11. Benson, G. K., Cowie, A. T., Folley, S. J., Tindal, J. S., in *Recent Progress in the Endocrinology of Reproduction,* C. W. Lloyd, ed., 1959, p480, Academic Press, N. Y.

12. Benson, G. K., Folley, S. J., *Nature,* 1956, v177, 700.

13. McCann, S. M., Mack, R., Gale, C., *Endocrinology,* 1959, v64, 870.

14. Reece, R. P., Turner, C. W., *Mo. Agr. Exp. Sta. Res. Bull.,* 1937, 266.

15. Grosvenor, C. E., Turner, C. W., PROC. SOC. EXP. BIOL. AND MED., 1958, v97, 463.

16. Meites, J., Turner, C. W., *Endocrinology,* 1942, v30, 711, 719, 726.

17. Meites, J., *Fed. Proc.,* 1959, v1, 103.

18. Moon, R. C., Turner, C. W., PROC. SOC. EXP. BIOL. AND MED., 1959, v101, 332.

19. Meites, J., Talk at 82nd Meeting, *Am. Physiol. Soc.,* Atlantic City, N. J., April 17, 1959.

20. Meites, J., Shelesnyak, M. C., PROC. SOC. EXP. BIOL. AND MED., 1957, v94, 746.

21. Meites, J., Webster, H. D., Young, F. W., Thorp, F., Hatch, R. N., *J. Animal Sci.,* 1951, v10, 411.

22. Sgouris, J. T., Meites, J., *Am. J. Physiol.,* 1953, v175, 319.

23. Meites, J., *Rev. Canad. de Biol.,* 1954, v13, 359.

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Metabolism of Sulfobromophthalein in Hepatectomized and Hepatectomized-Nephrectomized Dog.* (25167)

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Sulfobromophthalein (BSP), because it is preferentially removed by the liver, has been used extensively as a liver function test in clinical medicine. Recent studies have shown that BSP is metabolized by the liver(1-5) and excreted into the bile, in part at least, as a mercaptide with cysteine and possibly with a compound containing cysteine, glycine and glutamic acid(6). Previous studies of BSP metabolites were carried out in normal human subjects and animals(2-4), in isolated perfused livers(3) and in patients with liver disease(7). In this investigation the ability of tissues other than the liver to metabolize BSP was studied in hepatectomized and hepatectomized-nephrectomized dogs.

Methods. Two normal male dogs, weighing 16 and 23 kg respectively, were hepatectomized under pentobarbital anesthesia. The liver, isolated by the method of Dakin *et al.* (8), was removed, along with a short segment of the inferior vena cava. The resulting gap in the remaining inferior vena cava was bridged by a plastic catheter. Animals were maintained by continuous infusion of 20% glucose. The blood volume was restored when necessary by transfusion of dog blood. Control samples of urine and blood were collected. The animals were then given commercial BSP intravenously (5 mg/kg of body weight). Blood samples, taken at timed intervals thereafter, were analyzed for total BSP and BSP conjugates. Urine, collected from each animal during a 2 hr period after injection of BSP, was also analyzed for total and conjugated dye. Two additional dogs were hepatectomized and also nephrectomized. Fifteen to 30 min. later, control blood samples were obtained. Commercial BSP was then administered intravenously (5 mg/kg of body weight). Blood specimens were taken at timed intervals and analyzed for total and conjugated BSP. Concentrations of total BSP in serum and urine were determined by the method of Gaebler(9). To quantitate the proportion of conjugate, measured aliquots of serum and concentrated urine were extracted with acetone (1:3 v/v). The resulting supernatants were dried under vacuum. The material was taken up in small aliquots of water and chromatographed on Whatman 3 MM pa-

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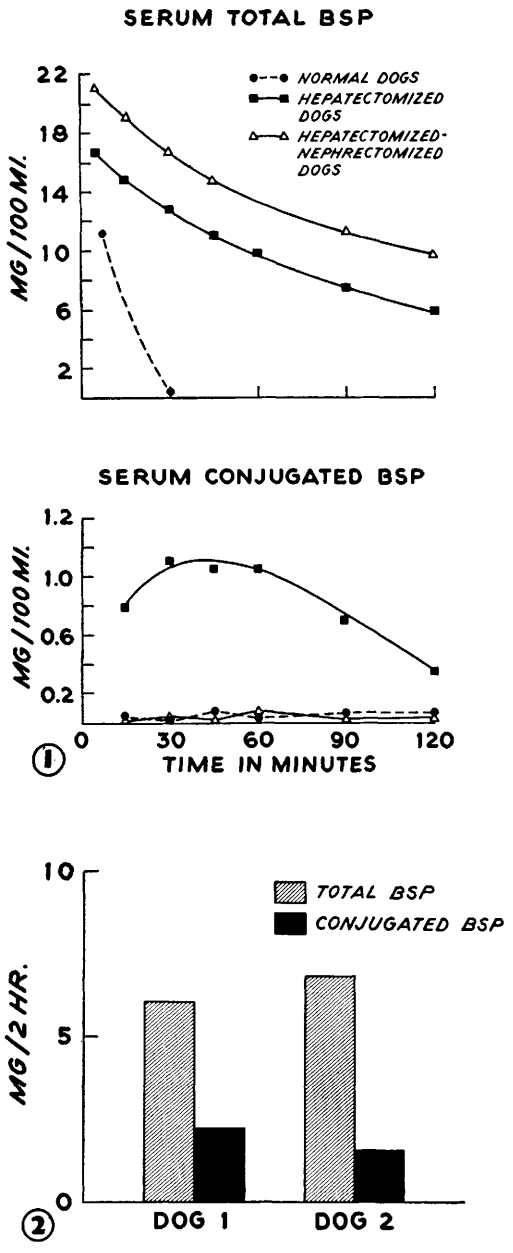

FIG. 1. Concentrations of BSP and BSP metabolites in serum after intravenous administration of dye into normal and surgically treated dogs.

FIG. 2. Excretion of BSP and its conjugates in urine of hepatectomized dogs.

per in an ascending system employing *t*-butanol/water (1.73:1 v/v) for 6 hr. BSP bands were developed by spraying them with freshly prepared 0.12 N NaOH in 95% ethanol. The metabolite bands (Rf approximately 0.65 and 0.23) were cut from the strip, pooled and macerated in 25 ml of 0.05 N NaOH. The mixture was centrifuged for 10 min. to remove the paper pulp. Samples of 6 ml of supernatant were read against a blank of 0.05 NaOH at 565 and 620 mμ. The unconjugated band (Rf approximately 0.88) was similarly extracted from the paper and quantitated. Standard deviation obtained after duplicate determinations on 15 different serum samples was 2.1%(7).

Results. Normal dogs. In 2 normal intact dogs, BSP was cleared rapidly (Fig. 1). After 45 min. only 0.36 and 0.13 mg/100 ml of the dye remained in circulation. Only traces of the BSP metabolites were observed. No appreciable quantities of BSP appeared in the urine of these animals.

Hepatectomized dogs. In hepatectomized dogs BSP was removed from the blood more slowly than in normal animals, but still at a significant rate (Fig. 1). Within 45 min. 35% of the dye present 7 min. after injection had been removed; by 2 hr. 65% had been cleared. During this interval the level of BSP metabolites rose to about 10% of total circulating pigment at 45 min.; thereafter it gradually decreased. After 2 hr. only about 10% of total BSP cleared could be accounted for in the urine. Approximately one-fourth to one-third of the urinary BSP, however, was in the conjugated form (Fig. 2).

Hepatectomized-nephrectomized dogs. Rate of disappearance of BSP from the blood of hepatectomized-nephrectomized dogs was similar to that of the hepatectomized animals (Fig. 1). Only trace amounts (less than 2%) of BSP metabolites, however, were found in the blood of the hepatectomized-nephrectomized dogs. These trace amounts were too small to measure quantitatively and technically were of questionable significance.

To demonstrate that the BSP metabolite bands did not arise as artifacts of chromatography or from nonspecific complex formation in blood and urine, specimens of serum and urine from both pairs of animals were incubated with commercial BSP for 1 hr. at 37°C and chromatographed. No significant levels of material corresponding in mobility to the BSP conjugates were found.

Discussion. Rapid removal of BSP from

the blood of the hepatectomized dog confirms the observation of Cohn *et al.*(10) in similarly treated animals that large amounts of the dye are removed from the blood during the first hour after injection. The appearance of significant quantities of BSP metabolites in the serum of these animals indicates that extrahepatic conjugation of BSP can occur. Since a higher percentage of metabolites was found in the urine than in the serum, the kidney apparently can either a) synthesize or b) preferentially excrete BSP metabolites. The almost complete absence of metabolites in the serum of the hepatectomized-nephrectomized animals favors the first possibility. The ability of the kidney to conjugate pigment has been demonstrated previously in the case of bilirubin(11). Furthermore, Mills and Wood (12) have shown that kidney slices contain the conjugative enzymes that may be required for BSP mercaptide synthesis.

Although the 2 hepatectomized dogs cleared 65% (52 and 75 mg) of injected BSP in 2 hr., only about 10% of the cleared dye appeared in the urine. Apparently the major portion of the dye in the hepatectomized animal is removed by uptake in peripheral extrarenal tissues. This conclusion is further supported by the fact that in the hepatectomized and the hepatectomized-nephrectomized dogs BSP was cleared from the blood at similar rates. The differences between the initial blood levels of BSP in the 2 pairs of animals may be attributable in part to differences in blood volume due to losses during surgical procedure or to rapid concentration of a portion of the pigment by the kidney in the animals which had only been hepatectomized.

A recent study(7) has shown that significant amounts of serum BSP conjugates are found in diseases of the liver and the biliary tract. In the normal organism in which BSP is rapidly removed by the liver, extrahepatic uptake and conjugation may play only a minor role. In severe liver disease, however, significant removal and conjugation of BSP by extrahepatic tissue may conceivably take place.

Summary. 1. Conjugation and rate of removal of sulfobromophthalein from the blood stream were studied in hepatectomized and hepatectomized-nephrectomized dogs. 2. Clearance of BSP from blood of hepatectomized dogs occurred at a slow but significant rate. Only 10% of cleared dye was excreted in urine. Conjugates of BSP appeared in serum and in greater percentage in urine. 3. Hepatectomized dogs after nephrectomy still cleared BSP at significant rate but had only questionable traces of metabolites in the serum. 4. It was concluded that the kidney is capable of conjugating BSP but that most of the dye clearance in the hepatectomized dog can be attributed to extrarenal mechanisms in the peripheral tissues.

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1. Grodsky, G. M., Fanska, R., Carbone, J. V., *The Physiologist,* 1958, v1, 29.

2. Krebs, J. S., Brauer, R. W., *Am. J. Physiol.,* 1958, v194, 37.

3. Grodsky, G. M., Carbone, J. V., Fanska, R., *Nature,* 1959, v183, 469.

4. Meltzer, J. I., Wheeler, H. O., Cranston, W. I., PROC. SOC. EXP. BIOL. AND MED., 1959, v100, 174.

5. Combes, B., *Science,* 1959, v129, 388.

6. Grodsky, G. M., Carbone, J. V., Fanska, R., *J. Clin. Invest.,* in press.

7. Carbone, J. V., Grodsky, G. M., Hjelte, V., *ibid.,* in press.

8. Dakin, R. L., Jew, J., Harper, H. A., McCorkle, H. J., *Arch. Surg.,* 1959, v78, 856.

9. Gaebler, O. H., *Am. J. Clin. Path.,* 1945, v15, 452.

10. Cohn, C., Levine, R., Streicher, D., *Am. J. Physiol.,* 1947, v150, 299.

11. Grodsky, G. M., Carbone, J., *J. Biol. Chem.,* 1957, v226, 449.

12. Mills, G. C., Wood, J. L., *ibid.,* 1954, v207, 695.

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