HEPATIC STORAGE AND EXCRETION OF SULFOBROMOPHTHALEIN SODIUM IN THE DOG *

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The use of a halogenated phthalein dye for the investigation of hepatic function was first proposed by Rowntree, Hurwitz and Bloomfield (1) in 1913. Their technique required the measurement of the fecal excretion of phenoltetrachlorphthalein following its parenteral administration. However, it was subsequently shown by Rosenthal and White (2) that intravenously administered phenoltetrabromophthalein disulfonate (BSP) is removed from the blood almost exclusively by the liver and that the function of the liver may therefore be evaluated by measuring the rate of disappearance of this dye from the plasma.

Various lines of evidence support the view that the removal of BSP (and presumably many other substances) from the plasma involves two processes: *1*) storage of the material in the liver and *2*) biliary excretion. Following the intravenous administration of BSP to dogs it was first shown by Wirts and Cantarow (3) that the output of BSP in the bile continues for over three hours after its virtual disappearance from the plasma, indicating that this material is first taken up by the liver and then gradually excreted. This observation was confirmed by Brauer and Pessotti (4), also in dogs. The existence of a similar hepatic BSP storage mechanism in man is evident from the studies of Mendeloff, Kramer, Ingelfinger and Bradley (5) in which a retardation of the BSP disappearance rate from plasma was noted when successive identical intravenous doses were administered 30 minutes apart. These authors postulated that "saturation" of the hepatic storage compartment occurs after a single injection of dye so that the rate of uptake of subsequent doses is limited by the biliary excretion mechanism. Fluoresence microscopy of living frog and rat livers by the technique of Grafflin and Bagley (6) has provided direct visualization of the storage phenomenon. Following intravenous injection both fluorescein and thioflavine-S accumulate in high concentration in hepatic parenchymal cells as well as in the bile canaliculi. Finally, the same process has been demonstrated after injection of I^{131}-labeled rose bengal by Taplin, Meredith and Kade (7) using an external counter over the liver. There is a rapid accumulation of isotope in the liver followed by a gradual disappearance as the dye is excreted. The latter phase can be blocked by acute common bile duct ligation in rabbits but the "storage" uptake is still apparent.

The rate of uptake of BSP from the plasma into hepatic storage may be very rapid immediately after an intravenous injection. In man, for example, the BSP concentration in plasma falls at the rate of 10 to 15 per cent per minute following a single injection (8) so that the initial rate of removal of a 300 mg dose must be of the order of 40 mg per minute. However, the liver is incapable of removing BSP this rapidly for an extended period of time. Constant BSP infusions at rates much over 3 mg per m^2 per minute in man (9) or 1.4 mg per 10 kg per minute in the dog (10) result in a continuously rising plasma concentration. Mason, Hawley and Smith (11) reported a higher figure of 4.4 mg per 10 kg per minute for dogs but arrived at the same general conclusion. These observations have led to the hypothesis that the biliary excretion of BSP is a rate-limited process and that the rate of uptake of BSP from plasma is ultimately limited by the maximal rate of excretion.

This mechanism was explored in more detail

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by Combes, Wheeler, Childs and Bradley (10) using I^{131}-labeled rose bengal to measure hepatic blood flow so that actual rates of hepatic BSP removal could be calculated from the splanchnic arteriovenous concentration difference. It was found that the removal rate of BSP reaches a limiting "maximum" during a BSP infusion which is sufficiently rapid to produce a continuously rising plasma concentration. This observation was interpreted as further evidence for the existence of a limited excretory transport mechanism, and the maximal rate of transport was designated "Tm." However, when a large "priming" dose of BSP was administered at the time that constant infusion was started, hepatic removal rates much higher than the apparent excretory "Tm" were observed transiently, suggesting rapid initial uptake of BSP into a "storage" compartment. It was postulated that the liver is capable of very rapid uptake of BSP from the plasma when the dye is entering the storage compartment but that the uptake approaches the excretory "Tm" once the storage compartment is "saturated."

The high plasma concentrations of BSP required for more detailed exploration of the storage mechanism interfered markedly with the removal of rose bengal making it impossible to obtain accurate measurements of hepatic blood flow. In the studies reported here a mechanical device (rotameter) was therefore employed for the measurement of flow in order to determine hepatic BSP uptake accurately over a wide range of plasma levels in the dog. The results have proved consistent with the view that biliary excretion of BSP involves a rate-limited process but that accumulation (or storage) of BSP in hepatic parenchymal cells is determined by plasma concentration.

METHODS

In 14 mongrel dogs (14 to 26 kg) anesthetized with pentobarbital (30 mg per kg) hepatic venous outflow was measured using the rotameter and optical recording system described by Shipley and Wilson (12). In the first 6 dogs hepatic venous blood was collected from the inferior vena cava below the liver and returned through a chest wound to the thoracic inferior vena cava (Figure 1). Respiration was maintained by means of a Starling pump. A femoral-to-jugular shunt was provided for return of venous blood from structures below the hepatic cannula. In the remaining 8 dogs the arrangement shown in Figure 2 was employed. Hepatic venous blood was led to the rotameter through a no. 20 Fr. woven nylon catheter threaded through a femoral vein and tied in place by a ligature around the vena cava above the renal veins. After passing through the rotameter the blood was delivered to a reservoir beneath the animal board from which it was returned by finger pump to an external jugular vein. Venous return from structures below the liver was collected from the opposite femoral vein and returned to the heart by way of the same reservoir system. A circulating warm water jacket around the

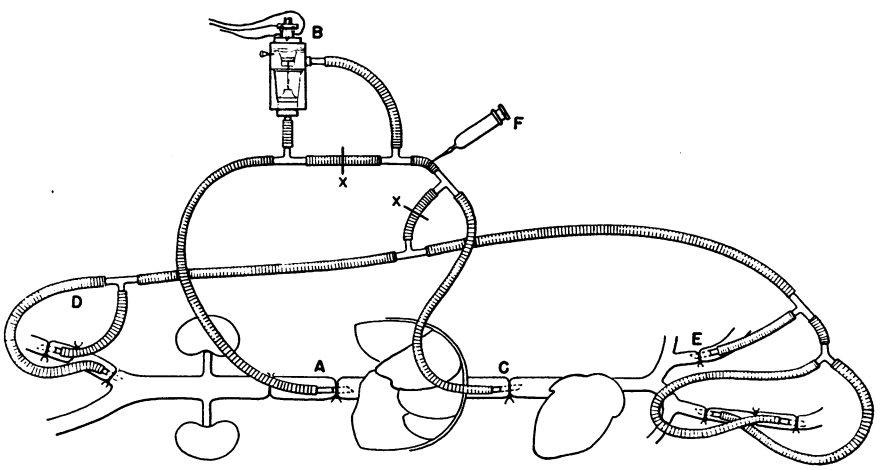

FIG. 1. SYSTEM FOR HEPATIC BLOOD FLOW MEASUREMENT USED IN FIRST SIX STUDIES. Hepatic venous blood was collected through cannula (A), delivered to rotameter (B) and returned to heart by way of cannula in thoracic inferior vena cava (C). Blood from structures below (A) was collected through femoral vein cannulae (D) and returned to heart via jugular cannulae (E). Mixed hepatic venous blood samples were obtained at F.

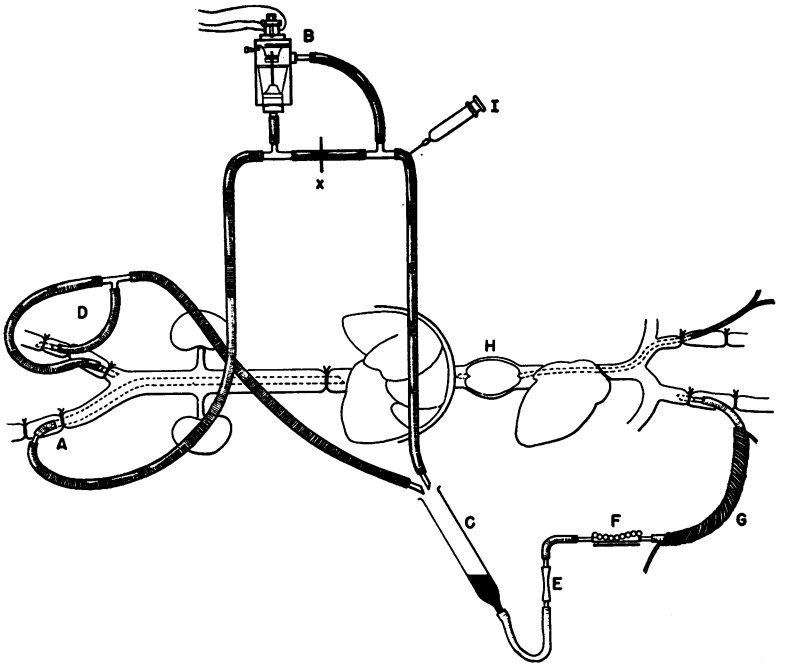

FIG. 2. ROTAMETER SYSTEM USED IN LAST EIGHT STUDIES. Hepatic venous blood was collected through catheter (A), passed through rotameter (B) and delivered to reservoir (C). Blood from structures below the liver was collected from the opposite femoral vein (D) and delivered to the same reservoir. A short section of Penrose drain (E) acted as a valve to prevent emptying of reservoir as blood was pumped (F) through return tubing (G) to the jugular vein. Warm water flowing through plastic tube jacketing (G) maintained body temperature. Balloon (H) directed hepatic venous blood through the rotameter. Mixed hepatic venous blood samples were obtained at I.

return tubing was regulated to maintain constant body temperature (measured by an esophageal thermistor). A cardiac catheter equipped with a balloon was inserted through the other external jugular vein and maneuvered into the thoracic inferior vena cava. Its position was determined by palpation through the intact diaphragm. Inflation of this balloon directed the entire hepatic venous outflow through the rotameter. Hepatic venous congestion did not occur, because the resistance of the tubing and rotameter to hepatic venous outflow was balanced by the siphoning effect of the blood delivered to the dependent reservoir.

In all studies heparin was used as the anticoagulant (10 mg per kg initially and 5 mg per kg every hour, intravenously). The external circuits were filled beforehand with 6 per cent dextran in saline to prevent sudden initial reduction in blood volume. The rotameter was calibrated frequently with dog blood at body temperature and in each of the last 8 studies this calibration was carried out immediately after completing the experiment. Sulfobromophthalein sodium (BSP) solutions were administered intravenously using a Bowman pump.

BSP concentration in arterial and hepatic venous plasma was measured in a Beckman DU spectrophotometer at 580 mμ after dilution of 1 ml of plasma with 10 ml of normal saline and addition of 0.2 ml. of 20 per cent potassium hydroxide. The optical density of a similarly treated alkalinized blank plasma (obtained before BSP administration) was subtracted. Standard concentrations for this determination were prepared in dog plasma. Frequent hematocrit determinations were carried out in Wintrobe tubes centrifuged 30 minutes at 2,000 rpm. These were not corrected for trapped plasma. Total plasma volume was measured after the external circuits were functioning, using I^{131}-labeled human serum albumin. A blood sample obtained 10 minutes after injection was compared with an aliquot of the injection solution diluted to standard volume with carrier plasma to avoid adsorption by glass. In the last 8 dogs this measurement was repeated near the end of the study and intervening values of plasma volume were estimated by interpolation. Splanchnic plasma volume was assumed to be 20 per cent of total plasma volume (13). The precision of this assumption has not been tested under the conditions of these experiments. However, as shown in the equation below, the value for splanchnic plasma volume is in-

volved in correcting for a comparatively small amount of BSP which is distributed in the splanchnic plasma so that only a reasonable approximation is required.

The hepatic BSP removal rate was calculated from the splanchnic arteriovenous concentration difference on the assumption that removal of BSP by splanchnic tissues other than the liver was practically negligible (14) except for changes in the BSP content of the splanchnic plasma itself. The following formula was employed:

$$R_H = F(A - V) - \left(\frac{\Delta P}{\Delta t}\right) \times SPV$$

where:

R_H = hepatic BSP removal rate, mg per minute

F = hepatic plasma flow, ml per minute = rotameter flow × (1 − hematocrit)

A = arterial plasma BSP concentration, mg per ml

V = mixed hepatic venous BSP concentration, mg per ml

$\frac{\Delta P}{\Delta t}$ = rate of change of arterial plasma concentration, mg per ml per minute

SPV = splanchnic plasma volume, ml = 0.2 × total plasma volume.

Measurements of flow and collection of blood specimens were carried out synchronously at intervals of 3 to 10 minutes and the values of F, A and V, obtained at the beginning and end of each interval, were averaged and substituted into the above equation to calculate R_H for the interval. The difference between the arterial concentrations at the beginning and end of each period was divided by the time interval to obtain $\frac{\Delta P}{\Delta t}$. During sudden changes in concentration following large injections of BSP (see Figures 4 and 5) continuous blood samples were drawn at a constant rate for 2 successive periods of 2 minutes' duration in order to obtain representative average values for A and V.

RESULTS

The first six studies demonstrate the effect of sudden alterations in arterial BSP concentration on the hepatic BSP removal rate. In the study illustrated in Figure 3 (Dog Hu) a constant BSP infusion of 3.8 mg per minute was administered. As anticipated from earlier work (10) the hepatic

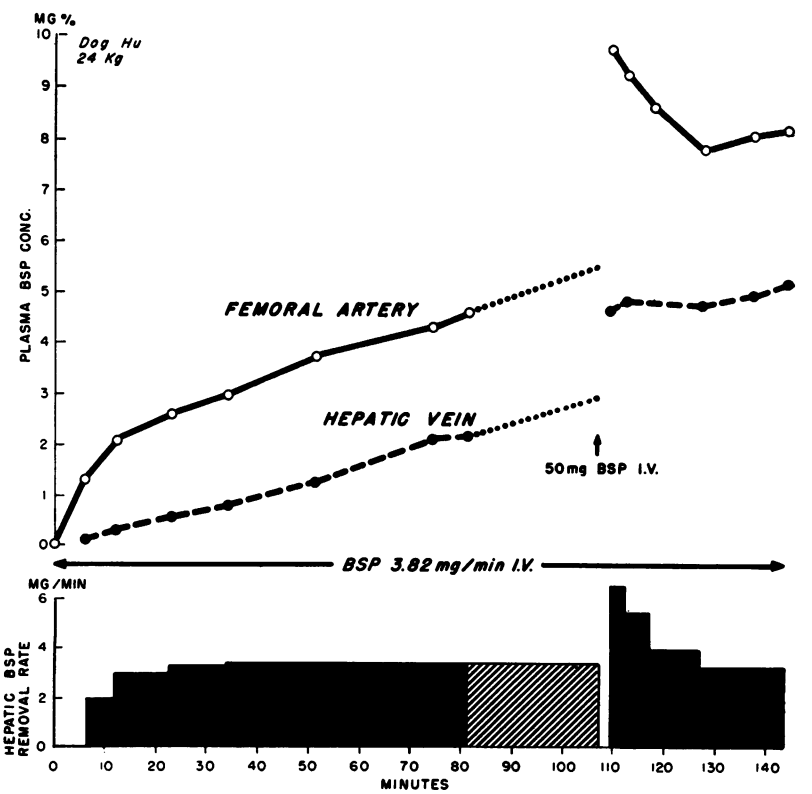

FIG. 3. EFFECT OF A SUDDEN INTRAVENOUS INJECTION OF BSP ON HEPATIC REMOVAL RATE. A BSP infusion of 3.82 mg per minute was started at zero time and continued throughout the study. Intravenous injection of 50 mg of BSP resulted in transient increase in arterial concentration and hepatic removal rate.

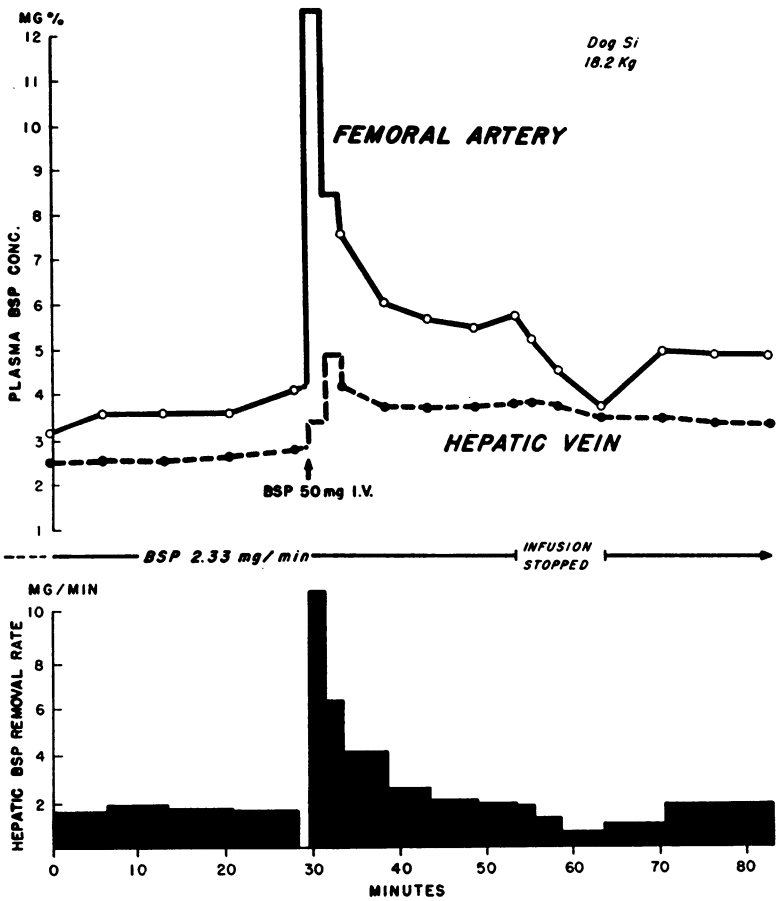

FIG. 4. EFFECT OF SUDDEN INCREMENT AND SUDDEN DECREMENT IN ARTERIAL BSP CONCENTRATION ON HEPATIC BSP REMOVAL RATE. A BSP infusion of 3.89 mg per minute was started 80 minutes before time zero and reduced to 2.33 mg per minute 10 minutes before time zero. During steady states hepatic removal rate was constant and independent of plasma concentration, but sudden increment and decrement in concentration produced, respectively, transient increase and decrease in hepatic removal rate representing net uptake and release of "stored" BSP.

BSP removal rate achieved a constant value (3.2 mg per minute) at about the time the arterial concentration reached 2.5 mg per 100 ml, while the latter continued to rise. After 107 minutes of steady BSP infusion, a sudden increment in arterial concentration was produced by the intravenous injection of 50 mg of BSP. There was a marked increase in hepatic removal rate to as high as 7 mg per minute followed by a gradual return during the next 20 minutes to the original rate of 3.2 mg. per minute. This phenomenon is repeated in the next illustration and the opposite effect is shown to occur after a sudden decrement in plasma BSP concentration (Figure 4). In this study a BSP infusion (4 mg per minute) was begun 80 minutes beforehand and slowed to 2.3 mg per minute 10 minutes before sampling. During the succeeding 30 minutes a relatively constant arterial level of 4 mg per 100 ml was thus obtained, and the hepatic BSP removal rate was constant at 2 mg per minute. After this control period an injection of 50 mg of BSP produced, as in the first study, a marked transient increase in hepatic removal rate followed by a gradual return to 2 mg per minute as the arterial concentration established a new level of 6 mg per 100 ml. The BSP infusion was then discontinued for a 10 minute interval. During the resulting decrement in arterial

concentration the hepatic removal rate decreased to as low as 0.7 mg per minute but returned again to 2 mg per minute after resumption of the infusion. This reduction in BSP removal rate occurred even though the absolute arterial BSP concentration was always higher than during the control period.

The other four studies in this series (Figure 5) confirm the observations described above. Sudden increments in plasma level produced either by instantaneous intravenous doses of BSP (Figure 5A, B, C) or temporary acceleration of infusion rate (Figure 5D) invariably resulted in significant transient increase in hepatic removal rate. Moreover, in Dog Sa (Figure 5D) the responses elicited by each of two successive concentration increments were identical. On the other hand, concentration decrements (Figure 5A, B, C) resulted in transient decreases in BSP removal rate in each case.

The subsequent eight studies were designed to measure the effect of more gradual but continuous changes in plasma concentration and were conducted as illustrated in Figure 6 (Dog Ot). A "priming" dose of BSP, approximately 12 mg per kg was administered, and this was followed by an infusion of approximately 0.15 mg per kg per minute. After a 30 to 40 minute equilibration period this rate of infusion usually produced a relatively constant or slowly changing arterial plasma level above 2.5 mg per 100 ml as in the illustration. After an initial phase of rapid "storage" uptake the BSP removal rate also became constant. Arterial and hepatic venous sampling was continued for an additional 30 minutes. The infusion rate was then doubled and measurements were continued at 10 minute intervals for another 50 minutes. At the high infusion rate the arterial concentration was observed to rise rapidly at first, but within approximately 20 minutes the rate of increase became less rapid and quite constant. Coincident with this the hepatic removal rate increased to a new constant value, considerably greater than the rate observed during the slower infusion. In the study illustrated the rate of change of arterial concentration with respect to time during the last 30 minutes of the initial BSP infusion was − 0.0014 mg per 100 ml per minute and achieved a new constant value of + 0.0346 mg per 100 ml per minute during the more rapid infusion. The corresponding hepatic BSP removal rates were 2.62 and 4.76 mg per minute, respectively. In the same manner values of arterial concentration change and corresponding hepatic removal rates were computed during each of two BSP infusion rates in the other seven studies (Columns 4 and 5 of Table I).

Extrahepatic removal rate of BSP was calculated in the last eight studies by means of the

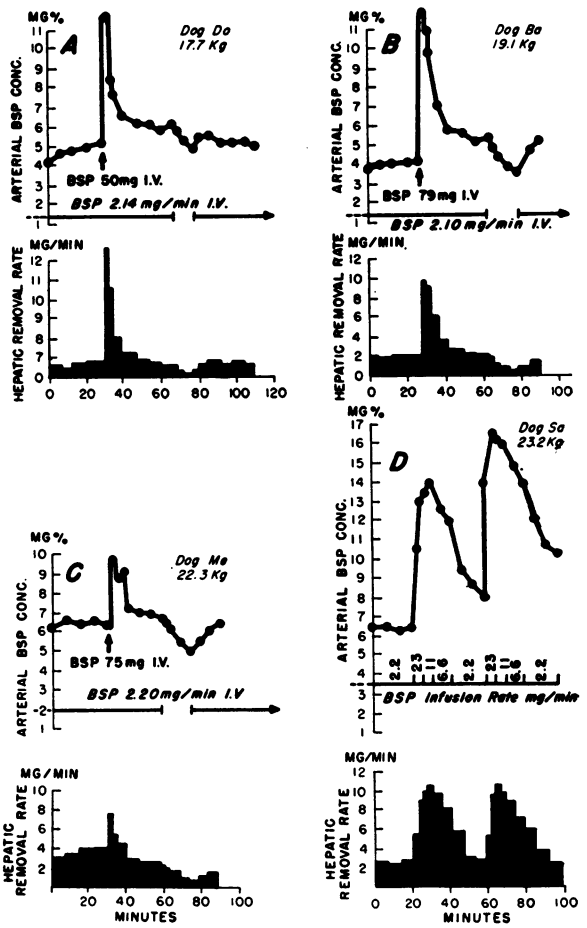

FIG. 5. EFFECT OF SUDDEN CHANGES IN ARTERIAL PLASMA BSP CONCENTRATION ON HEPATIC REMOVAL RATE IN FOUR DOGS. A BSP infusion of approximately 6 mg per minute was started about 70 to 100 minutes before time zero, and this was reduced to the indicated infusion rate 15 minutes before time zero. In each case increments in plasma concentration resulted in transient increase in hepatic removal rate, and decrements caused a transient decrease. In D an identical increase in removal rate accompanied each of two successive increments in plasma level indicating additional "storage" uptake with each increment.

TABLE I

BSP transport maximum and storage capacity

1	2	3	4	5	6	7	8	9	10	11	12	13
Dog	Weight	BSP infusion rate, I	Arterial concentration change, $\Delta P/\Delta t$	Hepatic removal rate, R_H	Excretory transport maximum, Tm	Tm	Relative storage capacity, S	S	Tm*	S*	10/6*	11/8*
	kg	*mg/min*	*mg/100 ml/min*	*mg/min*	*mg/min*	*mg/min/10 kg*	*mg/mg/100 ml*	*mg/mg/100 ml/10 kg*	*mg/min*	*mg/mg/100 ml*		
Ai	22.7	2.57 / 4.95	−0.0107 / +0.0192	2.43 / 4.56	3.19	1.41	71.2	31.4	3.44	66.9	1.08	0.94
Bi	14.3	3.12 / 6.22	+0.0189 / +0.1230	2.94 / 5.04	2.56	1.79	20.2	14.1	2.60	13.7	1.02	0.68
To	18.6	2.66 / 5.31	−0.0218 / +0.0261	2.55 / 4.40	3.38	1.82	37.8	20.3	3.87	43.4	1.15	1.15
Tr	14.5	2.53 / 5.08	−0.0180 / +0.0680	2.48 / 3.82	2.76	1.90	15.6	10.8	3.06	18.6	1.11	1.19
Ot	22.0	2.70 / 5.38	−0.0014 / +0.0346	2.62 / 4.76	2.70	1.23	59.4	27.0	2.79	61.1	1.03	1.03
Du	16.8	2.63 / 5.26	−0.0016 / +0.0488	2.34 / 4.00	2.39	1.42	32.9	19.6	2.71	37.5	1.13	1.14
Ia	15.9	2.73 / 5.28	0 / +0.0600	2.44 / 4.39	2.44	1.53	32.5	20.4	2.73	33.2	1.12	1.02
He	15.5	3.33 / 6.62	+0.0087 / +0.0910	2.96 / 4.95	2.75	1.77	24.2	15.6	2.99	28.2	1.09	1.16
				Mean		1.61		19.9			1.09	1.04
				Standard deviation		0.24		6.7				

* Tm and S in Columns 10 and 11 were calculated from indirect estimates of hepatic removal rate by Equation 2 (see text). These are compared in Columns 12 and 13 with the values of Tm and S calculated by Equation 1 from actual measurements of splanchnic removal rate.

following formula:

Extrahepatic removal rate

$$= I - \left[F(A - V) + \left(\frac{\Delta P}{\Delta t}\right) \times (ESPV)\right]$$

where:

I = infusion rate, mg per minute

F = hepatic plasma flow, ml per minute

A − V = splanchnic arteriovenous difference, mg per ml

$\frac{\Delta P}{\Delta t}$ = rate of change of arterial concentration, mg per ml per minute

ESPV = extrasplanchnic plasma volume, ml (assumed to be 0.8 × total plasma volume).

The magnitude of the extrahepatic removal rate, both in absolute terms and relative to total infusion rate, is shown in Table II. The average extrahepatic removal rate under the conditions of these studies was 9.2 per cent of the rate of infusion over a range of plasma BSP concentrations of approximatetly 3 to 16 mg per 100 ml.

TABLE II

Extrahepatic removal during estimation of BSP transport maximum and storage capacity

1	2	3	4	5
Dog	Infusion rate	Arterial concentration range	Extrahepatic removal rate	Per cent extrahepatic removal
	mg/min	*mg/100 ml*	*mg/min*	*4/2×100*
Ai	2.57	3.1– 3.8	0.14	5.4
	4.95	3.1– 5.3	0.18	3.6
Bi	3.12	5.8– 6.3	0.08	2.6
	6.22	6.3–12.2	0.59	9.5
	6.22	12.2–16.4	1.16	18.6
To	2.66	5.5– 5.8	0.36	13.5
	5.31	5.5– 8.1	0.48	9.0
Tr	2.53	7.4– 7.8	0.25	9.9
	5.08	7.4–12.0	0.50	9.8
Ot	2.70	2.7– 2.8	0.10	3.7
	5.38	2.8– 5.1	0.21	3.9
Du	2.63	5.8– 5.9	0.31	11.8
	5.26	5.8–10.2	0.83	15.8
Ia	2.73	5.4	0.29	10.6
	5.28	5.4– 9.2	0.39	7.4
He	3.33	6.4– 6.7	0.29	8.7
	6.62	6.7–13.3	0.80	12.1
			Average	9.2

DISCUSSION

A. Hepatic excretory and storage mechanisms. The movement of BSP from the plasma into the liver, as indicated in the introduction, depends upon both the excretory and the storage functions of this organ. Thus, in arithmetic terms, the hepatic removal rate of BSP at any instant must be equal to the sum of the hepatic excretory rate plus the rate of accumulation (or minus the rate of depletion) of BSP in the hepatic parenchyma. The various lines of evidence cited in the introduction, although indirect, strongly suggest that the BSP excretory process involves a rate-limited transport mechanism and that a constant maximal excretory rate (Tm) is established when a sufficient quantity of BSP is infused to maintain plasma concentrations above, approximately, 2 to 3 mg per 100 ml. This conclusion has been drawn, in part, from repeated observations (10) of the sort illustrated in the first portion of Figure 3. Here, for example, a constant hepatic BSP removal rate was achieved and maintained as the plasma concentration (and hence the available load) of BSP increased continuously. If one postulated a variable excretory rate, then the constancy of hepatic removal rate observed under these circumstances could only be explained by the unlikely assumption that every change in excretory rate is accompanied by an equal and opposite change in the rate of accumulation of BSP in the hepatic parenchyma. Thus it is assumed that BSP excretory rate is constant (and equal, by definition, to "Tm") whenever an adequate quantity of BSP is available for maximal transport (i.e., plasma concentrations consistently in the range of 2 to 3 mg per 100 ml or above). If these conditions are satisfied, increments or decrements in hepatic BSP removal rate can then be taken as an indication of changes in the rate of movement of dye into (or out of) hepatic parenchymal storage.

Application of this interpretation to the first six studies (Figures 3, 4, 5) leads to the conclusion that increments in plasma concentration result in an increase in hepatic BSP storage whereas

decrements in concentration are followed by a net depletion of stored BSP (since, in the latter case, sufficient BSP is certainly available in the liver to maintain a maximal excretory rate during the period of markedly reduced hepatic uptake from plasma). Apparently, then, the quantity of BSP which the liver can hold in storage is determined by the concentration of the dye in the surrounding plasma. The capacity of the liver to store BSP does not appear, within the limited range of plasma concentrations explored, to become "saturated" in an absolute sense, since successive increments in plasma concentration result in additional "storage" uptake of BSP by the liver (Figure 5D). Moreover, in the last eight studies the fact that hepatic removal rate is much higher throughout a period of rising plasma concentration than during a relatively steady state indicates that a continuously increasing quantity of BSP can be stored in response to a constantly increasing plasma concentration. Under these circumstances the hepatic removal rate includes a constant rate of movement of BSP into hepatic parenchymal storage and is, to this extent, greater than the excretory Tm. This also applies to all experiments of the type illustrated by the first portion of Figure 3, where the plasma BSP concentration is changing. While it does not modify the previous arguments in favor of a constant excretory Tm, the potential inequality between hepatic BSP removal rate and excretory Tm must obviously be recognized in any quantification of the latter which is based upon measurements of the former.

B. Quantification of hepatic storage and excretory Tm. Because changes in the quantity of BSP stored in the liver depend upon the difference between uptake from the plasma and excretion into the bile, the quantification of BSP storage requires a knowledge of both the hepatic removal rate and the excretory Tm. Theoretically, the Tm could be assumed to be equal to the hepatic removal rate observed toward the end of a protracted period of *constant* high plasma level, since the net quantity of stored BSP would ultimately achieve a

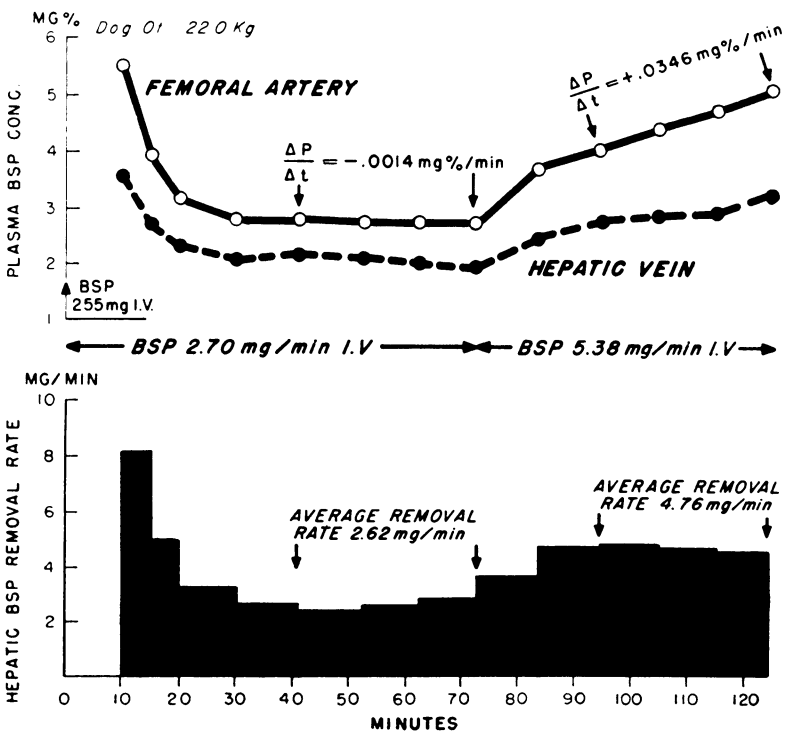

FIG. 6. RELATIONSHIP BETWEEN HEPATIC REMOVAL RATE AND RATE OF CHANGE OF PLASMA CONCENTRATION. A "priming" BSP injection was followed by two successive constant infusions of BSP. The rising plasma concentration during the more rapid infusion was associated with continuous uptake of BSP into "storage" as indicated by the higher hepatic removal rate.

constant value under these circumstances. In practice, however, this approach would require a knowledge of the Tm before it could actually be estimated. In other words, after administering a suitable "priming dose" of BSP one would have to find, by trial and error, the precise BSP infusion rate that would maintain a constant plasma level for an indefinite period. This laborious procedure can be avoided by the application of a general equation for hepatic removal rate based on the observed dependence of storage upon plasma concentration. If it is assumed, over a limited range of concentrations, that the quantity of stored BSP is directly proportional to the plasma concentration, then a proportionality factor can be defined to express the number of milligrams of BSP taken up into storage per mg per 100 ml of plasma concentration. This factor is designated "relative storage capacity" (S). The net rate of uptake of BSP into storage (in mg per minute) during a period of continuously changing plasma concentration is then equal to the product of S times the rate of change of plasma concentration (in milligrams per 100 ml per minute). The general equation for hepatic removal rate thus becomes:

$$\text{Equation 1: } R_H = Tm + S \times \left(\frac{\Delta P}{\Delta t}\right)$$

where:

R_H = hepatic removal rate, mg per minute

Tm = excretory transport maximum, mg per minute

S = relative storage capacity, mg per mg per 100 ml

$\frac{\Delta P}{\Delta t}$ = rate of change of plasma concentration, mg per 100 ml per minute.

Substitution into this general equation of the values of R_H and $\frac{\Delta P}{\Delta t}$, observed during two different rates of infusion, provides a set of simultaneous equations that can be solved for both Tm and S.

This calculation has been carried out using the values of R_H and $\frac{\Delta P}{\Delta t}$ shown in Columns 4 and 5 of Table I, and the resulting estimates of Tm and S are shown in Columns 6 and 8 of the same table. These have also been reduced to a standard body weight of 10 kg in Columns 7 and 9. The Tm values calculated in these dogs were not obviously different, per unit of body weight, from those estimated in studies reported previously (10). However, it must be emphasized that the present studies were carried out under conditions of anesthesia, severe trauma and often reduced hepatic blood flows. While these conditions produced no obvious progressive impairment of the mechanisms under investigation, the precise numerical values may not apply to normal animals.

C. Concentration of BSP in the liver. The relative storage capacity, as defined, has the dimensions of a volume, expressed in units of 100 ml. Thus, in a sense, an average "S" of 19.9 mg per mg per 100 ml per 10 kg means that the hepatic storage compartment of a 10 kg dog behaves as if it were an extension of the plasma compartment with a volume of 1,990 ml. Theoretically, therefore, one could define S as the hepatic "volume of distribution" of BSP, but compared with the known size of the liver the numerical values of S are absurdly large. Actually the dog's liver, exclusive of blood content, weighs only 235 g per 10 kg (an average figure for 14 dogs in which a prior injection of Cr^{51}-labeled red cells made it possible to estimate and subtract the blood content). In order to accommodate 19.9 mg of BSP for every mg per 100 ml change in plasma concentration, the liver must be capable, therefore, of storing BSP at more than eight times the concentration in the surrounding plasma. (If the material were stored at a concentration just equal to that in plasma, a 235 g liver would have a storage capacity of only 2.35 mg per mg per 100 ml.) Since part of the weight of the liver is attributable to comparatively inert supporting tissue, and since there is no assurance that dye is uniformly distributed through the remaining parenchyma, it is probable that concentration of BSP in the actual hepatic storage sites may achieve an even higher ratio with respect to plasma concentration.

D. Storage and transport mechanisms. The present studies provide no information regarding the nature of the mechanisms by which BSP is concentrated in the liver and transported into the bile nor of the possible relationship between the two processes. The schema presented here is undoubtedly oversimplified. It has been shown by Krebs and Brauer (15), for example, that BSP is

partially converted by the liver into a number of chromatographically separable molecular species, some of which have definitely lower extinction coefficients than the parent compound at 580 mμ. Whatever the behavior of these products with respect to storage and excretion their formation represents, in effect, a "loss" of BSP color from the body and would hence be included in the excretory Tm as calculated herein. However, the resulting overestimation of Tm compared with the actual rate of biliary excretion is probably not very great, since over 70 per cent of administered BSP is ultimately recoverable in the bile by simple colorimetry (3). Moreover, the major quantity of the derivatives is ultimately excreted in the bile, and to this extent properly belongs in the "Tm" calculation.

E. Simplification of technique. Even at the high plasma concentrations required for these studies the calculated removal of BSP by structures other than the liver was comparatively small. The figures shown in Table II tend, if anything, to overestimate the actual removal by extrahepatic tissues. They are undoubtedly magnified by the effect of blood loss and subsequent hemodilution (due to repeated sampling plus wound ooze after anticoagulation). Moreover, the hematocrits were not corrected for trapped plasma so that the values for hepatic plasma flow, and hence the hepatic removal rates, were subject to a systematic underestimation which would also exaggerate apparent extrahepatic removal.

If one ignores the effect of extrahepatic BSP removal altogether, then the values for Tm and S can be calculated without measurement of hepatic blood flow and hepatic vein concentration, as follows:

$$R = I - PV \times \left(\frac{\Delta P}{\Delta t}\right)$$

where:

R = BSP removal rate, mg per minute

I = BSP infusion rate, mg per minute

$\frac{\Delta P}{\Delta t}$ = rate of change of plasma BSP concentration, mg per 100 ml per minute

PV = plasma volume in hundreds of ml.

Substituting this expression into Equation 1:

$$\text{Equation 2: } I = Tm + (S + PV) \times \left(\frac{\Delta P}{\Delta t}\right)^{1}.$$

This equation may be solved for Tm and S by substitution of two sets of values for I and $\frac{\Delta P}{\Delta t}$. This calculation, in the eight studies shown in Table II, yielded values for Tm and S (Columns 10 and 11) which were quite comparable to the figures calculated from the actual hepatic removal rates. As shown in Columns 12 and 13, Tm was thus overestimated by an average of 9 per cent and S by 4 per cent when extrahepatic removal was ignored.

It is apparent from this that reasonable estimates of BSP transport maximum and storage capacity can be obtained by a relatively simple technique utilizing constant BSP infusions and multiple peripheral blood samples. Studies of this type are in progress (17); the results will be reported later in detail.

SUMMARY

The hepatic removal rate of sulfobromophthalein sodium (BSP) has been estimated in 14 dogs by means of measurements of hepatic blood flow (rotameter) and splanchnic arteriovenous concentration difference. During a constant BSP infusion hepatic BSP removal reaches a constant rate at an arterial concentration of 2 to 3 mg per 100 ml even when the latter continues to rise. Sudden increments in arterial concentration produce a marked transient increase in hepatic BSP removal rate and decrements produce a transient reduction. The hepatic removal rate is higher during continuously rising than during constant arterial BSP concentrations.

The data support the view that hepatic excretion of BSP depends upon a rate-limited transport mechanism and that the quantity of BSP stored in the liver parenchyma depends directly upon the concentration in the surrounding plasma. A method for calculating the excretory transport maximum (Tm) and the relative storage capacity (S) has been presented, based on ob-

[1] It should be noted that this equation is, in fact, the equivalent of one of the graphic methods proposed for estimation of the maximal BSP excretory rate by Lewis (16).

servations during two different constant rates of BSP infusion. The calculated values of storage capacity indicate that BSP in the hepatic storage compartment is maintained at a concentration more than eight times that in the plasma. In the range of plasma concentrations studied there was no evidence of "saturation" of the storage compartment. The extrahepatic removal rate of BSP is low enough so that Tm and S may be reasonably estimated from observations of peripheral plasma BSP concentrations during different rates of constant infusion, without resort to hepatic venous sampling or hepatic blood flow measurements.

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