In Vitro Binding of Mixed Micellar Solutions of Fatty Acids and Bile Salts by Cholestyramine (37259)

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Cholestyramine, a nonabsorbable anion-exchange resin, binds bile salts (1, 2) and other anions (3, 4) in the intestinal lumen and effectively decreases their absorption. Johns and Bates (5) found that cholestyramine has a relatively high affinity for bile acid anions, but an even greater affinity for long-chain fatty acid anions. Although they did not examine the effect of fatty acid anions on bile salt binding, their findings suggest that fatty acid anions, derived from digestion of dietary triglycerides, might reduce the *in vivo* bile salt sequestering ability of cholestyramine. The effects of fatty acids of various chain lengths and degrees of saturation on *in vitro* binding of bile salt to cholestyramine are described in this report.

Materials and Methods. Study 1. The binding of conjugated bile salts in isotonic saline to cholestyramine was examined in this study. Bile salt solutions (2–24 m*M*) were prepared by dissolving the sodium salt of taurocholic acid, glycocholic acid, taurodeoxycholic acid or glycodeoxycholic acid[1] in 0.15 *M* NaCl. Five milliliters of bile salt solutions were added to 16 × 100-mm screw-cap tubes and incubated on a rotary shaker with 20 mg of anhydrous cholestyramine[2] for 1 hr at 37°. Control tubes without resin were treated in the same manner. The contents of each tube were then transferred to 18 × 102 mm thick-walled centrifuge tubes and centrifuged at 30,000*g* for 10 min. The bile salt concentration in duplicate 0.2 ml aliquots of supernatant fluid was determined using hydroxysteroid dehydrogenase (6). All samples having a bile salt concentration > 2 m*M* were diluted with 0.15 *M* NaCl prior to analysis. Bile salt bound to resin was determined by difference between bile salt found in the resin-treated supernatant fluid and total bile salt in the original solution.

Study 2. The effects of fatty acid anions on binding to cholestyramine of a physiological level of individual conjugated bile salts in saline or a natural mixture of bile salts in diluted swine bile were examined in this study. Stock solutions of fatty acid (80 m*M*) were prepared by dissolving the sodium salts of $C_{8/0}$[1], $C_{9/0}$[3], $C_{10/0}$[1], $C_{11/0}$[3], $C_{12/0}$[1], $C_{18/1}$[1], or $C_{18/2}$[1] in deionized water. Stock solutions of bile salt (40 m*M*) were prepared by dissolving sodium taurocholate, glycocholate, taurodeoxycholate or glycodeoxycholate in 0.3 *M* NaCl. To 8 ml of bile salt solution were added 0, 1, 2, 4 or 8 ml of fatty acid solution and sufficient deionized water to make a total of 16 ml. Five milliliter aliquots of this mixture, containing 100 μmoles of bile salt (20 m*M*) and 0, 25, 50, 100, or 200 μmoles of fatty acid soap (0–40 m*M*) in 0.15 *M* NaCl were incubated with 20 mg cholestyramine for 1 hr at 37°. Bile salt bound to resin was determined by the procedure described for Study 1. The amount of fatty acid bound to the resin was determined only with oleate using the method of Kvam *et al.* (7).

A stock solution of swine gallbladder bile (approx 40 m*M* bile salt concentration) in 0.3 *M* NaCl was prepared by diluting 5 volumes swine gallbladder bile[4] with 3 volumes deionized water and 8 volumes 0.6 *M* NaCl. The effect of fatty acid soaps on binding of bile salt in swine bile to choles-

[1] A Grade, Calbiochem., Los Angeles, CA.
[2] Mead Johnson & Co., Evansville, IN.
[3] Eastman Chemical Co., Rochester, NY.
[4] Gallbladder bile from adult male Yorkshire-Hampshire swine.

tyramine was determined by the same procedure described above for pure bile salt solutions.

Study 3. The displacement by fatty acids of bile salt previously bound to cholestyramine was examined. Two and one-half milliliters of 40 m*M* taurodeoxycholate in 0.3 *M* NaCl was incubated with 20 mg cholestyramine in each of ten 18 × 102-mm tubes for 1 hr at 37°. Two of the tubes were centrifuged and the amount of bile salt in the supernatant fluid determined. To the remaining tubes, 2.5-ml aliquots of deionized water or 40 m*M* solutions of sodium caprylate, laurate, or linoleate were added in duplicate and the mixtures were incubated for an additional hour at 37°. These tubes were then centrifuged and taurodeoxycholate which remained bound to the resin was determined by difference as described above.

Results. Study 1. The relative affinities of the four conjugated bile salts for cholestyramine are shown in Fig. 1. Both the taurine and glycine conjugates of deoxycholate have higher affinities for cholestyramine than either conjugate of cholate. At bile salt concentrations ≦ 12 m*M*, over 90% of the conjugated dihydroxy bile salts were bound to the resin. Taurodeoxycholate was bound slightly better than glycodeoxycholate. At concentrations ≧ 16 m*M*, binding of dihydroxy bile salt was maximal. Presumably bile salt occupied all the available sites on the resin. Both of the conjugated trihydroxy bile salts had a lower affinity for cholestyramine than either of the dihydroxy conjugates; however, taurocholate had 50% greater affinity for the resin than did glycocholate.

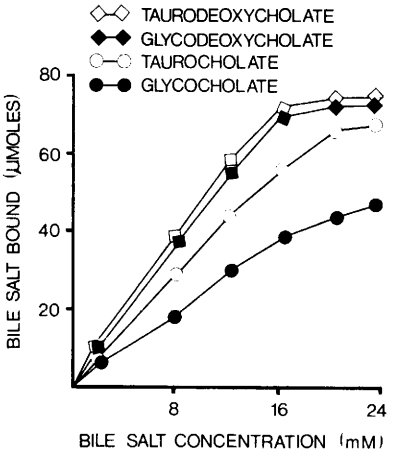

FIG. 1. Affinity of conjugated bile salts for cholestyramine. Each tube contained one of the conjugated bile salts dissolved in 5 ml 0.15 *M* NaCl and incubated for 1 hr at 37° with 20 mg cholestryramine (chloride exchange capacity of 90 μmole/20 mg).

Study 2. The inhibitory effects of fatty acid anions on bile salt binding are directly related to fatty acid chain length (Table I). The 200 μmole level of sodium caprylate ($C_{8/0}$) had practically no effect on binding of taurodeoxycholate, but reduced the binding of the other three bile salts by 19–25%. This level of pelargonate ($C_{9/0}$) and caprate ($C_{10/0}$) depressed taurodeoxycholate binding by 23 and 49%, respectively. Binding of the other bile salts was reduced by 43–45% by $C_{9/0}$ and 58–63% by $C_{10/0}$. The 200 μmole level of $C_{11/0}$, $C_{12/0}$, $C_{18/1}$, and $C_{18/2}$ depressed binding of all four bile salts by 65–80%. For each bile salt, the relative effects of these four fatty acids were similar.

When mixtures of each conjugated bile salt (20 m*M*) and graded levels of sodium oleate were incubated with cholestyramine, oleate was bound to the resin as bile salt was displaced. Binding of each anion was limited by the anion-exchange capacity of the resin (Table I).

When cholestyramine was incubated with diluted swine gallbladder bile, the competitive effects of $C_{8/0}$, $C_{10/0}$, $C_{12/0}$, and $C_{18/2}$ on bile salt binding to the resin were similar to their effects on binding of the pure bile salts in saline (Table I). Laurate ($C_{12/0}$) and linoleate ($C_{18/2}$) inhibited binding of bile salts in swine bile more than did either caprylate or caprate.

Study 3. Fatty acid anions (100 μmole of $C_{8/0}$, $C_{12/0}$, or $C_{18/2}$) were added to tubes containing cholestyramine that had previously been incubated with 100 μmole of taurodeoxycholate. Before addition of fatty acid, 74 μmole of taurodeoxycholate was bound to the resin. After addition of deionized water or solutions containing $C_{8/0}$, $C_{12/0}$, or $C_{18/2}$ followed by incubation for a second hour; 84, 80, 42, and 34 μmole of taurodeoxycholate were bound, respectively. Thus, the addition of fatty acid anions to resin containing previously bound taurodeoxychol-

TABLE I. Effect of Various Fatty Acid Anions on Bile Salts Bound by Cholestyramine.[a]

		Bile salt bound								Fatty acid bound
	Fatty acid added	None	$C_{8/0}$	$C_{9/0}$	$C_{10/0}$	$C_{11/0}$	$C_{12/0}$	$C_{18/1}$	$C_{18/2}$	$C_{18/1}$
	μmole					μmole				μmole
Taurocholate	0	66								
(100 μmole)	25		62	60	55	53	52	52	55	24
	50		58	56	47	43	42	46	44	42
	100		55	47	37	33	30	33	32	52
	200		50	37	28	17	18	16	24	66
Glycocholate	0	43								
(100 μmole)	25		39	39	34	32	38	38	35	23
	50		35	36	31	32	32	33	34	38
	100		31	30	25	22	23	23	24	48
	200		26	23	18	9	13	13	15	69
Taurodeoxycholate	0	73								
(100 μmole)	25		71	74	70	67	67	64	65	17
	50		70	73	67	58	56	50	54	34
	100		71	68	53	43	42	37	37	49
	200		69	56	37	19	22	24	22	61
Glycodeoxycholate	0	73								
(100 μmole)	25		67	69	63	57	56	58	57	19
	50		67	63	55	47	47	44	46	35
	100		62	55	42	34	33	29	30	48
	200		55	42	27	16	17	15	16	67
Diluted swine bile	0	61								
(117 μmole)	25		62		53		53		47	
	50		58		49		41		39	
	100		56		40		30		26	
	200		50		25		17		10	

[a] System: Five milliliters bile salt–fatty acid solution incubated with 20 mg cholestyramine for 1 hr at 37°. Each value is an average of duplicate analyses.

ate resulted in approximately the same binding equilibria as when equimolar amounts (100 μmole) of taurodeoxycholate and these fatty acid anions were added simultaneously to cholestyramine (Table I).

Discussion. These *in vitro* studies indicate that bile salt structure is an important determinant of the bile salt's affinity for cholestyramine. Taurine-conjugated bile salts have a higher affinity for the resin than do the glycine conjugates as shown in previous reports (8–10). This is thought to be related to a greater attraction of the fixed quaternary ammonium groups on the resin for the relatively stronger acidic sulfonic group of taurine conjugates than for the less acidic carboxyl group of glycine conjugates. The dihydroxy bile salts are more tightly bound to the resin than are the more polar trihydroxy bile salts, perhaps because of the greater nonionic attraction of the relatively hydrophobic dihydroxy bile salt nucleus for the polystyrene matrix of the resin. Furthermore, the interaction of dihydroxy bile salts with each other on the resin is probably greater than that between trihydroxy bile salts since dihydroxy bile salts have a greater tendency to associate in aqueous solution and form micelles (11).

The study with mixed bile salt–fatty acid solutions demonstrates that the long-chain fatty acid anions readily displace bile salts from binding sites on the resin and their effect on dihydroxy bile salt binding is more pronounced than their effect on trihydroxy bile salt binding. Fatty acid anions may depress bile salt binding not only by ionic competition, but also by reducing the nonionic attraction by which one bile salt may

reinforce the binding of another. Thus, fatty acid anions with chain lengths of 10 or less carbons only moderately inhibit bile salt binding; whereas those with 11 or more carbons are about equally effective in markedly reducing binding.

The relatively low affinity of medium-chain fatty acids for the resin may explain their essentially complete absorption when medium-chain triglyceride (MCT) is fed with cholestyramine (12, 13). Binding of long-chain fatty acids to cholestyramine may contribute to their malabsorption when long-chain fat diets are fed with the resin (12). Long-chain fat malabsorption may also be related to a lowered intraluminal concentration of bile salt in the presence of cholestyramine. Medium-chain fat, on the other hand, does not require bile salt for optimal absorption (14).

Bile salt binding is a reversible process, as indicated by the displacement of previously bound taurodeoxycholate from cholestyramine when fatty acids were subsequently incubated with resin. The final equilibrium established after incubation of the bile salt loaded resin with fatty acid solutions was similar to that achieved when fatty acid and bile salt mixtures were incubated simultaneously with the resin.

The significance of fatty acid competition as an *in vivo* effector of cholestyramine's bile salt binding ability is not known. On the one hand, Hofmann *et al.* (15) found that cholestyramine (16 g/day) effectively controlled bile salt induced diarrhea in patients with ileal resection only when fecal fat excretion was less than 20 g/day. In patients whose fat excretion exceeded 20 g/day, cholestyramine was ineffective in curtailing the bile salt induced diarrhea. When MCT was fed to these patients, the steatorrhea was eliminated and cholestyramine then effectively controlled the diarrhea. Thus, it is possible that fatty acid anions in the colon can compete with bile salt for binding sites on cholestyramine. These findings and the *in vitro* data above support the suggestion of Johns and Bates (5) that fatty acid anions derived from the hydrolysis of dietary fat could decrease the effectiveness of the resin as a bile salt sequestrant. On the other hand, recent findings in rats fed cholestyramine showed little difference in fecal bile salt excretion whether corn oil or MCT was fed as the dietary fat (16). Other factors, such as active transport of bile salt in the ileum (2), may also affect the binding of bile salt to cholestyramine when administered to man or experimental animals.

Summary. When cholestyramine was incubated with pure bile salts in isotonic saline, it preferentially bound taurine-conjugated bile salts and dihydroxy bile salts as compared with the respective glycine conjugates and trihydroxy bile salts. Addition of fatty acids to the incubation mixtures resulted in decreased binding of each bile salt, an effect directly related to concentration of fatty acid. Medium-chain fatty acids had relatively little effect on binding, whereas the longer chain fatty acids markedly depressed binding of each of four pure conjugated bile salts in isotonic saline and of naturally occurring mixed bile salts in swine bile.

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