Reduced Bile Flow After Sulfobromophthalein Administration in the Rat[1] (35054)

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The transport of organic anions into bile against a concentration gradient generally leads to an increase in bile flow due to the osmotic effect (1). Such a choleretic effect has been described in the rat for various dyes, including phenol red (1), fluoroscein (1), and sulfobromophthalein (BSP) (2). On the other hand, under certain circumstances, a diminution of bile flow has resulted from secretion of anionic dyes into the bile. Infusion or injection of indocyanine green (ICG) produces a dose-related diminution in bile flow in the rat and rabbit, although not noticeably so in the dog (3). In man, an increase in biliary BSP concentration has been reported to be correlated with a decrease in bile flow (4). We have observed diminution of bile flow in the rat when inhibition of BSP conjugation with glutathione (GSH) has led to elevated biliary concentration of unconjugated BSP (5, 6). Diminution of bile flow due to ICG and BSP excretion has also been observed in the isolated perfused rat liver (7).

In a series of experiments designed to study the interaction between biliary excretion pathways for BSP and related dyes, some further observations were made on the effect of BSP on bile flow.

Methods. Experiments were performed using Charles River CD strain rats (300–350 g) under pentobarbital (50 mg/kg ip) anesthesia. Body temperature was maintained at 37° throughout the experiments, to eliminate temperature-dependent changes in bile flow (8).

BSP (Dade Laboratories), its dibrominated analog, phenoldibromphthalein disulfonate (DBSP, Hynson, Wescott & Dunning) and synthetic BSP-GSH were dissolved in saline and injected or infused via a femoral vein cannula. Synthetic BSP-GSH was prepared by the method of Whelan *et al.* (9). Analysis by TLC on cellulose using the upper phase of *n*-butanol:acetic acid:water (4:1:2) as a developing solvent revealed only one colored contaminant, presumably BSP-$(GSH)_2$ (9), in amounts less than 10%; DBSP was chromatographically homogeneous in the same TLC system.

Bile flow was estimated gravimetrically in 10-min samples collected from a biliary cannula throughout the infusion period. Basal bile flow was estimated from two 5-min collections immediately prior to commencing the infusion of dye. After 60 min of infusion, BSP, BSP-GSH, or DBSP (100 mg/kg) was rapidly (30–60 sec) injected into the venous cannula and the infusion was continued for a further 60 min. Dye concentrations in 5-μl aliquots of bile were determined colorimetrically after separation of the components by TLC (5).

Results and Discussion. Figure 1 shows the patterns of bile flow observed in these experiments. Basal bile flow varied from rat to rat, therefore a representative curve from each experiment is presented, rather than the mean and standard error for each set of values. Table I summarizes the major alterations in bile flow seen after injection of BSP, BSP-GSH, or DBSP; as shown the effects were quite reproducible.

Rapid BSP injection consistently produced

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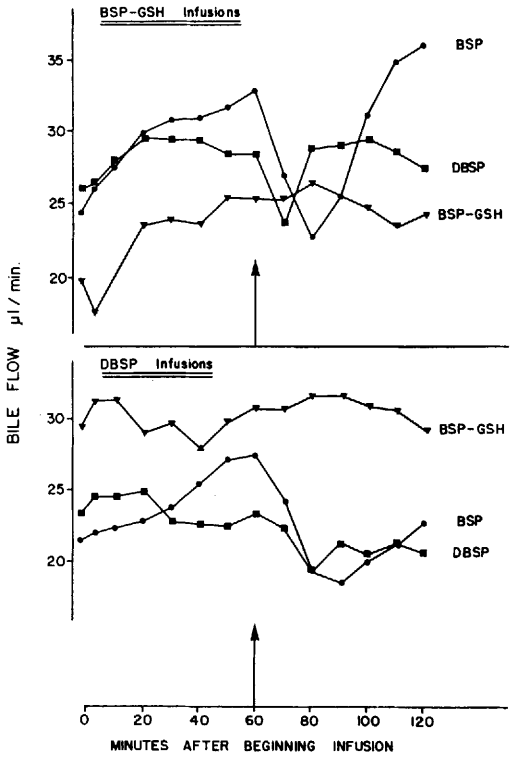

FIG. 1. Bile flow in individual rats infused for 2 hr with either BSP-GSH (3.5 mg/min/kg) or DBSP (5 mg/min/kg). After 1 hr, 100 mg/kg of BSP, BSP-GSH, or DBSP was rapidly injected (shown by arrow).

a sharp diminution of bile flow, followed by a somewhat varible increase. The tendency to return toward the preinjection bile flow was more marked in BSP-GSH infusion experiments. Rapid DBSP injection also produced diminution of bile flow, although the effect was less marked than that seen with BSP. No diminution of bile flow was observed when synthetic BSP-GSH was rapidly injected; in fact a very slight increase was noted.

The diminution of flow after BSP injection in BSP-GSH-infused rats correlated temporally with the appearance of unconjugated BSP in the bile (Table II), whereas no unconjugated BSP was detected in the bile after BSP-GSH or DBSP injection. Correlation of bile flow changes with changes in BSP-GSH, the major biliary component dye, seems less likely. Analysis of variance revealed that biliary BSP-GSH during BSP-GSH infusion was not significantly altered by BSP injection. This finding substantiates previously reported data (5, 6) which indicate that excretion of increased amounts of unconjugated BSP in rats results in reduced bile flow.

In DBSP-infused rats, the correlation is less clear. Maximal effects on bile flow were apparent 10–20 min after BSP injection, yet unconjugated BSP and BSP-GSH concentrations were still rising 40 min after injection. The correlation is probably complicated by the apparent effect of DBSP itself on bile flow (Fig. 1, Table I). Unlike BSP, the sole biliary component of DBSP appears to be the unconjugated moiety (10, 11). The results obtained in DBSP experiments suggest that the diminution in bile flow observed with BSP and DBSP are related to the properties of the unconjugated moieties.

Some experiments were also carried out where BSP was infused (2.5 mg/min/kg) instead of BSP-GSH or DBSP. However, the effects of rapidly injected BSP, BSP-GSH or

TABLE I. Percentage Change in Bile Flow After Rapid Injection of Dyes.[a]

Dye injected after 60 min	BSP-GSH infusions (3.5 mg/min/kg)	DBSP infusions (5 mg/min/kg)
BSP	−31 (20)	−33 (20)
	−31 (40)	−32 (30)
	−44 (20)	−28 (20)
	−55 (20)	−28 (20)
BSP-GSH	+ 4 (20)	+ 1 (20)
	+ 5 (20)	+ 2 (20)
		+ 5 (20)
DBSP	−14 (20)	
	−16 (10)	−17 (20)
	−16 (10)	

[a] Infusion of BSP-GSH or DBSP was continuous over 2 hr in each rat. Bile flow was calculated from serial 10-min samples. After the 1st hr of infusion, 100 mg/kg BSP, BSP-GSH, or DBSP was rapidly injected into the venous cannula. The percentage change in bile flow in each rat was calculated from the difference between flow just prior to injection (60-min sample), and the maximal observed effect on flow. Numbers in parentheses are the minutes after injection, when the maximum effect was observed in each rat.

TABLE II. Biliary Dye Concentrations Before and After BSP Injection.[a]

Infusion (min):		60	70	80	100
BSP-GSH infusions	U-BSP[b]	0	0.99 ± 0.09	2.17 ± 0.20	1.34 ± 0.17
	BSP-GSH[b]	10.06 ± 0.57	8.68 ± 0.56	8.34 ± 0.99	9.12 ± 1.10
DBSP infusions	U-BSP	0	0.18 ± 0.11	1.26 ± 0.05	1.34 ± 0.17
	BSP-GSH	0	0.39 ± 0.04	1.93 ± 0.20	3.52 ± 0.41

[a] Infusion of BSP-GSH or DBSP was continuous over 2 hr in each rat. After the first hour of infusion, 100 mg/kg of BSP was rapidly injected into the venous cannula. Each value represents the mean ± SE of 3–4 rats, expressed in mg/ml.

[b] U-BSP = unconjugated BSP; BSP-GSH = BSP glutathione.

DBSP were masked by a consistently declining bile flow from 40-50 min after infusion began until the end of the experiment. This phenomenon has been briefly reported elsewhere (6), and is presumably due to rising biliary concentrations of unconjugated BSP resulting from hepatic glutathione depletion.

Merely speculative explanations may be advanced for these phenomena at this stage. It seems unlikely that transport of bile salts would be sufficiently deranged to account for the effect on bile flow. O'Maille *et al.* (12) reported very little alteration in the biliary output of taurocholate under conditions of maximal BSP excretion in the dog. On the other hand, formation of micelles in the bile appears to be an important factor for the choleretic properties of bile salts. The "nonphysiological" bile salt derivative, sodium dehydrocholate, does not form micelles in the concentration range encountered in bile under experimental conditions, yet it is a more effective choleretic than sodium taurocholate, which does form micelles (1). One could speculate that unconjugated BSP might influence bile salt micelle formation *in vivo*, although this would be difficult to prove experimentally.

The effect on bile flow may be related to the colligative properties of the dyes themselves. The phenomenon is seen with ICG and DBSP, dyes which are excreted without conjugation, and with unconjugated BSP, but not BSP-GSH. Baker (13) has shown that ICG forms polymers in aqueous solution, a characteristic which could influence both its solubility and osmotic properties. Under the same *in vitro* conditions, BSP does not give any evidence of polymerization (13). Similar data for DBSP are not available.

Mediation of the effect by humoral influences also seems unlikely, although Homsher and Cotzias (14) have shown a depressive effect of pitressin on bile flow in the rat. However, demonstration of dye-induced changes on bile flow in the isolated perfused liver (7) tends to rule out an extrahepatic mechanism.

It is now generally recognized that active secretion of bile salts into bile is not the only factor determining bile flow. A second mechanism involves secretion of electrolytes and water across the biliary epithelium, and this mechanism is sensitive to an inhibitor of sodium transport (15). It remains to be shown that unconjugated BSP influences this factor while BSP-GSH does not.

While a specific mechanism for the dye-induced diminution of bile flow cannot be advanced at this stage, further investigation of this phenomenon may shed some light on mechanisms of intrahepatic cholestasis.

Summary. During continuous infusion of dyes into rats, rapid injection of BSP produced a transient depression in bile flow rate, whereas rapid injection of the synthetically conjugated moiety (BSP-GSH) produced no change in bile flow. Rapid injection of DBSP, a BSP analog excreted without biotransformation, produced a less marked change in bile flow. These findings suggest that colligative, or other properties specific to unconjugated dyes, may affect bile flow.

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1. Sperber, I., "The Biliary System," p. 457. Blackwell, Oxford (1965).

2. Klaassen, C. D., and Plaa, G. L., J. Pharmacol. Exp. Ther. **161**, 361 (1968).

3. Klaassen, C. D., and Plaa, G. L., Toxicol. Appl. Pharmacol. **15**, 374 (1969).

4. Schoenfield, L. J., McGill, D. B., and Foulk, W. T., J. Clin. Invest. **43**, 1424 (1964).

5. Priestly, B. G., and Plaa, G. L., Proc. Soc. Exp. Biol. Med **132**, 881 (1969).

6. Priestly, B. G., and Plaa, G. L., J. Pharmacol. Exp. Ther., **174**, 221 (1970).

7. Groszmann, R. J., Kotelanski, B., Kendler, J., and Zimmerman, H. J., Proc. Soc. Exp. Biol. Med. **132**, 712 (1969).

8. Roberts, R. J., Klaassen, C. D., and Plaa, G. L., Proc. Soc. Exp. Biol. Med. **125**, 313 (1967).

9. Whelan, G., Hoch, J., and Combes, B., J. Lab. Clin. Med. **75**, 542 (1970).

10. Javitt, N. B., Proc. Soc. Exp. Biol. Med. **117**, 254 (1964).

11. Klaassen, C. D., and Plaa, G. L., Amer. J Physiol. **215**, 971 (1968).

12. O'Maille, E. R. L., Richards, T. G., and Short, A. H., J. Physiol. (London) **186**, 424 (1966).

13. Baker, K. J., Proc. Soc. Exp. Biol. Med. **122**, 957 (1966).

14. Homsher, E., and Cotzias, G. C., Nature (London) **208**, 687 (1965).

15. Erlinger, S., Dhumeaux, D., and Benhamon, J. P., Nature (London) **223**, 1276 (1969).

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