Introduction

At the Virginia-Maryland Regional College of Veterinary Medicine (VMRCVM), use of the BSP 30-minute retention test of hepatic function continues despite the test's reputed disadvantages. The advantages of this test in our hospital have been a rapid turn-around time for results (our laboratory does not perform bile acid measurements and must send samples for this test to other laboratories), lack of toxicosis following administration of BSP, and a perceived accuracy in screening dogs for presence of hepatobiliary disease. Our subjective experience has been that BSP retention testing is as accurate as ammonia tolerance testing and bile acid determination for the detection of hepatobiliary disease in both dogs and cats.

This study was undertaken to determine objectively accuracy of the 30-minute BSP retention test in a large number of dogs with various types of hepatobiliary disease. The purpose of this study was to determine whether BSP retention is significantly different in dogs with and dogs without histopathologically confirmed hepatobiliary disease and to determine if BSP testing can distinguish between different types of hepatobiliary disease.

Literature Review

Development Of Hepatic Function Testing Using Cholephilic Dyes

Although cholephilic dyes have been studied since 1866[1], their use to evaluate hepatic function was not investigated until the early 20th century. Able and Rowntree[2], while studying the gastrointestinal purgative effect of phenoltetrachlorphthalein in 1909, discovered this dye was excreted in bile. Their work led to the suggestion that phenoltetrachlorphthalein might be of value in testing hepatic function, as it appeared to be excreted primarily by the liver.[1] In 1913, this suggestion was investigated by Whipple, et al.,[3] using experimental dogs, and Rowntree, et al.,[4] using human beings. In both studies, amount of dye excreted following intravenous injection was calculated by recovering phenoltetrachlorphthalein from feces and urine, and decreased dye excretion was interpreted to reflect decreased hepatic function.[3,5] Adverse effects associated with administration of phenoltetrachlorphthalein to human patients included thrombophlebitis, syncope, and chills.[6] Dye excretion was not evaluated in healthy human beings.

Due to the cumbersome task of fecal and urine collection[4,7], a method was developed in 1922 by Rosenthal whereby phenoltetrachlorphthalein could be colorimetrically quantitated in serum, leading to estimation of hepatic function by measuring rate of serum dye disappearance in normal dogs and dogs exposed to chloroform.[7] One year later, Rosenthal demonstrated that phenoltetrachlorphthalein retention in serum increased with decreasing functional hepatic mass by performing varying

degrees of hepatectomy in rabbits.[8] Bromsulfophthalein (phenoltetrabromphthalein sodium sulphonate, or BSP) was introduced by Rosenthal and White[9] in 1925 and found to be excreted in the bile of rabbits in higher quantities than phenoltetrachlorphthalein (85% versus 10%). This fact, combined with a lesser incidence of side effects upon intravenous injection when compared with phenoltetrachlorphthalein, led to BSP's use as a clinical test of hepatic function in human medicine.[9]

Other dyes, including methylene blue, azorubin S, and congo red, also were investigated as potential tests of hepatic function in the first half of the twentieth century. However, toxicosis, apparent insensitivity to hepatic dysfunction, and extrahepatic uptake made these dyes unsuitable for clinical use. Only indocyanine green and rose bengal appeared acceptable alternatives to BSP[1], although hemolysis was found to be an undesirable side effect of rose bengal injection.[6]

In 1925, Rosenthal and White recommended a BSP dosage of 2 mg/kg body weight given intravenously over one minute. Five and 30 minutes following injection, a blood sample was obtained and centrifuged. The serum was alkalinized using sodium hydroxide to show the blue color of the dye. The percent BSP remaining was determined by visually comparing the patients' sera to standard dilutions (comparator blocks) of BSP in sodium hydroxide. "Normal" values for BSP retention were established based on testing in 25 human beings, of whom 12 had acute or chronic gonorrhea, 2 had chronic nephritis, and 11 were convalescing from various non-digestive diseases. BSP retention ranged from 20 to 50% at 5 minutes post-injection (mean 35%). In all patients, amount of BSP remaining in serum at 30 minutes was insufficient to be colorimetrically estimated. The authors also performed BSP retention testing in 20 human beings that had various hepatic diseases and obtained values ranging from 3% to 99% retention at 30 minutes. They assumed that percent dye retention correlated directly with percent of dysfunctional hepatic mass (e.g., 10% retention was interpreted to mean 10% of the liver was dysfunctional).[9]

In 1928, Serby and Bloch evaluated the 30-minute BSP retention test in 76 human beings, of whom 32 underwent hepatic inspection by laparotomy or autopsy. The authors did not clarify whether hepatic histopathology was performed in addition to visual inspection. Increased 30-minute BSP retention was observed in 10/11 cases of pancreatic carcinoma, 2/9 cases of intra-abdominal malignancy, 10/12 cases of acute or subacute hepatitis and cholangitis, 2/3 cases of acute cholecystitis, 0/3 cases of chronic cholecystitis, 2/3 cases of common bile duct obstruction, 2/4 cases of hepatic cirrhosis, 0/8 cases of "blood disease", 1/9 cases of heart disease, 0/3 cases of bleeding gastric ulcer, 0/2 cases of late pregnancy toxemia, and 0/9 "control" cases (all of which suffered from various illnesses). The authors concluded that BSP retention is of limited value in cases of obstructive jaundice, as hyperbilirubinemia in these patients already indicates hepatic dysfunction. Adverse reaction to BSP, consisting of thrombosis at the injection site, was observed in 1 patient with polycythemia vera.[6]

Development Of the Modern BSP Retention Test

A study by MacDonald[10] in 1939 touted the value of measuring serial samples in performing BSP testing, arguing that the rate of BSP removal provided a more complete estimate of hepatic functional capacity than BSP retention at a single point in time. Although comprehensive pharmacokinetics were not discussed, MacDonald compared the shapes of BSP disappearance curves obtained by plotting serum BSP retention against time in both healthy and diseased human beings. On the basis of these findings, the 5 mg/kg dose of BSP was advocated.

In a 1943 review of hepatic function tests, Mateer, et al., evaluated 30-minute BSP retention in 30 healthy adult human beings ranging in age from 25 to 35 years in an attempt to define normal BSP retention more clearly. Initially, 25/30 subjects were given 2 mg/kg BSP intravenously. BSP was detected in the sera of all individuals at 10 minutes, in 11/25 individuals at 15 minutes, and in none at 20 minutes post-injection. Thirty subjects were then given 5 mg/kg BSP intravenously. BSP was detected in the serum of all individuals at 20 minutes, 21/30 at 25 minutes, 9/30 at 30 minutes, 4/30 at 35 minutes, 1/30 at 40 mintes, and in none at 45 minutes. The 2 mg/kg and 5 mg/kg doses also were evaluated in 46 human patients with hepatic disease. The higher BSP dose yielded abnormal retention in 62% more cases than the lower dose, leading the authors to conclude that testing with the higher dose resulted in greater sensitivity. Based on these findings, the authors advocated using the 5 mg/kg dose to screen patients for hepatic dysfunction and suggested 0% retention at 45 minutes was normal.[11]

In subsequent studies, Mateer and co-workers developed a BSP retention testing protocol using photoelectric colorimetric quantitation of BSP rather than the manual comparator blocks used previously. Less than 4% retention at 45 minutes was considered normal following injection of 5 mg BSP/kg. The advantage of this method of BSP quantitation was its ability to measure BSP in hemolyzed serum.[12,13] This protocol became the accepted method of performing BSP retention in human medicine.[14,15]

A study by Moses, et al., in 1948 compared 15-minute sampling with 45-minute sampling in performing BSP retention (using 5 mg/kg) in 20 human subjects with normal hepatic function (some of these had non-hepatic diseases), in 21 individuals with miscellaneous diseases, and in 17 chronic alcoholics. The authors observed increased 15-minute BSP retention in some subjects that became normal at 45 minutes. They concluded that both the 15- and the 45-minute samples should be obtained for the fullest evaluation of hepatic function, as they felt the 45-minute sample alone may not be sensitive enough to detect hepatic disease. BSP was quantitated using photoelectric colorimetry, and the authors suggested 25% retention at 15 minutes and 4% retention at 45 minutes as high normal values.[14]

In the 1950's, evaluation of hepatic function using cholephilic dyes extended beyond measuring percent retention to include more comprehensive pharmacokinetics. The advent of isotope-labeled cholephilic dyes made radionuclide scanning possible in addition to estimating hepatic function pharmacokinetically. Development of hepatic function testing using cholephilic dyes also resulted in development of cholecystographic contrast media for radiographic study of the biliary system.[1] BSP retention testing was used clinically in

human medicine primarily to screen non-icteric patients suspected of having hepatic disease and to follow patients convalescing from hepatic disease.[16] In a 1967 review, Charm stated BSP retention testing was most useful for documenting hepatic dysfunction in patients with inactive hepatic cirrhosis, early viral hepatitis, proximal gastrointestinal bleeding, and metastatic carcinoma in the liver.[15]

Metabolism And Pharmacokinetics Of BSP

Following intravenous injection in the dog, 60% of BSP is instantaneously bound to albumin and alpha$_1$-lipoprotein.[17] Protein-bound BSP is taken up by hepatocytes and conjugated with glutathione and other amino acids. Fifty to 80% of administered BSP is conjugated and secreted in bile. Conjugated BSP that is not secreted in bile may reflux back into the systemic circulation or diffuse into hepatic lymph, and some conjugated BSP undergoes enterohepatic circulation. BSP that is not protein bound is eliminated by the kidneys.[18]

Plasma volume is the volume of distribution of the cholephilic dyes, including BSP.[1] Distribution of BSP to blood, liver, and bile follows first-order kinetics.[19] Various mathematical models have been proposed to explain clearance curves of BSP and to determine whether decreased BSP clearance is due to impaired hepatocyte function (impaired uptake and conjugation) or due to cholestasis (impaired secretion into and elimination through bile). Two-, three-, four-, and six-compartment models of BSP clearance have been proposed and studied.[19-25] Studies done by Wheeler et al. in 1960 demonstrated that conjugated BSP secretion into bile is the rate-limiting step in BSP clearance from plasma.[26,27] Although measurement of BSP clearance provides a more sensitive test of hepatic function, clearance testing generally is not used clinically due to the need for serial blood sampling and more complex mathematical analysis.

In BSP retention testing in human beings, it is assumed that plasma volume equals 50 ml/kg body weight. The initial dose of 5 mg/kg BSP is assumed to give a zero-time concentration of 10 mg/dl, so that the percentage of BSP retained is obtained by multiplying the 45-minute plasma concentration by 10.[1] Various factors have been observed to alter BSP pharmacokinetics. The most clinically important of these, body weight, was evaluated in 1967 by Freston and Englert using 84 human hospital patients and employees given 5 mg/kg BSP. Mean BSP 45-minute retention was significantly greater in overweight than normal subjects (8.2% vs. 3.4%). As plasma volume does not increase with increasing body weight, it follows that the obese patients were "overdosed" relative to their plasma volume when dosed on a body-weight basis, leading to falsely high BSP retention. The authors suggested using 2.5 mg/kg as the dose in obese patients. Dye retention also was found to be significantly higher in patients over age 60, and the authors speculated that a defect in hepatic dye uptake could be responsible for this difference. Using multiple regression analysis, body weight was found to be a more significant variable than age in influencing BSP retention in the subjects of their study.[28]

Like obesity, ascites can alter BSP retention by affecting BSP dosing relative to plasma volume. BSP also may diffuse into ascitic fluid, however, causing accelerated BSP

removal from the plasma.[18] Decreased protein binding of BSP due to hypoalbuminemia or displacement from albumin by other drugs with a higher affinity for albumin decreases BSP retention by increasing renal elimination of unbound BSP.[29] Hepatic dysfunction may thus be underestimated. Other variables, such as fever, dehydration, congestive heart failure, and systemic hypotension, alter BSP pharmacokinetics by decreasing hepatic perfusion or by impairing hepatic uptake of BSP, causing falsely increased retention.[18,30] Numerous drugs and chemical substances have been reported to interfere with BSP pharmacokinetics by competing with BSP for uptake by hepatocytes or by interfering with BSP conjugation, including bilirubin, testosterone derivatives, estrogen, progesterone, cholecystographic agents, probenicid, novobiocin, phenothiazines, morphine, and codeine.[1,18,31,32]

Adverse Reactions To BSP In Human Beings And the Decline In BSP Use

Case reports of adverse reactions to BSP in human beings appear beginning in the late 1940s. All reported reactions occurred to BSP given intravenously at 5 mg/kg. Several patients were given multiple BSP injections over time periods ranging from a few days to several months, and some patients were apparently sensitized to BSP by having the drug extravasated on initial intravenous injection.

In a report from 1948, Chambers and Moister[33] described a systemic allergic reaction to BSP injection in a 35-year-old woman with a history of asthma and allergies who presented for evaluation of hepatomegaly. In 1949, Seivers, et al.,[34] reported urticaria following inadvertent perivascular injection of BSP in a 53-year-old white male. One month later this same patient developed a systemic allergic reaction following another BSP injection consisting of nausea, retching, weakness, abdominal cramps, tachycardia, generalized wheals, and pruritus.

The same year, Morey, et al.,[35] reported 2 additional cases of BSP hypersensitivity in a 25-year-old caucasian woman having infectious hepatitis and in a 19-year-old African American man admitted for umbilical hernia repair. The woman developed a local hypersensitivity reaction consisting of urticaria and severe phlebitis at the injection site following her sixth BSP injection into the same vein. The man developed a similar hypersensitivity reaction following his third injection of BSP into the same vein.

In 1950, Roth[5] reported anaphylaxis due to BSP administration in a 38-year-old caucasian man suffering from obesity, pyothorax, bacterial pneumonia and pleuritis, and hepatomegaly. The patient had a history of sensitivity to sulfadiazine, leading to the speculation that hypersentivity to the sulfur component of BSP was responsible for his reaction. In 1953, anaphylaxis was reported by McVay[36] in a 68-year-old African American man suffering from heart failure who had received his second BSP injection. A fatal reaction occuring in a 58-year-old Indian male suffering from hepatic cirrhosis and bronchitis was reported in 1957 by Walker and Koszalka.[37] Shock and sudden death occurred several minutes following injection of a second dose of BSP.

In a review of the human BSP literature in 1964, Katz and Scarf[38] cited 19 reported adverse reactions to BSP, 8 of which were fatal. In a 1965 letter discussing BSP

reactions in patients at the Johns Hopkins Hospital, Iber[16] reported localized reactions such as soft-tissue sloughing, thrombophlebitis, and urticaria at the injection site in 19/1500 BSP administrations (all at a dose of 5 mg/kg) over a 13-year period. Thirty-five of 233 physicians surveyed at Johns Hopkins Hospital in 1961 reported having witnessed BSP reactions following intravenous injection of the dye. In a review of the human literature, Iber cited 18 non-fatal and 14 fatal reactions to BSP administration.[16]

Such reports, combined with the advent of automatic chemical analyzers which facilitated performance of serum hepatic enzyme and bilirubin measurements, caused BSP retention testing to fall out of favor in human medicine beginning in the late 1960s.[39] In a 1969 letter, Weirum reported 27 hypersensitivity reactions and 15 deaths in human beings related to BSP administration.[39] BSP, which had been available as a 5% aqueous solution from Hynson, Westcott, and Dunning (Baltimore, MD), was discontinued as a commercially available drug in 1984. BSP currently is available in chemical form as a crystalline sodium salt from various manufacturers.

Use Of BSP In Veterinary Medicine

Numerous reports exist in the veterinary literature of BSP use in ruminant, equine, and avian species. Studies evaluating both pharmacokinetics and clinical use have been performed.[40-57] The first reports of BSP use in dogs and cats are experimental studies dating from the 1930s. BSP retention testing for diagnostic use in clinical small animal patients was not reported until 1950.[58]

In 1935, in a study correlating histopathological lesions of cholestasis with abnormalities in BSP retention and serum bilirubin concentration, Cantarow and Stewart determined BSP 30-minute retention using 2 mg/kg of the dye in 29 cats undergoing surgical ligation of the common bile duct. The cats were placed in one of 12 groups (not evenly divided between groups) and kept alive post-operatively for 1, 2, 3, 4, 6, 7, 8, 10, 11, 12, 15, and 16 days, respectively. BSP retention ranged from 0% to 100%, and the authors found no correlation between degree of dye retention and magnitude of histopathological changes in hepatic tissue and bile ducts. No correlation was observed between degree of dye retention and serum bilirubin concentration. Range of variation in BSP retention was greater than range of variation in serum bilirubin concentrations.[59] Statistical analysis was not performed.

In 1944, Drill and Ivy compared BSP retention, serum phosphatase concentration, prothrombin time, and intravenous galactose tolerance testing in 10 adult dogs poisoned with carbon tetrachloride. Each dog served as its own control. Although 5 mg/kg BSP was administered, the investigators used colorimetric comparator blocks standardized for the 2 mg/kg dose. BSP 30-minute retention of > 100% was thus obtained in some dogs. The authors suggested using 15% retention at 30 minutes as the upper limit of normal, and 10 to 15% retention at 30 minutes as suggestive of hepatic dysfunction. BSP retention prior to carbon tetrachloride administration ranged from 2 to 15% (mean 7.6%; median 9%). BSP retention following carbon tetrachloride administration ranged from 5% to 250% (mean 60%; median 90%). Multiple serial determinations were made in each dog over a period of 27 to 36 days, and BSP retention was abnormal in 7/10 dogs within 2

days of carbon tetrachloride administration. The authors concluded that, of the 4 tests evaluated, BSP was the most sensitive test of hepatic dysfunction.[60] Histopathologic evaluation of hepatic tissue was not performed in any dog.

In 1946, Svirbely, et al., compared BSP retention, rose bengal retention, serum phosphatase concentration, prothrombin time, icterus index, urine urobilinogen, and hepatic histopathology in 6 control dogs and 7 dogs poisoned by inhalation of a xylidine-air vapor mixture. Xylidine exposure was performed 5 days of every week for 15 weeks or until death of the animal, which occurred between 2 and 5 weeks in 6/7 exposed dogs. The remaining exposed dog was euthanized at 22 weeks. BSP was given at a dose of 5 mg/kg, and 30-minute percent retention was calculated based on BSP serum concentration at 5 and 30 minutes. BSP retention in the control dogs throughout the study ranged from 2.5% to 13.5% (mean 4.9%; median 4.2%). Pre-xylidine BSP retention in the exposed dogs ranged from 0% to 5.7% (median 2.7%). Post-xylidine BSP retention in the exposed dogs ranged from 1.9% (at week 1 in one dog) to 89% (at week 4 in another dog). BSP retention increased as duration of xylidine exposure increased, and all exposed dogs had histopathologically abnormal livers. All control dogs had histopathologically normal livers. The authors concluded that, of the tests evaluated, BSP was the most consistently abnormal and sensitive test of hepatic function. [61]

In 1950, Hoerlein and Greene evaluated BSP retention in 10 clinically normal dogs and in 16 dogs with various diseases.[58] Using 5 mg/kg BSP, serial blood samples were taken from the 10 normal dogs to evaluate serum dye disappearance. BSP was present in trace amounts in 3/10 dogs and absent in 7/10 dogs at 30 minutes. No BSP was detected in the serum of any normal dog at 35 minutes. BSP retention testing was abnormal in 6/16 diseased dogs and ranged from 10% (in 2 dogs with hepatitis and 2 dogs with distemper) to 100% retention (in 2 dogs with leptospirosis). Based on their findings, the authors recommended BSP 30-minute retention testing as a sensitive test of hepatic dysfunction in the dog. Histopathologic evaluation of the liver was not performed in any dog.

In 1960, Larson and Morrill studied correlation between 45-minute BSP retention and hepatic histopathologic changes in 85 dogs.[62] BSP was given at a dose of 5 mg/kg to 14 normal control dogs, 5 dogs poisoned with carbon tetrachloride, 20 dogs with naturally-acquired canine distemper, 6 dogs innoculated with infectious canine hepatitis virus, 5 pyrexic dogs, and 35 dogs given one of 5 hepatotoxic substances (streptovaricin, tolbutamide, benzoic acid, pyrazolidin, or methyl reserpate). All animals were euthanized and necropsied, and hepatic histopathology was performed. [62] BSP retention in control dogs was less than 1.5%, and all dogs had histopathologically normal livers. BSP retention in the 5 carbon tetrachloride-poisoned dogs ranged from 0.8 to 34.0% (mean 22%, median 23.6%), all of which had histopathologically abnormal livers. Six dogs given streptovaricin had BSP retention ranging from 1.5 to 9.0% (mean 3.8%, median 2.9%); of these, 4 had normal livers on histologic evaluation. Eight dogs given tolbutamide had BSP retention ranging from 0.8 to 4.3% (mean 2.15%, median 1.65%). Of these, 5 had normal livers. BSP retention in 9 dogs given benzoic acid ranged from 0.8 to 41.2% (mean 5.4%, median 0.8%), of which 6 had normal livers. BSP retention ranged from 6.2% to 21.0 % (mean 11.9, median 10.1%) in 6 dogs given pyrazolidin and all had hepatic lesions. In 6 dogs given methyl reserpate, BSP retention was normal in all (0.8%), and only 1 dog had hepatic lesions. In 20 dogs with canine distemper, BSP

retention ranged from 0.8% to 9.8% (mean 2.5%, median 1.5%). All but 3 dogs had hepatic lesions, and these 3 all had normal BSP retention (0.8% in each dog). In 6 dogs with infectious canine hepatitis BSP ranged from 0.8% to 12% (mean 4.1%, median 2.5%), and all but 1 had hepatic lesions. In 5 pyrexic dogs, BSP retention was normal in all (0.8% in each dog) and only 2 had hepatic lesions.

The authors concluded that dye retention and hepatic lesions were not consistent when BSP was < 5% or hepatic lesions were mild.[62] False positive BSP retention values occurred more often than false negative values. BSP retention was not quantitative in all cases (i.e., magnitude of BSP retention did not directly parallel magnitude of hepatic lesions in all dogs). The authors suggested < 1.5% retention at 45 minutes was the high end of normal for dogs, 1.5% to 4.0% retention as indicative of hepatic dysfunction, and > 4.0% at 45 minutes was definitively abnormal.[62]

In 1968, Van Vleet and Alberts evaluated hepatic enzyme concentrations, serum bilirubin, serum cholesterol, BSP retention, ICG clearance, urine bilirubin, urine urobilinogen, galactose tolerance testing, serum total protein, serum albumin:globulin ratio, and prothrombin time in 27 clinically normal dogs, 11 dogs before and after carbon tetrachloride administration, and 4 dogs before and after extrahepatic bile duct obstruction. The 15 dogs in the experimental groups served as their own controls.[63] BSP was administered at a dose of 5 mg/kg, and 30-minute retention ranged from 0% to 5% (mean 1.8%) in the control dogs. In those exposed to carbon tetrachloride, mean BSP retention of the 11 dogs ranged from 2.15% to 11.6% over the 10 days of the study. In those with surgically created biliary obstruction, mean BSP retention of the 4 dogs ranged from 1.64% to 18.0% over the 10 days of the study. Serial hepatic biopsies were taken from the 15 experimental dogs, and necropsies were performed at termination of the study. The authors felt that magnitude of BSP retention paralleled severity of structural hepatic changes observed in carbon tetrachloride poisoning, but that clinicopathologic changes preceded histologic changes in surgically-induced biliary obstruction.[63] Statistical analysis was not performe, and individual histopathologic findings and BSP retention values were not reported.

The protocol for BSP retention testing in dogs, horses, and cattle was reviewed in 1969 by Morgan, who cited less than 5% retention at 30 minutes as being the normal standard in dogs.[64] During a 1972 study correlating hepatic needle biopsy findings with clinicopathologic and necropsy findings in 15 dogs showing clinical signs of hepatic disease, Brobst and Schall reported using BSP retention in 5/15 dogs. BSP retention at 30 minutes was abnormal in all 5 dogs, ranging from 6% to 43% (mean 16.4%, median 10%). Diagnoses included hepatic lipidosis (2 dogs), lymphoma (1 dog), cholangiocellular carcinoma (1 dog), and hemangioma (1 dog).[65]

In 1974, Ewing, et al., evaluated clinical signs, clinicopathologic changes, and radiographic findings in 21 dogs with naturally-acquired portal vein anomalies. BSP retention testing at 30 minutes was performed in 20 dogs on 33 occasions and values ranged from 4.0% to 30.0% (mean 15.5%). Of these 20 dogs, only 1 dog had normal (< 5%) BSP retention values.[66]

In 1978, Strombeck reported clinicopathologic features of 8 dogs with primary hepatocellular carcinoma and 15 dogs with metastatic hepatic neoplasia. BSP 30-minute

retention was performed in 3 dogs and ranged from 1% to 11% (mean 5.7%).[67] Individual histopathologic diagnoses and albumin concentrations in these 3 dogs were not reported.

Meyer, et al., in a 1978 study evaluating usefulness of ammonia tolerance testing for diagnosis of portosystemic shunts, reported using BSP 30-minute retention in a group of 20 clinically normal adult dogs and 6 dogs with naturally-occurring portosystemic venous shunts (PSS). Following administration of 5 mg/kg BSP IV, all normal dogs had BSP retention at 30 minutes of less than 5%. One of 6 dogs with PSS had a normal BSP retention (4%) on one occasion and abnormal BSP retention (6%) on two other occasions. The other 5 dogs with PSS had BSP retention values ranging from 8% to 11.5% (mean 9.8%, median 9.5%). Overlap in fasting blood ammonia concentrations existed between the control dogs and dogs with PSS. However, following oral administration of 100 mg/kg ammonia, all dogs with PSS had increased blood ammonia concentrations, resulting in a statistically significant difference between dogs with PSS and control dogs. The authors concluded that fasting blood ammonia concentration alone was not reliable in differentiating dogs with PSS from normal dogs, while ammonia tolerance testing could reliably distinguish the two. The authors suggested that ammonia tolerance testing may be more sensitive for detection of altered hepatic blood flow than BSP retention testing, based on one dog with PSS that had the normal BSP retention value.[68]

In 1981, Meyer and Noonan used BSP 30-minute retention testing as part of a battery of laboratory tests evaluating hepatic function in 12 research dogs receiving anticonvulsant drugs (diphenylhydantoin and primidone). BSP was administered at a dose of 5 mg/kg, and each dog served as its own control. In the primidone-treated group, plasma glutamic-pyruvic transaminase (GPT) and alkaline phosphatase (ALP) activities increased significantly in 6/6 dogs after 2 and 3 weeks of treatment, respectively. In the diphenylhydantoin-treated group, GPT activity was mildly increased in 6/6 dogs; ALP was mildly increased in 5/6 dogs and markedly increased in 1/6 dogs. No significant changes were observed in GGT activity, bilirubin concentration, fasting bile acid concentration, or BSP retention in any dog. The authors concluded that GPT and ALP increase variably following anticonvulsant administration and that elevated concentrations of GPT may signal underlying hepatic disease. Individual BSP retention data were not presented and hepatic histopathologic examination was not performed in any dog.[69]

In a 1982 study of hepatic cirrhosis associated with long-term anticonvulsant administration in dogs, Bunch, et al., evaluated BSP retention, serum GGT activity, and fasting and post-prandial serum bile acid concentrations in 5 client-owned dogs receiving treatment for idiopathic epilepsy. Primidone and phenytoin were received by 2/5 dogs, primidone and phenobartital by 1/5, primidone alone by 1/5, and primidone, phenytoin, and phenobarbital by 1/5. At necropsy, hepatic cirrhosis was identified in 4/4 dogs. BSP retention was increased in 4/5 dogs (mean 16.6%, median 17.75%). In the remaining dog, BSP retention was performed twice and was normal on the second occasion (34% and 2.6%, respectively). Fasting bile acids were increased in 2/3 dogs (mean 75 μmol/L), and post-prandial bile acids were increased in 1/2 dogs (96 μmol/L). Hepatic enzyme activities were increased in all dogs. The authors concluded that variable increases in ALP and alanine aminotransferase (ALT) activities in dogs receiving anticonvulsants do not necessarily signal impending hepatic disease, but that the combination of increased GGT

activity, serum bile acid concentrations, and delayed BSP retention suggest reduced hepatic function and were an indication for hepatic biopsy [70]

In 1983, Center, et al., compared BSP and indocyanine green (ICG) pharmacokinetics in 19 healthy dogs. BSP was administered at a dose of 5 mg/kg, and 30-minute retention ranged from 0% to 4.2% (mean 1.9 ± 1.1%). BSP was cleared more rapidly than ICG.[71] In a companion article, Center, et al., compared BSP and ICG pharmacokinetics in 17 healthy cats. Administered at a dose of 5 mg/kg, BSP 30-minute retention ranged from 0% to 3.0% (mean 0.6 ± 0.8%). Based on these findings, 30-minute retention of < 3% was suggested as normal for the cat. As in the dog, BSP was cleared more rapidly than ICG.[72]

As ICG is restricted to the vascular compartment, has no extrahepatic uptake, is not biotransformed, does not undergo enterohepatic circulation, and does not diffuse into hepatic lymph, Center, et al., argued that ICG clearance reflects hepatic circulation and excretion more specifically than BSP and may therefore be a better test of hepatic function in both dogs and cats.[69,70] Despite these advantages, the clinical use of ICG did not become widespread due to logistical reasons such as difficult chemical analysis, the need for a special diluent, and the need for immediate injection following reconstitution.[18]

In 1983, DeNovo and Prasse, in a study attempting to differentiate cholestasis-induced increased alkaline phosphatase (ALP) concentration from corticosteroid-induced increased ALP concentration, performed BSP retention testing in 20 adult dogs. Dogs undergoing treatment with dexamethasone had significantly higher BSP retention at days 6 and 12 of the experiment than either normal control dogs, sham-operated control dogs, or dogs undergoing surgical hepatic duct ligation. Based on these findings, the authors speculated that increased BSP retention in the dexamethasone-treated group was either the result of decreased BSP uptake by hepatocytes due to competition with dexamethasone or the result of hepatocyte dysfunction from corticosteroid-induced hepatopathy.[32]

In 1984, in a second study evaluating compromised hepatic function in anticonvulsant-treated dogs, Bunch, et al., performed BSP retention testing and other laboratory tests in 48 dogs receiving anticonvulsant drugs for 6 months or longer. BSP retention was increased in 10/20 dogs treated with primidone alone, 2/7 dogs treated with phenytoin, and 7/13 dogs treated with combinations of anticonvulsant drugs. Fasting bile acid concentrations were increased in 3/14 dogs receiving primidone, 2/7 dogs receiving phenytoin, and 4/17 dogs receiving combinations of anticonvulsant drugs. Individual laboratory data were not presented. Hepatic cirrhosis was identified at post-mortem examination in 3/3 dogs. The authors concluded that, although concern over hepatotoxicity of anticonvulsant drugs was justified, risk of hepatotoxicity appeared small when compared with risk of death from intractable seizure activity.[73]

In a third article on the same subject, Bunch, et al., in 1985 published a study evaluating hepatoxoticity of experimental anticonvulsant administration in 29 healthy female Beagle dogs. Thirty-minute BSP retention following injection of 5 mg/kg was greater than 5% only in dogs given 55 mg/kg primidone and 198 mg/kg phenytoin in combination (mean BSP >10%). Although individual data were not presented, this group reportedly had a high mean BSP retention due to an icteric dog in the group with a BSP retention at 9 weeks of 40.2%. In all other groups, BSP retention was less than or equal to 5% in both

treated and control dogs. At 20 weeks a statistically significant difference in BSP retention was present between treated and control dogs (treated dogs had higher BSP retention, although their BSP retention was less than or equal to 5%). Fasting and post-prandial bile acid concentrations were variable within all groups (treatment and controls), and magnitude of increase in post-prandial bile acid concentrations did not change consistently in any group in relation to treatment. Hepatic biopsies were performed in all dogs and showed mild, multifocal hepatocellular lipidosis and scattered single hepatocyte necrosis in all treated dogs. Three dogs that became clinically ill during the study developed intrahepatic cholestasis. The authors concluded that hepatic disease associated with anticonvulsant administration does occur, and that high drug dosage and/or combination with phenytoin were important factors in development of hepatic disease.[74]

In 1985, Center, et al., compared serum bile acid testing with ammonia tolerance and BSP testing in 18 dogs and 4 cats with naturally-acquired portosystemic venous anomalies. BSP 30-minute retention, performed in 13/18 dogs, was normal in 2 dogs (4.2% and 2.2%, respectively) and ranged from 6.0% to 23% (mean 11.3%; median 9.8%) in the others. Fasting blood ammonia concentration was increased in 11/12 dogs (mean 251.5 µmol/L). Ammonia tolerance testing was abnormal in 7/7 dogs at 30 and 60 minutes following administration of ammonium chloride (mean post-challenge 361.6 µmol/L at 30 minutes). Fasting serum bile acid concentrations were increased in 14/18 dogs (mean 61.7 µmol/L), and post-prandial bile acid concentrations were abnormal in 15/18 dogs (mean 229.9 µmol/L). BSP 30-minute retention, performed in 3/4 cats, was normal in 2 cats (2.0% and 2.5%, respectively) and abnormal in 1 cat (5.4%). Fasting blood ammonia concentrations were abnormal in 3/4 cats (mean 160.8 µmol/L). Ammonia tolerance testing was performed in 3/4 cats, one of which had a normal fasting ammonia value. Ammonium chloride challenge resulted in increased blood ammonia concentrations in all 3 cats. Fasting bile acid concentrations were abnormal in 4/4 cats (mean 24.4 µmol/L), and post-prandial bile acid concentrations were abnormal in 2/2 cats (mean 120.6 µmol/L). The authors concluded that bile acid evaluation was comparable to ammonia tolerance testing and more sensitive than serum enzyme measurement and BSP retention testing for the detection of hepatobiliary insufficiency in both dogs and cats.[75]

In 1986, Meyer compared serum bile acid testing with 30-minute BSP retention and ammonia tolerance testing in 11 dogs with portosystemic venous shunts. BSP retention was normal in 1 dog (4%) and abnormal in 10 dogs (range 7% to 19%; mean 13.1%; median 15%). Fasting plasma ammonia concentrations were increased in 11/11 dogs (mean 246.9 µg/dl). Ammonia tolerance testing was abnormal in 7/7 dogs (mean post-challenge plasma ammonia 510.7 µg/dl). Fasting serum bile acid concentrations were abnormal in 11/11 dogs (mean 78.8 µmol/L), and post-prandial serum bile acid concentrations were abnormal in 8/8 dogs (mean 177.0 µmol/L). Meyer concluded that serum bile acid measurement was a reliable test of hepatic function. No conclusions were drawn about diagnostic efficacy of BSP retention testing. [76]

In a 1988 study by Aguilera-Tejero, et al., hepatic function testing was evaluated in 15 healthy laboratory dogs given carbon tetrachloride. Fasting and post-prandial bile acid concentration, lactate dehydrogenase concentration (LDH), and 45-minute BSP retention were measured, and dogs served as their own controls. Fasting bile acid concentrations rose significantly following carbon tetrachloride administration, and the authors concluded that determining post-prandial values did not increase diagnostic capacity of the test because the baseline values were already abnormal. LDH activity and BSP

retention also increased markedly in all dogs following carbon tetrachloride administration. The authors concluded that BSP retention was more useful in identifying hepatic dysfunction in early stages of hepatic disease, whereas bile acid determination was more useful in determining hepatic dysfunction in more advanced stages of disease.[77] The basis for this conclusion is not clear.

BSP and ICG pharmacokinetics were further evaluated in cats in a 1989 study by Middleton and Watson. BSP was cleared more slowly at a dose of 10 mg/kg than at doses of 2 and 5 mg/kg. At 5 mg/kg, < 3.6% retention was considered normal. The authors reported vomiting in 1 cat (once when given 5 mg/kg and once when given 10 mg/kg), trembling and hyperesthesia in the same cat given 2 mg/kg, and trembling and hyperesthesia in another cat receiving 5 mg/kg. All reactions occurred immediately following BSP injection, and both cats were given BSP on later occasions without complications.[78] The only other report of adverse BSP reactions in small animal species is by Wheeler, et al. During a pharmacokinetic study using 6 research dogs that had undergone splenectomy, cholecystectomy, and installation of a duodenal fistula as part of a previous study, these authors reported vomiting in one dog and vomiting and death in 2 dogs 4 hours following BSP administration. All dogs were given 1500 to 1800 mg of BSP in a continuous intravenous infusion over 4 hours.[27]

BSP retention testing was performed along with other laboratory tests in a 1989 study by Hardy, et al., evaluating periportal hepatitis in 13 client-owned dogs associated with diethylcarbamazine-oxibendazole use. Thirty-minute BSP retention was abnormal in 7/7 dogs (mean 31.4 %, median 31%) and fasting blood ammonia concentration was increased in 7/8 dogs (245 µg/dl, median 257 µg/dl). All dogs had increased ALP and ALT activities. Histopathologic examination of hepatic biopsies revealed periportal hepatitis associated with vacuolar hepatopathy in 12/13 dogs. The authors concluded that diethylcarbamazine-oxibendazole should be used only in dogs with confirmed coexisting heartworm and hookworm infestations, and recommended that the drug not be given to dogs with known hepatic disease.[79]

In 1991, Bayrell-Hart, et al., retrospectively evaluated BSP retention and other laboratory tests in 18 phenobarbital-treated dogs to determine whether hepatotoxicity is associated with chronic phenobarbital use. Nine of 18 dogs had received other drugs, and 4/18 dogs had received other anticonvulsants. BSP retention was reported to be abnormal in 9/11 dogs, fasting bile acid concentrations were abnormal in 6/11 dogs, post-prandial bile acid concentrations were abnormal in 8/11 dogs, fasting ammonia concentration were abnormal in 2/5 dogs, and ammonia tolerance testing was abnormal in 2/2 dogs. Individual data and group means were not reported. Histopathologic examination of the liver revealed bridging portal fibrosis in 9/10 dogs, inflammation in 8/10 dogs, and nodular regeneration in 9/10 dogs. The authors concluded that toxicosis due to phenobarbital administration occurs in some animals and recommended serial laboratory evaluation of dogs receiving this drug at 6-month intervals.[80]

In two studies from 1992 and 1994, Boothe, et al., evaluated ICG, antipyrine, and caffeine disposition in 24 Beagles having experimental dimethylnitrosamine (DMNA) -induced hepatotoxicity. BSP 30-minute retention was performed in all dogs as part of a battery of laboratory tests. BSP retention was abnormal in all treated dogs and correlated with severity of histologic abnormalities (dogs with more severe histologic abnormalities

had higher BSP retention). The authors concluded that, although the disposition kinetics of ICG, antipyrine, and caffeine were altered in dogs given DMNA compared with control dogs, they were not sensitive enough to distinguish between moderate and severe hepatic disease in their study. No conclusions were drawn regarding the diagnostic efficacy of BSP or any other laboratory tests of hepatic disease that were performed.[81,82]

After 1990, BSP retention testing was no longer routinely used for the diagnosis of hepatobiliary disease in small animal species. Center, in a 1990 article discussing hepatic function tests in the diagnosis of portosystemic vascular anomalies, stated that BSP retention testing in cases of portovascular anomaly had been replaced by ammonia tolerance testing and serum bile acid determination, as the latter are more sensitive and dependable tests of hepatic function in this disorder.[83] In a 1995 review of hepatic function testing using cholephilic dyes, Center listed risk of hypersensitivity reactions, inaccuracy due to the large number of variables influencing dye metabolism, and the cumbersome nature of clearance studies as the major reasons why BSP testing has fallen out of favor for clinical use in small animal medicine.[84]

Histopathologic Changes Associated With Major Disease Mechanisms of Canine Liver

Histopathologic criteria for portosystemic shunt have been widely described and include hepatocellular atrophy (reduction in cell volume but not number) leading to small lobules and portal tracts that are relatively too close together.[85] Sinusoids in zone 1 may be narrowed, while sinusoids in zone 3 may be widened. In addition, portal tracts may lack recognizable portal veins and contain prominent hepatic arterioles, and abnormal structures resembling vessels and bile ducts may be present.[85] Bile duct proliferation and fatty infiltration of hepatocytes also have been reported.[86,87] Multifocal areas of hydropic degeneration may be observed, especially in zone 3 hepatocytes, and the central veins may be surrounded by increased connective tissue.[85] In some dogs, sections may show increased hepatocellular iron content, and lipogranulomas (foci of iron-laden macrophages) may occasionally be present.[85,88]

Based on a 1986 study by van den Ingh et. al, extrahepatic cholestasis in dogs is characterized by edema, neutrophil infiltration, and concentric periductal fibrosis of the portal triad. Ohlsson et. al reported similar changes in a 1970 study.[89] Dogs with both intra- and extrahepatic cholestasis show bile accumulation characterized by bile pigment in the canaliculi (as bile thrombi), in Kupffer cells (as phagocytosed thrombi), and in hepatocytes (as fine, granular pigment). Hepatocytes in cholestatic areas may show hydropic degeneration and macrophages may be observed. Proliferation of bile ductules is seen in chronic cases.[90]

Various patterns of hepatic necrosis and acute hepatic injury have been described, and the distribution of affected hepatocytes is dependent upon the etiology.[85] In human beings, chronic hepatitis is defined as hepatic inflammation continuing without improvement for at least 6 months. Chronic hepatitis is divided into 2 morphologic

13

categories, chronic persistent hepatitis and chronic active hepatitis.[91,92] Chronic persistent hepatitis is characterized by portal tract expansion and inflammatory cell infiltration, focal hepatocyte necrosis, and the presence of acidophilic bodies. Lobular architecture remains intact and piecemeal necrosis (i.e., focal necrosis at the junction of portal tracts or septa and parenchyma) and fibrosis are slight or absent. Chronic active hepatitis is characterized by portal tract inflammation (predominantly lymphocytic and plasmacellular), alteration of lobular architecture, and fibrosis. Piecemeal necrosis, bridging necrosis (i.e., necrosis linking central to central, central to portal, or portal to portal areas), or multilobular necrosis may be present. Chronic active hepatitis carries a worse prognosis than chronic persistent hepatitis.[91]

Chronic hepatitis in the dog is an umbrella term encompassing a variety of inflammatory hepatopathies.[92] Inflammatory cells (usually lymphocytes) and fibrosis are initially seen in portal areas but may extend into the hepatic parenchyma in severe cases.[85] Reported morphologic abnormalities in dogs with chronic hepatitis have included periportal fibrosis, bridging fibrosis, varying degrees of hepatoctye necrosis, piecemeal necrosis, hepatocyte hypertrophy, micro- and macronodular change, inflammatory cell infiltrates (predominantly lymphocytic and plasmacellular, but neutrophilic inflammation was present in some instances), and cholestasis.[93-96] Periportal copper and iron accumulation have been reported in some Doberman Pinschers with chronic hepatitis.[97]

Corticosteroid-induced hepatopathy is characterized by hepatocyte swelling and vacuolation due to glycogen accumulation.[85,98] Moderately to grossly distended hepatocytes (2 to 20 times their normal size) may be seen, and changes may be severe and diffuse or multifocal and random.[99,100] In very distended hepatocytes, cellular organelles may be displaced to the cell periphery.[98,101] Sinusoid size is reduced due to hepatocyte swelling. Single cell necrosis, focal necrosis, and multifocal aggregations of neutrophils may be seen in some animals. Necrosis, if present, is typically minor and randomly distributed.[85]

While the term cirrhosis has been used interchangeably with fibrosis and chronic hepatitis, the three are distinct morphologic entities. Fibrosis, defined as the presence of excess collagen, occurs following necrosis and may be localized, multifocal, or diffuse. Chonic hepatitis may entail fibrosis, but fibrosis is not a diagnosis in itself and occurs following a variety of hepatic insults. [91] In a 1977 review of human hepatic histopathology, Anthony et al. defined cirrhosis as characterized by diffuse hepatic involvement, presence of fibrosis, and conversion of normal hepatic architecture to structurally abnormal nodules (sometimes called "regenerative" nodules). Presence of fibrosis in combination with nodular degeneration is the hallmark of cirrhosis. In human beings, cirrhosis is morphologically divided into micronodular cirrhosis, in which nodules are less than 3 mm in diameter, macronodular cirrhosis, in which nodules are greater than 3 mm in diameter, and mixed cirrhosis, in which small and large nodules are present in roughly equal proportions. Micronodules may contain portal tracts but generally lack normal hepatic structures. Macronodules may contain portal structures and efferent veins, but these are abnormally related to one another. Macronodules can be further classified as "incomplete septal" (also known as "post-hepatic") or "post-collapse" (also known as "post-necrotic") based on the fibrotic pattern.[91]

14

Hepatocellular adenoma (hepatoma) is characterized by relatively normal-looking hepatocytes arranged in cords or tubules. Hepatocytes may contain lipid. Portal triads and acini are not discernable.[102] Cells of hepatocellular carcinoma may be well-differentiated, closely resembling normal hepatocytes or may be highly anaplastic.[85] Neoplastic hepatocytes may have a granular acidophilic cytoplasm, a large nucleus with a distinct membrane, and acidophilic nucleoli. Giant cells containing large nuclei, multilobed nuclei, or multiple nuclei may be seen.[102]

One of several histologic patterns that may be observed in hepatocellular carcinoma is formation of neoplastic trabeculi. Neoplastic trabeculi differ from normal trabeculi in that they are several cells thick and Kupffer cells and sinusoids are not seen. A second histologic pattern consists of acinus-like arrangements of neoplastic hepatocytes. The acinar lumen may contain proteinaceous material that is PAS positive. A third histologic pattern consists of a compact, solid, or scirrous arrangement of neoplastic hepatocytes growing in solid sheets. Sinusoids and fibrous tissue are absent.[85]

Intrahepatic tumors of biliary origin are histologically divided into tubular carcinomas or bile cystadenocarcinomas.[103] Tubular carcinomas consist of tubular structures lined with cuboidal or columnar cells and dissected by diffuse fibrous stroma. Bile duct adenocarcinomas differ from primary hepatocellular carcinomas in that they have more fibrous stroma. The luminal border of malignant cells may have microvilli, and mucus secretion may be seen in some cells. Cystadenocarcinomas are characterized by many cysts lined with single or multiple layers of epithelials cells. Papillary structures lined with cuboidal or columnar epithelium may fill cystic spaces.[103] Hepatocellular or biliary-origin neoplasms may be difficult to distinguish from metastatic disease.[85]

Among neoplasms which metastisize to the canine liver, lymphoma and hemangiosarcoma are common, but various other tumors may metastasize there as well. Various metastatic carcinomas, sarcomas, and leukemias have been reported.[104]

Passive congestion results in hypoxia of zone 3 hepatocytes, producing histologic change that ranges from hydropic degeneration in this area (if early) to focal necrosis and fibrosis in zones 3 and 2 if advanced. Sinusoids in zone 3 are distended with blood.[85,105]

Copper-induced hepatopathy results in a spectrum of histopathologic change that varies with chronicity and severity of copper accumulation. Changes range from excessive hepatic copper concentrations without morphologic change to severe alteration of hepatic architecture and cirrhosis.[106-110] Copper-induced hepatopathy is characterized by mild inflammatory cell infiltrates (predominantly neutrophilic) in portal areas, focal necrosis, accumulation of copper granules within hepatocytes, and lipid vacuolization of hepatocytes.[85,102] Vacuolization of hepatocytes due to hydropic change and glycogen accumulation is a consistent feature of this disorder.[107,111]