THE DISTRIBUTION AND EXCRETION OF S^{35}-LABELED SULFO-BROMOPHTHALEIN-SODIUM ADMINISTERED TO DOGS BY CONTINUOUS INFUSION [1]

By R. W. BRAUER,[2] R. L. PESSOTTI,[2] AND J. S. KREBS [2, 3]

(From the Department of Pharmacology and Experimental Therapeutics, School of Medicine, Louisiana State University, New Orleans, La.)

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It has been known for some time that continuous intravenous infusion of sulfo-bromophthalein-sodium (referred to below as BSP) in dogs as well as in man results eventually in a steady state in which blood and bile concentrations, and usually the rate of biliary excretion of the dye, remain constant (1). Bradley and co-workers have pointed out that such a steady state can only be achieved at infusion rates resulting in blood levels of BSP lower than a certain liminal value (20 mg. per L. in man [2] or 40 mg. per L. according to other workers [3]). The present authors (4) have pointed out in a previous communication that during such steady state conditions, the rate of biliary excretion of the dye is consistently below the rate of infusion, indicating that considerable amounts of sulfo-bromophthalein-sodium are being stored in the body. This is in accord with observations by Cantarow and Wirts (5) who showed that, following the administration of single doses of sulfo-bromophthalein-sodium, dye excretion into bile lagged by a considerable period of time behind the disappearance of most of the dye from the blood stream; they concluded that this period must represent storage of the dye.

The actual sites of storage of this "lost BSP" could not be determined with certainty in either of these investigations; indications were obtained that a considerable amount of dye is retained in the liver prior to excretion. Further investigation of this point seemed indicated for two reasons: First, application of BSP to clinical studies of hepatic blood flow depend, to a considerable extent, upon assumptions regarding the storage of the dye in tissues supplied by the splanchnic circulation; second, BSP is a most useful prototype substance for theoretical studies of bile formation and bile secretion, and knowledge of its actual sites of deposition is essential if serious errors of interpretation are to be avoided in this connection.

The preparation of S^{35}-labeled BSP (6) provides a tool which permits a direct approach to these questions. The present communication deals with the application of S^{35}-labeled BSP to the study of the distribution of BSP when administered to dogs by continuous intravenous infusion.

MATERIALS AND METHODS

Mongrel dogs under sodium pentobarbital anesthesia were studied after cholecystectomy and preparation of common bile duct fistulae. When desired, liver injury was induced by means of CCl_4 administered by stomach tube (0.5 cc. per Kg., 72 and again 24 hours before operation). BSP[4] was administered intravenously by means of a continuous infusion pump. For details of animal selection and maintenance, of induction of liver injury and surgery see (4).

S^{35}-labeled BSP was prepared by sulfonation of tetrabromphenolphthalein and purification of the product by chromatography and recrystallization (6). Colorimetric analyses for BSP were undertaken in the cases of the blood and bile samples only. The procedure employed, a modification of that described in (7), cf. also (8), consisted of diluting the samples with water, then acetone, in such fashion as to attain final protein concentrations between 0.8 and 1.2 per cent, and acetone concentrations of 60 per cent by volume. Blood plasma samples were then stored in the refrigerator for one hour and the protein precipitate removed by centrifugation; bile samples could be used without such intermediate treatment. Aliquots of the solutions were collected, 0.1 cc. of 10 per cent NaOH added to 2 cc. of solution, and suitable amounts transferred to the 10 mm. plunger microcell of

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[2] Present Address: U. S. Naval Radiological Defense Laboratory, San Francisco 24, California.

[3] NRC-AEC fellow in the Biological Sciences.

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an Evelyn photoelectric colorimeter. BSP concentrations were determined from the optical densities (580 mμ filter) by means of a calibration curve. In the presence of plasma protein, BSP recoveries of 90 to 100 per cent can be attained readily, the average loss in a series of experiments with different plasma samples being 3.5 per cent over the range of BSP concentrations of interest here. Table 1 shows a typical experiment in which BSP loss in the precipitate is determined at three levels of BSP concentration covering the range of values observed in the present series. In calculating blood BSP concentrations one may either use an aqueous solution calibration curve and correct for BSP loss using the above values (cf. Table 1), or, where extreme accuracy is essential, set up individual plasma calibration curves for each experiment. For the present series the former procedure was resorted to. In the case of the bile samples, preparation for colorimetry does not involve any material loss, so that equivalent BSP concentrations can be calculated directly from the optical density. The addition of bile in the amounts here involved to solutions of BSP does not alter the absorption coefficient of this dye at 580 mμ.

Analyses for S^{35} content were carried out on tissue homogenates as well as on blood and bile specimens. Blood and bile S^{35} determinations were carried out using the same solutions as employed for colorimetry. One- to two-tenths cc. of these were plated directly on tissue-paper-covered aluminum planchets, dried, and counted using a 1.4 mg. per cm^2 end-window Geiger-Muller Counter. The count rate was converted to equivalent BSP concentrations by comparison with a calibration curve using identically prepared planchets containing known amounts of the S^{35} BSP preparation used for the particular ex-

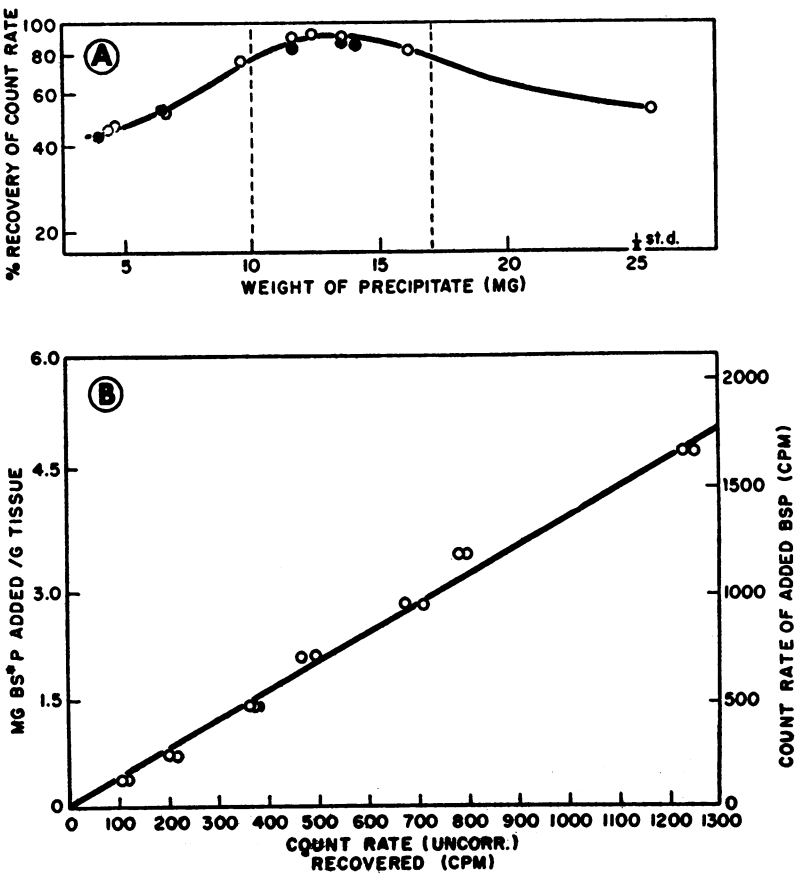

FIG. 1. S^{35} RECOVERY IN TISSUE ANALYSES

a. Relation between BSP-S^{35} recovered after addition of constant amounts of dye to varying amounts of dog liver, and weight of precipitate collected. Region between dashed lines represents conditions acceptable for analytical purposes.

b. Relation between amount of BSP-S^{35} added to a constant amount of dog liver, and S^{35} recovery.

TABLE 1

RECOVERY OF BSP FROM DOG BLOOD PLASMA AFTER ACETONE DEPROTEINIZATION

CONCENTRATION OF BSP IN PLASMA -- MG/L	CONCENTRATION AFTER ACETONE DEPROTEINIZATION*	% RECOVERY
15.8	15.2	96.4
37.4	36.2	96.8
68.4	65.0	95.3

* After correction for dilution, and using the proper acetone-saline-BSP calibration curve.

periment. Recovery of added BSP-S^{35} from blood plasma is controlled by the same factors which control recovery of colorimetric BSP; recoveries, therefore, are the same as shown in Table 1. Bile samples were prepared without material loss; S^{35} recoveries, after correction for self absorption, are 100 ± 1.0 per cent. Specific activity determinations are independent of BSP recovery in any case, since the same final solution was used for colorimetry and for radioassay. Standard deviations for specific activity determinations are about ± 2 per cent provided duplicate determinations are employed.

S^{35} in the various tissue homogenates was determined by the alkaline permanganate combustion procedure described in (9), followed by collection of the precipitates on filter paper in a precipitation apparatus, drying to constant weight, and counting as above. Recoveries of count rate are influenced by two factors varying in opposite direction with the weight of precipitate collected: Incomplete recovery of precipitate on the filters, and self absorption. Figure 1a shows a typical calibration curve obtained by adding the same, known, amount of S^{35} BSP to varying amounts of liver tissue. Again, using varying amounts of BSP with a constant amount of tissue the count rate obtained is proportional to the amount added (Figure 1b). Assuming such a linear relation, the standard deviation of individual points from the least square line is 10.7 per cent. Table 2 shows the recovery of known amounts of BSP added to different tissues and calculated from the actual count rates using the calibration curve of Figure 1a. Since recovery does not change excessively with precipitate weights in the range of 10 to 17 mg., determinations of tissue BSP-S^{35} should be carried out within this range of sample sizes. Experience has shown that the nature of the analytical procedure employed here is such that consistent results can be expected if, but only if, all details of the entire procedure are carried out identically in the minutest degree. Best results were obtained by carrying an entire series of determinations, including unknown samples and samples set up for determination of the calibration curve, through the entire series of steps in parallel and at the same time. Each dog experiment reported below was treated in this fashion and the estimates of standard deviation for each point derived from the above data apply. Using triplicate samples, each individual tissue concentration has a standard deviation of the mean of ± 6.7 per cent.

RESULTS

A. Color and activity relations in blood and bile

After a period of one to two hours of constant infusion of S^{35}-labeled BSP, in all dogs blood levels of the dye were attained which varied by no more than ± 10 per cent with continued infusion at the same rate. In normal dogs, bile levels also had become steady (± 8 per cent) at this time; in CCl_4 treated dogs, three hours of constant infusion might be required before bile levels reach a plateau. Figure 2 shows representative experiments indicating the course of blood and bile BSP concentrations as calculated from S^{35} content, as well as the ratio of BSP—calculated from S^{35} content—to BSP determined colorimetrically. Relevant data concerning this ratio, and its components, during the steady state in a series of experiments, are summarized in Table 3. In all normal dogs the S^{35} content of the blood plasma agreed within the limits of experimental error with values predicted on the basis of the colorimetric determinations of BSP in the same samples. $\left(\frac{\text{BSP-}S^{35}\text{ Activity}}{\text{BSP color}}\right.$ ratio equal to one.$\left.\right)$ In bile, on the other hand, an excess of S^{35} over that which could be accounted for as colorimetrically determined BSP was found in each case. This excess was found quite variable, ranging from 15 per cent in one case to as much as 62 per cent at the other extreme. No correlation was found between

TABLE 2

RECOVERY OF BSP-S^{35} ADDED TO VARIOUS TISSUES

TISSUE	TISSUE BS^{35}P CONCENTRATION -- MG/KG	BSP-S^{35} RECOVERED -- %
LIVER	(547 to 3750	97.0 ± 2.8*)
SPLEEN	114 135 198	101 91.6 90.4
KIDNEY	135 135 135	92.6 79.1 83.8
MUSCLE	135 124	89.3 91.0
DUODENUM	138 138 138	78 102 100
COLON	121 132 190	104 125 110
STOMACH	128 190	104 89
MEAN ± ST.D.M. ST.D. SINGLE DETN.		95.7 ± 3.0 ± 11.7

*Values from Figure 1b; standard deviation for single determination ± 10.8.

TABLE 3
RELATIONS BETWEEN COLORIMETRICALLY DETERMINED BSP, AND S^{35} DURING THE STEADY STATE PHASE OF CONTINUOUS INFUSIONS OF S^{35} BSP IN DOGS

DOG #	RATE OF S^{35} BSP INFUSION MGM/KGM/MIN	BLOOD PLASMA		BILE			
		S^{35} CONCENTRATION (AS BSP)—MGM/I	S^{35} (AS BSP) / COLORIM. BSP	S^{35} CONCENTR. (AS BSP) GM/I	S^{35} (AS BSP) / COLORIM. BSP	RATE OF EXCRETION % OF INFUSION RATE COLORIM. BSP	S^{35}
		NORMAL DOGS					
50-26	0.076	5.3	1.00	4.4	1.51	33.0	49.8
50-21	0.085	9.9	1.01	3.0	1.35	27.6	37.0
50-24	0.085	14.5	1.03	4.6	1.62	39.2	45.0
50-5	0.150	22.0	1.00	10.2	1.18	43.2	52.1
50-6	0.183	38.0	1.05	8.8	1.18	58.8	69.2
51-4	0.196	37.6	1.02	10.6	1.15	48.7	57.0
51-9	0.196	14.9	1.06	16.9	1.58	42.4	66.2
51-7	0.236	22.4	1.01	10.5	1.26	45.8	58.0
51-3	0.261	21.1	0.99	16.6	1.54	50.7	77.0
		CCl_4 TREATED DOGS					
50-16	0.090	234.	0.95	2.9	1.00	12.1	12.1
50-25	0.112	109.	1.06	2.9	1.19	3.9	4.7
50-9	0.144	220.	0.95	5.1	1.03	11.8	12.0
51-22	0.173	102.	1.00	7.5	1.46	2.9	4.3
51-21	0.204	47.3	1.02	16.5	1.31	35.3	46.4

the magnitude of excess of S^{35} and the infusion rate.

Inert sodium sulfate was added to several samples of bile containing S^{35} BSP. Separation of this carrier sulfate by means of acetone precipitation indicated that less than 2 per cent of the S^{35} in such bile was present as inorganic sulfate. The S^{35} in bile was not extractable with ether.

B. Retention of S^{35} BSP

In spite of the fact that the biliary concentration of S^{35} was greater than could be accounted for in terms of colorimetric BSP, the rate of S^{35} excretion into bile was still found to be significantly below the rate of BSP-S^{35} infusion in all experiments (Table 3, last column). Urine formed during the infusion of BSP in these dogs contained either no detectable BSP (seven cases) or else contained small amounts of BSP (two cases), never in excess of 1.5 per cent of the total S^{35} infused. Thus, considerable amounts of BSP are being stored in the bodies of these animals even during the steady state phase. The ratio between BSP-S^{35} infused and BSP-S^{35} excreted into bile approached more closely to 1.0 at the higher rates of infusion. Since, according to Bradley (2), excessive rates of infusion result in increasingly high apparent hepatic plasma flow, *i.e.*, increasing relative importance of extrahepatic BSP extraction in hepatic blood flow determinations by the BSP method, this finding in the dogs indicates that the range of infusion rates employed here cannot have been outside the acceptable limits for such studies.

C. Distribution of BSP-S^{35} in dogs after continuous infusion to steady state

The amount of BSP-S^{35} retained in the carcasses of dogs could be calculated as the difference between the amount infused and the amount excreted into bile with a minor correction for urinary BSP-S^{35}. The distribution of the material retained was determined in four normal and two CCl_4 treated animals by direct determination of S^{35} concentrations in the tissues. The results of these analyses are presented in Table 4. The figures for individual tissue concentrations of S^{35} were converted to "estimated total fraction of BSP-S^{35} retained" by multiplication either with directly determined tissue weight (liver, kidney, intestine, urinary bladder, heart, spleen, lung, adrenal, pancreas, and brain) or with the tissue weight as estimated from mean values given in (10) (skeletal muscle, fat); these values also are included in Table 4. An over-all survey of the results obtained is presented in Table 5 which indicates that between 34 and 43 per cent of the BSP-S^{35} infused was retained in the carcasses of these animals; between 48 and 75 per cent of BSP retained was stored in the liver; the concentration of BSP in the liver was between 30 and 57 times as high as the concentration in the rest of the carcass.

The amount of BSP not retained by the liver appeared to be loosely related to plasma BSP levels: The ratio of rest carcass BSP-S^{35} concentrations to plasma BSP-S^{35} concentrations ranged from 0.39 to 0.59.

TABLE 4
THE DISTRIBUTION OF S^{35} CALCULATED AS BSP THROUGH THE BODY OF DOGS AFTER CONTINUOUS INFUSIONS OF S^{35} BSP

DOG #	51-4		51-9		51-7		51-3		51-22		51-21	
WT., SEX	10.2 KGM		9.0 KGM		7.0 KGM		10.2 KGM		13.2 KGM		8.2 KGM	
NORMAL OR CCl_4	NORMAL		NORMAL		NORMAL		NORMAL		CCl_4		CCl_4	
RATE OF INFUSION – MGM BSP/KGM/HR	0.196		0.196		0.236		0.261		0.173		0.205	
TOTAL INFUSED-MGM	625		616		396		410		530		548	
S^{35} CALC. AS BSP IN:	MGM/KGM	% TOTAL INFUSED	MGM/KGM	% TOTAL INFUSED	MGM/KGM	% TOTAL INFUSED	MGM/KGM	% TOTAL INFUSED	MGM/KGM	% TOTAL INFUSED	MGM/KGM	% TOTAL INFUSED
BLOOD PLASMA	37.6	1.7	14.9	0.6	22.4	1.2	21.1	1 5	102	7.1	47.3	1.9
LIVER	390.	21.8	360.	25.3	525.	20.0	305.	23.5	625	46.0	579.	33.0
BILE	16,680.	58.0	15,600.	66.1	10,500.	57.5	10,590.	56.8	7,460	2.9	16,500.	28.4
STOMACH	165.	3.2	12.0	0.1	30.0	0.5	84.0	2.7	–	–	–	–
SMALL INTESTINE	49.0	2.6	16.0	1.1	31.1	2.4	36.0	2.9	–	–	–	–
LARGE INTESTINE	20.1	0.2	15.0	0.2	18.9	0.5	16.0	0.2	–	–	–	–
CONTENTS SM. INTEST.	–	–	7.0	<0.1	5.0	<0 1	–	–	–	–	–	–
CONTENTS L. INTEST.	6.0	<0.1	9.0	<0.1	10.0	0 1	4.0	<0.1	–	–	–	–
KIDNEY	36.5	0.3	90.0	1.2	72.3	1.0	37.0	0.5	400.	5.3	118.	1.0
URINARY BLADDER	–	–	22.0	0.0	16.0	<0.1	–	–	–	–	–	–
URINE	NIL	0.0	320.	0.8	NIL	0.0	44.4	0.2	20.7	0.2	267.	1.7
SKEL. MUSCLE – RECT. ABD. OR QUADRUCEPS*	12.2	8.6	5.3	3.0	13.2	10.0	3.3	3.6	–	–	–	–
INTERCOSTAL	32.0	–	22.0	–	–	–	41.0	–	–	–	–	–
HEART	12.0	0.2	14.0	0.2	13.9	0.2	26.0	0.6	–	–	–	–
SPLEEN	11.1	0.1	–	–	16.0	0.1	5.7	0.1	–	–	–	–
LUNG	4.0	0.1	–	–	2.7	0.1	12.0	0.3	–	–	–	–
ADRENAL	6.0	0.0	–	–	–	–	11 0	0 0	–	–	–	–
PANCREAS	–	–	14.0	0.1	53.0	0.2	67.0	0.2	–	–	–	–
BRAIN	–	–	1.5	0.0	1.3	0 0	–	–	–	–	–	–
FAT**	(8)	(2.5)	8.5	2 3	8.2	2 7	8.4	3.9	–	–	–	–
TOTAL IN TISSUES SAMPLED	–	99.4	–	100.1	–	96.6	–	97 1	–	–	–	–

*MEAN OF QUADRUCEPS, RECTUS ABDOMINIS, OCCIPITAL AND EXTERNAL OBLIQUE MUSCLES. ESTIMATED AS 40 3% OF BODY WEIGHT
**ESTIMATED AS 19% OF BODY WEIGHT.

The detailed distribution of extrahepatic BSP can be seen from Table 4. Tissues showing relatively high concentrations of BSP compared to the plasma levels were: stomach and small intestine, pancreas, intercostal muscle, and especially kidney. The appearance of BSP in urine is sporadic; to date no way has been found of predicting the occurrence of urinary BSP. There does not appear to be any correlation with plasma levels nor with BSP-S^{35} concentrations in the kidney. The concentrations of BSP in the urine of those animals which did show urinary BSP were considerably higher than in blood plasma, thus indicating that under certain circumstances

TABLE 5
SUMMARY OF DATA REGARDING DISTRIBUTION OF INFUSED BSP S^{35} IN DOGS

DOG #	51-4	51-9	51-7	51-3	51-22	51-21
% OF INFUSED S^{35} RETAINED IN BODY	NORMALS 42.0	33.9	42.5	43.2	CCl_4 TREATED 97.1	71.6
% OF INFUSED S^{35} IN LIVER AND BILE	80.5	92.1	77.5	80.0	48.1	61.0
% OF S^{35} (RETAINED IN BODY) FOUND IN LIVER	53.2	74.7	47.5	54.1	46.5	46.4
S^{35} CONCENTRATION (AS BSP) IN BLOOD PLASMA – MGM/KGM	37.6	14.9	22.4	21.1	102.	47.3
DTO. IN CARCASS EXCLUDING LIVER (CALC.)	12.8	6.3	13.3	8.4	25.6	21.9
RATIO OF S^{35} CONCENTRATION $\frac{\text{LIVER}}{\text{PLASMA}}$	10.4	24.2	23.5	14.5	6.2	12.2
DTO. $\frac{\text{REST CARCASS}}{\text{PLASMA}}$	0.34	0.42	0.59	0.39	0.25	0.46
DTO. $\frac{\text{LIVER}}{\text{REST CARCASS}}$	30.7	57.0	39.7	37.4	24.8	26.5

the kidney is capable of concentrating BSP. Extremely low concentrations of S^{35} were found in the brain in the two cases examined, and low concentrations were found in intestinal contents.

The largest reservoir of extrahepatic BSP appears to be the skeletal musculature. This is due primarily to the large mass represented by this tissue. Concentrations of S^{35} in various large muscles were found relatively constant and low. By contrast the respiratory muscles, in particular the intercostal muscles, tended to show high concentrations of BSP-S^{35} relative to blood plasma. Cardiac muscle took an intermediate position following more closely values found in the blood plasma. For calculation of the total amount retained in skeletal muscle the mean of concentrations in various large muscles was employed; respiratory muscles—which alone showed higher concentrations—account for no more than 5 per cent of the total muscle mass; their contribution to the extrahepatic BSP storage should be less than 1 per cent of the total infused. Finally, attention may be called to the surprisingly large contribution to the total BSP storage made by body fat; *in vitro,* BSP has been found insoluble in fat solvents at all physiological pH values. The total contribution due to fat is probably over-estimated since it is based on the figures of reference (10) which were obtained by total extraction, and thus, in all probability, include lipids extracted from tissue other than the fat depots.

D. Continued accumulation of BSP-S^{35} in depots during steady state phase

Comparison of the figures for BSP-S^{35} concentrations in respiratory muscles, on the one hand, and in the large muscles of the various dogs, on the other hand, suggests that the latter at any rate have not exhausted their capacity to take up BSP. To test this point, an experiment was conducted in which duplicate biopsy specimens of corresponding portions of the right and left rectus abdominis were taken, the first shortly after the establishment of steady blood levels, and the second two hours later. S^{35} contents of these tissue calculated as BSP were 2.75 and 2.57 mg. per Kg. after 67 minutes of infusion, and 5.07 and 6.02 mg. per Kg. after 189 minutes of infusion (Experiment 51–9). It appears, therefore, that

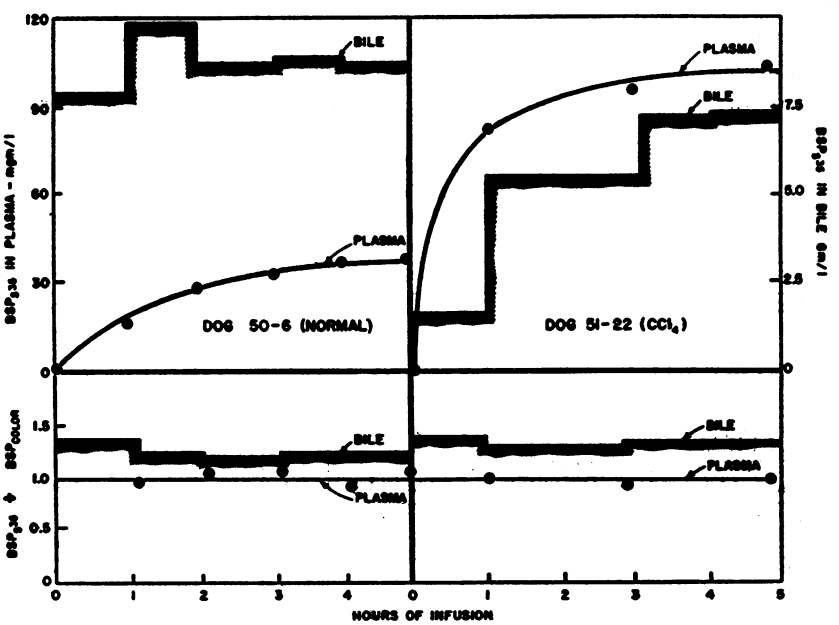

FIG. 2. CONTINUOUS INFUSION OF BSP INTO FEMORAL VEIN OF DOGS UNDER SODIUM PENTOBARBITAL ANESTHESIA

Dog 50-6, ♂ 8.2 Kg., Infusion rate 0.183 mg. per Kg. per min. Dog 51-22, ♂ 13.2 Kg., Infusion rate 0.173 mg. per Kg per min., pretreated with CCl_4.

BSP uptake, by the large skeletal muscles at least, continues during the steady state.

E. BSP-S^{35} distribution in CCl_4 treated dogs

As indicated in Figure 2, a steady state of blood and bile BSP concentrations was obtained more slowly in these animals than in the normals. BSP activity to color ratios in plasma and bile of these dogs did not differ significantly from those of normal dogs although the data of Table 3 suggest that a larger series might show that activity to color ratios in the bile of the CCl_4 treated dogs may be slightly lower than in normal dogs. Liver injury was reflected most clearly in low excretion rates and high plasma BSP concentrations considering the infusion rates employed (Table 3).

As a result of the high proportion of BSP-S^{35} retained in these dogs, the rest carcass concentrations of S^{35} were several times as high in those as in normal dogs; the ratio of rest carcass S^{35} to plasma S^{35} concentrations was found normal in the case of one and slightly subnormal in the case of the second animal. Renal S^{35} concentrations were high but no more so than would be predicted as a result of the high plasma or rest carcass concentrations. Hepatic BSP-S^{35} concentrations, relative to the carcass were normal, or at worst slightly below normal. As a result of the low biliary excretion, however, there was a significant reduction below normal in the proportion of infused dye which could be recovered from liver and bile.

DISCUSSION

Transfer of acid dyes from blood to bile was conceived of by early investigators as a relatively simple process, involving secretory activity of the hepatic (or possibly the Kupffer) cells to effect the manifold concentration of these dyes in the bile. More recently this picture has been complicated somewhat by data indicating a temporal separation between disappearance of the dye from the blood stream and its reappearance in the bile (5). Finally, dye balance studies during continuous BSP infusion (4) rendered the old picture altogether untenable: Only about 60 per cent of BSP infused can be recovered from liver and bile, and even during the so-called steady state a sizeable gap persists between infusion and excretion rates.

These studies, based on colorimetric assay, are supplemented by the present data involving BSP labeled with S^{35} in the sulfonate groups. Bile of anesthetized, acute bile fistula dogs treated with this material invariably contains more S^{35} than can be accounted for by colorimetric assay. These observations establish the fact that BSP undergoes some measure of metabolic transformation before being excreted.

That the fate of BSP does not include desulfonation (and hence, loss of its S^{35} label) emerges from several observations: In the present report it was shown that the excess S^{35} of bile does not occur as inorganic or as ethereal sulfate. In the blood stream, even after prolonged infusion no unaccounted S^{35} has been observed. These indications are strongly supported by more extensive investigations of the metabolism of BSP (to be reported elsewhere); all of the S^{35} activity administered to dogs as S^{35} BSP was recovered in the form of sulfonated phthaleins identical with or derived from BSP. These observations justify the use of S^{35} determinations as the basis of a study of the distribution of infused S^{35} BSP throughout the body.

The gap between the amount of BSP infused, and the amount recovered from liver and bile is narrowed considerably if the recovery of S^{35}, rather than that of colorimetric BSP is made the basis of consideration: Almost 80 per cent of the total dye removed from the circulation in normal dogs can now be accounted for in terms of hepatic extraction. This figure agrees well with an earlier estimate reached by different means (4) and with recent direct determinations of hepatic blood flow in comparison with estimated hepatic blood flow calculated from BSP data (11). The remainder of the infused dye is distributed throughout the tissues, as indicated by Tables 4 and 5.

Several aspects of the observed distribution of extrahepatic BSP are of theoretical interest: The high concentrations of BSP in tissues like kidney and upper gastrointestinal tract; the efficacy of the blood-brain barrier; the surprisingly high concentration of this virtually lipid insoluble dye in the fatty tissue; and the inferred low concentrations in the skeleton and skin. Particular interest attaches to the uptake of BSP by the skeletal muscles. From theoretical considerations (8) it would appear that the equilibrium concentration

in this tissue should approximate closely that of the blood, or even exceed it. This, indeed, is the situation encountered with respiratory muscle and with cardiac muscle. The large skeletal muscles, on the other hand, show concentrations of BSP considerably below those coexisting in the blood plasma; thus, this tissue apparently has not reached equilibrium with the blood even after several hours of BSP infusion. By direct experiment it could be shown that this conclusion is correct, and that throughout the period of infusion the concentration of BSP in at least one of the great muscles increases continuously, presumably towards an equilibrium value not too different from that in the respiratory muscles. Because of its great relative mass, this tissue accounts for a large proportion of the entire extrahepatic BSP store; the slowness with which the muscle mass is saturated with BSP leads one to predict that elimination of the uptake of BSP by this tissue cannot be achieved by an initial booster dose. BSP uptake by the large muscles thus remains at a significant level throughout the course of an infusion experiment, and constitutes the major extrahepatic sink for BSP.

Carbon tetrachloride poisoning does not greatly alter the distribution of retained BSP between liver and rest carcass. This would have been predicted from experiments with BSP uptake by liver slices *in vitro* (8), as well as from histological observations *in vivo* based on the related dye rose bengal (12). Apparently, the ability of the liver to store and to concentrate BSP and related dyes is unaffected by CCl_4 injury. The excretion of such dyes, on the other hand, is greatly decreased under these conditions. Since the total BSP extracted by the liver is the sum of BSP (or BSP derivatives) excreted into bile, and BSP stored in the liver, CCl_4 injury results in a markedly reduced hepatic BSP extraction under continuous infusion conditions, and in a greater proportion of extrahepatically retained dye. Only if the infusion rate could be slowed until excretion can keep pace with infusion (if, indeed, such a condition is possible in the presence of parenchymal injury) may a BSP distribution comparable to the normal be expected.

Finally, one may briefly consider the effect of the anesthesia and biliary tract surgery involved in the present experiments upon the results obtained. Both conditions have been claimed to affect BSP clearance after single injections; however, it was found in constant infusion experiments (4) that laparotomy (including preparation of a bile fistula as employed in the present series) did not result in blood BSP levels differing from those of dogs merely anesthetized, but receiving BSP at the same infusion rate.

In the isolated rat liver it has been shown (13) that Nembutal® does not directly interfere with either bile formation or BSP clearance. An indirect effect, mediated in the intact animal *via* changes in hepatic blood flow, is possible. Such an effect, however, would not alter the validity of the essential conclusions derived from the present experiments. Even if such an effect obtained, the result would be to render the conclusions derived from the study of "normal" dogs in the present series unduly conservative—the hepatic contribution to BSP extraction might actually be larger in the unanesthetized animal. In the presence of a functionally impaired liver, on the other hand, regardless of whether this is due to CCl_4 treatment alone, or compounded by anesthesia and surgery, the present data show that extrahepatic BSP uptake may become important.

CONCLUSIONS

The application of S^{35}-labeled sulfo-bromophthalein-sodium in the present report has provided definite evidence for the occurrence of chemical changes in the molecule of this dye, associated with the process of excretion into bile. Whether these reactions are an integral part of the mechanism of dye excretion by the liver or whether, like dye storage in the hepatic cells, they are only an incidental accompaniment of such excretion, remains to be established by future investigations. With regard to the distribution of infused BSP, the present results in anesthetized, acute bile fistula dogs confirm previous impressions regarding the otherwise normal dog. Hepatic BSP uptake accounts for a very large proportion of administered dye and errors in calculations based on the assumption that all of the BSP leaves the circulation on passage through the liver are relatively small. At the same time, findings of continued uptake of BSP by skeletal muscle point to this tissue as the major site of sustained extrahepatic dye extraction.

In dogs with toxic hepatitis the present findings allow a clearer formulation of ideas previously expressed concerning errors of quantitative interpretation caused by neglect of extrahepatic BSP uptake. It has been shown that the reduction in the hepatic BSP extraction is accounted for almost entirely in terms of decreased BSP excretion; if infusion conditions allowing BSP excretion to keep pace with BSP infusion could be attained in the face of parenchymal injury these should yield results comparable in reliability to those in the normal dog.

Finally, the present results clarify the nature of the so-called lost BSP observed previously in experiments confined to colorimetric assay alone.

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