THE SULFOBROMOPHTHALEIN (BSP) LIVER FUNCTION TEST FOR SHEEP

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There is a growing need for the quantitative measurement of hepatic integrity in the ruminant. Situations often arise in experiments with ruminants where various treatments may involve changes in liver function. High concentrate diets, known to increase liver abscesses in cattle (Jensen, 1960; Wise et al., 1968), may possibly stress liver function. Many drugs and hormones depend on the liver for detoxication and excretion via the bile (Streeten, 1959; Flock et al., 1951) which implies that changes in liver function alter the effective dosage of growth stimulants and products to control estrus. High urea diets may need to be examined for hepatotoxic effects which are possibly due to a specific protein deficiency or excess ammonia production. The adequacy of the liver in chronic bloat or chronic founder is not defined. Since older animals are seldom kept until death and liver injuries in meat animals are most commonly discovered by postmortem observations, liver disease per se is not a commonly recognized problem in ruminants as in man. Thus, methods for evaluating the liver have not been widely applied to sheep and cattle.

The organic dye sulfobromophtalein (BSP) has been successfully used for many years as a liver function test in clinical medicine (Rosenthal and White, 1925; Mateer et al., 1943. Metabolism of the dye occurs almost exclusively in the liver of the dog (Javitt et al., 1960), rat (Combes, 1959), man (Grodsky, Carbone and Franska, 1959) and other domestic animals (Cornelius and Kaneko, 1963). The sensitivity of the BSP test to changes in hepatic blood flow, liver mass and the integrity of the parenchymal tissue and the biliary excretory system suggests that the test would be valuable for ruminants. In view of the limited data for BSP tests in sheep, a series of studies was conducted to determine if data from other species concerning optimum dosage, manner of blood clearance and route of excretion would apply to sheep. A further study was conducted to determine if the test was responsive in sheep to a known hepatotoxic agent.

Experimental Methods

The BSP, obtained as the amorphous dye from the Baker Chemical Company in aqueous solution gave the extinction coefficient at 580 mμ: E 0.1% = 78. A 5.0% solution, cm prepared for intravenous injection, was autoclaved at 1.06 kg per cm² for 15 minutes. Colorimetric estimation and recovery of BSP were obtained for hemolyzed and non-hemolyzed blood plasma. The nonhemolyzed plasma was diluted 1:9 with distilled water, alkalized with 0.02 ml of saturated sodium hydroxide to develop color and read at 580 millimicrons. The hemolyzed plasma required acidification of the 1:9 diluted plasma with 0.01 ml of concentrated hydrochloric acid to destroy the heme and establish a usable reference for reading the alkalized BSP at 580 mμ.

To determine the optimum dosage, levels of 5, 10, 15 and 20 mg per kilogram of body weight were injected into the jugular vein of four groups of four wethers each, with an average weight of 38 kilograms. Blood clearance of BSP was determined from jugular blood sampled at approximately 2- or 4-min. intervals (the exact time seconds of each blood sample was recorded) for 24 min. and BSP concentration was determined from the heparinized plasma as described above.

To estimate the extent of urinary excretion, four wethers weighing 37 to 59 kg were placed in metabolism crates and one was injected with 20 mg per kilogram in a single dose and three were injected with 30 mg per kilogram in two doses 10 min. apart. Urine was collected for 24 hr. and urinary BSP was quantitatively estimated. The low concentration of BSP and the abundance of urinary pigments required special colorimetric procedures. The

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urine was centrifuged and decanted to remove the clear supernatant. To determine if urinary pigments would selectively interfere with the BSP measurement, the absorption spectrum was obtained for the acidified urine between 550 and 650 millimicrons. After making the urine alkaline, the absorption spectrum was again obtained and the optical difference between the baseline and the BSP maxima at 580 m~ was used to quantify the urinary BSP.

BSP metabolism and excretion by the liver was studied in a 38 kg wether that had been surgically prepared with a biliary cannula below the pancreatic juncture. The cannula was exteriorized with 4 mm o.d. tygon tubing and connected via re-entry tube to the small intestine. The wether had fully recovered from surgery prior to this study, bile flow appeared to be normal. BSP was administered through a jugular catheter and bile was collected in a fraction collector. Each fraction of bile was measured by volume and an aliquot diluted to an appropriate volume for colorimetric estimation of BSP. A second trial was conducted on the same animal on the following day.

Chromatography of the biliary BSP, free BSP and in vitro BSP-bile (BSP mixed with previously collected bile) was adapted from procedures of Javitt et al. (1960) by applying these substances to a 47 x 57 cm sheet of Whatman no. 1 filter paper and developing in water saturated butanol:acetic acid (4:1 v/v) for 20 hours. The characteristic purple spots of BSP were developed by spraying the chromatograph with a dilute sodium hydroxide solution. The spots were outlined by pencil, acidified to remove color and sprayed with ninhydrin to develop color with amino nitrogen. Additional spots were cut out and the BSP dissolved in water for quantitative estimation of the various fractions.

To prove the sensitivity of the BSP test in sheep, the plasma BSP clearance was measured before and after carbon tetrachloride poisoning in wethers. Normal liver function was determined as in the dosage determinations. The wethers were then dosed via rumen tube with 0.6 ml per kilogram of carbon tetrachloride. The BSP plasma clearance rate was again determined at 24 and 48 hr. after the poisoning.

Results and Discussion

The recovery of BSP added to nonhemo-

lyzed plasma was 100% but varied from 95 to 105% with hemolyzed plasma. Every effort was made to prevent hemolysis in subsequent studies.

Although 5 mg per kilogram body weight has been suggested as a suitable dosage for sheep by Cornelius and Kaneko (1963), at the BSP dosage of 5 or 10 mg per kilogram of body weight in this study, less than 3% of the injected BSP remained in circulation after 5 minutes. As noted by Cornelius and Kaneko (1963), BSP clearance appeared to be much more rapid for sheep than for other species. The authors consider the two lower dosages inadequate to plot a dependable BSP blood clearance rate. BSP clearances at the two higher dosages are shown in figure 1. At the 15 or 20 mg per kilogram dosage, BSP clearance to as low as 1 mg per 100 ml approached a straight line with a plot of the logarithm of concentration vs. time. Wheeler et al. (1958) have indicated that liver storage of BSP is in equilibrium with

Figure 1. Average BSP clearance rates of four wethers dosed with 15 or 20 mg per kilogram of body weight. Percent mean clearance at 15 min. = 97.7±0.38 and 85.6±2.6, respectively.
plasma BSP. In the current study, the higher dosage appeared to more nearly saturate liver storage without a proportionate increase in biliary excretion since plasma clearance rate was reduced. The clearance rate, average of four wethers, for the 15 mg per kilogram dosage was: log $C = -2.5189 - 0.1205t$ and for the 20 mg per kilogram dosage: log $C = -2.6807 - 0.0670t$ where $C$ equals concentration of BSP and $t$ equals time after injection.

Although the 15 mg per kilogram dosage was three times that used in clinical medicine for human liver tests (Rosenthal and White, 1925; Mateer et al., 1943), higher dosages have been given to rats and rabbits (Klaassen and Plaa, 1967, 1968). The satisfactory appearance of the blood clearance plot of the 15 mg per kilogram dosage led to the selection of this dosage for subsequent liver function studies with sheep.

Urinary pigments contributed greatly to the optical density of urine, but there were no peaks near the 580 m~ maximum absorption of BSP. Since the urinary excretion of BSP, shown in table 1, at the 20 mg per kilogram dosage was only 0.03% of the injected dose, higher dosages were investigated. The average urinary excretion of the three wethers injected with 30 mg per kilogram was 0.83% of the dose. Urinary excretion at this higher dosage was also small, which indicated that urinary excretion could be ignored in similar liver studies with sheep.

In the metabolism and excretion study, biliary excretion from the cannulated bile duct in the two successive trials gave recoveries of 82 and 83% within 56 min. after the BSP injections (figure 2). Concentrations of BSP and BSP metabolites in the bile were 200 to 300 times the plasma concentration of free BSP which suggests that the excretory process was against a concentration gradient and would require an active transport mechanism. Plasma clearance appeared to be nearly complete within 15 min., but only about 50% of the injected BSP had been excreted into the bile during the same time period. Presumably, the remaining BSP was in the ducts of the biliary tree or bound in liver storage.

Development of the chromatograph resolved three fractions from the biliary BSP and one fraction each from free BSP and the in vitro BSP-bile (figure 3). Of the three fractions from the biliary BSP, fraction I had the same $R_f$ (0.19) as free BSP and constituted 13.4% of the total biliary BSP. Fractions II and III were ninhydrin positive with $R_f$ values of 0.09 and 0.04, constituting 58.5 and 28.0% of the total biliary BSP, respectively. These data suggest that fractions I and II were conjugated with amino acids or other amine containing compounds. Since the bile duct cannula in this wether was below the pancreatic juncture, the bile could have contained pancreatic products that yielded coincidental ninhydrin-positive spots with the BSP fractions. However, BSP is known to be excreted as a conjugate with amino acids or glutathione in the rat (Combes, 1959) and dog (Javitt et al., 1960), which may be true in sheep.

The effects of liver poisoning with carbon tetrachloride on BSP clearance in sheep are shown in figure 4. Mean BSP clearances for the four wethers in percent of the injected dose at 15 min after injection were 94.6, 53.3 and 53.8 for successive tests before, 24 and 48 hr. after carbon tetrachloride poisoning. In all of the poisoned wethers there was a dramatic decrease in blood BSP clearance at 24 and 48 hours. The wethers immediately went off feed and were depressed for a period of 3 to 5 days. However, subsequent recovery was without incident or visible consequences. If liver dysfunction in ruminants resulting from other hepatotoxic agents or nutritional deficiencies affects BSP clearance as does carbon tetrachloride poisoning, determining the status of the ruminant liver under many physio-

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<th>TABLE 1. URINARY EXCRETION OF BSP IN SHEEP</th>
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<td>Avg of 30 mg dose</td>
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LIVER FUNCTION TEST FOR SHEEP

1193

Figure 2. Plasma and biliary clearance of BSP in a wether with a bile duct cannula.

Figure 3. Chromatography of BSP metabolites from a wether with a bile duct cannula. Chromatograph developed in n-butanol-acetic acid (4:1 v/v).

Summary

Studies were conducted to adapt the sulfobromophthalein (BSP) liver function test to sheep. The dosage of BSP required to accurately measure blood clearance of this dye in sheep was found to be 15 to 20 mg per kilogram of body weight, which is about three times the dosage used in clinical medicine. From 82 to 83% of the injected dye was excreted in the bile within 60 min. after injection while less than 1.0% appeared in the urine. Chromatography revealed three fractions of BSP in the excreted bile. One of these fractions chromatographed as free BSP, whereas two of the fractions appeared to be conjugated with ninhydrin-positive compounds. Direct liver injury by carbon tetrachloride poisoning greatly reduced the ability of the ovine liver to excrete BSP. These findings suggest that the BSP liver function test for sheep is useful if the dosage is increased sufficiently to compensate for the rapid clearance by this species.

logical and biochemical stresses may be simple, rapid and of great value.
TUCKER, MITCHELL, JR. AND LITTLE

Figure 4. Plasma BSP clearance of four wethers before and 24 hr. after carbon tetrachloride poisoning. Percent mean clearance at 15 min. = 94.6 ± 1.9 and 53.3 ± 9.7 for the initial and 24 hr. tests, respectively.

Literature Cited


