JCI The Journal of Clinical Investigation

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J Clin Invest. 1973;52(5):1230-1236. https://doi.org/10.1172/JCI107290.

Research Article

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Effect of Glycine-Conjugated Bile Acids with and without Lecithin on Water and Glucose Absorption in Perfused Human Jejunum

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ABSTRACT Perfusion studies were performed in healthy volunteers to test whether the secretory effect of conjugated bile acids, previously shown for the colon, was also present in the jejunum. A perfusion system with a proximal occlusive balloon (and continuous aspiration of duodenal secretions) was used; isotonic test solutions contained glycine-conjugated bile acids with or without lecithin. Fluid movement was measured by changes in the concentration of polyethylene glycol (PEG, mol wt 4,000). Conjugated dihydroxy bile acids inhibited electrolyte and fluid absorption and, at higher concentrations, evoked secretion of an isotonic fluid. Glucose absorption continued, despite fluid secretion, but its rate decreased. The secretory effects of bile acids were abolished by the addition of lecithin to the bile acid solutions. A trihydroxy bile acid (cholylglycine) had no effect on jejunal absorption. Small amounts (6–9%) of conjugated bile acids were absorbed in the jejunum; lecithin was well absorbed (72–90%). The results indicate that dihydroxy bile acids influence salt and water transport in the human jejunum but that this effect may be abolished when a polar lipid such as lecithin is present. We speculate that this effect of bile acids may modify fluid movement in the small intestine postprandially after fat absorption has occurred.

INTRODUCTION

The concept of cholerheic enteropathy (1) stimulated interest in the effects of bile acids on intestinal water absorption. Supporting evidence was provided by the demonstration that dihydroxy bile acids inhibit water absorption in the canine colon (2) and induce secretion in the human colon (3). A similar secretory phenomenon was observed in the perfused hamster jejunum (4), suggesting that these effects of dihydroxy bile acids on absorption were not confined to the colon.

Here, we describe the effects of glycine-conjugated bile acids on fluid and electrolyte movement in the perfused proximal jejunum of man. Glycine-conjugates were chosen since they predominate in the normal human small intestine. In addition, the effects of combining lecithin, a major lipid constituent of bile, with a dihydroxy bile acid were studied. A double-lumen perfusion system with a proximal occlusive balloon (5) was used to exclude endogenous bile acids from the perfused segment. All perfusion fluids contained glucose, so that its absorption together with that of water and electrolytes could be quantified; in addition, absorption of bile acids and lecithin was measured.

METHODS

Preparation of perfusates. Glycine-conjugated bile acids were prepared and their purity (95%) was determined by methods previously described (3). Bile acids were obtained from the following sources: deoxycholic acid (Schuchardt, Munich, Germany), cholic acid (Matheson, Coleman and Bell, East Rutherford, N. J.), and chenodeoxycholic acid (Weddell Pharmaceuticals, London, England). Bile acids were purified by crystallization and were then conjugated with glycine methyl ester. The reaction product was extracted into ethyl acetate-benzene, 1:1 (vol/vol), which was washed three times with an equal volume of 1 M sodium carbonate and three times with equal volumes of 1 M sodium chloride to remove unreacted free acid and glycine methyl ester. The washing procedure was repeated; the ethyl acetate phase was then reduced to dryness on a rotary evaporator and saponified in ethanol-1 N NaOH, 1:1 (vol/vol), for 2 h at room temperature. The saponification mixture was extracted three times with equal volumes of petroleum hydrocarbon to remove tri-*n*-butylamine. The

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Received for publication 5 June 1972 and in revised form 6 October 1972.

conjugated bile acids were then isolated as described previously. The sodium salt of chenodeoxycholylglycine (CDC-G) [1] was obtained by freeze-drying; the sodium salts of cholylglycine (C-G) and deoxycholylglycine (DC-G) were precipitated with diethyl ether from a methanolic solution. All bile acids were dried to constant weight in a vacuum desiccator. Lecithin (chromatographically pure, Schwarz/Mann Div., Becton, Dickinson and Co., Orangeburg, N. Y.) was used as purchased.

Control solutions were pH 8.0 and contained the following concentrations of ions (in meq/liter): Na^+, 140; K^+, 5; Cl^-, 100; HCO_3^-, 45. In addition, *D*-glucose was added to a concentration of 200 mg/100 ml (11.2 mM). Polyethylene glycol (PEG, mol wt 4000) was the nonabsorbable marker; each liter of perfusate contained 5 g of stable PEG and 5–10 μCi of ^{14}C-labeled PEG (New England Nuclear, Boston, Mass.). In initial studies, stable and ^{14}C-labeled PEG were compared as nonabsorbable markers and no differences between them were found (6). Test solutions also contained bile acids, with or without lecithin (see below). The sodium concentration in test solutions was up to 10 meq/liter greater than that in control solutions. Osmolality of control solutions was 284 mosmol/kg and that of test solutions was 289–299 mosmol/kg.

Perfusion technique. Subjects were healthy human volunteers (postmenopausal women, or men more than 21 yr old) who gave written informed consent.

The perfusion tube has been described (5). The tube was passed at 8 a.m., after the subject had fasted overnight. The location of the balloon was assessed fluoroscopically; when it reached the ligament of Treitz it was inflated with 35 ml of air and its inflation port was sealed. Occlusion, confirmed by the absence of bile staining in the perfusate, was signaled by a sensation of slight epigastric distention in the subject. Inflation of the balloon was adjusted so that the sensation was present but minimal. The perfusate at 37°C was then delivered at a constant speed of 10 ml/min and sampled from the test segment (25 cm) by siphonage. Intermittent suction was applied to the proximal aspiration lumen to remove duodenal secretions. During the study, the subjects remained fasting and recumbent, but were allowed to read, converse, watch television, or sleep.

Each perfusion fluid was delivered for 90 min; a single study consisted of four consecutive 90-min experiments. Perfusate samples were collected in 10-min portions. In each perfusion, the first 50 min were allowed for equilibration, and the last four 10-min periods were taken to represent sampling of a steady state; these assumptions appeared to be justified in practice.

Analytical methods. Sodium and potassium were estimated by flame photometry; chloride was measured by electrometric titration with a silver nitrate solution. Glucose was measured by an enzymatic hexokinase method (Boehringer Mannheim Corp., New York). Bile acids were estimated by a modification of the method of Iwata and Yamasaki (7). The PEG[^{14}C] in 0.2 ml of perfusate, mixed with 15 ml of a scintillation "cocktail," was counted by liquid scintillation spectrometry; quench correction was made by external standardization. Phospholipid concentrations were determined on the chloroform phase after Folch extraction.

Calculations. Absorption and secretion of water and solutes, relative to PEG, were calculated by standard methods (6). Comparisons between means were made by calculation of Student's *t* test for unpaired values; linear regression analysis was by the method of least squares.

Experimental design

A control and three test solutions were perfused in random order in each subject. Three groups of studies were performed.

Group 1: Effect of conjugated bile acid. Trihydroxy Bile Acid. Three solutions, 2.5, 5.0, or 10.0 mM in C-G, and control solutions without bile acids were used in each of three subjects. The order of perfusion in each subject was drawn from a table of random numbers.

Dihydroxy Bile Acids. A control solution and test solutions containing 2.5, 5.0, or 10 mM dihydroxy bile acid (either DC-G or CDC-G) were used in each subject. A 4 × 4 Latin square was used for perfusion order so that each concentration of each of the two bile acids was administered at a different point in the perfusion sequence in each of the eight subjects. This design allowed analysis of the data for comparison between control and test solutions, for the effects of sequential perfusion, and for the reversibility of the effects of test perfusions.

Group 2: Effect of unconjugated bile acid. To test the possibility that the effects found with conjugated dihydroxy compounds might be due to contamination with unconjugated bile acid, which is known to be more potent in the hamster (4), two subjects were perfused alternately with glucose-electrolyte solution containing 2.5 mM C-G (which had no effect on fluid movement) and the same solution containing 0.25 mM sodium deoxycholate (DC). Each subject received each solution twice, and the perfusion sequence was reversed for the second set of perfusions.

Group 3: Effect of dihydroxy bile acid in presence of lecithin. This group of studies also utilized a 4 × 4 Latin square design in four subjects and was performed to determine whether the effects of a dihydroxy conjugated bile acid would be modified by the presence of phospholipid. Each subject received: (*a*) glucose-electrolyte solution; (*b*) glucose-electrolyte solution with 5 mM DC-G; (*c*) glucose-electrolyte solution with 5 mM DC-G and 1.25 mM lecithin; and (*d*) glucose-electrolyte solution with 5 mM DC-G and 2.5 mM lecithin. This choice of solutions permitted comparison, by solutions 1 and 2, with the results of group 1 studies. Evaluation of dose-response to lecithin at three different concentrations (0, 1.25, and 2.5 mM) was possible; the effect of perfusion sequence was eliminated.

TABLE I

*Effect of Perfusion Sequence on Water and Glucose Movements**

Substance	Perfusion period 1	2	3	4
Water movement				
All perfusions (N = 44)	0.7±0.4	0.7±0.3	1.1±0.4	0.7±0.4
Controls only (N = 11)	1.4±0.2	1.7±0.4	1.7±0.3	2.2±0.1
Glucose movement				
All perfusions	13.1±0.9	14.4±0.9	15.3±1.1	15.8±0.8
Controls only	14.3±1.3	16.6±1.9	19.1±0.3	17.9±1.2

* Water fluxes are expressed as ml/min per 25 cm jejunum; glucose fluxes are mg/min per 25 cm jejunum. Data are shown as means ±SE.

[1] *Abbreviations used in this paper:* CDC-G, chenodeoxycholylglycine; C-G, chloylglycine; CMC, critical micellar concentration; DC, deoxycholate; DC-G, deoxycholylglycine; PEG, polyethylene glycol.

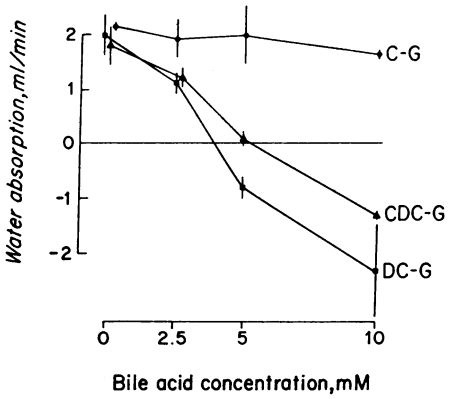

FIGURE 1 Fluid absorption from 25 cm segment of human jejunum (mean ±SE; $N = 3$ or 4) perfused with isotonic electrolyte solution and with conjugated trihydroxy (C-G) or dihydroxy (DC-G, CDC-G) bile acid solutions. Negative values represent fluid secretion.

RESULTS

Effect of perfusion sequence. Absorption rates of water and glucose were influenced little by the sequence of perfusion (Table I). When only control perfusions are considered, a small but statistically significant ($P < 0.05$) mean increase of water absorption, 0.2 ml/min for each hour of perfusion, was noted. Thus, during the 5 h of perfusion there was a trend for water absorption to increase slightly. This trend was small relative to the changes in water movement induced by bile acid perfusions.

Effect of bile acids on water absorption. Dihydroxy bile acids (DC-G, CDC-G) inhibited water absorption (Fig. 1). This effect was concentration related: at higher concentrations, fluid was secreted. Infused concentrations of DC-G (and CDC-G) and net fluid movement were closely related ($P < 0.001$). DC-G appeared more potent but the dose-response relationships were not significantly different. Changes in water movement occurred rapidly and were fully reversible; one such study is illustrated in Fig. 2.

TABLE II
*Effect of Unconjugated Deoxycholate on Net Absorption**

Substance	Perfusate: 2.5 mM C–G	Perfusate: 2.5 mM C–G with 0.25 mM DC
Water (ml/min per 25 cm)	1.8±0.2	1.4±0.3
Glucose (mg/min per 25 cm)	13.4±1.8	12.1±1.5
Sodium (meq/min per 25 cm)	241 ±36	178 ±39

* Means ±SE; $N = 4$. Differences not statistically significant.

There was no significant depression of water absorption by the trihydroxy bile acid (C-G), even at 10 mM. The relationship between water movement and C-G concentration was not significant.

Effect of unconjugated deoxycholate. If the DC-G used in earlier studies was 95% conjugated and 5% unconjugated, the 5 mM DC-G solution would have been 0.25 mM in unconjugated deoxycholic acid. Since there was no effect on water absorption when C-G containing 0.25 mM unconjugated deoxycholate was perfused (Table II), the effect of DC-G on absorption was unlikely to be caused by free deoxycholate present as a contaminant.

Effect of lecithin on water secretion induced by deoxycholylglycine. The addition of lecithin reversed the secretory effect of DC-G: water absorption with 2.5 mM lecithin was close to that from the control solution (Fig. 3). The rapidity of the effect and the reversibility of the effect of lecithin were similar to those found with perfusions of bile acids without lecithin (Fig. 4).

Relationships between water and electrolyte movements. Net movements of sodium and chloride were closely related to water movement. However, chloride absorption was always less than sodium absorption, suggesting concomitant absorption of another anion (bicarbonate). The relationship between ion and water movements indicated that the net fluid that was absorbed or secreted was isotonic, and was predominantly

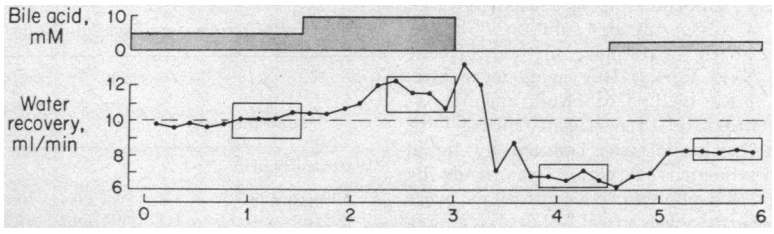

FIGURE 2 Water recovery in sequential 10-min samples from jejunum perfused at 10 ml/min, showing rapid onset of inhibition of water absorption induced by dihydroxy bile acid (DC-G), with rapid reversibility of this effect. Rectangles mark samples taken as steady-state observations. Water recovery values below broken line are net absorption; those above the line are net secretion.

TABLE III

Effect of Bile Acid Concentration on Glucose Absorption

Bile acid conc.	Glucose absorption (mg/min per 25 cm jejunum)*		
	C–G	CDC–G	DC–G
mM			
0	18.0±0.7	18.7±0.8	17.8±1.1
2.5	14.6±0.9	14.5±2.6	16.1±0.7
5.0	16.7±0.5	12.7±1.0	13.1±0.6
10.0	15.2±1.7	11.2±0.9	9.2±0.5

* Mean ±SE; $N = 3$ or 4 for each perfusate. Glucose infusion rate was 20 mg/min.

a sodium chloride solution. Detailed analysis of these changes will be described elsewhere.[2]

Glucose absorption. More than 85% of the perfused glucose was absorbed from the control glucose-electrolyte solution. There was a progressive decrease in glucose absorption (to approximately 50%) with increasing concentration of DC-G and CDC-G but not with C-G (Table III). Decreased glucose absorption was not due to a lowering of luminal glucose concentration by secreted fluid, since effluent glucose concentrations were always higher when glucose absorption was decreased. With dihydroxy acids at 10 mM, glucose was absorbed when fluid was secreted. Glucose absorption and water movement were related ($P < 0.001$).

Absorption of bile acid and lecithin. Small but significant absorption of DC-G occurred (Table IV). There was a significant correlation between bile acid absorption and water movement, the uptake of bile acid increasing with water absorption ($r = 0.627$; $P < 0.005$). Lecithin was well absorbed from DC-G perfusates.

Side-effects of perfusion. Colicky pain in the upper abdomen, nausea, or vomiting was observed during the perfusion of 5 mM or 10 mM dihydroxy bile acids in 8 of 16 subjects. Pain was sufficiently severe to necessitate interruption of the study in two instances. PEG recovery was less in those studies with side-effects (34.8±3.9%) than in those without side-effects (55.8±5.6%; $P < 0.02$), suggesting that the symptoms resulted from the passage of dihydroxy bile acids into the intestine distal to the test segment. However, there was no correlation between the degree of fluid secretion and the occurrence of symptoms. Replacement of the test solution by a bile acid-free solution containing glucose and electrolytes was associated with prompt disappearance of symptoms. No symptoms occurred during cholylglycine perfusions.

[2] Wingate, D. L., H. S. Mekhjian, and S. F. Phillips. Unpublished observation.

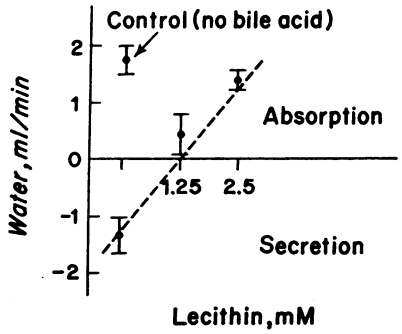

FIGURE 3 Influence of added lecithin on fluid secretion (mean ±SE; $N = 4$) induced by 5 mM DC-G. Addition of 2.5 mM lecithin blocked the secretory effect of bile acids, and net water absorption was similar to control values (no bile acid, no lecithin). Negative values represent fluid secretion.

DISCUSSION

Secretory effects of dihydroxy bile acids. The present study is the fourth to show a rapid, reversible, secretory effect of conjugated dihydroxy bile acids in vivo. The dose-response curves, although incomplete, are similar to those reported for hamster jejunum and human and canine colon (2–4). Forth, Rummel, and Glasner

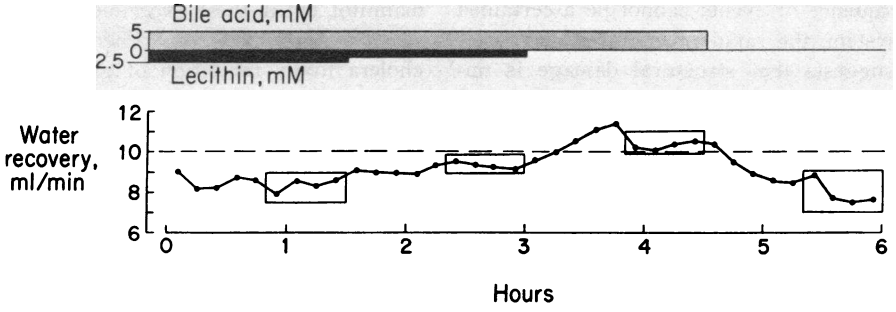

FIGURE 4 Water recovery in sequential 10-min samples from jejunum perfused at 10 ml/min, showing modifying influence of lecithin on secretion induced by bile acid (DC-G). Rectangles mark samples taken as steady-state observations. Water recovery values below broken line are net absorption; those above the line are net secretion.

TABLE IV

Absorption of Deoxycholylglycine and Lecithin

Starting conc.		Absorption			
		Bile acid		Lecithin	
DC–G	Lecithin	μmol/min per 25 cm*	%	μmol/min per 25 cm*	%
	mM				
5	0	2.8±0.5	5.6	—	—
5	1.25	3.3±0.5	6.6	11.3±2.0	90
5	2.5	4.6±0.2	9.2	18.0±3.1	72

* Mean ±SE; $N = 4$ for each perfusate.

(8) had shown, in the rat jejunum in vitro, a similar effect of unconjugated bile acids but conjugated bile acids had no effect.

A fundamental assumption of these studies is that PEG serves as a valid marker for fluid movement. Wilkinson (9) has shown that PEG administered orally to healthy man is recovered totally in the feces. In our studies, the jejunum was exposed to physiologic concentrations of bile acids. Furthermore, PEG was not absorbed when bile acids were perfused into the human colon (3), and PEG recovery was complete when fluid secretion was induced by bile acids in the hamster jejunum (4). Our perfusion system has also been shown to isolate the jejunum from endogenous biliary secretion (5). Thus, it seems reasonable to propose that PEG dilution was caused by fluid secretion induced by the perfused bile acids.

In vivo perfusion systems demonstrate phenomena; their value in the elucidation of mechanisms is limited. The observed effects were not osmotic because solutions were essentially isotonic and small osmotic gradients do not inhibit jejunal absorption in the presence of glucose (10). Moreover, identical concentrations of the trihydroxy compounds had no effect.

Bile acids can disrupt cell membranes (11). Although the exact time sequence of events cannot be ascertained by a perfusion system, the rapid reversibility of the secretory effects suggests that structural damage is unlikely. Moreover, in the hamster jejunum (4), secretion was induced by conjugated bile acids without histologic changes. Nonetheless, we cannot exclude a subtle change of membrane structure sufficient to alter mucosal permeability. However, it is not clear how alteration per se in pore size could reverse net flow across the mucosa. Glucose absorption persisted and the secreted fluid was mainly a sodium chloride solution. Rohde and Chen (12) were unable to demonstrate a change in apparent pore size in the jejunum when secretion was induced by cholera toxin.

Bile acids might influence certain chemical mechanisms proposed for ion transport, by activation of the adenyl cyclase system or by inhibition of Na, K^+-activated ATP-ase. Glycine-conjugated dihydroxy and trihydroxy bile acids have been shown to influence intestinal ATP-ase in vitro, but the effects of individual bile acids on ATP-ase correlated imperfectly with their effects on water absorption in vivo (13).

Bile acids are surface active, and dihydroxy compounds possess greater surface activity than do trihydroxy compounds; however, correlations between physical and pharmacologic properties are unjustified until a greater variety of bile acids are tested for a secretory effect.

Abolition of secretion by lecithin addition. Addition of lecithin, which was used in physiologic concentrations (14), would be anticipated to decrease markedly the critical micellar concentration (CMC) of the dihydroxy bile acids (15). By definition, a decrease in CMC would decrease the concentration of bile acid in molecular form. The CMC of glycine-conjugated dihydroxy bile acids in the absence of lecithin is 3–4 mM (16); in the presence of lecithin, it is lower (15). Lecithin, which decreases "activity" of dihydroxy bile acids chemically, might have also decreased the pharmacologic activity. However, we cannot exclude a more direct effect of lecithin on membrane transport processes.

Glucose movements. Glucose absorption occurred in all experiments, but its magnitude diminished during perfusion with the bile acids which induced secretion. Levitt, Hakim, and Lifson (17) partitioned glucose absorption in the dog into three components: active transport, diffusive transport, and convective transport. The simplest explanation of our observations is that the decrease in glucose absorption associated with water secretion represented decreased convective transport. Diffusive transport is unlikely to have decreased, since intraluminal concentrations were greater when secretion occurred. Whether or not active transport was altered cannot be ascertained. However, glucose absorption was not decreased (17, 18) during fluid secretion induced by mannitol, but in these experiments the amount of induced secretion was much less. When secretion was induced by cholera toxin, inhibition of glucose absorption was not observed (19); but in these experiments the glucose concentration was unphysiologic (60 mM).

Glucose absorption occurred simultaneously with movement of sodium into the lumen when fluid secretion was induced. Two recent reports (20, 21) have described glucose absorption concomitant with movement of sodium into the lumen when intestinal loops were perfused with isotonic, sodium-free solutions containing glucose. These observations and our own are in marked contrast to in vitro studies in which a stoichiometric relationship between glucose and sodium absorption has been ob-

served. However, induced secretion may occur from a different mucosal site (for example, crypts) than the site of glucose absorption (villi) (22). Thus, our data neither support nor refute the hypothesis that sodium and water absorption is induced by active glucose absorption. Our results do indicate that stoichiometry, claimed for glucose and sodium absorption in vitro (23), is not applicable to our findings which showed glucose absorption during secretion of a sodium-containing fluid.

Lecithin absorption. Absorption of lecithin has not been studied in this manner before. The hydrolysis of dietary lecithin to 1-lysolecithin and fatty acid has been clearly shown in man (24), although whether or not biliary lecithin is hydrolyzed similarly is unclear. We did not ascertain if lecithin was hydrolyzed during absorption in our experiments; but the only lipid present in the recovered fluids was lecithin, indicating that, if hydrolysis had occurred, the products were completely absorbed.

Bile acid absorption. Absorption of DC-G was much less than that of lecithin, and the presence of lecithin did not decrease the rate of bile acid absorption. According to current concepts, bile acids are absorbed in the jejunum by passive nonionic diffusion of the protonated form (25). This mechanism is strongly pH-dependent, since the ionized species should diffuse very slowly due to a reflection coefficient of close to 1 (25). The rate of absorption, extrapolated from our studies at pH 8.0, from 150 cm of jejunum would be about 1 to 1.5 mmol/h. This finding is consistent with earlier observations, in man (26, 27) and in primates with ileal resection (28), that passive jejunal absorption of glycine-conjugated dihydroxy bile acids occurs in health and disease. However, studies of bile acid absorption during digestion of a meal are needed.

Physiologic significance. These results imply that dihydroxy bile acids inhibit water absorption at low concentrations and cause water secretion at higher concentrations in both the small and the large intestine, provided that lecithin is not present. In health, phospholipid and lipolytic products are absorbed in the jejunum (29) whereas bile acids are absorbed preferentially in the distal ileum. Deoxycholic acid evokes water secretion from the canine ileum in vivo,[3] so the secretory effect of bile acids is present in the entire gastrointestinal tract. Since the concentration of bile acids in the distal small bowel is high (30) and unconjugated bile acids are found there in health (31), bile acids may influence water movement in this portion of the intestine. Whether this secretory effect of bile acids has any physiologic significance is as yet uncertain. In disease, bile acid-induced secretion in the colon appears to be important in the diarrhea that may complicate ileal resection (32).

[3] Ammon, H. V., and S. F. Phillips. Unpublished observation.

ACKNOWLEDGMENTS

This investigation was supported in part by Research Grant AM-6908 from the National Institutes of Health, Public Health Service, and by grants from the Share Foundation and the Mead Johnson and Company.

Note added in proof: Further studies (H. V. Ammon and S. F. Phillips, unpublished) have indicated that the rate of absorption of lecithin from a micellar solution may be much slower than that reported here. A possible reason for this discrepancy could be continuing hydrolysis of lecithin to lysolecithin during sample handling; in our hands, lysolecithin is not completely extracted by the chloroform-methanol extraction procedure.

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