Preferential Retention of Taurine-Conjugated Bile Salts by Cholestyramine in the Rat Ileum (36079)

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(Introduced by H. P. Sarett)

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Bile salt absorption occurs primarily in the distal ileum via an active transport system and to a lesser degree in the duodenum and jejunum by passive diffusion (1–4). Cholestyramine, a quaternary ammonium anion exchange resin, sequesters bile salts in the intestine, reduces their absorption, and thereby increases fecal bile salt excretion. The loss of bile salt elicits a compensatory increase in cholesterol catabolism (5) and results in a decrease in serum cholesterol levels in some species (5, 6).

The effectiveness with which cholestyramine interrupts the enterohepatic circulation of bile salts may depend on the type of amino acid conjugate and the number of hydroxyl groups of each bile salt. *In vitro* studies show that cholestyramine preferentially binds taurine conjugates of both dihydroxy and trihydroxy bile salts over the corresponding glycine conjugates, and binds dihydroxy bile salts of either conjugate type more effectively than the trihydroxy bile salts (7, 8). Furthermore, dietary taurine, which increases the proportion of taurine-conjugated bile salts in the gallbladder bile of humans (9, 10) and hamsters (11), augments fecal bile salt excretion in rats and hamsters fed cholestyramine (11).

Present results demonstrate that, even in the presence of cholestyramine, some of the bile salts are absorbed from the ileum and that, in this system as *in vitro*, the resin retains taurine conjugates more effectively than glycine conjugates and dihydroxy bile salts more effectively than trihydroxy bile salts.

Methods. Male rats of Wistar derivation,[1] 200–400 g, were used in all studies. The animals were housed individually in screen-bottom cages in an air-conditioned room and allowed to consume a purified diet (11) and water *ad libitum* for at least 10 days prior to the experiments. All surgery was performed with rats under light ether anesthesia.

To study exogenous bile salt absorption from the ileum without using radioactive compounds, it was necessary that essentially all endogenous bile salts be absent. In the first experiment, conditions were defined under which endogenous bile salt absorption was essentially complete. Exogenous bile salt absorption was measured in the second experiment.

Expt. 1. Twelve rats, 230–300 g, were fasted for 16 hr prior to surgery. A midline laparotomy was made and a ligature was placed around the terminal ileum 1 cm proximal to the ileocecal junction. Then 1 ml of 0.9% NaCl was instilled into the lumen of the ileum in a caudal direction from a point 30 cm proximal to the ileocecal junction. The intestine was ligated at the point of saline instillation. Groups of 3 rats each were sacrificed immediately or returned to their cages for 2, 4, or 6 hr after closure of the abdominal incision with wound clips. Rats regained consciousness within a few minutes after surgery.

Sacrifice was by cervical dislocation. After removal of the small intestine from the rat, one end of the ileum was slipped over the delivery tip of a Repipet[2] and the intestinal contents were flushed into a calibrated test tube with 7 ml of 0.5 *N* HCl in 50% ethanol. Fluid remaining in the intestine was carefully expressed with a soft rubber roller

[1] Obtained from Harlan Industries, Cumberland, IN.

[2] Labindustries, Berkeley, CA.

and added to the contents of the test tube.

The fluid in each test tube was diluted to 10 ml with 0.5 *N* HCl in 50% ethanol and centrifuged at 34,000*g* for 10 min. Aliquots of the supernatant fluid were analyzed for total bile salt content with hydroxysteroid dehydrogenase (12) using a purified cell-free enzyme preparation.[3]

Expt. 2. These studies were conducted to determine the effects of cholestyramine on the absorption of pure bile salt solutions from the ileum.

Four hour prior to ileal instillation of the bile salt solutions, 1 ml of saline was instilled into the ileum of each rat to enhance endogenous bile salt absorption as described in Expt. 1.

Solutions containing physiological concentrations of bile salt (20–25 μmoles/ml) were prepared in 0.9% NaCl, using commercially obtained[4] sodium salts of taurocholate (TC), glycocholate (GC), taurochenodeoxycholate (TCDC) and glycochenodeoxycholate (GCDC). The solutions were incubated alone or equilibrated with 10 mg of cholestyramine/ml for at least 1 hr at 37° prior to instillation. The cholestyramine suspensions were carefully agitated immediately before being drawn into the syringe. Instillation was done immediately to avoid settling of the cholestyramine–bile salt complex.

A loose tie was made around the ileum about 1 cm distal to the point where saline had been instilled 4 hr earlier. A 22 gauge needle on a plastic disposable syringe which contained 1.0 ml of bile salt solution with, or without, cholestyramine was inserted into the lumen of the ileum in a caudal direction. With the needle in the lumen of the ileum, the ligature was tightened around the intestine and, after slow instillation of the bile salt solution (30 sec), the needle was withdrawn. Saline was instilled into the ileum of control animals and the amount of endogenous bile salt remaining in the ileum was determined.

To obtain 0 hr values, the intestine of some animals was removed and flushed within 3–5 min after instillation of the saline or bile salt solutions. The abdominal incisions of the other animals were closed with wound clips and they were returned to a screen-bottom cage for 1 or 2 hr. The rats were then killed and the contents of the ileum were obtained and analyzed for total bile salts as described in Expt. 1.

TABLE I. Absorption of Endogenous Bile Salts from the Ligated Rat Ileum.

	Bile salt (μmoles) in ileum[a]; time after ileal ligation and saline instillation (hr):			
	0	2	4	6
Mean	24.3	4.9	0.0	0.4
Range	14.6–30.1	3.8–5.7	0.0–0.1	0.0–1.3

[a] Values from 3 animals at each time interval.

In order to determine the amount of bile salt which was bound to cholestyramine before instillation, duplicate 1.0 ml aliquots of each solution were centrifuged at 34,000*g* for 10 min. The resin-free supernatant fluid was decanted, made to a volume of 10 ml with 0.5 *N* HCl in 50% ethanol and assayed for bile salts. The cholestyramine precipitate was resuspended in 5 ml of 0.5 *N* HCl in 50% ethanol to elute the bile salts bound to the resin and then centrifuged. The supernatant fluid was decanted, made to volume, and assayed. It was found that 96 to 97% of the dihydroxy bile salts were bound to cholestyramine prior to intra-ileal infusion compared with 79% for glycocholate and 88% for taurocholate.

Results. Expt. 1. Endogenous bile salts were rapidly absorbed from the ligated ileum (Table I). Eighty percent of the bile salts in the ileum at the time of saline instillation were absorbed within 2 hr and absorption was essentially complete within 4 hr. Therefore, in Expt. 2, saline was instilled 4 hr preceeding bile salt instillation to enhance endogenous bile salt absorption.

Expt. 2. Ileal administration of saline 4 hr prior to instillation of the pure bile salt solutions with, or without, cholestyramine resulted in essentially complete absorption of endogenous bile salts from the ileum by the time the pure bile salt solutions were instilled (Table II).

Of the bile salts instilled without choles-

[3] Worthington Biochemical Corporation, Freehold, NJ.

[4] A grade, Calbiochem, Los Angeles, CA.

TABLE II. Effect of Cholestyramine on Retention of Exogenous Bile Salts in the Ligated Rat Ileum.[a]

Cholestyramine	Solution instilled (μmoles)		Bile salt in ileum (μmoles); time after bile salt instillation (hr): 0	1	2
—	NaCl[b]	154	0.9 ± 0.6 (11)	—	—
—	TCDC	22.4 ± 0.6 (6)	17.6 ± 1.7 (5)	7.1 ± 1.6 (6)	1.9 ± 0.9 (6)
—	TC	22.5 ± 0.8 (6)	18.4 ± 0.7 (6)	3.5 ± 2.4 (6)	1.6 ± 0.7 (6)
—	GCDC[c]	21.9 ± 0.8 (6)	18.5 ± 0.5 (6)	7.5 ± 1.7 (6)	2.8 ± 1.3 (6)
—	GC	23.3 ± 1.8 (6)	18.8 ± 0.9 (5)	4.4 ± 2.7 (6)	2.5 ± 1.8 (6)
+	TCDC	20.3 ± 1.1 (5)	19.2 ± 0.9 (6)	14.5 ± 1.6 (6)	10.2 ± 1.5 (6)
+	TC	19.8 ± 0.9 (5)	17.8 ± 2.4 (4)	10.0 ± 3.9 (6)	8.0 ± 1.7 (6)
+	GCDC	21.1 ± 1.0 (4)	18.3 ± 2.0 (6)	10.2 ± 1.6 (6)	7.2 ± 2.9 (5)
+	GC	20.8 ± 1.3 (6)	19.9 ± 2.0 (6)	7.7 ± 2.6 (6)	5.0 ± 1.9 (6)

[a] Values represent micromoles ± 1 standard deviation for the number of observations shown in parentheses.

[b] One milliliter of 0.9% NaCl was instilled into the ileum of each rat 4 hr prior to exogenous bile salt instillation to promote endogenous bile salt absorption. Eleven rats received another 1.0 ml of 0.9% NaCl at 0 hr and residual endogenous bile salts were measured.

[c] GCDC, glycochenodeoxycholate; TCDC, taurochenodeoxycholate; GC, glycocholate; TC, taurocholate.

tyramine, 79–84% were recovered from the intestines of the 0 hr animals; whereas recovery was 87–96% when the bile salt–cholestyramine complex was instilled. This indicates that bile salt absorption proceeded rapidly during the initial 3–5 min after instillation, but was retarded by cholestyramine.

In the absence of cholestyramine, slightly less taurine-conjugated than glycine-conjugated bile salts remained in the ileum both 1 and 2 hr after instillation. This suggests that the active transport system for bile salt in the ileum has a slightly greater affinity for taurine conjugates than for the corresponding glycine conjugates.

With each bile salt studied, the amounts remaining in the ileum both 1 and 2 hr after instillation were greater when given with cholestyramine than when given alone (Table II). The order of ileal bile salt retention in the presence of cholestyramine was TCDC> GCDC≧ TC>GC at 1 hr after instillation, and TCDC>TC>GCDC>GC at 2 hr after instillation.

In the presence of cholestyramine 25–45% of the exogenous trihydroxy bile salts and 39–53% of the dihydroxy salts in the ileum at 0 hr remained there 2 hr after instillation, indicating that a portion of the bile salts can be removed from the resin in the ileum and subsequently are absorbed.

Discussion. In the first experiment, ileal absorption of endogenous bile salts was found to be quite rapid. Preliminary studies in our laboratory indicated no absorption of bile salts from the jejunum with or without cholestyramine.[5] These observations are in agreement with reports of several other investigators (1–4, 13, 14). Dietschy (1) has presented evidence for jejunal absorption of bile salts by nonionic passive diffusion and related this to the type of conjugate and pK_a of each bile salt. Rat bile salts are primarily taurine conjugated ($pK_a = 2$) and, thus, almost totally ionized at intestinal pH. Therefore, little absorption would be expected to occur in the jejunum.

In Expt. 2, the trihydroxy bile salts were absorbed from the ileum slightly faster than the dihydroxy salts in the absence of cholestyramine. Similar results have been reported by Lack and Weiner (14). Furthermore, both TC and TCDC were absorbed slightly faster than their glycine counterparts when instilled without resin. This extends the obser-

[5] Cook, D. A., Hagerman, L. M., and Schneider, D. L., unpublished observations.

vations of Dietschy (1) who calculated higher serosal to mucosal ratios for the taurine conjugates than for the glycine conjugates from the data of Lack and Weiner (14).

Intestinal reabsorption of bile salts is normally about 97% efficient (15). The results presented here indicate that the mechanism by which cholestyramine administration increases fecal bile salt excretion probably involves retardation of the normal bile salt absorption process in the ileum. Both *in vitro* (7, 8) and in the ileum (Table II) cholestyramine has a greater affinity for taurine-conjugated bile salts than for the corresponding glycine conjugates. Oral administration of taurine increases the proportion of taurine-conjugated biliary bile salts in man (9, 10) and in hamsters (11). Thus, dietary taurine may have augmented fecal bile salt excretion in animals fed cholestyramine (11) by increasing the effectiveness with which the resin interrupts ileal bile salt absorption.

A greater affinity of cholestyramine for dihydroxy bile salts than for the corresponding trihydroxy salts has been demonstrated *in vitro* (7, 8) and in the ileum (Table II). Preferential binding of dihydroxy bile salts by this anion exchange resin may explain the decrease in the proportion of deoxycholate in human gallbladder bile during cholestyramine administration (16, 17).

These experiments also demonstrate that trihydroxy bile salts are removed from cholestyramine in the ileum more readily than dihydroxy salts. The more rapid absorption of cholate conjugates from the ileum in the presence of cholestyramine may be a factor in regulating the activity of certain hepatic enzymes involved in bile salt synthesis. Johansson (18) reported that the 12α-hydroxylation of 7α-hydroxy-4-cholesten-3-one, an important reaction in hepatic cholate synthesis, was stimulated 5-fold in bile duct fistulated rats but only 2-fold in rats fed cholestyramine. If cholate regulates its own synthesis via feedback control at the site of 12α-hydroxylation of 7α-hydroxy-4-cholesten-3-one, then the results of Johansson (18) may be due to the incomplete interruption of the enterohepatic circulation of cholate by cholestyramine.

Summary. In the absence of cholestyramine both glycine- and taurine-conjugated bile salts were rapidly absorbed from the rat ileum. Equilibration of bile salts with the resin prior to ileal instillation retarded, but did not completely interrupt, bile salt absorption. Cholestyramine retained the taurine conjugates of both the dihydroxy and the trihydroxy bile salts in the ileum more effectively than the corresponding glycine conjugates, and preferentially retained dihydroxy bile salts compared with trihydroxy bile salts.

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