Effects of Certain Drugs on the Mechanism of Biliary Excretion of Sulfobromophthalein: Sodium dehydrocholate, Sodium hippurate, Sodium salicylate and Liver hydrolysate

Minoru MIZUTA, Kinya MURATA, Kenzo NAGAI and Katsushi TAMURA

First Division, Department of Internal Medicine, (Director: Prof. Teruo Fujita) Yamaguchi University School of Medicine, Ube, Japan

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The sulfobromophthalein (BSP) retention test is generally regarded as one of the most widely used clinical measurements of liver function. Many steps are involved in the movement of BSP from blood to bile following the intravenous administration of this compound. It is effected by the interrelated functions of hepatic uptake, storage, conjugation, and biliary excretion, each of which may be influenced by such dynamic factors as blood circulation and protein availability. The detailed mechanism of the biliary excretion of BSP, however, has not been clearly established.

The present studies were undertaken to examine the alterations of steps involved in the transfer of BSP from blood to bile after an intravenous administration of sodium dehydrocholate, sodium hippurate, sodium salicylate or liver hydrolysate.

Sodium dehydrocholate is a hydrocholeretica which inhibits the biliary excretion of phenolsulfonphthalein (PSP) following an intravenous administration of PSP into bilaterally nephrectomized rabbits [1].

Both sodium salicylate, while it is toxic to the liver when massive doses are administered for a long period, [2][3] and sodium hippurate, which is non-toxic to the liver [2], are known to increase the biliary excretion of PSP following an intravenous administration of PSP into bilaterally nephrectomized rabbits.

Liver hydrolysate is one of the therapeutic agents of liver disease which accelerates hepatic metabolism and regeneration [4].

METHODS

1. Biliary excretion of BSP following a single intravenous administration of BSP.

A polyvinyl tube, 0.31 or 0.62 mm in diameter, was inserted into the common

bile duct of an adult male Whistar rat anesthetized with an intraperitoneal administration of 10 mg sodium thiopentalate.

Ten mg BSP per 100 gm body weight was administered intravenously to the rat and bile was collected for one hour. Prior to the administration of BSP one of the following drugs, sodium dehydrocholate, 20 mg per 100 gm body weight, sodium hippurate, 7 mg per 100 gm body weight, sodium salicylate, 6 mg per 100 gm body weight, or liver hydrolysate (Proheparum, Nordmark-Werke-Kaken), 0.03 ml per 100 gm body weight, was administered intravenously. The amount of BSP excreted in the bile was determined spectrophotometrically and its percentage to the total amount injected was calculated.

2. Experiments with a constant infusion technique of BSP.

Using a constant infusion pump, BSP in normal saline solution was infused intravenously during one hour at a constant rate of 5 to 20 mg per hour per 100 gm body weight into rats in which the common bile duct had been cannulated. Either sodium dehydrocholate (10 mg per 100 gm body weight), sodium hippurate (3.5 mg per 100 gm body weight), sodium salicylate (3 mg per 100 gm body weight) or liver hydrolysate (0.03 ml per 100 gm body weight) was also infused during this period dissolved in the solution of BSP.

The bile obtained during 50 minutes following the start of the BSP infusion was used for determination of the conjugation rate of BSP. The maximal biliary transport rate of BSP (Tm) was estimated from the bile obtained between 50 to 60 minutes following the beginning of the BSP infusion.

The rats were sacrificed through cardiac puncture at 60 minutes following the start of the BSP infusion, blood was obtained for determination of serum BSP concentration, and the livers were removed for determination of their BSP content.

3. Estimation of BSP.

(1) Concentration of BSP in serum was estimated by Duebler's method[5].

(2) For analyzing the BSP in bile, samples were made alkaline with 10 ml of 0.1 N KOH and diluted 1,000 or 2,000 times with distilled water. Then the concentration of BSP in these diluted samples was determined by the same method that used in the determination of serum BSP.

(3) For analyzing BSP in the liver, triplicated samples of liver weighing approximately 1 gm each were homogenized with 2 ml of ice cold acetone in a homogenizer with a glass pestle. Three ml of 80 per cent acetone were added to the contents of each tube and the contents were then transfered to a glass-stoppered conical centrifuge tube. The contents were thoroughly mixed and the tube was centrifuged for 10 minutes at 4,500 rpm. The supernate was removed and the precipitate was extracted twice with 80 per cent acetone. The supernates were pooled, and the volume was recorded. Duplicated aliquots of the combined acetone extract were mixed with 0.1 N KOH to a final volume

of 4.15 ml, and the optical density was determined using 0.1 N KOH as a blank in a spectrophotometer first at 580 mμ and then at 620 mμ. The optical densities of 150 μl of BSP standards mixed with 4 ml of 0.1 N KOH were similarly determined. The alkalinized acetone extracts exhibited varying amounts of turbidity. The optical density at 580 mμ of similarly turbid alkalinized acetone extract of homogenate averaged 1.20 times the optical density at 620 mμ. Thus BSP extracted per gram of liver wet weight was calculated as follows :

$$\frac{\text{O.D. } 580 - 1.20 \text{ O.D. } 620 \text{ unknown}}{\text{O.D. } 580 - 1.20 \text{ O.D. } 620 \text{ standard}} \times \frac{\text{Volume of standard (150 } \mu\text{l) in 4.15 ml}}{\text{Volume of unknown (in } \mu\text{l) in 4.15 ml}} \times$$

Concentration of standard in mg per 100 ml ×
Volume of acetone extract in fractions of 100 ml /
Weight of liver sample in grams.

The data were expressed as mg BSP contained per gm liver wet weight per 100 gm body weight. Since recovery of unknown amounts of BSP from liver averaged 70 per cent, the above values were multiplied by 100/70.

(4) Chromatography of BSP.

The chromatographic technique and the methods of quantitating BSP on chromatograms were the same as have been described by Combes and Stakelum,[6] although an ascending chromatoraphic technique instead of decending one was used.

4. Effects of drugs on BSP-glutathione conjugating system.

(1) Experiments on liver slices.

Half a gram of liver slices, in 5.0 ml of 0.1 M pyrophosphate buffer, pH 7.4, were incubated in test tubes with 1×10^{-4} M BSP, 2×10^{-4} M glutathione, and other substrates at 37°C for 30 minutes under oxygen supply. The substrates were 1×10^{-4} M, 3×10^{-4} M and 5×10^{-4} M of sodium dehydrocholate, sodium hippurate and sodium salicylate, and 1.0 ml and 3.0 ml of liver hydrolysate.

Incubation was terminated by the addition of 0.36 ml saturated ammonium sulfate and 5 ml of absolute ethanol. After thorough mixing and standing for 20 minutes at room temperature, the tubes were centrifuged at 2,500 rpm for 10 minutes.[7] Then the supernates were chromatographed to estimate the conjugation rate of BSP.

(2) Experiments on liver supernatant.

Enzyme fraction which conjugates BSP with glutathione was prepared from rat liver according to the method of Booth and his co-workers.[8] Then the effects of drugs on the above enzyme fraction were examined by the same incubation method as that used in the experiments on liver slices.

5. Effects of drugs on BSP-binding affinity of plasma albumin.

Crystalline bovine serum albumin was made 100 mg per 100 ml solution of

1/15 M, pH 7.4 phosphate buffer.

Equilibrium dialysis was performed by a method similar to that described by Klotz and his co-workers[9]. Cellophane dialysis bags (20/32″, Visking Co.) were filled with 10 ml of bovine serum solution. The bags were suspended in test tubes containing 50 ml of solution of BSP (10 mg per 100 ml) and one of the other substrates in 1/15 M, pH 7.4 phosphate buffer. The substrates were 2 ml of 1/4 M sodium dehydrocholate, sodium hippurate, and sodium salicylate, or 2 ml of liver hydrolysate.

Dialysis was carried out for 17 hours at 4°C while the tubes were shaken constantly by means of a motor-driven shaker. The concentration of BSP in the contents of the bags and in the outside buffer solution was determined at the end of the dialysis period. The concentration of protein-bound BSP in the bags was calculated by substracting the value of BSP concentration in the outside solution from the BSP concentration in the solution inside the bag.

6. Effects of drugs on BSP release from BSP-bound rabbit serum.

Two ml of 5 per cent BSP solution and 43 ml of 1/15 M, pH 7.4 phosphate buffer were added to 5 ml of rabbit serum. The serum was thoroughly saturated with BSP by shaking for 30 minutes at 4°C. Then unbound BSP remaining in the serum was dialyzed after shaking for 10 to 12 hours at 4°C.

Cellophane dialysis bags were filled with 5 ml of the BSP-bound protein solution and 5 ml of substrate solution in 1/15 M, pH 7.4 phosphate buffer, which has been described above. Dialysis was carried out by shaking constantly for 17 hours at 4°C. The optical density of the outside buffer solution at 575 mμ was determined spectrophotometrically.

RESULTS

I. Biliary excretion of BSP following a single intravenous administration of BSP.

As shown in Figs. 1 and 2, the volume of bile output during one hour in the control rats with a biliary canula of 0.31 mm in diameter and that of those with a biliary canula of 0.62 mm in diameter was 0.38 and 0.46 ml, respectively. The biliary excretion rate of BSP in the control rats in the former was 18.2 per cent and in the latter was 23.9 per cent.

The volume of bile output was increased by an intravenous administration of sodium dehydrocholate, sodium hippurate, sodium salicylate or liver hydrolysate as much as 4.7, 1.6, 1.4 and 1.6 fold, respectively.

Biliary excretion rate of BSP was increased by each intravenous administration of sodium dehydrocholate, sodium hippurate, sodium salicylate or liver hydrolysate as much as 1.3, 1.5, 1.5 and 1.8 fold, respectively.

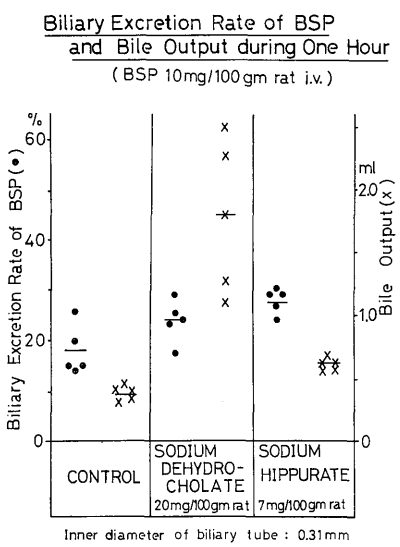

Fig. 1.

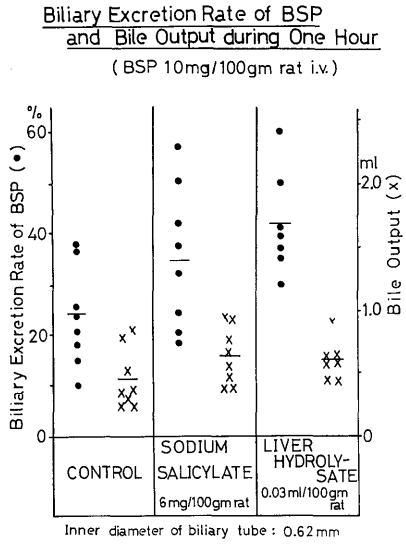

Fig. 2.

II. Experiments with a constant infusion technique of BSP.

1. Relationship between serum BSP concentration and biliary BSP excretion (Estimation of biliary transport maximum, Tm).

As shown in Figs. 3 and 4, biliary excretion of BSP per minute reached a maximum at the serum BSP level of 20 to 30 mg per 100 ml. When the maximal point of biliary excretion of BSP was regarded as the maximal transport rate of BSP into the bile (biliary BSP-Tm), an intravenous administration of sodium dehydrocholate elevated the BSP-Tm 1.5 times and sodium hippurate elevated it 2.1 times. Neither sodium salicylate nor liver hydrolysate altered it.

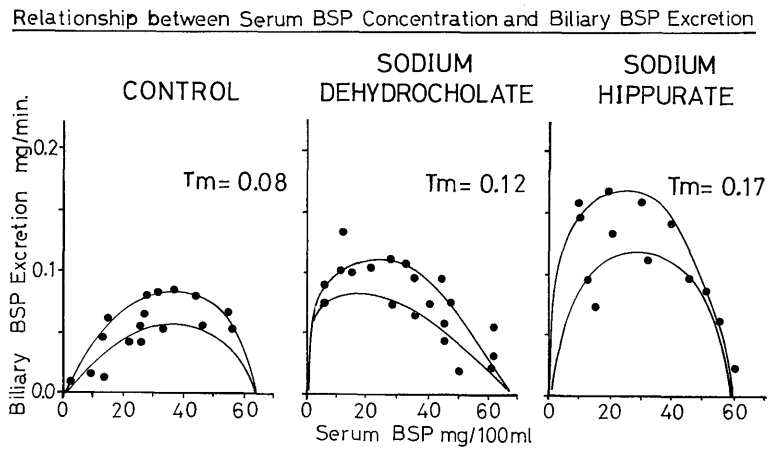

Fig. 3.

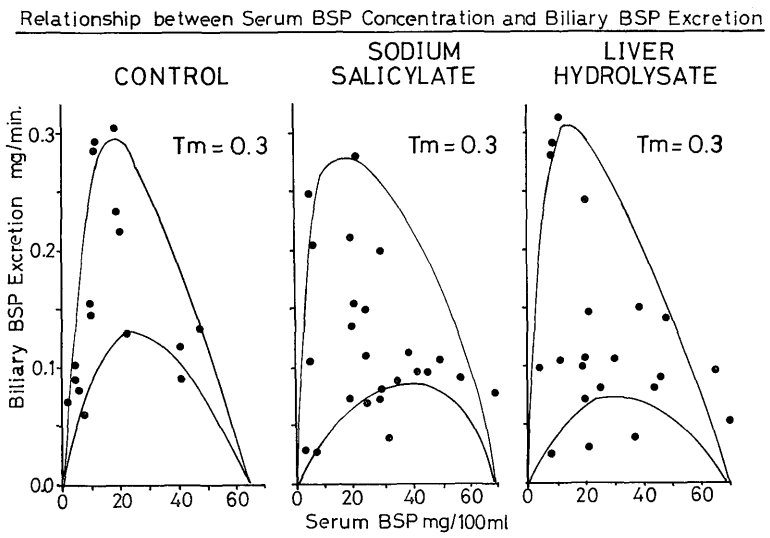

Fig. 4.

2. Relationship between biliary content of BSP and conjugation rate of biliary BSP.

As shown in Figs. 5 and 6, conjugation rate of biliary BSP in the control rats with a biliary canula of 0.31 mm in diameter ranged from 60 to 85 per cent regardless of biliary BSP concentration and that of those with a biliary canula of 0.62 mm in diameter ranged from 65 to 90 per cent.

Neither sodium dehydrocholate nor sodium hippurate altered the conjugation rate of biliary BSP, but both sodium salicylate and liver hydrolysate reduced it to some extent.

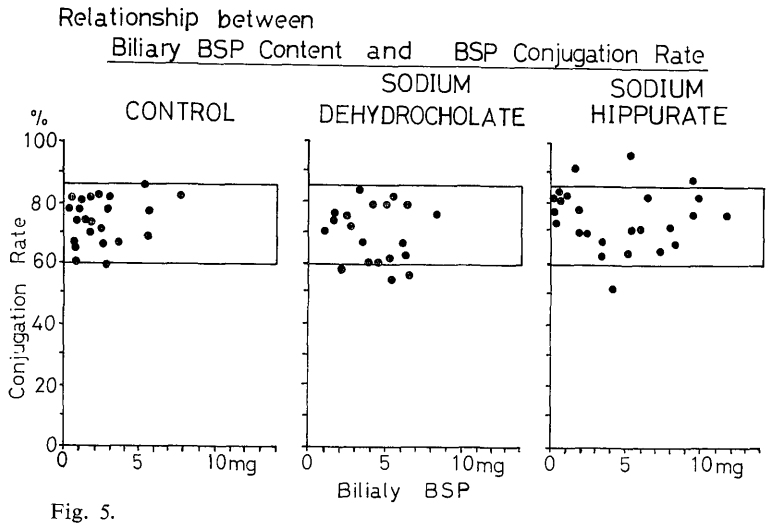

Fig. 5.

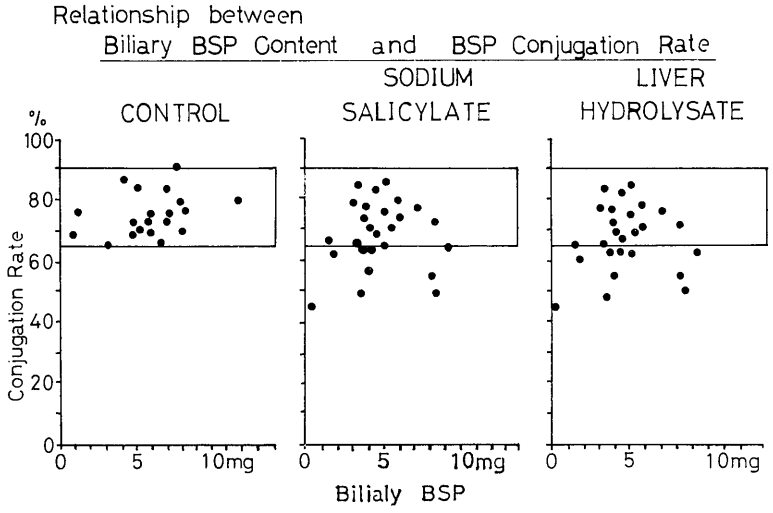

Fig. 6.

3. Relationship between serum concentration of BSP and hepatic content of BSP.

As shown in Figs. 7 and 8, hepatic content of total BSP (BSP storage) was almost directly proportional to the serum concentration. Hepatic content of conjugated BSP was also almost directly proportional to the hepatic content of total BSP.

Hepatic BSP storage was increased greatly as a result of marked increase in the amount of free BSP through either intravenous administration of sodium hippurate, sodium salicylate or liver hydrolysate, whereas it was decreased distinctly accompanied with both marked decrease in the amount of free BSP and slight decrease in conjugated BSP by an intravenous administration of sodium dehydrocholate.

4. Effects of drugs on BSP-glutathione conjugation system.

As shown in Fig. 9, conjugation of BSP with glutathione in the liver slices experiments was greatly accelerated by the addition of sodium dehydrocholate. Furthermore, it was accelerated more as the concentration of the drug was increased. Addition of sodium hippurate, sodium salicylate or liver hydrolysate did not increase the conjugation of BSP.

As shown in Fig. 10, conjugation of BSP with glutathione in the liver supernatant experiments was greatly accelerated by the addition of either sodium

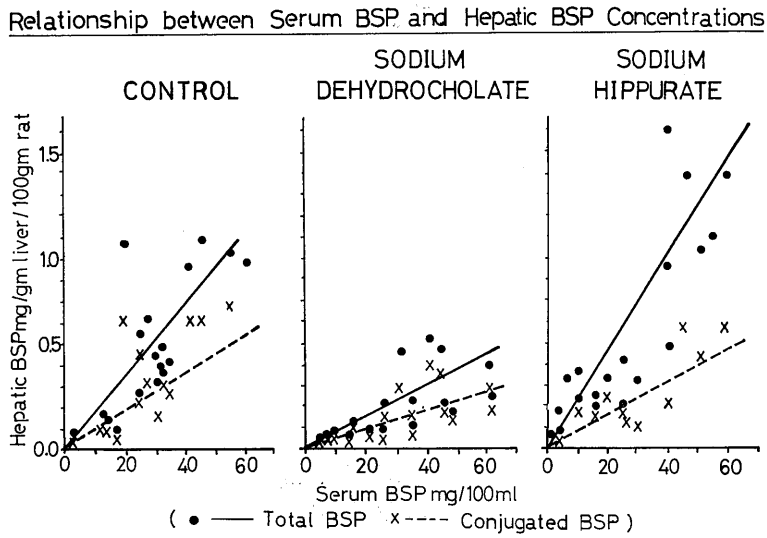

Fig. 7.

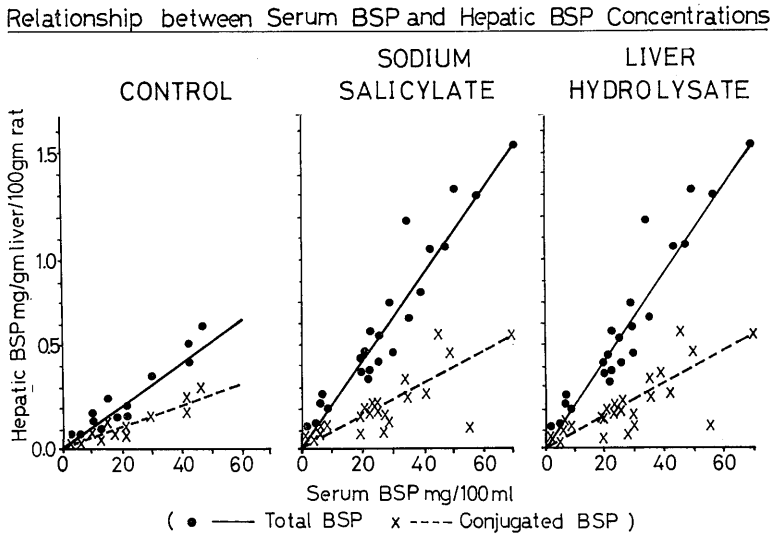

Fig. 8.

dehydrocholate or sodium hippurate, and acceleration increased as the concentration of the drug was increased. An addition of either sodium salicylate or liver hydrolysate did not increase the conjugation of BSP.

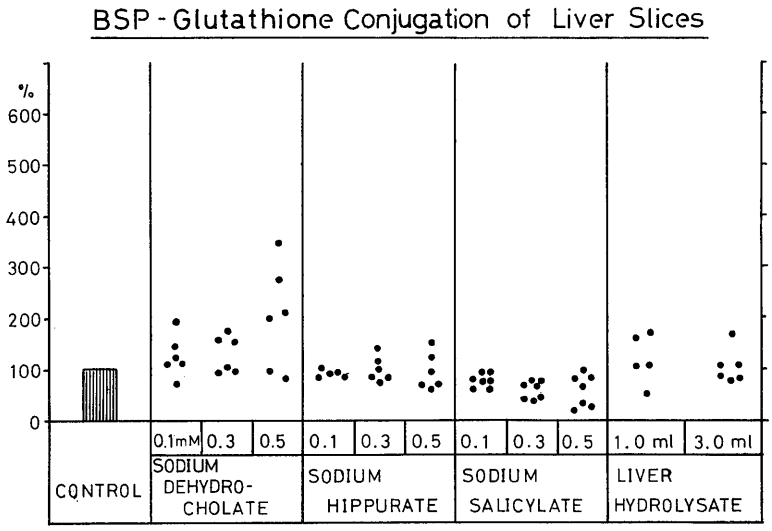

Fig. 9.

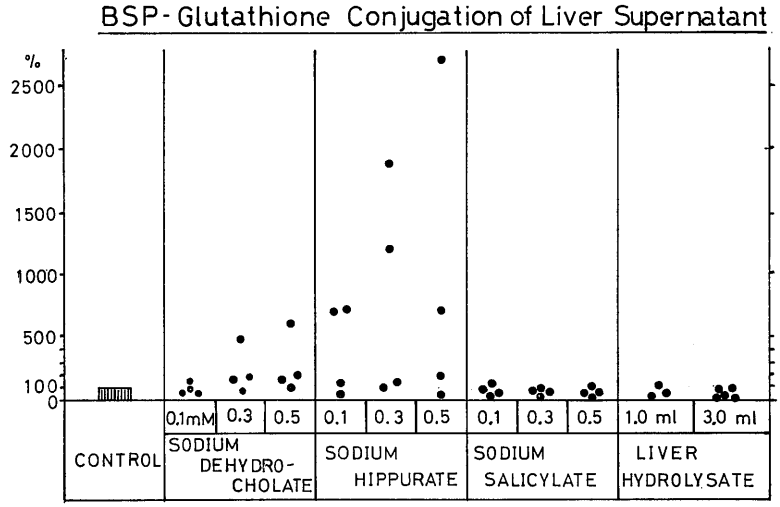

Fig. 10.

5. Effects of drugs on BSP-binding affinity of serum albumin.

As shown in Fig. 11, the BSP binding affinity of bovine serum albumin was reduced markedly by the addition of either sodium dehydrocholate or sodium salicylate and reduced slightly by the addition of sodium hippurate, but it was not influenced by the addition of liver hydrolysate.

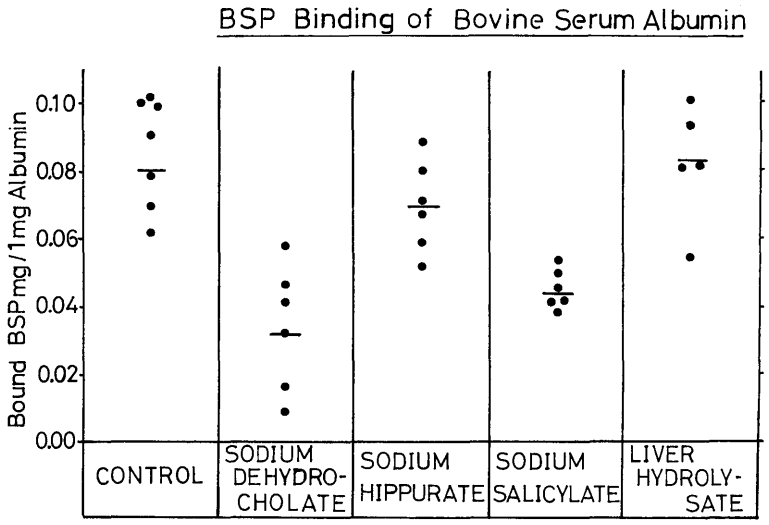

Fig. 11.

6. Effects of drugs on BSP-release from BSP-bound rabbit serum.

As shown in Fig. 12, a large amount of BSP was released from BSP-bound rabbit serum by the addition of sodium salicylate. A small amount of BSP was released from the serum by the addition of either sodium dehydrocholate, sodium hippurate or liver hydrolysate.

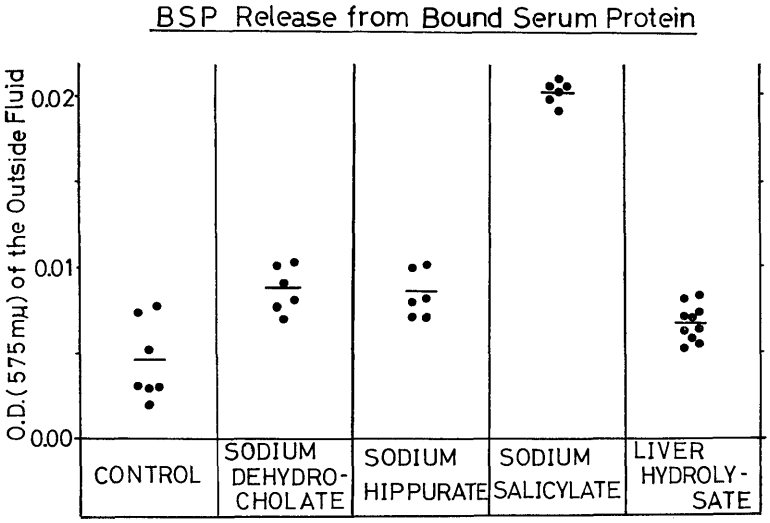

Fig. 12.

DISCUSSION

In rat experiments, biliary excretion of BSP was increased by the intravenous administration of sodium dehydrocholate, sodium hippurate, sodium salicylate or liver hydrolysate. The present studies were undertaken to exmine the altered steps involved in biliary excretion of BSP following an intravenous administration of each of these drugs. The summarized data is shown in Table 1.

Table 1. Summary of the Experimental Data

	Biliary excretion of BSP	Volume of bile output	Biliary BSP-Tm	Conjugation rate of biliary BSP	BSP-storage of the liver	BSP-glutathione conjugation system		Protein-BSP binding system	
						Liver slices	Liver supernatant	BSP-binding affinity	BSP release
Sodium dehydrocholate	increased ×1.3	increased ×4.7	elevated ×1.5	unchanged	decreased	activated	activated	inhibited	slightly increased
Sodium hippurate	increased ×1.5	increased ×1.6	elevated ×2.1	unchanged	increased	unchanged	activated	slightly inhibited	slightly increased
Sodium salicylate	increased ×1.5	increased ×1.5	unchanged	slightly decreased	increased	unchanged	unchanged	inhibited	increased
Liver hydrolysate	increased ×1.8	increased ×1.6	unchanged	slightly decreased	increased	unchanged	unchanged	unchanged	slightly increased

Important factors which increase biliary excretion of BSP by the administration of sodium dehydrocholate were a distinct increase of bile output, a distinct acceleration of BSP-glutathione conjugating enzyme activity and a moderate elevation of biiary BSP-Tm. In addition, an inhibition of BSP-binding affinity of serum protein might have a role in this mechanism, although BSP could not be released from BSP-bound serum. Storage of BSP in the liver was reduced significantly associated with a marked decrease in the amount of free BSP and a moderate decrease in conjugated BSP, suggesting an increased permeability of the hepatic cell membrane especially on the excretory side.

Most authors have reported that sodium dehydrocholate has inhibited the biliary excretion of BSP[10)11)12)] and we also found a similar phenomena in the excretion of phenolsulfonphthalein.[1)] The contradictory data which was observed in this experiment seems to be explained by the fact that a different sized dose of sodium dehydrocholate was administered in this experiment and that the phase of inhibition in biliary excretion of BSP might have been demonstrated in the early stage following the administration of the drug.

The increase in biliary excretion of BSP is possibly brought about by an increase of hepatic circulation as sodium dehydrocholate increases hepatic blood flow.[13)] Dopans and his co-workers,[14)] however, did not observe any increase in BSP clearance though they perfused an isolated pig liver at higher flow rate.

After intravenous administration of sodium hippurate, a distinct elevation of biliary BSP-Tm, a significant increase of BSP storage in the liver associated with a marked increase in the amount of free BSP, and a moderate degree of choleresis were demonstrated. A slight degree of inhibition of BSP-binding affinity of serum protein and release of BSP from BSP-bound serum was demonstrated by the addition of sodium hippurate.

In the experiment using liver supernatant, in contrast to that using liver slices, a significant acceleration of BSP-glutathione conjugating enzyme activity was demonstrated by the addition of sodium hippurate. The conjugation of BSP, however, is not important in the steps of biliary excretion of BSP.

The mechanism causing the elevation of the biliary BSP-Tm is not clear but an increase of permeability on the excretory side of hepatic cell membrane may be an important factor involved in it. Thus, the above facts suggest that an increased permeability of the hepatic cell membrane on both uptake and excretory side is a single factor which brings about an increased biliary excretion of BSP following an intravenous administration of sodium hippurate.

A significant increase of free BSP storage in the liver and a moderate degree of choleresis were demonstrated after intravenous administration of sodium salicylate. However, the most important factor to increase biliary excretion of BSP seems to be a competition of sodium salicylate against BSP in the mechanism of protein binding, resulting in an increased hepatic uptake of unbound

BSP. No acceleration of BSP-glutathione conjugating enzyme activity and elevation of biliary BSP-Tm was observed.

Schmid and his co-workers[15] have demonstrated that sodium salicylate inhibited the bilirubin-binding affinity of plasma albumin, resulting in a distinct decrease of hyperbilirubinemia. A similar mechanism may exist in the case of biliary excretion of BSP following administration of sodium salicylate.

A significant increase of free BSP storage in the liver and a moderate degree of choleresis were demonstrated after intravenous administration of liver hydrolysate. No acceleration of BSP-glutathione conjugating enzyme activity, elevation of biliary BSP-Tm, inhibition of BSP-binding affinity of serum albumin and release of BSP from BSP-bound rabbit serum were demonstrated.

The above facts suggest that the most important factor to increase biliary excretion of BSP following an intravenous administration of liver hydrolysate may be an increased hepatic storage of BSP, namely, increased permeability of hepatic cell membrane especially on the uptake side from the blood stream.

Conjugation of BSP with glutathione has been regarded as a major pathway in the transfer of BSP from plasma to bile.[16] The evidence, however, obtained in this experiment tends to depreciate its role, because unconjugated BSP, with or without a minor increase in conjugated BSP, is easily transfered from plasma to bile by the administration of certain drugs.

CONCLUSION

Animal experiments were undertaken to examine the alterations of steps involved in the transfer of BSP from blood to bile after the intravenous administration of sodium dehydrocholate, sodium hippurate, sodium salicylate or liver hydrolysate.

1) Biliary excretion of BSP was increased by intravenous administration of all the drugs.

2) Through the intravenous administration of sodium dehydrocholate, a distinct increase of bile output, a distinct acceleration of BSP-glutathione conjugating enzyme activity, and an inhibition of BSP-binding affinity of serum albumin were observed. Storage of BSP in the liver was reduced distinctly associated with a marked decrease in the amount of free BSP and a moderate decrease in conjugated BSP.

3) Through the intravenous administration of sodium hippurate, a distinct elevation of biliary BSP-Tm, a significant increase of unconjugated BSP storage in the liver and a moderate degree of choleresis were demonstrated. A slight degree of inhibition of BSP-binding affinity of serum albumin and release of BSP from BSP-bound serum was demonstrated. A significant acceleration of

BSP-glutathione conjugating enzyme activity was demonstrated.

4) Through the intravenous administration of sodium salicylate, a significant increase of unconjugated BSP storage in the liver and a moderate degree of choleresis were demonstrated. Neither acceleration of BSP-glutathione conjugating enzyme activity nor elevation of biliary BSP-Tm was demonstrated. Inhibition of BSP-binding affinity of serum albumin and release of BSP from BSP-bound serum were distinctly demonstrated in vitro.

5) Through the intravenous administration of liver hydrolysate, a significant increase of free BSP storage in the liver and a moderate degree of choleresis were observed. An acceleration of BSP-glutathione conjugating enzyme activity, elevation of biliary BSP-Tm, inhibition of BSP-binding affinity of serum albumin and release of BSP from BSP-bound serum were absent.

6) It was concluded that each of the above factors is individually involved in the steps of the increased movement of BSP from blood to bile.

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