PROCEEDINGS OF THE SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE **157**, 306–311 (1978)

Alterations in Bile Flow and Na^+-K^+ Biliary Excretion Induced by Theophylline and Ethacrynic Acid (40043)

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Hepatic canalicular bile production is currently held to be generated by two mechanisms, a bile salt-dependent (BSDF) and bile salt-independent (BSIF) process (1-3). Bile salts are actively transported into the bile canaliculi and subsequent development of osmotic gradients and passive movement of water into the canalicular lumen has been proposed as the means by which bile salts generate bile flow (4). There is more speculation as to the mechanism by which BSIF is generated. Some investigators have implicated cyclic 3′-5′-adenosine monophosphate (cAMP). Theophylline, a phosphodiesterase inhibitor which decreases breakdown of cAMP, has been shown to increase BSIF in the dog, an animal in which very low rates of BSIF have been found (5-7). Observations of the effects of theophylline on BSIF in animal models with high rates of BSIF such as rodents have not been reported. Other investigators have suggested that inhibition of membrane-bound Na^+-K^+-activated adenosine triphosphatase (Na^+-K^+-ATPase) is a factor in BSIF production (8-10). Ethacrynic acid, a diuretic which has been shown to inhibit Na^+-K^+-ATPase in the kidney (11), has been reported to both inhibit (10) and stimulate (12, 13) bile flow without significant change in bile salt output. These studies have been interpreted to support a role for Na^+-K^+-ATPase in the generation of BSIF. In a search for agents which would increase *in vivo* BSIF in the rat, both theophylline and ethacrynic acid were studied. Results of these studies showed that both agents significantly enhanced BSIF. However, both agents also produced alterations in bile salt-dependent flow which suggest they influence multiple steps in the bile secretory process.

Materials and methods. Male Walter Reed rats weighing 250–350 g were used in all experiments. In conducting the research described in this report, the investigator adhered to the Guide for Laboratory Animal Facilities and Care as promulgated by the Committee on the Guide for Laboratory Animal Resources, National Academy of Sciences, National Research Council. During ether anesthesia, external biliary fistulae were constructed and cannulation of both the femoral artery and vein was accomplished with PE-10 polyethylene tubing (Clay Adams, Inc.). Bilateral nephrectomy was performed. Animals were then allowed to recover in restraining cages and had free access to food and water for the remainder of the experiments. Studies were performed on the day following surgery after overnight depletion of the bile salt pool. During experiments, body temperature was monitored and maintained at 37 ± 1° with heating pads (14), and animals were perfused with 0.45 ml/hr normal saline. [^{14}C]-Erythritol (Amersham-Searle) was diluted to 3 μCi/ml with sterile normal saline. Grade A sodium taurocholate (Calbiochem) solutions containing 5, 15 and 30 mg/0.45 ml normal saline were prepared on the day of experiment and used for bile salt perfusion. Theophylline and ethacrynic acid were obtained from Vitarine, Inc.

Three microcuries of [^{14}C]erythritol were given through the femoral vein. After a 90 min equilibration period, bile samples were collected at 15 min intervals for 1 hr during the bile salt-depleted state with serum samples obtained midway through the individual bile collection periods via the femoral artery. Each animal then received infusions of taurocholate at 5, 15 and 30 mg/hr; the order of infusion was varied throughout the experiments and did not differ significantly among the three groups. One hour was allowed for equilibration of serum bile salt levels and bile flow between each different

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rate of bile salt infusion. After each equilibration period, bile samples were again collected at 15 min intervals for 1 hr and serum obtained midway through each collection period. Experimental animals treated with theophylline received 20 mg/kg intravenously 15 min before the start of bile collection. Ethacrynic acid-treated animals received 10 mg/kg intravenously 15 min prior to the start of bile collection and again halfway through the 1 hr collection period. Control animals received a comparable volume of normal saline. The amounts of drug used in these experiments were selected after monitoring maximum change in bile flow and duration of effect produced by each drug in doses from 10 to 40 mg/kg.

Following each experiment, animals were sacrificed and liver wet weight determined gravimetrically. Bile volume was determined gravimetrically. Bile salt concentrations were measured using the hydroxysteroid dehydrogenase method as modified by Admirand and Small (15). Sodium and potassium concentrations in bile were measured by an IL Flame Photometer, Model 343 (Instrumentation Laboratory, Inc.). Radioactivity in bile and plasma was determined in a Beckman LS-345 scintillation system using the external standard ratios to correct for quenching. Biliary clearance of [^{14}C]erythritol was calculated as the products of bile flow and the ratio of bile/plasma [^{14}C]radioactivity. Correlations between biliary [^{14}C]erythritol clearance versus bile flow and bile salt output and bile flow versus sodium and potassium excretion were calculated for individual animal experiments [Control $n = 5$, Ethacrynic acid $n = 7$, Theophylline $n = 5$], using the method of least squares (16). The y-intercept of the regression line calculated for the relationship of erythritol clearance to bile acid output is used to describe BSIF while the slope of the regression line for this relationship represents the amount of choleresis/unit of bile acid (BSDF). Similar terminology has been used in the interpretation of the regression lines calculated for bile flow versus biliary Na$^+$ and K$^+$ output. The unpaired student t test was used to compare differences in mean slopes and y-intercepts between two animal groups in which linear regression lines were calculated from data attained from each individual animal in the group. Analysis of covariance was also used to compare differences in the composite regression lines obtained for these comparisons between the control group of animals and the two drug-treated groups (16).

Results. Erythritol clearance showed a linear relationship to total bile flow in control animals and in both the theophylline (TH) and ethacrynic acid-treated (EA) groups (Fig. 1). Bile/plasma [^{14}C]ratios approximated unity and were not significantly different among the three groups (Control 1.006 ± 0.040, TH 0.960 ± 0.084, EA 0.973 ± 0.048). These data suggest that bile flow was originating from the hepatocyte canaliculus in all three experimental groups and that there was little alteration of canalicular bile by ductular secretion or reabsorption. However, TH and EA both altered the relationship between erythritol clearance and bile salt output as compared to controls (Fig. 2 and Table I). Both agents significantly increased the fraction of erythritol clearance which was independent of bile salt output ($P < 0.001$). In addition, theophylline produced a significant decrease in the erythritol clearance generated per unit of bile salt secreted as compared to the

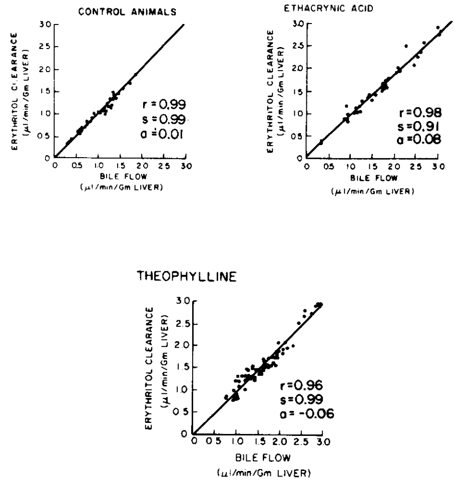

FIG. 1. Relationship of erythritol clearance to bile flow in control and both experimental groups is linear with a slope which approximates unity. No significant difference exists for this relationship between control and either experimental group.

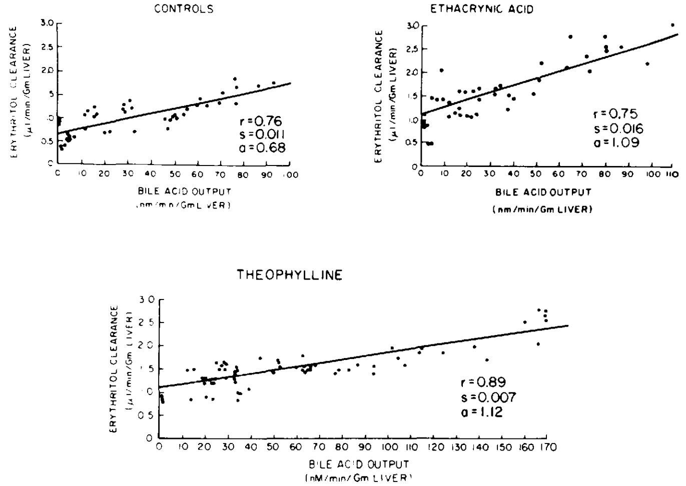

FIG. 2. Linear regression lines showing the relationship of erythritol clearance to bile acid output in control and experimental groups. Bile salt-independent flow (y-intercept) is significantly increased by both EA and TH treatment ($P < 0.001$). The bile flow generated per unit bile acid secretion (slope) is significantly increased by EA ($P < 0.025$) and significantly decreased by TH treatment ($P < 0.001$).

TABLE I. MEAN VALUES FOR SLOPES AND Y-INTERCEPTS OF REGRESSION LINES CALCULATED FROM COMPARISON OF ERYTHRITOL CLEARANCE VERSUS BILE ACID OUTPUT AND BILE FLOW VERSUS BILIARY NA$^+$ AND K$^+$ EXCRETION IN CONTROL, ETHACRYNIC ACID AND THEOPHYLLINE TREATMENT GROUPS.[a]

	Erythritol clearance versus bile acid output[b]		Bile flow versus biliary Na$^+$ excretion		Bile flow versus biliary K$^+$ excretion	
	Slope	Y-intercept	Slope	Y-intercept	Slope	Y-intercept
Control ($n = 5$)[c]	0.0120 ± 0.0011	0.585 ± 0.217	0.5385 ± 0.0749	0.1297 ± 0.0968	0.8685 ± 0.1352	0.1667 ± 0.0884
EA ($n = 7$)	0.0145 ± 0.0016 ($P < 0.025$)	1.254 ± 0.159 ($P < 0.001$)	0.5583 ± 0.0497	0.1587 ± 0.1087	1.0183 ± 0.1010	0.3535 ± 0.1291 ($P < 0.05$)
TH ($n = 5$)	0.0062 ± 0.0007 ($P < 0.001$)	1.148 ± 0.027 ($P < 0.001$)	0.5006 ± 0.0543	0.3120 ± 0.1130 ($P < 0.05$)	1.2129 ± 0.2215 ($P < 0.05$)	0.4116 ± 0.1815 ($P < 0.05$)

[a] P values listed in parentheses below mean when significantly different from control group.
[b] Mean ± SD.
[c] Number of animals in group.

control experiments ($P < 0.001$). Bile acid outputs for the theophylline experiments were also somewhat higher than those seen during similar bile salt perfusion rates in the control and EA treatment groups (see Fig. 2). It is possible that TH has some effect on the transport of bile acids such that given levels of serum and hepatic bile acids result in higher biliary bile acid outputs after TH in comparison to control or EA treated animals. Recalculation of the linear regression line for erythritol clearance versus bile acid output in the TH group using only values for bile acid output similar to those seen in the control and EA groups (<100 nM/min/Gm liver) resulted in a slope and y-intercept which differed insignificantly from those obtained using all the TH data. Ethacrynic acid produced the opposite effect of theophylline with more erythritol cleared per unit bile salt secretion than was seen in controls ($P < 0.025$). Separate linear regression lines for erythritol clearance versus bile acid output during the different rates of bile salt perfusion were calculated for the ethacrynic acid and theophylline groups. This was done to insure that the changes in erythritol clearance per unit bile salt secretion were not due to variations in degree of BSIF alteration by EA and TH during different rates of bile salt perfusion. The calculated bile salt-independent choleresis induced by both EA and TH was similar for each rate of bile salt perfusion, indicating that differences in the amount of bile generated per unit bile salt secretion produced by these agents is not due to changes in magnitude of BSIF at varying rates of bile salt perfusion.

Since changes in active transport of Na$^+$ and K$^+$ have been proposed as a mechanism by which BSIF is generated, Na$^+$ and K$^+$ biliary excretion were plotted against bile flow in the three experimental groups (Figs. 3, 4, Table I). Bile flow was linearly related to total Na$^+$ biliary excretion in the controls and in both the TH and EA animals. There was no significant difference in the amount of bile flow generated per unit of Na$^+$ excretion among the three treatment groups. Little bile flow was independent of biliary Na$^+$ excretion in the control group. However, an increased amount of bile flow appeared to be independent of biliary Na$^+$ excretion in both the TH and EA groups, and this increased Na$^+$ independent bile flow in the theophylline group differed significantly from that seen in the control group ($P < 0.05$). Both TH and EA produced similar changes from the control group in relationship of bile flow to biliary K$^+$ excretion. Significantly more bile was secreted per unit of biliary K$^+$ excreted in the theophylline group as compared to the control group ($P < 0.05$), and this comparison approached significance in the ethacrynic acid-treated group ($P > 0.05$). Both agents produced a significant increase in K$^+$-independent bile flow ($P < 0.05$).

Discussion. In this study both theophylline, a phosphodiesterase inhibitor which would be expected to elevate intracellular

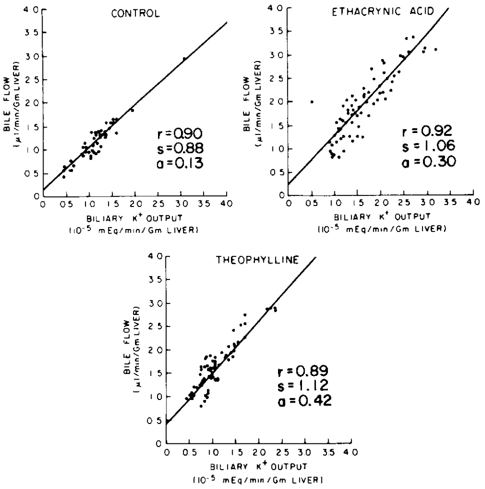

FIG. 4. Linear regression equations for bile flow versus biliary K$^+$ excretion show that the bile flow per unit K$^+$ excretion (slope) is increased by both EA ($P > 0.05$) and TH ($P < 0.05$). Both agents also produced an increase in bile flow independent of biliary K$^+$ excretion ($P < 0.05$).

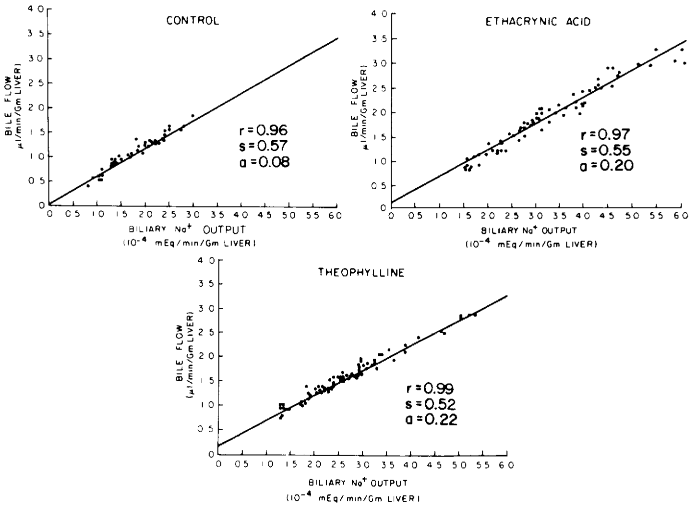

FIG. 3. Comparison of the relationship of bile flow to biliary Na$^+$ excretion shows no significant differences among the control and experimental groups for the volume of bile flow generated per unit Na$^+$ excreted. Bile flow independent of biliary Na$^+$ excretion (y-intercept) was increased in both the EA and TH groups, and this increase was statistically significant for TH ($P < 0.05$).

levels of cAMP, and ethacrynic acid, a Na$^+$-K$^+$-ATPase inhibitor, produced approximately equivalent increases in *in vivo* BSIF in the rat. While biliary excretion of TH and EA was not measured in this study, it is unlikely that the osmotic effect of either drug or its metabolites accounts for these increases in BSIF and alterations in biliary electrolyte excretion. Measurement of biliary TH in dogs indicates that amounts excreted into bile are insufficient via their osmotic effects to account for the increase in bile flow produced by this agent (5, 6). A recent report suggested an osmotic mechanism for EA-induced choleresis in the rat (17), but a comprehensive study of hepatic metabolism and biliary excretion of EA in the rat showed asynchronism between the choleretic effect of EA and its appearance in bile (18).

Proposed mechanisms involved in the generation of BSIF have centered primarily around cAMP and Na$^+$-K$^+$-ATPase. Proponents of a role for cAMP point to the increased BSIF induced by TH in dogs (5, 6) and increased bile flow in both dogs and humans in response to glucagon which enhances intracellular concentration of cAMP (19, 20). Since active Na$^+$ transport in other cell membranes is associated with changes in Na$^+$-K$^+$-ATPase and this enzyme has been isolated from rat liver homogenates (21, 22), other investigators favor alterations in Na$^+$-K$^+$-ATPase as the mechanism responsible for changes in BSIF (8–10). Both TH and EA increased BSIF in this study and both agents produced similar changes in the relationship of bile flow to biliary K$^+$ excretion which included an increase in bile flow per unit K$^+$ excretion and an increase in bile flow independent of K$^+$ excretion. This suggests that at least some part of the increased BSIF produced by both agents is due to alterations in electrolyte transport. It is conceivable that TH induces changes in Na$^+$-K$^+$-ATPase to account for its stimulatory effect of BSIF or that EA produces alterations in intracellular cAMP levels. It remains to test these drugs in this regard. However, it is quite likely that production of BSIF is dependent on multiple mechanisms and that both the cAMP and Na$^+$-K$^+$-ATPase systems are important in the generation of bile flow independent of bile salt secretion.

In addition to its effect on BSIF, TH also altered bile salt-dependent bile flow by decreasing the volume of bile secreted per unit of bile salt secretion. Several possible mechanisms could explain this change. Theophylline can produce vasoconstriction with resultant reduction in hepatic plasma flow. While generation of BSIF appears to be independent of wide fluctuations in hepatic plasma flow (23), the effects of decreased hepatic plasma flow on generation of BSDF have not been carefully examined. Theophylline-induced alteration of bile salt-cation interrelationships might also explain the decreased bile flow generated per unit bile salt secretion. It has been shown that activity coefficients for Na$^+$ and K$^+$ in bile salt solutions are less than the corresponding values one could expect in NaCl and KCl solutions (24). Loss of osmotic activity resulting from changes in bile salt-cation interaction produced by TH could account for the decreased bile flow generated per unit bile salt secretion. In contrast to theophylline, ethacrynic acid produced an opposite effect with increased bile flow per unit bile salt secretion when compared to controls. In a study of the effects of EA on bile flow in multiple animal species, Shaw also found an increased bile flow per unit bile salt output (13). However, no erythritol clearances were performed in this study and the data was not subjected to statistical analysis. These data suggest that the mechanism for generation of BSDF may not be as simplistic as has been previously postulated.

Summary. Both theophylline, a phosphodiesterase inhibitor, and ethacrynic acid, a Na$^+$-K$^+$-ATPase inhibitor, produced similar increases in BSIF in the rat. Whereas theophylline significantly decreased BSDF, ethacrynic acid increased BSDF. These results suggest that the mechanism responsible for generation of both BSIF and BSDF are multifactorial and further study will be necessary to define them.

Portions of this work were performed in the Division of Hematology, Walter Reed Army Institute of Research, Washington, D.C., and were supported by the Research and Development Command of the U.S.

Army. The author expresses his appreciation to Ms. Carol Bell and Mr. David Brooks for technical assistance; to Mr. James S. McNeil and Mr. Roy Spangler who performed Na$^+$, K$^+$, Cl$^-$, HCO$_3^-$ and osmolality determinations, and to Ms. Paula Wojick, Ms. Carol Riemen and Ms. Marge Smith for their help in the preparation of this manuscript.

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Received November 29, 1976. P.S.E.B.M. 1978, Vol. 157.