Materials And Methods

Retrospective Study Of Hospitalized Dogs

Medical records of dogs examined at the Virginia-Maryland Regional College of Veterinary Medicine (VMRCVM) between 1986 and 1996 were reviewed. Records were identified by searching the medical records department database for dogs with hepatobiliary disease, pharmacy records for animals having BSP charged to their accounts, clinical pathology logbooks for animals having BSP retention testing performed, and endoscopy and surgery logbooks for animals having hepatic biopsies performed. Dogs were included in the study if they had undegone BSP 30-minute retention testing and histopathologic examination of the liver, and if the histopathological tissue section was available for retrospective review. Hepatic samples were obtained by means of surgical, laparoscopic, or percutaneous ultrasound-guided biopsy or during necropsy. One-hundred fifty dogs were included in the study. The histopathologic slides were reviewed by two of the authors (Flatland, Sponenberg) without prior knowledge of clinical or biochemical results. The medical records were then reviewed, and information concerning signalment, BSP concentrations, serum bile acid concentrations, plasma ammonia concentrations, and final clinical diagnoses was recorded.

BSP has not been an FDA-approved drug since 1984. An Investigational New Animal Drug Application (INADA) is not currently required for clinical diagnostic use, and BSP may be purchased in its chemical form from the manufacturer.* BSP retention testing of dogs evaluated at the VMRCVM was performed using a 5% solution (50 mg/ml) of bromosulfophthalein sodium salt in sterile water at a dosage of 5 mg BSP/kg body weight IV. The calculated dose of BSP solution was administered intravenously and a heparinized venous blood sample was collected 30 minutes later. Blood samples were centrifuged at 3000 rpm for 10 minutes,** and the plasma was removed by pipetting. The BSP assay was performed according to Kaneko and Cornelius.[112] One ml of plasma was added to 4 ml of 0.1N NaOH in a spectrophotometer cuvette, covered with parafilm, and mixed. The sample and a blank consisting of 1 ml plasma mixed with 4 ml of 0.1N HCl, were placed into a spectrophotometer*** and read at 580 nm. The standard curve for spectrophotometric measurement of BSP was obtained by diluting BSP solution to concentrations of 0, 5, 10, 20, 25, and 50% and measuring BSP concentration (see Appendix 1). The concentration of BSP in the sample was calculated by the spectrophotometer and reported as % BSP retained in serum.

Evaluation Of Control Dogs

A 5% solution (50 mg/ml) of BSP was made as described above. A BSP 30-minute retention test using 5 mg/kg IV was performed in 30 adult dogs that were part of a

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* sulfobromophthalein sodium salt, Sigma Chemical Company

** Jouan CR412 Centrifuge, Jouan

*** Shimadzu UV-160A Recording Spectrophotometer, Shimadzu Corporation

terminal junior surgery laboratory at the VMRCVM. Dog use was approved by the Animal Care and Use Committee of Virginia Tech. Thirteen female and 17 male dogs were evaluated. Of these, 22 were mixed-breed dogs, 5 were beagles, and 3 were various pure breeds (1 each of Australian Shepherd, Labrador Retriever, and Keeshond). The mean body weight was 15.7 kg (range 6.4 to 36.4 kg). Age and neutering status was unknown. BSP retention testing was performed the morning of surgery (following a 12-hour fast during which dogs were allowed access to water) by injecting the calculated dose of BSP solution intravenously into a cephalic vein and obtaining a heparinized venous blood sample from a jugular vein 30 minutes later. BSP retention was measured as described above. Blood samples were assayed within 1 hour of being received by the laboratory. Hepatic biopsies were obtained from each dog immediately post-mortem. A ventral midline incision opening the cranial half of the abdominal cavity was made using a number 10 scalpel blade and handle. A lobe of liver was grasped and exteriorized, and a section measuring 1 to 2 by 1 to 2 cm was removed using the scalpel. One biopsy was obtained from each dog, and biopsy sites were chosen at random. Hepatic tissue was fixed in 10% buffered formalin, embedded in paraffin, stained with hematoxylin and eosin, and sectioned at 5 microns.

Histopathologic Review

Tissue sections were reviewed by two authors (Flatland, Sponenberg) without prior knowledge of the dog's signalment or clinical or biochemical findings. All tissue sections were grouped into one of 11 predermined categories: normal liver [group 0] , portosystemic shunt [group 1], cholestasis [group 2], acute hepatitis [group 3], chronic hepatitis [group 4], corticosteroid-induced hepatopathy [group 5], "other" hepatopathy [group 6], cirrhosis [group 7], primary (hepatocellular or biliary) neoplasia [group 8], secondary (metastatic) neoplasia [group 9], and unclassifiable [group 10]. Histopathologic criteria for each category were established prior to beginning the review, and categories were chosen to reflect major disease mechanisms of canine liver. Tissue sections were classified according to the predominant histopathologic change present. The criteria for portosystemic shunt, cholestasis, corticosteroid-induced hepatopathy, cirrhosis, and hepatic and metastatic neoplasia were defined according to criteria in the literature. A diagnosis of unclassifiable was made if small sample size precluded classification into one of the above categories. The normal histologic structure of hepatic parenchyma is reviewed in Appendix 2.

Tissue sections were classified as acute hepatitis if neutrophilic inflammation and hepatocyte necrosis were the predominant changes, and histologic changes suggesting chronicity (bile duct proliferation, fibrosis, and lymphocytic or plasmacellular inflammation) were absent. The category chronic hepatitis was assigned if the predominant histopathologic change was an inflammatory infiltrate (neutrophilic, lymphocytic, plasmacellular, or mixed) and hepatocellular necrosis together with histologic changes suggesting chronicity (fibrosis and bile duct proliferation).

The category of "other" hepatopathy was used to classify non-specific degenerative hepatic change (such as hepatocyte swelling, hepatocyte vacuolation, or hydropic degeneration) that could not be clearly classified into one of the other categories. Our criteria for "other" hepatopathy were consistent with Center's description of "reactive"

hepatitis: mild mononuclear infiltrates in the periportal area, minor foci of hepatic cell necrosis associated with small infiltrates of leukocytes, increased numbers of Kupffer cells, and hepatocellular vacoluar change.[85] Changes were mild to moderate, and significant inflammation (neutrophilic, lymphocytic, plasmacellular, or mixed), fibrosis, vascular abnormalities, and neoplasia were not present. Chronic passive congestion and copper-induced hepatopathy were included in the "other" hepatopathy category because, despite the spectrum of histologic change which can result from these conditions, a consistent feature is hepatocelluar degeneration characterized by vacuolar change.

Evaluation BSP Retention Test Efficacy

Results were evaluated by calculating descriptive statistics, sensitivity, specificity, positive predictive value, and negative predictive value of the test for the specific categories of hepatobiliary disease and for all categories together. Histopathologic examination was the gold standard test to which BSP retention was compared, and the above indices were calculated as proportions and expressed as percentages. Indices were calculated by creating 2 by 2 tables in which A is number of true positive (dogs having both abnormal BSP retention and abnormal livers based on histopathologic examination), B is number of false positives (dogs having abnormal BSP retention but normal livers), C is number of false negatives (dogs having normal BSP retention but abnormal livers), and D is number of true negatives (dogs having both normal BSP retention and livers). The sum (A + C) is the total number of dogs having abnormal livers based on histopathological examination, (B + D) is the total number of dogs with normal livers, (A + B) is the total number of dogs with abnormal BSP retention, and (C + D) is the total number of dogs with normal BSP retention.[113] Formulas are shown in Appendix 3.

Sensitivity and specificity are proportions calculated based on numbers of dogs having hepatic histopathology performed. Sensitivity refers to the ability of a test to identify a diseased patient and is defined as the proportion of dogs with hepatobiliary disease that had an abnormal BSP retention test result [A/(A + C)]. High sensitivty corresponds to a low number of falsely negative results. Specificity refers to a test's ability to identify disease-free patients and is defined as the proportion of dogs without hepatobiliary disease that had a negative (normal) BSP retention test result [D/(B + D)]. High specificity corresponds to a low number of falsely positive values.

Predictive values are proportions calculated based on numbers of dogs having the BSP retention test performed. Predictive values are prevalence-dependent. High prevalence results in a higher positive predictive value and lower negative predictive value. Positive predictive value reflects the accuracy of a positive test result and is defined as the proportion of dogs with an abnormal BSP retention test that actually had hepatobiliary disease [A/(A + B)]. Negative predictive value reflects the accuracy of a negative test result and is defined as the proportion of dogs having a normal (negative) BSP retention test that did not have hepatobiliary disease [D/(C + D)].

Other Hepatic Function Tests

Serum bile acid and ammonia tolerance test results for hospitalized dogs were retrieved from the medical records. Sensitivites were calculated and compared with that of BSP retention testing.

The bile acid test was performed following a 12-hour fast. Venous blood was obtained for the fasting samples, and dogs were fed a high-fat meal (Hill's k/d or p/d[#]). Two hours later, venous blood was obtained for the post-prandial sample. The bile acid assay is not performed at VMRCVM, and samples were sent to Cornell University. Normal values for fasting and post-prandial serum bile acid concentrations were considered < 13 µmol/L and < 30 µmol/L, respectively.

Fasting plasma ammonia concentrations were also measured following a 12-hour fast. Post-challenge samples were obtained by administering 100 mg/kg of 5% ammonium chloride orally or rectally and obtaining a heparinized venous blood sample 30 minutes later. The ammonia assay is performed at VMRCVM by colorimetric assay and less than 32 µmol/L was considered normal for both fasting and post-challenge samples. Prior to 1989, our laboratory used a different chemistry analyzer than the one currently being used, and ammonia concentrations were reported in units of µg/dl. For medical records reporting ammonia in µg/dl, concentrations were converted to µmol/L by multiplying the value by 0.5872.[114]

Statistical Analysis

BSP retention was evaluated as both a continuous and dichotomous variable. Descriptive statistics (mean and median retention, minimum and maximum retention, and standard deviation) were calculated for all histopathologic groups together and within individual histopathologic groups.[115] As BSP retention data did not appear to be normally distributed based on a normal probability plot of the measurements (Wilk-Shapiro rankit plot[115]), BSP retention time ranks were compared between groups using the non-parametric Kruskal-Wallis test.[115]

For calculation of epidemiologic indices of test efficacy, dogs were classified as being either BSP test positive or BSP test negative based on given cut-off values for "normal" retention. Chi squared testing[116] was used to compare proportions of dogs with positive results and negative results (i.e., used to compare true positive and false positive rates) between dogs known to have hepatic disease and control dogs. This comparison was also made between histopathologic groups of hospitalized dogs (leaving out control dogs) to see whether BSP testing could distinguish between types of hepatobiliary disease (true positive and false positive rates were compared between hospitalized dogs with a given disease and all other hospitalized dogs not having that disease). P values as well as sensitivities and specificities obtained during these comparisons were considered in deciding whether BSP testing was of value for telling groups apart. For all comparisons,

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[#] Hill's Pet Nutrition, Topeka, KS

Fisher's exact testing[116] was used when groups were too small to permit analysis with the chi squared test, and p values < 0.05 were considered significant.

McNemar's chi squared test[116] was used to compare the sensitivities of BSP testing to bile acid and ammonia testing among hospitalized dogs. For each comparison, only dogs undergoing both tests were evaluated.

Ninety-five percent exact binomial confidence intervals were calculated for sensitivity, specificity, and predictive values of BSP retention testing for detection of hepatic disease (including all histopathologic groups).[116] Ninety-five percent confidence intervals also were calculated for individual sensitivities of BSP retention testing for each histopathologic group. A receiver operator characteristic (ROC) curve was used to compare sensitivities and specificities at various cut-off values for "normal" retention.

Results

Histopathological Review

One hundred seventy-three slides were reviewed from the 150 hospitalized dogs. Of these, 78 came from surgical biopsies, 46 came from necropsies, 29 came from laparoscopic biopsies, and 19 came from percutaneous ultrasound-guided biopsies. The biopsy method was not recorded in 1 record. Twenty-two of 150 cases had multiple hepatic samples evaluated; 21/22 had 2 samples evaluated, and 1/22 had 3 samples evaluated. In 14/22 cases, all slides from the individual dogs were classified in the same histopathologic category. In 8/22 cases, different histopathologic categories were identified. In these cases, the dog was classified according to the category of the liver sample collected chronologically closest to the BSP retention test. No cases were classified as having cholestasis. Group 8 [primary neoplasia], which contained only 1 dog, was combined with group 9 [metastatic neoplasia] for calculations and statistical evaluation. Group 10 [unclassifiable], which also contained only 1 dog was excluded, leaving 149 hospitalized dogs for analysis. Of the 30 adult laboratory dogs evaluated, 25 had histopathologically normal livers and were used as controls. Results of the histopathologic review are detailed in Table 1, and data from evaluation of the random source dogs are listed in Appendix 4.

Signalments

A variety of breeds were represented in the hospitalized dog group. Most common were mixed breeds, miniature schnauzers, doberman pinschers, golden retrievers, Yorkshire terriers, cocker spaniels, and German shepherds. Breeds and frequency are listed in Table 2. Females and neutered animals predominated. Eighty-two dogs were female (14 sexually intact, 68 neutered), and 68 dogs were male (38 sexually intact, 30 neutered). Gender results and frequency are detailed in Table 3. Age was known in

149/150 dogs. The remaining dog was listed simply as "adult". The mean age of the 149 dogs was 6.2 years (range 0.17 to 17 years). The mean body weight was 18 kg (range 1.7 to 60.0 kg). Age and body weight distribution for hepatic disease groups is shown in Figures 1 and 2.

BSP 30-Minute Retention Test Results

No adverse reactions to BSP were documented in any of the medical records. Of 149 hospitalized dogs, 35 (23.5%) had BSP retention of less than or equal to 5.0% and 114 (76.5%) had BSP retention of greater than 5.0%. Mean BSP retention for all hospitalized dogs was 13.9% (standard deviation 11.5%, median 10.0%), with a range of 0.08 to 58.0% (Figure 3). Of 25 control dogs, 22 (88%) had BSP retention of less than or equal to 5.0%, and 3 (12%) had BSP retention of greater than 5.0%. The 3 abnormal values were slightly above this reference value at 5.1%, 5.4%, and 6.0%, respectively. Mean BSP retention of control dogs was 3.19% (standard deviation 1.36%, median 2.76%), with a range of 1.19 to 6.0%. BSP retention of hospitalized dogs (all groups combined) was significantly different from that of control dogs ($p < 0.0001$).

Within histopathologic groups, mean BSP retention was highest for dogs with chronic hepatitis (23.1%), followed by cirrhosis (21%), neoplasia (14.2%), "other" hepatopathy (12.3%), portosystemic shunts (11.9%), acute hepatitis (11.8%), and corticosteroid-induced hepatopathy (9.7%). BSP retention of all groups except group 3 [acute hepatitis] was significantly different from that of controls ($p < 0.0001$). Lack of significant difference between dogs of group 3 and controls likely reflects the small number of dogs in group 3. Descriptive statistics for BSP retention are presented in Table 4. Data are graphed in Figures 3 and 4.

Evaluation Of Test Accuracy (Hospitalized Dogs Vs. Controls)

Using the historical reference value of 5.0% retention as normal, the sensitivity of the BSP 30-minute retention test for all histopathologic groups combined (i.e., for the detection of hepatobiliary disease in general) was 76.4%. Specificity was 88%, positive predictive value was 97.4%, and negative predictive value was 38.6%. If the cut-off value for normal BSP retention was raised to 6.0%, then specificity and positive predictive value became 100%. However, using the value 6.0% retention as normal resulted in somewhat lowered sensitivity (69.6%) and negative predictive value (35.7%). Statistical analysis using the chi squared test showed that the proportion of dogs testing positive was significantly different between dogs with histologically normal livers and dogs with liver disease ($p < 0.0001$), indicating that BSP retention was able to distinguish diseased and disease-free dogs. Data are listed in Table 5 and calculations are shown in Appendix 5.

Evaluation of a receiver operator characteristic (ROC) curve, which plots the false positive rate versus the true positive rate at various cut-off values for BSP retention, confirmed that the highest possible sensitivity while still having a specificity of 100% was obtained using a cut-off value of 6.0%. The curve was relatively flat, and small changes in

sensitivity resulted in large corresponding changes in specificity. The curve is shown in Appendix 5.

Evaluation Of Test Accuracy Comparing Histopathologic Groups (Hospitalized Dogs)

Using 5.0% retention as the cut-off value for normal, statistical analysis with chi squared and Fisher's exact tests revealed no significant difference in the false positive and true positive rates for BSP retention testing when hospitalized dogs with a specific hepatobiliary disease were compared to all other hospitalized dogs (i.e., comparison of false positive and true positive rates between group X and non-group X). Thus, BSP retention testing was not able to distinguish between different types of hepatobiliary disease. Calculations and p values are shown in Appendix 5.

When 6.0% was used as the cut-off value for normal retention, statistical analysis with chi squared and Fisher's exact tests again revealed no significant difference in the false positive and true positive rates for BSP retention testing when hospitalized dogs with a specific hepatobiliary disease were compared to all other hospitalized dogs. The only exception was group 5 [corticosteroid-induced hepatopathy]. Comparing the true positive and false positive rates of group 5 dogs with non-group 5 dogs yielded a significant p value of 0.0453 (indicating a statistically significant difference between the proportion of dogs testing positive in group 5 and the proportion testing positive in all other groups combined). Although statistically significant, this difference was biologically meaningless. The sensitivity and specifity obtained showed that a smaller proportion of group 5 dogs had a positive BSP test when compared with non-group 5 dogs (i.e., group 5 dogs were less likely than non-group 5 dogs to have high BSP retention). This is likely because corticosteroid-induced hepatopathy is a mild disease which rarely causes overt hepatobiliary dysfunction. To be biologically meaningful, group 5 dogs should have been more likely to have increased BSP retention. The test was therefore not a good one for telling these groups apart, despite the statistical significance of the comparison. Calculations and p values are shown in Appendix 5.

Sensitivities calculated by comparing histopathologic groups of hospitalized dogs to control dogs. Data and 95% exact binomial confidence intervals are shown in Table 6.

Evaluation Of Other Hepatic Function Tests

Bile acid testing was performed in 46 dogs (Table 7). Forty-four dogs had both fasting and post-prandial concentrations measured. In 3/44 dogs, the fasting concentration was not detectable (considered normal) but a value was obtained for the post-prandial concentration. Two dogs had only a baseline concentration measured. Of the 44 dogs having both fasting and post-prandial serum bile acid concentrations measured, 21 had abnormal values, 12 had normal values, 7 had an abnormal baseline value but a normal post-prandial value, and 4 had a normal baseline value but an abnormal post-prandial value. The sensitivity of measuring fasting bile acids was 63% (29/46), and that of measuring post-prandial bile acids was 56.8% (25/44). The sensitivity of requiring both fasting and

post-prandial serum bile acid concentrations to be out of the reference range for a positive test result was 47.7% (21/44). The sensitivity of accepting either the fasting or the post-prandial concentration out of the reference range as a positive result was 72.7% (32/44).

Seventy-six hospitalized dogs had plasma ammonia concentrations measured (Table 8). Of these, 14 also had post-challenge plasma ammonia concentrations measured (i.e., had ammonia tolerance testing performed) following administration of 100 mg/kg ammonium chloride PO. One dog having only a fasting plasma ammonia concentration measured was not included in the analysis because its hepatic specimen was unclassifiable [group 10], leaving 75 dogs for evaluation. Abnormal fasting plasma ammonia concentrations were identified in 46/75 dogs (sensitivity 61.3%). Abnormal post-challenge plasma ammonia concentrations were identified in 10/14 dogs (sensitivity 71.4%). In dogs having ammonia tolerance testing performed, 9 dogs had both concentrations out of the reference range, 1 dog had a normal fasting value but an increased post-challenge value, and 4 dogs had both values within the reference range. The sensitivity of requiring both the fasting and post-challenge ammonia concentrations to be out of the reference range for a positive test result was 64.3% (9/14). The sensitivity of accepting either value was out of the reference range as positive was 71.4% (10/14). The sensitivities and 95% confidence intervals of BSP retention testing, serum bile acid testing, and plasma ammonia testing are listed in Table 9.

Discussion

Defining "Normal" BSP Retention

BSP 30-minute retention was significantly different in control dogs and hospitalized dogs ($p < 0.0001$). The control group's mean of 3.19% is consistent with previous reports of BSP retention in clinically normal dogs.[61,63,71]

The basis for the historical standard of ≤ 5% BSP retention at 30 minutes being normal in the dog is unclear. Drill and Ivy in 1944 stated that 2 to 12% retention at the end of 30 minutes is normal, but this statement is not referenced and the range did not appear to come from their data.[60] Svirbely et al. stated that up to 10% retention could be expected in normal dogs based on their study.[61] The value of less than 5.0% retention at 30 minutes being normal in dogs first appeared in the literature beginning with the 1968 study by Van Vleet and Alberts[63] and is reiterated by Morgan in his 1969 review.[64] The source of this reference range is not apparent in either article. The only previous report of BSP retention being evaluted in a large number of dogs and correlated with hepatic histopathologic findings is the 1960 report by Larson and Morrill, in which 45-minute BSP retention was evaluated in 85 dogs. Fourteen of 85 dogs had histopathologically normal livers, and all had a 45-minute BSP retention of less than 1.5%.[62]

It has been argued that BSP retention is a poor test relative to other tests of hepatic function due to lower sensitivity, and that other tests of hepatobiliary function, such as serum bile acid and ammonia testing, are better tests due to greater sensitivity. [83,84] High

sensitivity (a low rate of false negatives) is most important for initial screening tests used to detect presence or absence of disease. When trying to identify disease, it is obviously not desirable to have many false negatives and allow patients with undetected disease to remain in the general population. High specificity (a low rate of false positives) is less important for such screening tests because falsely positive patients incorrectly identified during initial screening are detected by further testing after the initial screening has been completed. It follows that high specificity is of value for testing patients already suspected of having the disease in question, where more invasive or risky diagnostic procedures or treatments are being considered. It obviously is not ideal to perform risky procedures on patients that do not actually have the disease.

Hepatic function tests are not often used as initial screening tests. BSP retention and serum bile acid and ammonia concentrations are typically performed in dogs already suspected of having hepatobiliary disease based on history, physical examination, serum biochemical evaluation, and abdominal imaging studies. Hepatic function tests are performed to confirm presence of hepatobiliary disease and to assess its severity. For this reason specificity is as important as sensitivity in determining the value of these tests.

Choosing a "normal" value for a given test depends in part upon whether having false positives or false negatives is the worse error to make, a decision is based upon the test's purpose. Because BSP retention testing is not typically used as an initial screening test, but is more commonly used to evaluate dogs already suspected of having hepatobiliary disease, false positive errors are more costly than false negative errors. Accordingly, a high specificity (low false positive rate) is desirable. Sensitivity (the false negative rate) is still important, but specificity should be given higher priority in selecting the cut-off value for "normal".

Based on this study, 6.0% appears to be a better cut-off value for normal BSP retention at 30 minutes than the traditionally used 5.0%. Based on the ROC curve, using 6.0% as the cut-off value yields an acceptable sensitivity of 69.6% and results in a specificity of 100%. Cut-off values higher than 6.0% still yield 100% specificity but cause sensitivities of lower than 69.6%, resulting in a lower overall test accuracy. Cut-off values below 6.0% do not give 100% specificity. In this study, any dog with a BSP retention of greater than 6% had a 100% chance of having a histopathologically abnormal liver. Clinically, this means that hepatic biopsy is indicated when BSP retention is greater than 6%. This degree of confidence is invaluable when contemplating a procedure as potentially risky as hepatic biopsy, particularly in compromised patients.

Effect of Disease Prevalence On Predictive Values And Interpretation Of Test Results

Because hepatic function tests are most often used to further evaluate dogs already suspected of having hepatobiliary disease, prevalence of hepatobiliary disease in dogs that undergo BSP retention testing is high. As a result, the positive predictive value of this test is very good (i.e., a positive test result is believable), but negative predictive value is not (i.e., a negative test result does not rule out disease). This concept may be illustrated by an example. Formulas used for the following calculations are listed in Appendix 3.

If BSP testing is performed in 100 dogs and the prevalence of hepatobiliary disease among them is 80%, then 80 dogs have hepatobiliary disease and 20 dogs do not. Using 6.0% as the cut-off value for normal retention in our study yielded a sensitivity of 69.8%. This means that 56 dogs with hepatobiliary disease will test positive (0.698 x 80 = 55) and that 24 dogs with hepatobiliary disease will test negative (80 - 56 = 24). The total number of dogs testing negative is 44 (20 truly negative dogs + 24 falsely negative dogs), and 55% of dogs testing negative actually had disease. The positive predictive value in this example is 100% (55/55), and the negative predictive value in this example is 45.5% (20/44).

If the prevalence of hepatobiliary disease among these dogs were only 50%, then negative predictive value improves. With 50% prevalence, 35 dogs with hepatobiliary disease will test positive (0.698 x 50 = 35) and 15 dogs with hepatobiliary disease will test negative (50 - 35 = 15). The total number of dogs testing negative is 65 (50 truly negative dogs + 15 falsely negative dogs), and only 23% of dogs testing negative actually had disease. The positive predictive value in this example is still 100% (35/35), but the negative predictive value is 76.9% (50/65).

Ultimately, hepatic biopsy is the diagnostic test which best guides treatment of hepatobiliary disease and assessment of prognosis. To the clinician and pet owner, BSP retention testing is of value in that a positive result may facillitate the decision to perform hepatic biopsy. It may be easier for a clinician to recommend biopsy to an owner if the BSP retention is greater than 6%. However, BSP test results should not be interpreted in a vacuum, and it is important for pet owners to understand that a negative result does not rule out hepatobiliary disease. In all dogs, but particularly those testing negative, clinicians must also consider historical and physical examination findings, other laboratory data (and trends in these data), and abdominal imaging studies in considering whether and when to perform hepatic biopsy.

Factors That Might Affect BSP Retention

Hyperbilirubinemia was present in 30/142 (21%) hospitalized dogs. A plasma bilirubin concentration greater than 2.5 mg/dl was present in 3 dogs, and the highest bilirubin concentration was 5.3 mg/dl. Hyperbilirubinemic dogs had a mean BSP retention of 20.1%, and dogs with normal bilirubin concentrations had a mean BSP retention of 12.3%. This difference is likely due to the presence of more severe hepatobiliary disease in the hyperbilirubinemia group, although competitive inhibition of BSP metabolism by bilirubin may be a factor.

Hypoalbuminemia was present in 81/145 (56%) hospitalized dogs. Hypoalbuminemic dogs had a mean BSP retention of 15.3%, hyperalbuminemic dogs had a mean retention of 9.1%, and dogs with normal albumin concentrations had a mean retention of 12.9%. This difference is likely due to the presence of more severe hepatic disease in the hypoalbuminemic group.

Ascites was present in 18/149 (12%) hospitalized dogs. Whether ascites was present was unknown in 1 dog. Dogs with ascites had a mean BSP retention of 18.8%, and dogs without ascites had a mean retention of 13.1%. This difference is likely due to

the presence of more severe hepatobiliary disease in dogs with ascites, although overdosing BSP in dogs with ascites due to increased body weight relative to plasma volume may be a factor.

Statistical comparison of the above differences was not possible in this study. As hepatobiliary disease can raise serum bilirubin concentration, lower albumin concentration, and may cause ascites, the effect of these variables on BSP retention is difficult to ascertain due to confounding. Statistical evaluation would require multivariate analysis controlling for type and severity of hepatobiliary disease. As no index of disease severity was used in our study, we were unable to perform such an analysis. The impact of obesity on BSP retention in dogs of this study was not assessed for this reason and because, although body weights were known in all dogs, the body condition of dogs was unknown. No dogs received medications reported to interfere with BSP metabolism.

Comparing BSP Retention Testing With Other Tests Of Hepatic Function

Unfortunately, not enough data were generated by this study or available in the literature to compare specificity of BSP retention testing with that of ammonia tolerance or serum bile acid testing. In a 1991 study, Center et al. reported a specificity and positive predictive value of 100% at cut-off values of 20 µmol/L and 25 µmol/L for fasting and post-prandial serum bile acid concentrations, respectively. [117] Currently, Cornell's reference range is ≤ 13 µmol/L for fasting serum bile acids and ≤ 30 µmol/L for post-prandial bile acids.[##] Specificity of serum bile acid testing at these cut-off values is not reported. In other reports in which serum bile acid and ammonia tolerance testing were performed, not enough data from individual dogs were presented to permit calculation of specificity.[32,69,74,77,83]

Based on our study, BSP retention had similar sensitivity to serum bile acid and ammonia testing (Table 9). Sensitivity of BSP retention testing in this study was 76.5% using 5.0% retention as normal and 69.6% using 6.0% retention as normal. Sensitivity of serum bile acid testing in this study was 72.7% if having either the fasting or post-prandial value out of the reference range was considered abnormal. Sensitivity of ammonia tolerance testing was 71.4% if having either the fasting or post-challenge value out of the reference range was considered abnormal. Statistical comparison using a McNemar's chi squared test revealed no significiant difference between the sensitivity of BSP testing and serum bile acid testing ($p = 0.3$) or between BSP testing and plasma ammonia testing ($p = 1.0$) in hospitalized dogs undergoing both tests. For these comparisons, an "abnormal" bile acid or ammonia result was defined as having either the baseline or the post-prandial (or post-challenge) value out of the reference range. This definition of "abnormal" was chosen because this is how these tests are most often interpreted clinically. Comparing the sensitivity of bile acid testing to ammonia testing was not performed, as only 4 dogs underwent both pre- and post-parandial bile acid and ammonia tolerance testing. Based on these findings, BSP 30-minute retention was as good a test as either serum bile acid or ammonia tolerance testing in the hospitalized dogs.

[##] Cornell University College of Veterinary Medicine Laboratory Report Form

Comparison Of BSP Data To That Reported In the Literature

Data of this study were considered consistent with previous reports if the sensitivities calculated from data in the literature fell within the 95% exact binomial confidence intervals calculated for sensitivities from our data.

Sensitivities obtained for BSP testing in this study (Tables 6 and 9) are consistent with those calculated from data in the literature. Of 125 BSP retention tests reported in dogs with hepatobiliary disease [65-68,70,73,75,76,79,80], 85 were abnormal (sensitivity 68%). Most BSP tests reported were performed either in dogs with portovascular anomalies or dogs with hepatobiliary disease secondary to anticonvulsant administration. Most dogs (approximately 60 %) had hepatobiliary disease confirmed by means of hepatic biopsy, necropsy, surgical exploration, angiography, or venous portography.

Fifty-four of 125 BSP tests were performed in dogs with portovascular anomalies. [66,68,75,76] All but 1 dog had a diagnosis of portosystemic shunt confirmed by means of angiography, venous portography, surgery, and/or necropsy. Forty of 54 tests showed greater than 5.0% retention, yielding a sensitivity of 74.1%. This value is somewhat lower than the sensitivity calculated for the portosystemic shunt group [group 1] in our study (87.5% when 5.0% retention was considered normal), although it falls within the 95% confidence interval calculated for sensitivity of the group 1 dogs and is therefore consistent with our results.

Fifty-six of 125 reported BSP tests were done in dogs receiving anticonvulsant medication.[70,73,80] These 56 tests were performed in 55 dogs, and cirrhosis was diagnosed in 17/55 following histopathologic examination of the liver. The remaining 38 dogs were not biopsied and were assumed to have hepatobiliary disease (but not necessarily cirrhosis) based on other serum biochemical data and clinical findings. Thirty-two of 56 tests showed greater than 5.0% retention (sensitivity of 57.1%). This sensitivity is lower than that calculated for the 7 dogs with cirrhosis [group 7] in our study (100% when 5.0% retention was considered normal) and does not fall within the 95% confidence interval. However, the number of dogs with cirrhosis in our study was small, and the extent of hepatic disease in 38 reported dogs is unknown, making comparison difficult. Although in a 1985 study Bunch et al. reported performing hepatic biopsies and BSP retention testing in 29 Beagles experimentally given anticonvulsant drugs, not enough data from individual dogs were reported to permit the calculation of BSP testing sensitivity.[74]

Comparison Of Serum Bile Acid Data To That Reported In the Literature

Sensitivity of fasting serum bile acid testing in this study (63%; Table 11) was was comparable to that calculated from the literature. Forty of 77 reported fasting bile acid concentrations were abnormal[70,73,75,76,80] yielding a sensitivity of 51.9%. This value falls within the 95% confidence interval for sensitivity calculated from the hospitalized dogs and is consistent with our results. Sensitivity of the post-prandial bile acid test calculated from our data (56.8%) was lower than that calculated from previous reports. Twenty of 23 reported post-prandial serum bile acid concentrations were abnormal, yielding a sensitivity of 87%.[75,76,80] This value falls outside the 95% confidence interval (41.0% to 71.6%)

for sensitivity calculated from the hospitalized dogs and is not consistent with our results. This may be due to a higher proportion of dogs with mild hepatobiliary disease (i.e., steroid hepatopathy and "other" hepatopathy) in our study. Previous studies in which bile acids were measured contained a higher proportion of dogs with portosystemic shunts and dogs given anticonvulsants, some of which had histologically-confirmed cirrhosis.

Among reports of dogs with portosystemic shunts, serum bile acid testing results are consistent with our study and fall within the 95% confidence interval calculated for sensitivity of group 1 dogs. Sensitivity of previously reported fasting serum bile acid testing was 80% (20/25 tests abnormal).[75,76] That of previously reported post-prandial serum bile acid testing was 84.6% (11/13 tests abnormal).[75,76] In our study, sensitivity of both was 100% (7/7 fasting values abnormal and 7/7 post-prandial values abnormal), with a 95% confidence interval of 59% to 100%.

Among reports of dogs receiving anticonvulsants, sensitivity of fasting serum bile acid testing was 38.5% (20/52 tests abnormal).[70,73,80] That of post-prandial bile acid testing in these dogs was 90% (9/10 tests abnormal).[70,73,80] Again, histologic evaluation of the liver was not performed in all dogs and significance of these findings is difficult to evaluate. No dogs with cirrhosis in our study had serum bile acid testing performed.

Comparison Of Plasma Ammonia Data To That Reported In the Literature

Sensitivity of ammonia tolerance testing calculated in this study for all histopathologic groups combined (61.3% for fasting ammonia and 71.4% for post-challenge ammonia) is not consistent with previous reports. Sensitivity calculated using data from previous reports was higher than and did not fall within the 95% confidence interval for sensitivities calculated from our data (Table 11). Of 39 fasting plasma ammonia concentrations reported in dogs with hepatobiliary disease, 33 were abnormal (sensitivity of 84.6%).[70,75,76,79,80] Of 17 post-challenge ammonia concentrations reported in dogs with hepatobiliary disease, 16 were abnormal (sensitivity 94.1%).[68,70,75,76,80] Of these, 2 were post-prandial ammonia samples rather than samples taken following ammonium chloride administration.[70] Histopathologic examination of hepatic tissue was performed in 24/39 reported dogs. The higher sensitivities calculated from data in the literature may be due to a higher proportion of dogs with portosystemic shunts in these previous reports.

Among reports of dogs with portosystemic shunts, the sensitivity of performing a fasting plasma ammonia concentration was 95.6% (22/23 tests abnormal), and that of performing a post-challenge ammonia concentration was 100% (13/13 tests abnormal).[68,75,76] Most dogs had the diagnosis confirmed by angiography or portal venography rather than histologic examination of the liver. Histologic examination of the liver was performed in 1 dog[75] and laparotomy was performed in 2 dogs (whether histologic examination of the liver was performed in these 2 dogs is not reported, although gross shunting was observed)[76]. In our study, sensitivity of ammonia testing in dogs with portosystemic shunts was 100% (14/14 fasting concentrations abnormal and 4/4 post-

challenge concentrations abnormal, with 95% confidence intervals of 76.8% to 100% and 39.7% to 100%, respectively).

Among reports of dogs receiving anticonvulsants, sensitivity of fasting plasma ammonia was 50% (4/8 tests abnormal) and that of post-challenge ammonia was 75% (3/4 tests abnormal).[70,80] In our study, sensitivity of ammonia testing was 100% among dogs with cirrhosis (2/2 fasting values abnormal and 1/1 post-challenge values abnormal, with 95% confidence intervals of 15% to 100% and 2.5% to 100%, repectively). Again, because the number of dogs having cirrhosis in our study was small and because not all reported dogs receiving anticonvulsants underwent histopathologic examination of the liver, comparison between these populations is difficult.

Adverse Reactions To BSP Administration

In addition to sensitivity, a second concern that has been raised regarding the use of BSP is the risk of side effects following BSP administration. Although anaphylactoid and perivascular reactions have occurred in humans, side effects have been infrequently reported in small animal species[26,78] and no adverse reactions were recorded in the 150 medical records reviewed from this hospital. BSP toxicosis may be dose-dependent. BSP toxicosis reported in human beings often involved multiple BSP injections given to the same individual, and the only report of BSP toxicosis in dogs in the veterinary literature also involved large doses of dye. Whether a species difference between human beings and dogs in susceptibility to BSP toxicosis exists is unknown. A single dose of BSP at 5 mg/kg does not appear toxic in dogs based our study.

Choosing An Hepatic Function Test

Results of one hepatic function test should not be interpreted in a vacuum, and no test of hepatobiliary function is perfect. The liver is intimately associated with the body's blood volume and is therefore subject to a variety of metabolic derangements. In addition, the liver has a large functional reserve capacity, and hepatic lesions do not always result in altered hepatic function. Judicious use of hepatic function tests must be combined with careful evaluation of other parameters to rule in or out the presence of significant hepatobiliary disease and to guide therapy.

BSP's biggest disadvantage is limited availability due to the fact that it is not a commercially available drug. Advantages of the 30-minute BSP retention test include technical ease of the test, brevity of the test, lack of toxicosis, low expense, a stable blood sample for assay, and a simple spectrophotometric assay. In contrast, serum bile acid testing is a 2-hour test requiring feeding (which may be difficult in anorectic dogs) and the bile acid assay may give inconsistent results.### Measuring a plasma ammonia concentration requires that the blood sample be processed immediately or shipped on ice and processed within 2 to 3 hours, as ammonia is volatile.[118] Ammonia tolerance testing

Personal communication with H Bender, DVM, PhD, DACVCP and W. R. Chickering, DVM, PhD, June 1997.

may result in vomiting (if given orally) or defecation (if given rectally) of ammonium chloride, making this test difficult to perform in some patients.[119] Rarely, ammonia administration may potentiate hepatic encephalopathy.[120]

Which hepatic function test is chosen to evaluate a given patient should be decided based on available laboratory services and on a case by case basis, and more than one hepatic function test may be required for complete evaluation. Based on this study, BSP appears non-toxic in dogs at the 5 mg/kg dose. Its sensitivity is comparable to that of serum bile acid and ammonia tolerance testing, and BSP retention data from this study were consistent with that of earlier reports. Previous arguments discouraging BSP's use do not seem valid based on our results, and VMRCVM's continued use of the BSP 30-minute retention test is justified.

Areas that warrant further investigation include comparing the specificities of serum bile acid and ammonia testing to that of BSP retention and determining whether cetain levels of BSP retention are associated with prognosis or clinical outcome. Such studies would be best accomplished using a prospective study design evaluating dogs with histopathologically normal and abnormal livers, in which all dogs undergo each of the three hepatic function tests.

Conclusions

BSP administration was non-toxic in dogs of this study. Based on dogs of this study, the sensitivity of the BSP 30-minute retention test is comparable to that of serum bile acid and ammonia tolerance testing. It is therefore as good a screening test for hepatobiliary dysfunction as these tests. Based on the dogs of this study, the BSP 30-minute retention test has a high specificity, making it a good test for evaluating dogs already suspected of having hepatobiliary disease in which more invasive or risky procedures are being considered. BSP testing could not distinguish between types of hepatobiliary disease.