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Increased biliary excretion of pentobarbital with bile salt-induced hepatic choleresis

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Hepatic excretion of drugs metabolized by the liver is a complex process dependent on organ blood flow, hepatocyte uptake, biotransformation within the liver cell, and secretion of either a metabolite or the unmetabolized compound into the biliary canaliculus. When high plasma concentrations of sulfobromophthalein (BSP) and bilirubin are produced, secretion is the limiting process which determines the maximum hepatic capacity to excrete these two frequently studied organic anions [1, 2]. Bile salts are potent choleretic agents whose infusion increases bile flow in all species studied including man [3]. Manipulation of this bile salt-dependent fraction of bile flow increased the maximal hepatic excretory capacity for both BSP and bilirubin [4–6]. The purpose of this study was to determine if bile salt-induced increases in bile flow might also enhance biliary excretion of pentobarbital, an organic anion whose ingestion is frequently abused.

[^{3}H]5-ethyl-5-(1-methylbutyl)-barbituric acid (pentobarbital) was obtained from New England Nuclear and mixed with unlabeled pentobarbital sodium (Vitarine Co., Inc.) and 0.9% saline to give a concentration of 14 mg/ml and a specific activity of 0.08 μCi/mg. Grade A sodium salt of taurocholic acid was obtained from CalBiochem. Male Walter Reed rats (250–350 g) were anesthetized with ether, and external biliary fistulae were constructed and the femoral veins catheterized with PE-10 tubing.* Animals were then placed in restraint cages for overnight biliary drainage to deplete bile salt pools. During this period, animals were infused with 1.5 ml/hr of 0.9% saline to prevent dehydration and were allowed free access to laboratory chow. On the morning after the operation, 40 mg/kg of pentobarbital was given intravenously and bile was collected hourly for 4 hr to monitor biliary excretion of pentobarbital and its metabolites. Rectal temperatures were maintained at 37 ± 1° by heating pads [7]. Control animals were perfused with 1.5 ml/hr of 0.9% saline during the collection period, while experimental animals were perfused with equal volumes of saline containing either 10 or 30 mg sodium taurocholate/1.5 ml.

Bile volume for each collection period was determined gravimetrically. Bile salt outputs were measured using the hydroxysteroid dehydrogenase method as modified by Admirand and Small [8]. Duplicate 25-μl aliquots of bile were counted with a Beckman LS-345 scintillation system. The external standard ratios method was used to correct for quenching. The degree of pentobarbital metabolism and the effect of bile salts on hepatic biotransformation were determined by sequential extractions of bile with petroleum ether and ethyl acetate after addition of 2.0 M, pH 5.0, acetate buffer [9]. Extracts were taken to dryness under nitrogen and chromatographed on Silica gel G in chloroform–acetate (9:1, v/v) [10]. Unmetabolized pentobarbital and pentobarbital metabolites were quantitated by the amount of radioactivity in the various extractions and chromatographic spots. Significance of difference between the three treatment groups was determined, employing Student's *t*-test [11].

Total biliary excretion of pentobarbital and its metabolites in both saline-perfused, bile salt-depleted animals and bile salt-perfused animals showed a linear relation to bile flow (Fig. 1). Table 1 summarizes the effects of bile salt depletion and the two different rates of bile salt perfusion on bile flow, bile salt output, and pentobarbital biliary excretion in the three treatment groups. Pentobarbital biliary excretion was higher in the group perfused with 10 mg/hr of taurocholate than in the bile salt-depleted group but did not reach statistical significance ($P > 0.10$). Excretion was significantly increased in the group perfused with

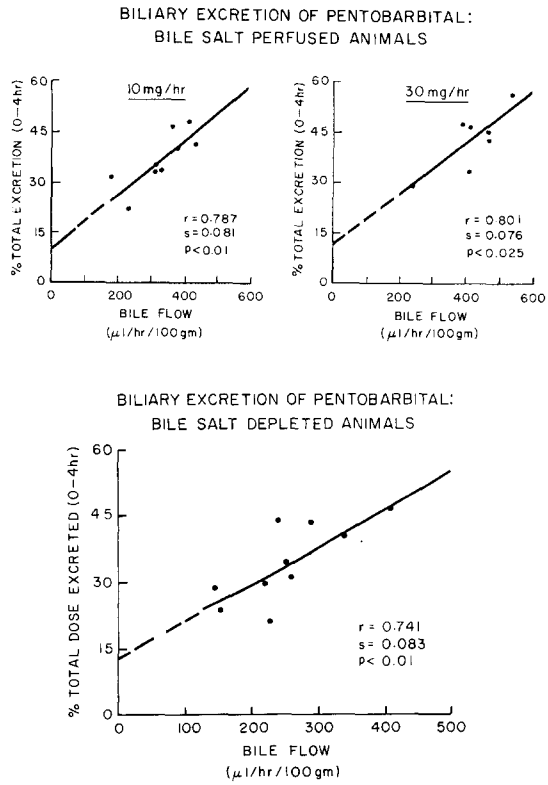

Fig. 1. Relationship of pentobarbital biliary secretion to bile flow. The bottom graph portrays data from saline-perfused, bile salt-depleted animals, while the upper two graphs show results in the animals perfused with 10 or 30 mg/hr of taurocholate. Each point represents an individual animal. The linear regression lines were calculated by the method of least squares; the dotted portions of the lines represent extrapolated rather than experimentally derived data.

* In conducting the research described in this report, the investigators adhered to the "Guide for Laboratory Animal Facilities and Care" as promulgated by the Committee on the Guide for Laboratory Animal Facilities and Care of the Institute of Laboratory Animal Resources, National Academy of Sciences-National Research Council.

Table 1. Effect of bile salt perfusion on bile flow, bile salt output and pentobarbital biliary excretion in the rat

	Bile flow* (μl/hr/100 g)	Bile salt output (μM/hr/100 g)	Pentobarbital biliary excretion (% total dose, 0–4 hr)
Bile salt depletion (n = 10)†	252 ± 25	0.72 ± 0.18	34.35 ± 2.82
Perfusion (10 mg) (n = 9)	326 ± 27 (P > 0.05)	6.35 ± 0.46 (P < 0.001)	40.38 ± 2.76 (P > 0.10)
Perfusion (30 mg) (n = 7)	448 ± 23 (P < 0.001)	18.98 ± 1.79 (P < 0.001)	45.91 ± 3.08 (P < 0.05)

* Mean ± S. E. M.
† Number of animals.

Table 2. Effect of bile salt perfusion on hepatic metabolism of pentobarbital

Treatment	Unmetabolized pentobarbital*	Ethyl acetate metabolites	Non-extractable metabolites
Bile salt depletion (n = 5)†	1.6 ± 0.5	16.6 ± 4.0	81.8 ± 4.3
Perfusion (10 mg) (n = 4)	1.5 ± 0.6	14.4 ± 3.3	84.1 ± 3.8
Perfusion (30 mg) (n = 4)	1.8 ± 0.5	13.6 ± 3.9	84.6 ± 4.4

* Mean percentage of total [^{3}H]biliary secretion ± S. D.
† No significant difference when compared to either bile salt-perfused group.

30 mg/hr ($P < 0.05$). Since large individual variations in bile flow among rats might obscure some of the effects of bile salt perfusion on pentobarbital biliary excretion, paired experiments were conducted in which pentobarbital biliary excretion was measured in each animal both during a saline-perfused, bile salt-depleted period and during taurocholate perfusion with 10 or 30 mg/hr. No significant spontaneous changes in bile flow or pentobarbital excretion occurred during a 2-day study period in which three animals were studied during the saline-perfused, bile salt-depleted state on both days. However, as an added control, half of the animals in each perfusion group were studied during the saline-perfused, bile salt-depleted state on day 1 with the taurocholate perfusion study performed on day 2 while the sequence of study was reversed in the remaining animals. Figure 2 compares the changes in bile flow rate and pentobarbital biliary excretion when individual animals were perfused with either 10 or 30 mg/hr of taurocholate as opposed to perfusion of the same bile salt-depleted animal with saline. Statistically significant increases

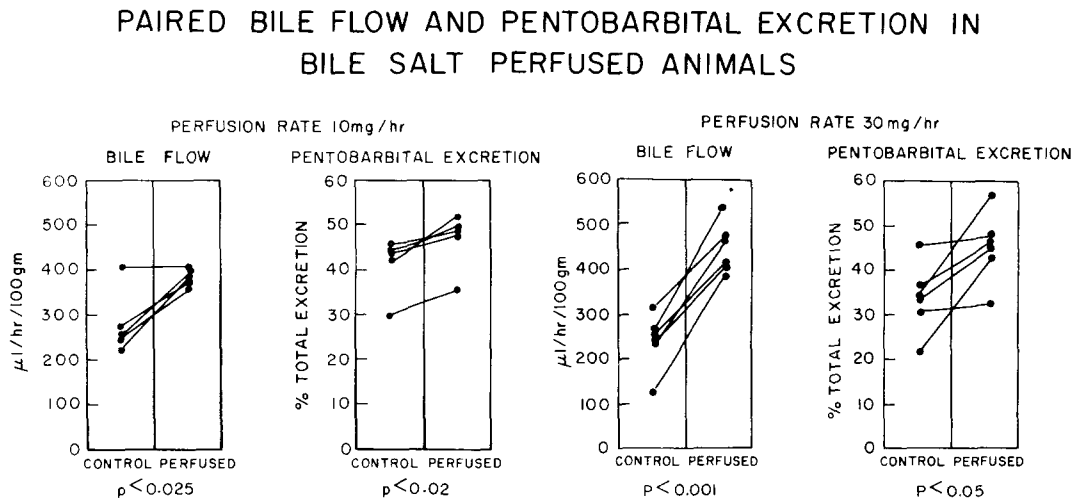

Fig. 2. Effect of bile salt perfusion on bile flow and pentobarbital biliary excretion in paired experiments using each animal as his own control. Pentobarbital biliary excretion was measured in each animal on 2 successive days. During the control experiments, the bile salt-depleted animals received saline infusions. During the perfused part of the experiment, animals were infused with 10 mg/hr or 30 mg/hr of taurocholate. The left two graphs show results of bile flow and pentobarbital excretion for the 10 mg/hr perfusion group; the right two graphs show similar data in the 30 mg/hr perfusion group. Results obtained in each animal for bile flow and pentobarbital excretion under the two experimental conditions are connected by lines.

in pentobarbital excretion were seen in both the 10 mg/hr and 30 mg/hr taurocholate-perfused groups.

Taurocholate perfusion had no significant effect on the form in which pentobarbital was excreted into bile (Table 2). Less than 2 per cent of total biliary radioactivity remained as unmetabolized pentobarbital. Metabolites extracted into ethyl acetate and those metabolites remaining in the bile-buffer mixture after ethyl acetate extraction did not move from the origin when chromatographed on Silica gel G in chloroform–acetone (9:1, v/v). Further identification of pentobarbital metabolites was not attempted.

Biliary excretion of pentobarbital metabolites was increased in the rat by bile salt perfusion. Interdependence between bile flow rates and biliary excretion could explain the increased pentobarbital excretion in response to taurocholate perfusion. Such interdependence has been shown for digitoxin [12], propylthiouracil [13], and cholecystography contrast agents [14]. In addition, hypothermia produced pronounced decreases in bile flow and markedly reduced excretion of pentobarbital into bile in the isolated perfused rat liver [15]. Direct interaction between pentobarbital metabolites and taurocholate is a second possibility. Physicochemical interaction between BSP and taurocholate to form a large macromolecular complex has been demonstrated *in vitro* [16], and it has been postulated that the increased T_m for BSP during bile salt infusion represents a specific effect of the bile salt rather than being attributable to bile salt-induced choleresis. It is possible that taurocholate combines with pentobarbital metabolites to form a complex which is then secreted into the biliary canaliculus. Whether enhanced biliary excretion of active pentobarbital metabolites by bile salt-induced choleresis actually decreases pentobarbital toxicity remains to be determined. Accumulation of a primidone metabolite has been shown to reduce primidone metabolism in the isolated perfused rat liver [17], so depletion of intrahepatic pools of metabolites may play some role in facilitating metabolism of parent compounds. Numerous other potentially toxic compounds such as digitoxin and chlorpromazine are excreted in bile as the unchanged parent compound or as pharmacologically active metabolites [12, 18]. If bile salt-induced choleresis can be shown to increase hepatic drug metabolism by depletion of intrahepatic metabolites or to enhance biliary excretion of unchanged parent compounds or active metabolites, it may be a useful means of rapidly manipulating hepatic excretory function.

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Department of Hematology,
Walter Reed Army Institute of Research,
Walter Reed Army Medical Center,
Washington, D. C. 20012, U.S.A.

ROBERT G. KNODELL†
EDWARD E. HOLLOWAY

† Reprint requests should be sent to RGK, Division of Gastroenterology, University of New Mexico School of Medicine, Albuquerque, N.M., 87131.

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Comparison of the inhibition of 5-hydroxytryptamine uptake by methadone and its congeners in human platelets

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Recent studies have indicated that methadone is a potent inhibitor of 5-hydroxytryptamine (5-HT) uptake by rabbit brain synaptosomes with a K_i of approximately 10^{-9} M [1]. Despite this low K_i, no depletion of brain 5-HT during treatment of animals with methadone has been reported [2, 3]. Apparently, the rate of endogenous synthesis of 5-HT in the brain is sufficient to maintain the normal levels.

In contrast to 5-HT-containing neurons in the brain, it is thought that platelets derive most of their 5-HT stores from exogenous sources [4]. Consequently, it is possible to deplete platelet 5-HT in man by uptake blockers, such as imipramine [5]. Although methadone has also been found to inhibit the uptake of 5-HT by human platelets *in vitro*, the concentration of methadone reported to cause a 50 per cent inhibition of uptake (2.1×10^{-5} M) [6] appears to be much greater than the concentration required to inhibit uptake by synaptosomes [1]. The relatively large concentration of methadone reportedly required to inhibit human platelet 5-HT uptake [6] would