Effects of Ethanol and Taurocholate on Bromsulphthalein Excretion by the Isolated, Perfused Liver[1] (35748)

J. KENDLER, H. BASSAN,[2] AND H. J. ZIMMERMAN

Medical Service, and Liver and Metabolic Research Laboratory, Veterans Administration Hospital, Boston; and Departments of Medicine and Biochemistry, Boston University School of Medicine, Boston, Mass.

Previous studies have shown that perfusion of the isolated rat liver with ethanol leads to a marked reduction of bile flow and bromsulphthalein (BSP) biliary excretion (1). It was further demonstrated that these phenomena closely paralleled each other permitting the inference that ethanol could act on the liver as a direct "cholestatic" agent thereby decreasing the transport of foreign dye into the bile. It has been proposed that BSP effectively competes with bile salts for excretion in bile (2–5). In the perfused rat liver, BSP added as a single bolus significantly decreases the rate of bile flow (6) suggesting perhaps a direct competition with the bile acids.

Taurocholate has been shown to increase both the rate of bile flow and the biliary excretion of BSP by dogs *in vivo* (5) and in the isolated rat liver (7). The present study demonstrates that perfusion of the isolated liver with taurocholate and ethanol, leads to a dissociation between the rate of bile flow and the biliary excretion of BSP.

Experimental. Female, unfasted Sprague–Dawley rats (220–280 g) were anaesthesized with Sodium Pentobarbital (Nembutal, Abbott, 5 mg/100 g body weight). Following cannulations of the bile duct and the portal vein, the liver was excised and perfused according to methods that have been described (1). The perfusion medium consisted of Krebs–Henseleit buffer, gassed with a mixture of O_2:CO_2 (95:5, vol %, pH = 7.4 and contained, per 100 ml, 240 mg glucose, 2.5 g bovine albumin (35% sol., Pentex Biochemicals), and 3,000 USP units of heparin (Liquaemin, Organon). 250 mM/liter of ethanol were added 30 min after beginning the perfusion. An infusion of simultaneous Sodium Taurocholate (Mann Research Labs.) and BSP (Hynson, Westcott, & Dunning, Inc.) was started 45 min later, using a calibrated constant rate pump (Sage Instruments #255-2) that delivered 0.17 μM of BSP/min and 1.0 μM of sodium taurocholate/min. In some experiments, BSP was added at the time of the taurocholate infusion in a separate single dose of 130 μM/liter of perfusate. Samples of perfusate and bile were collected every 5 min for the determination of BSP. Bile was collected in 20 μl pipettes at intervals that were carefully timed. Determinations of BSP were carried out as described previously (1). The mean weight of the livers was 10.0 $\pm$ 0.3 g (SE). Analysis of variance was calculated according to Snedecor (8).

Results and Discussion. A prompt choleresis followed the administration of taurocholate to the perfused untreated livers. The rate of flow of bile increased, within 10 min, from control values of 11 μl/min to a peak of 18 μl/min (Fig. 1) and slowly decreased to 13 μl/min after 45 min of perfusion. Similar increases of bile flow have been reported in the rat liver perfused with blood (7). The slow decrease in the rate of bile flow of control livers, after the peak value, that occurred despite continual infusion of taurocholate, could be attributed to the concurrent presence of BSP in the perfusate and in the liver. Perfusion of livers with ethanol led to a reduction in bile flow, as has previously

[1] Partially supported by Licensed Beverage Industry, Inc.

[2] Present address: Central Hospital, Afula, Israel.

[3] Reprint requests: Dr. H. J. Zimmerman, Chief, Medical Service, Veterans Administration Hospital, Washington, D. C.

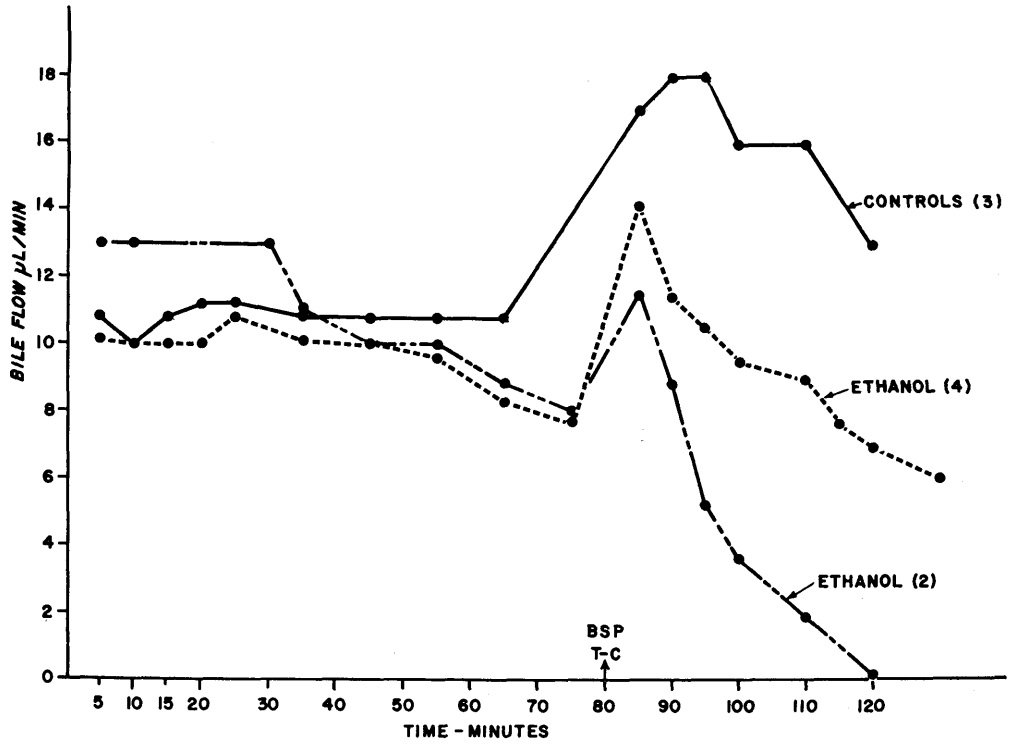

FIG. 1. Excretion of bile by the perfused liver. Taurocholate and BSP were simultaneously infused, in controls and ethanol-treated (4) preparations. In two experiments (the lowest curve) BSP was added as a single bolus. Numbers of experiments are shown in brackets.

been reported (1). However, taurocholate infusion markedly reversed the ethanol-produced "cholestasis" from low values of 7.8 µl/min to a peak flow of 14 µl/min. The flow subsequently declined, paralleling the decrease in the control values but separated from those by a significant difference ($p<0.01$) of 6–7 µl/min. The choleretic effect of taurocholate was of a short duration when BSP was added to the perfusate of ethanol-treated livers in a single dose of 130 µ*M*/liter.

The biliary excretion of BSP in the control perfusions reached peak transport concentrations of 60 µg/min after 30 min of infusion and slowly declined thereafter (Fig. 2).

The total BSP excreted was 2,135 mg, representing approximately 30% of the total dose infused. Livers that were perfused with 250 m*M*/liter concentration of ethanol excreted little BSP in their bile, showing maximum values of only 10 µg/min and maintaining this level of excretion throughout the experiment. Total excretion of dye from these livers accumulated to 0.163 mg or approximately 2.5% of total infused dose. When BSP was given as a single load to the perfusate, during perfusion with ethanol, a higher rate for a shorter period led to a total quantity of BSP in the bile approximately equal to that of the experiments in which the dye had been slowly infused.

The results of this study suggest that the choleretic effect of taurocholate infusion in this *in vitro* system may not change the biliary output of BSP, as the concentration of this dye in the bile of control livers was similar to those found in the absence of taurocholate (1, 7). Taurocholate infusion, however, permitted demonstration of a dissociation between the effects of ethanol on BSP excretion and bile flow. Without taurocholate, the effects of ethanol on BSP excretion and of BSP on bile flow merged to pro-

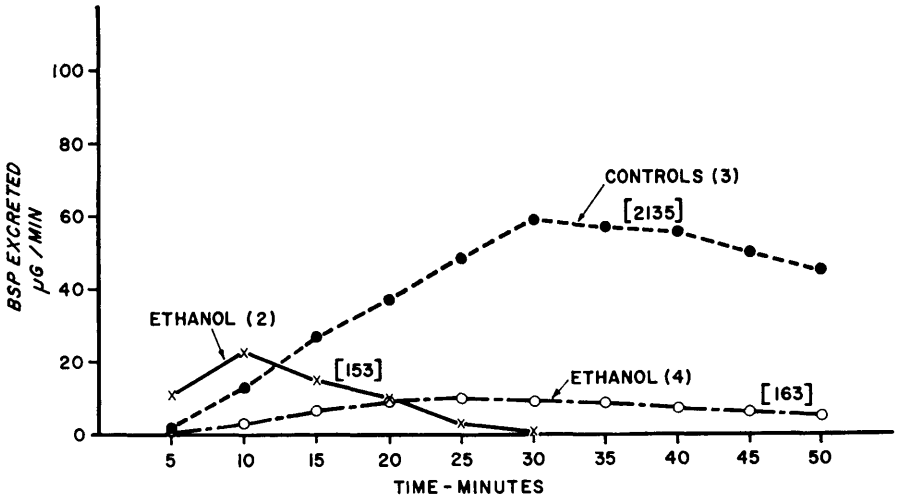

FIG. 2. Rate of BSP biliary excretion by the perfused liver. Total dye excretion is shown in brackets on the respective curves.

duce a pattern of decreased BSP excretion and reduced bile flow. The choleretic effect of taurocholate permitted bile flow to continue at an essentially normal rate, despite which an ethanol-induced impairment of BSP excretion could be demonstrated. Recent studies (9, 10) have indicated that a major portion of the bile excreted by the liver may not be dependent on the canalicular secretion of bile salts. It would appear that in the presence of ethanol, the biliary BSP transport is largely performed by the bile salt-independent fraction of canalicular bile production.

1. Kotelanski, B., Groszmann, R. J., Kendler, J., and Zimmerman, H. J., Proc. Soc. Exp. Biol. Med. 132, 715 (1969).

2. Mendeloff, A. I., Ingelfinger, F. J., and Bradley, S. E., Gastroenterology 13, 222 (1949).

3. Mills, M. A., and Dragstedt, C. A., Arch. Intern. Med. 62, 216 (1938).

4. Wheeler, H. O., and Ramos, O. L., J. Clin. Invest. 39, 161 (1960).

5. O'Maille, E. R. L., Richards, T. G., and Short, A. H., J. Physiol. (London) 186, 429 (1966).

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

7. Boyer, J. L., Scheig, R. L., and Klatskin, G., J. Clin. Invest. 49, 206 (1970).

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

9. Boyer, J. L., Gastroenterology 60, 185 (1971).

10. Klaassen, C. D., Gastroenterology 60, 196 (1971).

Received March 26, 1971. P.S.E.B.M., 1971, Vol. 137