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STUDIES ON BILIARY EXCRETION MECHANISMS OF DRUGS—IV

INHIBITORY STUDIES ON SULFOBROMOPHTHALEIN AND GLUCURONIDES IN THE RAT

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Abstract—After the administration of 50 μmoles sulfobromophthalein (BSP) or BSP glutathione conjugate (BSP-GSH) to rats, the maximal dye excretion was approximately 50 per cent higher for the latter as compared to the former. BSP or BSP-GSH and three glucuronides, thiamphenicol glucuronide (TPG), chloramphenicol glucuronide (CPG) and phenolphthalein glucuronide (PPG), depressed each other's bilary excretion. Competition between BSP or BSP-GSH and these glucuronides was not observed in the first phase of disappearance from blood. Transport of these drugs from blood to bile occurs rapidly. From these results, it can be inferred that in the transport of these drugs from blood to bile at least one common step is involved, but that this step is not the uptake from blood to liver. The binding affinity of these organic anions to hepatic cytoplasmic proteins was examined by Sephadex G-75 gel filtration and by ultrafiltration. In the former experiment, BSP showed an apparent binding affinity to the cytosol fraction containing ligandin and Z protein. BSP-GSH showed a lower affinity than that of BSP. On the other hand, three glucuronides were not eluted with the fraction. In the latter experiment, 41 per cent of BSP was bound to the cytosol fraction, whereas only 10 per cent of BSP-GSH and PPG and none of CPG and TPG were bound. These results also suggest that the competition in biliary excretion observed between BSP or BSP-GSH and the glucuronides does not occur at the binding sites of ligandin or Z protein, and that binding to these proteins may not be required for the overall transport of these glucuronides from blood to bile.

Sulfobromophthalein (BSP) is rapidly taken up by the liver in several animal species and excreted against a concentration gradient into the bile mainly as the glutathione conjugate (BSP-GSH). A much higher rate of excretion of dye into the bile is observed after the administration of conjugated BSP rather than free BSP in the rats and guinea pigs [1, 2]. Although this may indicate that conjugation facilitates dye transport into the bile and is rate limiting in the overall transport from blood to the bile of these animals, other interpretations of these results are possible [3]. The observations that free BSP also appears in the bile and competitively inhibits the transport of conjugated BSP into the bile [4] further complicate the problem.

Thiamphenicol glucuronide (TPG), chloramphenicol glucuronide (CPG) and phenolphthalein glucuronide (PPG) are transferred from blood into bile against a large concentration gradient in rat and their transport processes are saturable. The excretion of these glucuronides is inhibited by probenecid [5]. However, no definite mechanisms have been defined for the overall transport of these organic anions from blood to bile. The purpose of the present report was to investigate the interaction between BSP or its major metabolite, BSP-GSH, and these glucuronides with regard to disappearance from blood and biliary excretion, and to discuss their hepatic transport mechanisms.

In recent years, two hepatic cytoplasmic protein fractions, designated as ligandin (or Y protein) and Z protein, have been described [6, 7]. It has been proposed that binding to these proteins, especially ligandin, is an important event in the hepatic uptake and storage and/or transport into bile of cholephilic agents [6, 8, 9]. As a second phase of this project, the binding affinity of these organic anions to the cytoplasmic proteins was compared, and their possible roles in the overall transport of these anions are discussed.

MATERIALS AND METHODS

Drugs. TPG, m.p. 190–192°, was isolated from guinea pig urine and bile by a method described elsewhere [10]. CPG was isolated from human urine by the method of Uesugi *et al.* [11]. PPG and D-saccharic acid-1,4-lactone were obtained from the Sigma Chemical Co. BSP was obtained from K & K Laboratories Inc. BSP-GSH was isolated from rat bile by the methods of Combes [12] and Grodsky *et al.* [13].

Biliary excretion. Male Wistar rats weighing 340–360 g were anesthetized with sodium pentobarbital i.p. (40 mg/kg). Through an abdominal incision, the renal pedicles were ligated and the bile duct was cannulated with polyethylene tubing (18 cm in length). A thermometer probe was placed on the surface of the liver, and body temperature was maintained at 38° with a heating lamp throughout the experiments. After the incision had been closed, bile was collected for 10 min prior to administration of drugs. The drugs (50 μmoles) were then injected into the femoral vein over a 1.5-min period, and bile was collected for 90 min at 10-min intervals. TPG, CPG and PPG in

bile were determined by the methods reported previously [5, 10], and BSP was determined in bile by measuring the absorbance at 578 nm after alkalinization with 0.1 N NaOH. Determination of these glucuronides was not disturbed by the presence of BSP or BSP metabolites. For the determination of BSP metabolites, a portion of bile was chromatographed on Toyo No. 51 paper in a descending system employing *n*-propanol–water–acetic acid (10:5:1) for 5 hr [12]. After the position of the fractions had been determined with ammonia, each band was cut out and the dye was eluted with 20 ml of 0.5 N NaOH by shaking for 10 min. The BSP content of the eluate was measured colorimetrically. Two spots corresponding to free BSP and BSP-GSH and two spots of unknown metabolites were observed by this method. The fraction of the unknown metabolites can be separated to three spots by the method of Whelan and Plaa [14]. In the present study, we measured the sum of these unknown metabolites.

Blood samples. Blood samples (0.2 ml) were drawn from the common jugular vein into heparinized syringes at 2, 4, 6, 8, 10, 15, 20 and 30 min after the start of injection of drug over a 1.5-min period, and diluted with distilled water to a volume of 10 ml. For the determination of CPG and TPG, 4 ml of the diluted blood sample was deproteinized with 1 ml of 0.5 N NaOH followed by 1 ml of 10% $ZnSO_4 . 7 H_2O$ solution, and the supernatant was treated by the methods reported previously [10]. PPG was determined in blood samples by the method reported previously [5], after hydrolysis of 4 ml of the diluted sample with 1 ml of conc. HCl at 100° for 60 min. BSP was determined in blood using DEAE-cellulose column as reported previously [15].

Gel filtration. Heparinized male rats weighing 340–360 g were anesthetized with sodium pentobarbital, and the livers were perfused *in situ* through the hepatic vein with ice-cold 0.9% NaCl solution to remove residual blood. The perfused livers were rapidly excised and washed with the perfusion fluid. A 25% homogenate was prepared in 0.25 M sucrose in 0.01 M phosphate buffer (pH 7.4). The homogenate was centrifuged at 2° at 110,000 *g* (max) for 120 min. The supernatant fraction was separated from the pellet and surface lipid. BSP (2.4 μmoles), BSP-GSH (2.4 μmoles), TPG, CPG or PPG (20 μmoles) and 20 μmoles of D-saccharic acid-1,4-lactone as an inhibitor of β-glucuronidase were added to 2 ml of the supernatant fraction containing about 36–43 mg of protein and thoroughly mixed. After standing at 4° for 20 min, the mixture was applied to the bottom of a Sephadex G-75 column (2.2 × 100 cm) equilibrated with 0.01 M phosphate buffer at 4°. Elution was performed using the same buffer and a pump-driven, upward flow system. Fractions of 4.4 ml of eluate were collected. The flow rate was 31.2 ml/hr. The absorbance at 280 nm was determined for each fraction, and protein content was estimated by subtracting the absorbance at 280 nm of drug itself from the absorbance of the mixture measured at 280 nm, because of considerably high absorbance at 280 nm of the drugs themselves. Determination of drugs in the fractions was made by the methods described above for the bile samples and was not affected by protein. Protein concentration in the supernatant fraction was estimated by the method of Lowry *et al.* [16].

Binding of drugs to rat liver supernatant fraction in vitro. Three ml of solution in 0.01 M phosphate buffer containing 5 μmoles drug and 10 μmoles D-saccharic acid-1,4-lactone was mixed with 1 ml of the supernatant fraction prepared above in a Visking tube (6.4 mm in diameter), and ultrafiltered at 1500 g for 3 hr at 4°. Another 3 ml of drug solution was mixed with 1 ml of buffer solution, instead of the supernatant fraction, and treated as described above. The concentration of drugs in the filtrate was determined by the same methods described above.

Statistical analysis. Data were analyzed by a group comparison Student's *t*-test; $P < 0.05$ was considered significant.

RESULTS

Biliary excretion of BSP and BSP-GSH. In the first experiment, BSP and BSP-GSH were injected in two groups of rats with ligated renal pedicles and their rates of biliary excretion of total dye were compared. In Fig. 1 it is seen that peak excretion was 40 min after injection of BSP and 20 min after injection of BSP-GSH. Subsequently, the excretion rate after BSP-GSH injection fell more rapidly than was seen after the injection of BSP. One possible explanation for this observation is that the formation of BSP-GSH is the rate-limiting step in the excretion of BSP and accounts for the delay in the excretion peak observed after injection of BSP [1, 2, 17].

Interaction of BSP and glucuronides in biliary excretion. In the previous studies of this series [5], it appeared that TPG, CPG and PPG were transferred from blood to bile against a large concentration gradient and that the transport process was saturable. The excretion was influenced by probenecid. Therefore, it was postulated that these glucuronides and BSP would influence each other's biliary excretion. This indeed was seen in the experiments illustrated in Fig. 2 and Table 1. The biliary excretion of these

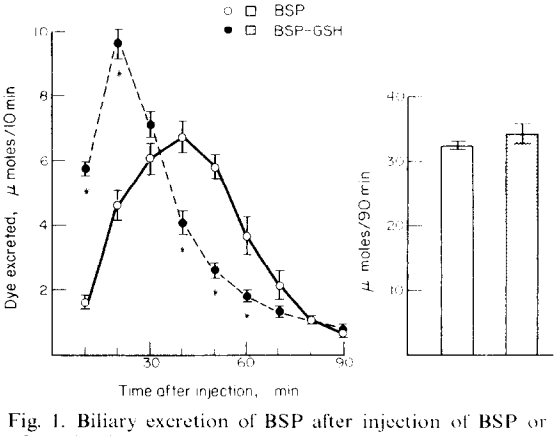

Fig. 1. Biliary excretion of BSP after injection of BSP or BSP-GSH in rat. Rats with ligated renal pedicles received BSP (50 μmoles) or BSP-GSH (50 μmoles) intravenously. Bile was collected in 10-min periods for 90 min. Results are given as the mean ± S.E.M. for six animals receiving BSP and four animals receiving BSP-GSH. An asterisk (*) indicates significantly different ($P < 0.05$) from the group injected with BSP.

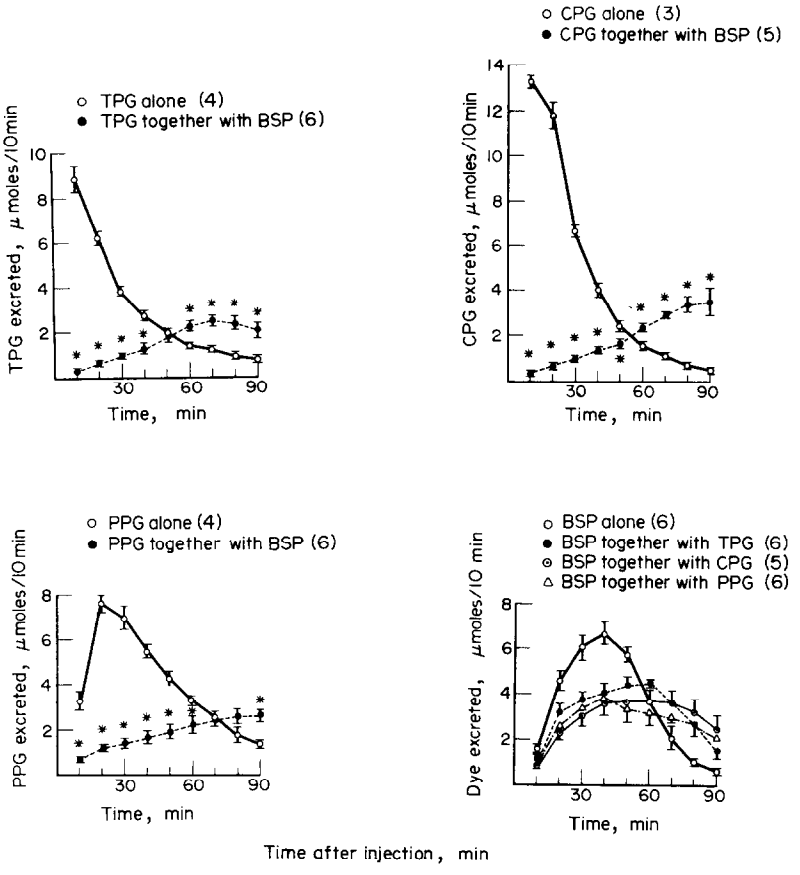

Fig. 2. Effect of BSP on biliary excretion of TPG, CPG and PPG. Rats with ligated renal pedicles received glucuronide (50 μmoles) alone, or glucuronide together with BSP (50 μmoles) intravenously. Bile was collected in 10-min periods for 90 min. The number of experiments is shown in parentheses. Results are given as the mean ± S.E.M. An asterisk (*) indicates significantly different ($P < 0.05$) from the group injected with each drug alone.

glucuronides was markedly depressed by the simultaneous administration of BSP. The excretion of BSP was also significantly ($P < 0.05$) depressed by these glucuronides. It appeared that the biliary excretion of BSP was influenced most by PPG and least by TPG under these experimental conditions.

Table 1. Interaction of BSP or BSP-GSH and TPG, CPG and PPG in biliary excretion

Drug	Biliary excretion (μmoles/90 min ± S.E.M.)
TPG alone	28.17 ± 0.76
+ BSP	14.29 ± 1.37*
+ BSP-GSH	18.13 ± 1.75*
CPG alone	42.13 ± 1.58
+ BSP	17.57 ± 1.17*
+ BSP-GSH	24.21 ± 1.92*
PPG alone	36.65 ± 1.85
+ BSP	16.81 ± 2.26*
+ BSP-GSH	25.24 ± 0.53*
BSP alone	32.32 ± 0.61
+ TPG	28.25 ± 0.50*
+ CPG	26.62 ± 1.30*
+ PPG	25.91 ± 1.81*
BSP-GSH alone	34.15 ± 1.55
+ TPG	30.93 ± 1.17
+ CPG	30.86 ± 1.22
+ PPG	30.33 ± 0.30

* Significantly different ($P < 0.05$) from the group injected with each drug alone.

Effect of PPG on excretion of BSP metabolites. In another set of experiments, the effect of PPG on the excretion of BSP metabolites was determined. Table 2 shows the biliary excretion of BSP metabolites after injection of BSP alone. Rats with ligated renal pedicles were given i.v. injections of 50 μmoles BSP, and 30-min bile samples were collected for 90 min. It was confirmed by paper chromatography that free BSP,

Table 2. BSP and its metabolites excreted in rat bile after injection of BSP*

Fraction	Amount excreted (μmoles) 0–30 min	30–60 min	60–90 min
Free BSP	2.79 ± 0.39	1.78 ± 0.02	0.68 ± 0.16
Unknown metabolites	0.49 ± 0.10	2.04 ± 0.02	1.03 ± 0.17
BSP-GSH	8.97 ± 0.39	12.35 ± 0.03	2.17 ± 0.11

* Chromatography was carried out on bile collected from rats with ligated renal pedicles after i.v. administration of 50 μmoles BSP. Results are expressed as the mean ± S.E.M. for five animals.

Table 3. Effect of PPG on excretion of BSP and its metabolites after injection of BSP*

Fraction	Amount excreted (μmoles) 0–30 min	30–60 min	60–90 min
Free BSP	1.99 ± 0.08	2.70 ± 0.32	1.46 ± 0.33
Unknown metabolites	0.24 ± 0.01	0.98 ± 0.04	1.78 ± 0.03
BSP-GSH	3.50 ± 0.15	6.79 ± 0.46	5.67 ± 0.04

* Bile was collected after simultaneous i.v. administration of 50 μmoles each of BSP and PPG. Results are expressed as the mean ± S.E.M. for three animals.

BSP-GSH and unknown metabolites were in the bile. It can be seen in Table 2 that, at all time periods, the percentage of total dye excretion as free BSP was less than that of total BSP metabolites and that the percentage excreted as BSP-GSH was higher than that of other fractions. The percentage of excreted dye in the unknown metabolites fraction was the lowest of all fractions during the first 30-min period but increased with time.

Table 3 shows the effect of PPG on the biliary excretion of dye after injection of BSP. PPG (50 μmoles) was injected together with 50 μmoles BSP, and 30-min bile samples were collected for 90 min. The results show that the excretion of free BSP in the first 30-min period was substantially ($P < 0.05$) lower than that of control (Table 2), although it was significantly increased in the later periods. The excretion of BSP-GSH and unknown metabolites was markedly ($P < 0.05$) depressed by PPG in the first and second periods, and significantly increased in the last 30-min period. Consequently, the total recovery of unknown metabolites was statistically unchanged. However, the total recovery of BSP-GSH was substantially ($P < 0.05$) lower than that of control. These results may explain the inhibition by the glucuronides on the biliary excretion of dye after injection of BSP, seen in Fig. 2 and Table 1.

Interaction of BSP-GSH and glucuronides. It can be seen in Fig. 3 and Table 1 that the simultaneous injection of BSP-GSH significantly decreased the peak excretion rate and the total amount excreted in 90 min of TPG, CPG and PPG. However, there was no delay in the peak excretion of these glucuronides as was seen with BSP (Fig. 2). The inhibitory effect of BSP-GSH on the excretion of these glucuronides was apparently less than that of free BSP. On the other hand, the peak excretion rate of BSP-GSH was depressed significantly by the simultaneous injection of PPG and CPG. PPG caused a delay in the peak excretion of BSP-GSH, whereas CPG did not cause such a delay. TPG did not significantly depress the

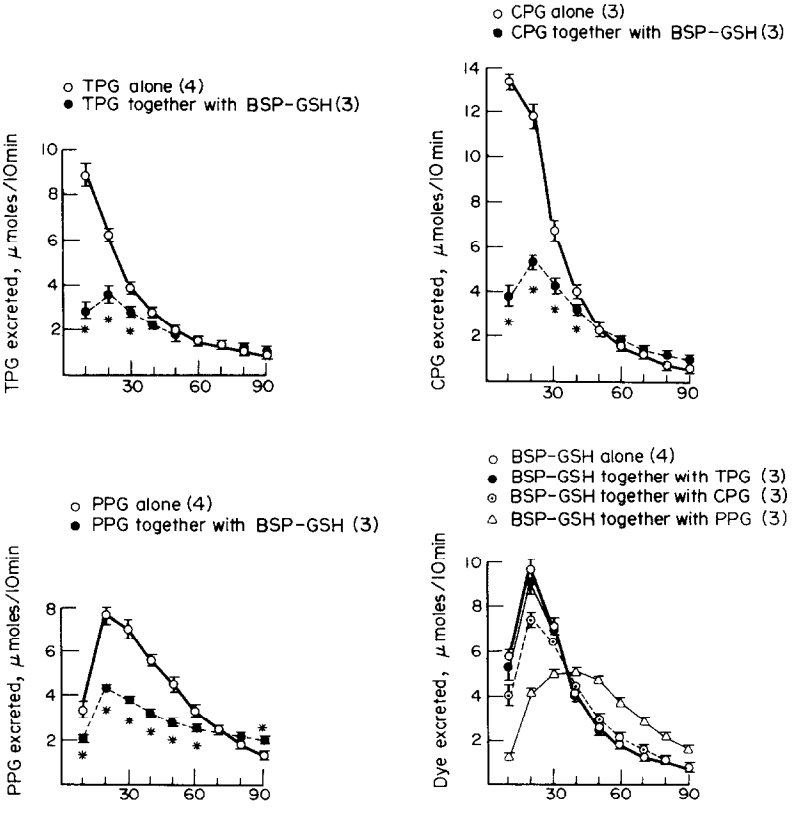

Fig. 3. Effect of BSP-GSH on biliary excretion of TPG, CPG and PPG. Rats with ligated renal pedicles received glucuronide (50 μmoles) alone, or glucuronide together with BSP-GSH (50 μmoles) intravenously. Bile was collected in 10-min periods for 90 min. The number of experiments is shown in parentheses. Results are given as the mean ± S.E.M. An asterisk (*) indicates significantly different ($P < 0.05$) from the group injected with each drug alone.

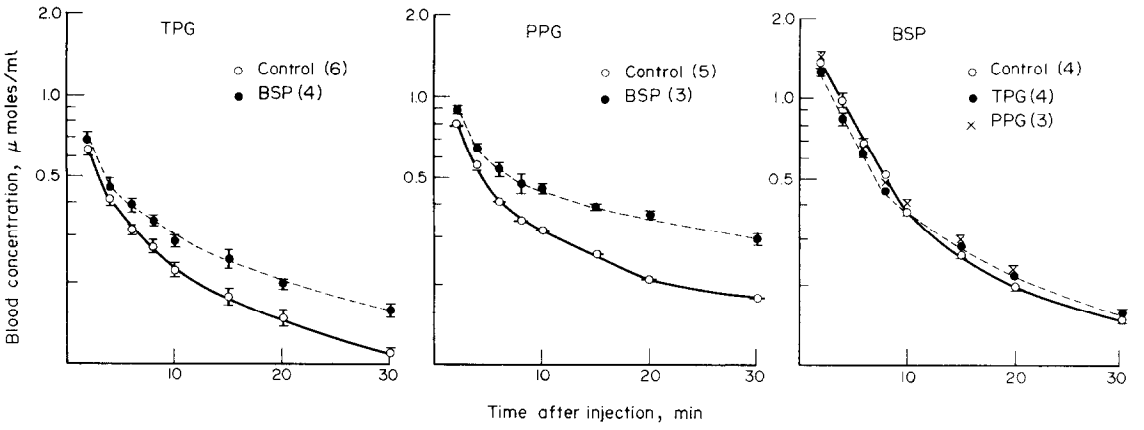

Fig. 4. Effect of BSP on blood disappearance of TPG and PPG. Blood (0.2 ml) was collected from the common jugular vein at 2, 4, 6, 8, 10, 15, 20 and 30 min after the start of i.v. injection (femoral vein) of glucuronide (50 μmoles) alone, or glucuronide together with BSP (50 μmoles) over a 1.5-min period. The number of experiments is shown in parentheses. Each point represents the mean ± S.E.M.

excretion of BSP-GSH at any time period, although the glucuronide significantly suppressed dye excretion after BSP injection and caused a delay in its peak excretion (Table 1 and Fig. 2).

Effect of BSP and BSP-GSH on blood disappearance rate of glucuronides. The effect of BSP on the blood disappearance of TPG and PPG was minimal during the first phase but more evident later on. The disappearance of BSP from blood was not influenced by TPG and PPG at any time during the course of the experiment (Fig. 4). In Fig. 5, it is seen that the disappearance of PPG from blood is not influenced by BSP-GSH during the first phase but some inhibition is apparent during the second phase. The disappearance of BSP-GSH from blood was not influenced during either phase by PPG, although among three glucuronides PPG caused the greatest depression of the biliary excretion of BSP-GSH (Fig. 3).

Binding of drugs to rat liver supernatant fractions in vitro. Levi *et al.* [6, 18–21] have suggested that ligandin is a major determinant of the net flux of organic anions such as BSP, bilirubin and indocyanine green from plasma into liver. A role for hepatic cytoplasmic proteins has also been suggested in the hepatic transport of acid azo dyes and bromphenol blue [8]. From these observations, it was considered possible that BSP-GSH and three glucuronides would also bind to cytosol proteins.

In the first set of experiments, binding of the drugs to cytosol proteins was examined according to the method of Levi *et al.* [6]. The presence of the organic anions binding proteins, ligandin and Z protein, in the supernatant fraction of a liver homogenate was demonstrated by the elution profile of BSP from Sephadex G-75, shown in Fig. 6. These results were in accord with the findings of Levi *et al.* [6]. BSP was also eluted at two low molecular weight regions centering around tubes 95 and 120. The peak at tube No. 120 is thought to represent unbound BSP. Tubes 70–100 absorbed at 280 nm but contained no protein detectable by the method of Lowry *et al.* [16]. Measurement of these peaks at 280 and 260 nm [22] suggested the presence of nucleic acids, proteins with low molecular weight, or peptides. Since BSP was not eluted in this region when BSP alone was applied to a Sephadex G-75 column under the same condi-

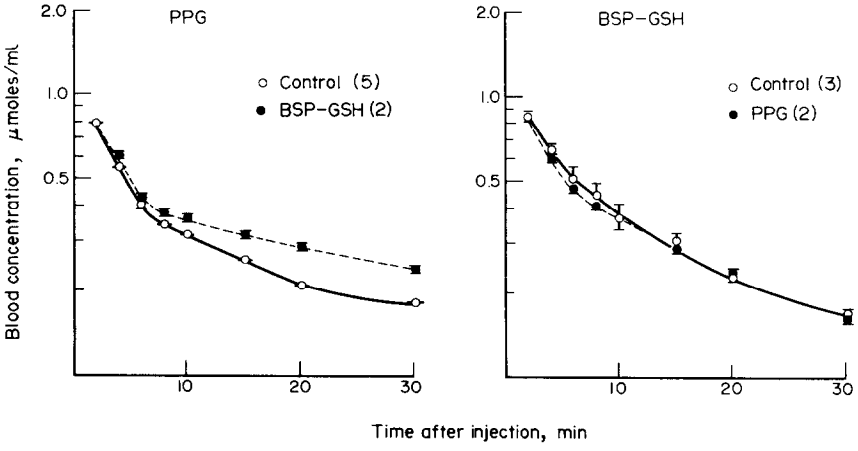

Fig. 5. Effect of BSP-GSH on blood disappearance of PPG. Blood (0.2 ml) was collected from the common jugular vein at 2, 4, 6, 8, 10, 15, 20 and 30 min after the start of i.v. injection (femoral vein) of PPG (50 μmoles) alone, or PPG together with BSP-GSH (50 μmoles) over a 1.5-min period. The number of experiments is shown in parentheses. Each point represents the mean ± S.E.M.

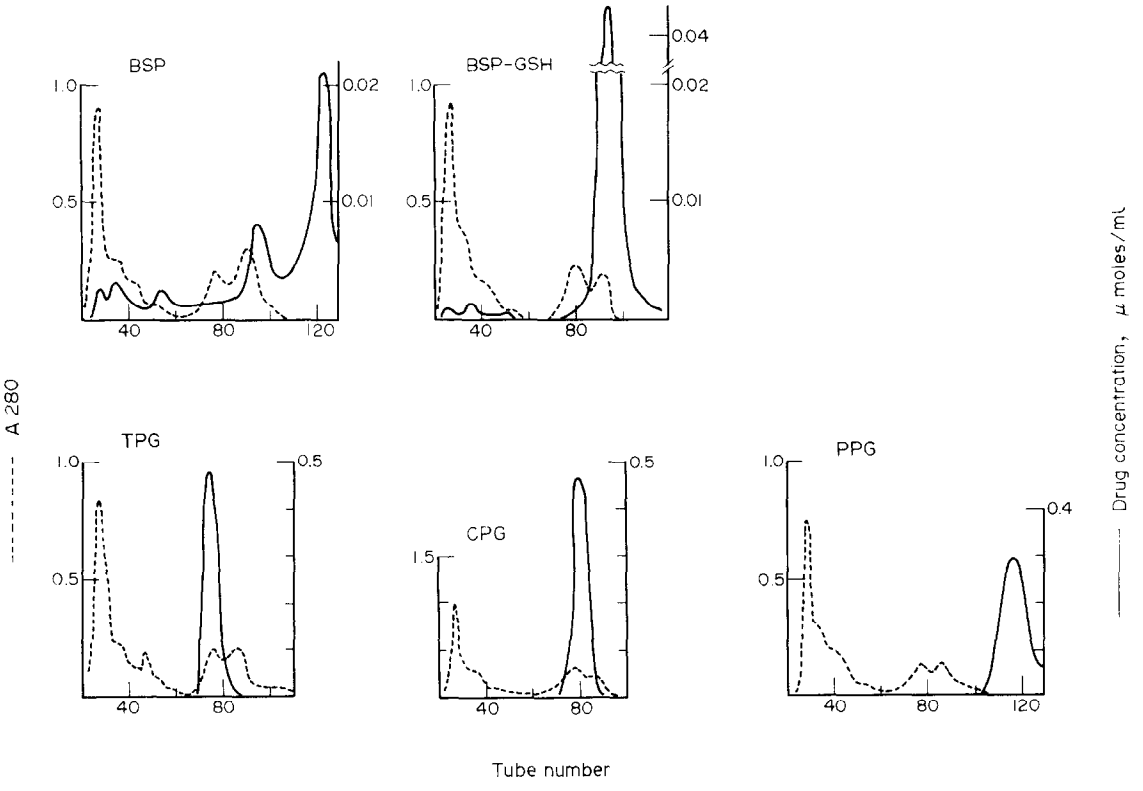

Fig. 6. Elution patterns of rat liver supernatant fraction with drugs added *in vitro* using Sephadex G-75. Two ml supernatant (110,000 *g*. max), containing 35.5 to 43.2 mg protein, from 2 g of rat liver was added to BSP or BSP-GSH (2.4 μmoles) or glucuronide (20 μmoles) *in vitro*, and eluted from a 2.2 × 100 cm Sephadex G-75 column in 0.01 M phosphate buffer (pH 7.4). Flow rate was 31.2 ml/hr and collections were 4.4 ml/tube. A_{280} was corrected by deducting the absorption calculated at 280 nm of drug itself from the absorption of the mixture measured at 280 nm.

tions, it was inferred that BSP was truly bound to some low molecular components. As shown in Fig. 6, BSP-GSH was bound to ligandin and Z protein fractions only to a slight extent. TPG and CPG appeared not to be associated at all with these fractions, although they were in the low molecular weight region of tubes 70–90. On the other hand, PPG was not eluted with any protein fraction. It should be pointed out that the amount of each glucuronide (20 μmoles) applied to the column was about eight times as much as of BSP or BSP-GSH (2.4 μmoles). However, interaction between the drug and gel may occur, especially with BSP, BSP-GSH and PPG in view of their affinity for liver supernatant shown below. Interaction with the gel could give the impression of little or no protein binding.

Table 4. Binding of drugs to rat liver supernatant fraction *in vitro**

	Per cent of binding
TPG	0.01 ± 0.01
CPG	0.02 ± 0.01
PPG	9.57 ± 2.15
BSP-GSH	9.90 ± 0.64
BSP	40.73 ± 0.75

* Results are given as the mean ± S.E.M. for three animals.

In the second experiment, binding was examined by ultrafiltration, and BSP showed considerable affinity for the rat liver supernatant fraction (Table 4). BSP-GSH and PPG showed much lower affinity than did BSP, and TPG and CPG were not bound at all.

DISCUSSION

BSP is conjugated with glutathione in the liver [12, 13, 23–25]. A number of investigators have reported that the conjugation of BSP facilitates dye transport from the parenchymal cell into bile in rats [1, 2, 17, 26] and guinea pigs [2]. Our results corroborate these findings: after the administration of 50 μmoles BSP or BSP-GSH to rats the maximal dye excretion was approximately 50 per cent higher for the latter compared to the former (Fig. 1). However, whether the conjugating step of BSP is rate limiting in the overall transport of BSP from blood to bile is highly controversial [3]. Whelan and Combes [4] have reported that when excretion of conjugated BSP reached a constant level during BSP infusion into guinea pigs and rats, conjugated BSP was present in the liver in quantities sufficient to result in much higher rates of excretion, had only conjugated material been present in the liver. Furthermore, they observed that the rate of excretion of conjugated BSP

during infusion of conjugated BSP alone was markedly depressed when BSP was added to the infusion. From these observations they concluded that the lower maximal rate of excretion of dye into the bile for BSP compared to conjugated BSP was not due to a rate-limiting conjugation step but reflected the competition between BSP and conjugated BSP. In experiments using single i.v. injections of free BSP, however, more conjugated than free BSP is excreted into the bile over a wide range of doses [1, 2] (Table 2). At least two processes, conjugation and excretion, are involved in the transport of dye into bile after injection of BSP. Conjugated BSP is much less readily bound to the proteins of the supernatant fraction of rat liver than is free BSP [1] (Table 4), and we have shown that the excretion rate of conjugated BSP is higher than that of free BSP (Fig. 1). Varga *et al.* [26] demonstrated that the hepatic concentration of free BSP was approximately seven times higher than that of BSP-GSH after administration of 200 mg/kg of free BSP or an equimolar dose of BSP-GSH in diethylmalate-treated rats which have low conjugation ability. After a single BSP injection, hepatic concentration of free BSP high enough to inhibit the transport of BSP-GSH can probably be maintained only a short time after injection, because free BSP is rapidly conjugated and excreted into the bile [1] (Table 2). The results from these experiments using single i.v. injections of BSP or conjugated BSP have given us the impression that conjugation may be the rate-limiting step in the overall transport of BSP from blood to bile.

It is well known that organic anions of varying structure influence each other's biliary transport [5, 27–30]. It has now been found that BSP, BSP-GSH and three glucuronides (TPG, CPG and PPG) depress each other's biliary excretion (Figs. 2 and 3, and Table 1). The biliary excretion of these glucuronides was markedly depressed by the simultaneous injection of BSP or BSP-GSH. On the other hand, the peak excretion rate and total amount of BSP excreted in 90 min were significantly depressed by these glucuronides. Only the peak excretion of BSP-GSH was suppressed by PPG and CPG. Although TPG significantly suppressed dye excretion after BSP injection, this glucuronide did not depress the excretion of BSP-GSH. As possible explanation for the different effects of three glucuronides on the excretion of dye after BSP or BSP-GSH injection was offered. A high concentration of conjugated BSP at the transport site in liver is undoubtedly reached more rapidly after BSP-GSH injection than after free BSP injection, because the amount of conjugated BSP in the liver in the latter case is dependent upon the rate of BSP conjugation. Therefore, the glucuronides may be competing with less BSP-GSH and thus may inhibit more efficiently the transport of conjugated BSP after injection of free BSP. This possibility is supported by the results shown in Table 3. The simultaneous administration of PPG and BSP resulted mainly in the inhibition of excretion of BSP-GSH rather than of free or unknown metabolites. This suggests that the glucuronide suppression of dye excretion after BSP injection may be due mainly to the competition between the glucuronides and BSP-GSH, while the delay in the excretion of glucuronides may be attributable to the strong inhibitory effect of free BSP. It is not apparent whether the competitive inhibition occurs at the level of uptake, transport through the hepatocyte, excretion or some combination. In our study, there was no apparent inhibition during the early phase of blood disappearance (Figs. 4 and 5). Thus, the level of inhibition probably is not hepatic uptake. The subsequent suppression at the second phase may be due to competition for active transport within the liver or from liver to bile. The decreased biliary excretion may also lead to a greater efflux of drug into the blood.

Recently, it was shown that ligandin, one of the cytoplasmic proteins, was identical to glutathione s-transferase B, an enzyme catalyzing the conjugation of glutathione with such electrophiles as BSP, ethacrynic acid and 1-chloro-2,4-dinitrobenzene [31]. This protein is also capable of binding some neutral compounds such as methylcholanthrene, certain steroids [7] and iodomethane [31]. Indocyanine green is not only an ineffective substrate but is a competitive inhibitor for the enzyme [31]. These facts suggest that binding affinity of the protein is directed toward electrophilic sites rather than limited to organic anions [31]. BSP has a very high affinity for the cytoplasmic proteins. On the other hand, BSP-GSH and PPG have lower affinity for the proteins than does BSP, and TPG and CPG have little affinity (Fig. 6 and Table 4). From these observations, it is inferred that competition for biliary excretion observed between BSP, BSP-GSH and the glucuronides does not occur at the binding sites on the cytoplasmic proteins. In view of the low affinity, these proteins may not play a role in the transport of these glucuronides and BSP-GSH from blood to bile, but rather may play a role as a storage site [1] of some cholephilic compounds such as BSP, billirubin and indocyanine green which have electrophilic sites.

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