phosphatase levels should await further experimentation. Such tests if performed should be interpreted with great uncertainty.

Fig. 4. Method of calculating T₁/₂ (half-time) values of Bromsulphalein disappearance.

Case Report

Case 1: A horse was presented with symptoms of central nervous system lesions. In addition to the stumbling and staggering, a mild icterus was present. The total serum bilirubin level was 7.2 mg. per cent as compared to a normal mean value of near 1.0 mg per cent. A Bromsulphalein test was performed to confirm a tentative diagnosis of hepatolenticular degeneration. A T₁/₂ (half-time) value of 3.5 minutes was reported, which indicated liver pathology most likely did not exist. At autopsy, the diagnosis of equine encephalomyelitis confirmed the BSP test. Since intestinal stasis was present, catarrhal duodenitis might possibly explain the hyper-bilirubinemia by partial closure of the common bile duct in the absence of a gallbladder.
Case 2(11): A horse with mild icterus (serum bilirubin = 6.8 mg per cent) exhibited a BSP T½ of 11.6 minutes. The prolonged clearance of the dye suggested hepatic lesions. Upon autopsy, a marked hemosiderosis of the liver was observed.

SUMMARY
1. The clinical significance of the enterohepatic circulation of bile pigments is presented.
2. Indications for and the classification of liver function tests are summarized for the dog and horse.
3. The clinical and laboratory diagnosis of hepatic disease and icterus is discussed.

BIBLIOGRAPHY

Book Review
HOW TO WRITE SCIENTIFIC AND TECHNICAL PAPERS

This volume is an outgrowth of two earlier books: “Preparation of Scientific and Technical Papers” and “The Scientific Paper, How to Prepare it, How to Write It.”

The book deals with all the factors necessary in the preparation of technical papers. Topics which are beyond the scope of the book are treated lightly, then the author suggests references for those who find it necessary to study the particular topic more thoroughly.

It briefly covers the choosing of a research problem, use of libraries and guides to the literature. There is a short section on statistical methods and the reliability of measurements. The writing and arrangement of the subject matter is well covered. Methods are suggested which will help make the paper more interesting. For those who are preparing a manuscript to be published, the correct method of preparing copy and estimating the length of the printed material is given. Only a few rules of grammar and rhetoric are included. Considerable detail is given on how to prepare and present organized data in the form of tables. Techniques are given on the preparation of graphs, drawing and photographs for the paper.

This book will be a valuable aid for students and research workers who are preparing illustrated papers, scientific reports, or manuscripts for publication in journals.