Effect of Sulfobromophthalein and Indocyanine Green on Bile Excretion (34294)

R. J. GROSZMANN,[1] B. KOTELANSKI,[1] J. KENDLER, AND H. J. ZIMMERMAN[2]

Medical Service, Veterans Administration Hospital and Department of Medicine, Boston University School of Medicine, Boston, Massachusetts 02130

The few reports describing the effects on the rate of bile excretion of dyes, used in the evaluation of the liver function, have yielded inconsistent results. Sulfobromophthalein (BSP) has been reported to induce changes in bile flow of man and other animals. These reports have consisted of descriptions of reduction in man (1), increase in rat (2) or sheep (3), and no significant changes in bile flow in the dog (4). On the other hand, Klaassen and Plaa (2) found that indocyanine green (ICG) depressed the bile flow in the rat. Evidence is presented in this paper to indicate that these dyes reduce the bile flow excretion in the isolated perfused rat liver.

Materials and Methods. Animals. Female Sprague-Dawley rats (300–350 g) were used as donors of blood and livers. The animals were kept under standard environmental and dietary conditons and were not fasted prior to experimentation.

Perfusion medium. Blood was drawn from the ventral aorta into heparinized[3] syringes and mixed in a solution of Krebs–Ringer–bicarbonate buffer (pH = 7.4) to form a 17% suspension (v/v) with an hematocrit of 12%. A constant volume of 65 ml was maintained in the system throughout the experiment.

Preparation. The perfusion method of Penhos *et al.* (5) was used with slight modification. Cannulations of the common duct with PE 10 tube and the portal vein with PE 205 (Intramedic, Clay-Adams) were performed under pentobarbital anesthesia (5 mg/kg of body wt). This was followed by hepatectomy, and the isolated liver was then transferred to a perfusion chamber (Metaloglass, Boston).

The entire procedure, from initial cannulation until transfer to the chamber required 4–6 min. To avoid transient anoxia during excision of the liver, oxygenated heparinized buffer was passed through the portal cannula before transfer of the liver to the chamber.

Perfusion. The chamber was thermostatically maintained, at 38 ± 0.5° during the experiment. Blood flow, measured through a calibrated bypass was 45–50 ml/min. Care was taken to keep the hydrostatic pressure of the portal vein at 17 cm of water. Oxygenation was ensured by passing a constant flow of air mixture (O_2:CO_2/95:5%). The liver was perfused for 30 min of equilibration before the addition of BSP[4] or ICG[4] to the system. The BSP was administered in a concentration of 0.15 mg/ml and 0.015 mg/ml of perfusion medium and ICG in a concentration of 0.015 mg/ml. Bile was collected continuously into 20 μl pipettes, and the periods of collection were carefully timed. A total of 18 perfusions were performed. Analysis of variance was carried out according to Snedecor (6).

Results. Effects of dyes on bile excretion rate. Perfusion with a medium containing BSP in a 20 μ*M* concentration led to a significant reduction in the rate of bile excretion. This effect was present only for the first 15 min after the BSP was added to the perfusion system (Fig. 1). Thereafter, bile

[1] Present address: Veterans Administration Hospital, Section of Haemodynamics, Medical Service, Washington, D.C.

[2] Requests for reprints should be addressed to: Dr. H. J. Zimmerman, Chief, Medical Service, Veterans Administration Hospital, Boston, Massachusetts 02130.

[3] Heparin sodium; Upjohn.

[4] Sulfobromophthalein and Cardiogreen, Hynson, Westcott & Dunning, Inc., Baltimore, Maryland.

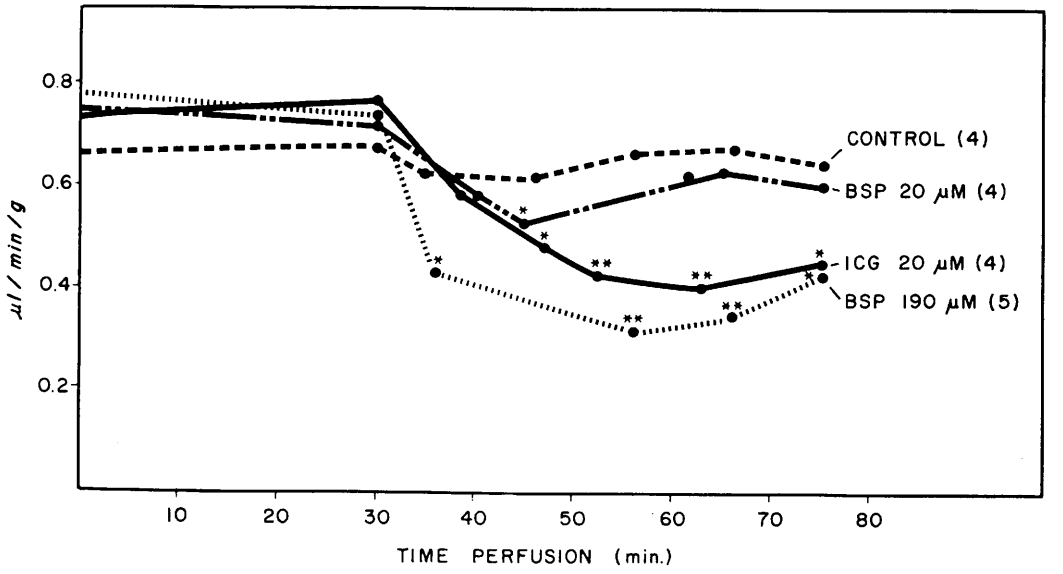

FIG. 1. Effect of BSP and ICG on the mean rate of bile excretion (µl/min/g); numbers of experiments per treatment or control are shown in parentheses. Differences from controls were significant: $p<0.05$ (*); or $p<0.01$ (**).

excretion returned to the previous level. Perfusion with a medium containing 190 µmoles/liter of BSP, however, caused a highly significant depression of the bile flow rate throughout the entire period of perfusion (Fig. 1).

Perfusion with a medium containing ICG in a 20 µ*M* concentration also reduced significantly the rate of bile excretion (Fig. 1). The effect of higher concentrations of this dye could not be evaluated accurately since they led to precipitation of the dye. The decrease in rate of bile flow induced by perfusion with media containing ICG, in a concentration of 20 µmoles/liter, or BSP in a concentration of a 190 µmoles/liter, was also demonstrated by the significantly reduced volume of bile, as measured after 45 min of perfusion (Table I). For the 20 µ*M* concentration of BSP, however, the reduction in "total" volume of bile was too little to be significant. Presumably this reflected the relatively short period (15 min) during which this concentration of BSP depressed bile excretion.

Discussion. It is currently accepted that the primary event in the formation of bile is the active transport of bile acids from hepatocytes into the bile. Presumably, it is the osmotic effect of these anionic substances which results in the passage of water and solutes into the bile canaliculi (7). Many other organic anions are excreted presumably by the same, or similar, active transport mechanism(s) since they appear to complete

TABLE I. Effect of Dyes on Bile Volume and Rate of Excretion.

Treatment	Conc of dye (µ*M*)	*n*	45-min bile vol (µl/g)	Rate of decrease[a] (µl/min/g)
Control	—	5	29.6 ± 1.0	0.04 ± 0.005
ICG	20	4	18.0 ± 0.9[b]	0.208 ± 0.01[b]
BSP	20	4	25.0 ± 2.0	0.205 ± 0.02[b]
	190	5	11.5 ± 1.0[b]	0.325 ± 0.02[b]

[a] Rate of decrease was calculated for the first 10 min after addition of dyes.

[b] Means ± SEM were significantly different ($p<0.005$) from controls.

with each other for excretion into bile (7). For example, the excretion of cholate is inhibited by dehydrocholate (8); and, more pertinent to the present study, the excretion of BSP (or its metabolites) is inhibited by dehydrocholate and cholate (9).

There is strong evidence that the excretion of the anions BSP and ICG is by their transport into bile in a manner similar to that of bile acids (7). The data of the present study indicate that both dyes reduce bile flow in an *in vitro* model. This effect appears to be dose-related (at least for BSP, Fig. 1). These observations are consistent with those of O'Maille *et al.* who showed that BSP competes reciprocally for its excretion with taurocholate (10).

The interference by BSP and ICG with bile flow may be a direct competitive inhibition of transport of bile acids. In this case, the dye would act as an "antagonist" (11) ("competitive inhibitor") (12). Alternatively, these dyes may be replacing the bile acids at the transport sites; and, having entered the canaliculi, they may have a lesser effect on bile flow, thus behaving as "partial agonists" (11). Such agonist rather than simply antagonist behavior is consistent with the reports (2, 3) that continuous infusion of a high concentration of BSP led to increased rate of bile flow.

Indocyanine green, administered in equimolar concentration to BSP appeared to induce a more prolonged reduction of the rate of bile excretion. It is possible that these two exogenous anions behave differently with respect to the physicochemical determinants in their capacity as osmotically-active agents; analogous to the differences in osmotic behavior among the bile acids (13).

Summary. The administration of sulfobromophthalein (BSP) and indocyanine green (ICG) produced a decrease in bile flow, in the isolated perfused rat liver. A dose-related effect is shown for BSP while ICG, in equimolar concentrations, produced a more prolonged inhibitory effect on bile flow and rate of excretion. Possible mechanisms for the effect of these dyes on bile excretion are discussed.

The authors are grateful to Mrs. A. Harinasuta for technical assistance and to Dr. J. Penhos for advice.

1. Schonfield, L. J., McGill, D. B., and Faulk, W. T., J. Clin. Invest. **43**, 1424 (1964).

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

3. Alpert, S., Mosher, M., Shanske, A., and Arias, I. M., J. Gen. Physiol. **53**, 238 (1969).

4. Cantarow, A., Wirts, C. W., Snape, W. J., and Miller, L. L., Am. J. Physiol. **154**, 211 (1948).

5. Penhos, J. C., Wu, C. H., Daunas, J., Reitman, B. A., and Levine, R., Diabetes **15**, 740 (1966).

6. Snedecor, G. W., "Statistical Methods," 5th ed. Iowa State Univ. Press, Ames, Iowa (1956).

7. Schanker, L. S., *in* "Handbook of Physiology" (C. F. Code, ed.), Vol. 5, p. 2433. Williams and Wilkins, Baltimore, Maryland (1968).

8. Bradley, W. B. and Ivy, A. C., Proc. Soc. Exptl. Biol. Med. **45**, 143 (1940).

9. Wirts, C. W., Jr., Cantarow, A., Snape, W. J., and Delserone, B., Am. J. Physiol. **165**, 680 (1951).

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

11. Fingl, E. and Woodberry, D. M., *in* "The Pharmacological Basis of Therapeutics" (L. S. Goodman and L. Gilman, eds.) p. 18. Macmillan, New York (1965).

12. Dixon, M. and Webb, E. C., "Enzymes," p. 316. Academic Press, New York (1964).

13. Sperber, I., Pharmacol. Rev. **11**, 109 (1959).

Received June 25, 1969. P.S.E.B.M., 1969, Vol. 132.