Effect of Dietary Taurine on Fecal Bile Salt Excretion in Rats and Hamsters Fed Cholestyramine (36001)

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Oral administration of cholestyramine, a quaternary ammonium anion exchange resin, increases fecal bile salt excretion in animals (1, 2) and in man (3, 4). The loss of bile salt elicits a compensatory increase in cholesterol catabolism (4, 5) and results in a subsequent decrease in serum cholesterol levels in those species in which the rate of cholesterol catabolism appears to exceed its rate of biosynthesis (2–6).

Studies by Johns and Bates (7) and experiments in our laboratory[1] have shown that cholestyramine *in vitro* has a greater affinity for taurine-conjugated bile salts than for the corresponding glycine conjugates. The work of Roe (8) and Wood *et al.* (9) in human subjects suggests preferential excretion of taurine-conjugated bile salts during cholestyramine administration. Since dietary taurine markedly increases the proportion of taurine-conjugated bile salts in human bile (10, 11), dietary taurine in conjunction with cholestyramine might increase fecal bile salt excretion more than cholestyramine alone. The present results demonstrate that dietary taurine significantly increases fecal bile salt excretion in rats and hamsters fed diets containing cholestyramine.

Methods. Expt. 1. Ninety male weanling rats of Wistar derivation[2] were allotted to 9 groups of 10 animals each, on the basis of body weight. They were housed individually in screen-bottom cages in an air-conditioned animal room and were fed basal diets (Table I) containing 0, 2, or 4% cholestyramine in combinations with 0, 0.5 or 1.0% taurine in a 3 × 3 factorial experiment. Cholestyramine and taurine were added at the expense of the total diet. Feces were collected from each animal for 7 days beginning with day 22 on test and analyzed for total fecal bile salts. Bile salts were extracted from feces with 0.5 *N* HCl in 50% ethanol. Fatty acids were removed from the alcohol extract by liquid–liquid extraction with petroleum ether. An aliquot of the fatty acid-free alcohol extract was assayed for total fecal bile salts by the hydroxysteroid dehydrogenase method of Hurlock and Talalay (12) using a purified cell-free enzyme preparation.[3]

Liver taurine levels were determined on group-pooled samples (13). Plasma cholesterol levels were determined after 2 and 4 weeks on experiment (14). Growth, food con-

TABLE I. Composition of Basal Diets.

Ingredient	Diet; (g/100 g) Rat	Hamster
Casein, ANRC[a]	20.00	27.0
Partially hydrolyzed cornstarch[b]	61.62	57.5
Corn oil	10.00	5.0
Fiber[c]	4.00	5.0
Salt mix	4.00[d]	5.0[e]
Vitamin mix	0.35[f]	0.5[e]
Oleum percomorphum	0.015	—
33% α-Tocopherol acetate	0.015	—

[a] Sheffield Chemical Co., Norwich, N.Y., 90% protein. Meets specifications of Animal Nutrition Research Council.

[b] Amidex, Corn Products Co., New York.

[c] Nonnutritive cellulose, General Biochemicals, Inc., Chagrin Falls, OH.

[d] Jones, J. H., and Foster, C., J. Nutr. 24, 245 (1942).

[e] Dam, H., Z. Ernaehr. 6, 97 (1965).

[f] Sarett, H. P., and Snipper, L. P., J. Nutr. 52, 525 (1954). Ascorbic acid omitted.

[1] Hagerman, L. M., Cook, D. A., and Schneider, D. L., unpublished results.

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

[3] Worthington Biochemical Corp., Freehold, NJ.

sumption, and selected organ weights were also measured.

Expt. 2. Ninety young adult male Golden Syrian hamsters[4] were used, employing the same experimental design as in Expt. 1. Feces were collected twice weekly throughout the 28-day experimental period and group pooled on a weekly basis for bile salt analyses. At the end of the experiment bile was obtained from the gallbladder of each hamster after an overnight fast. The relative proportion of glycine-conjugated and taurine-conjugated bile salts was determined, utilizing the thin-layer chromatography system of Kelly and Doisy (15) and hydroxysteroid dehydrogenase (12).

Expt. 3. Two-hundred male rats, 80–90 g, were fed the basal diet (Table I) for a 14-day pretest period. Groups of 10 rats each were then selected on the basis of body weight and given diets containing 0.0, 0.035, 0.35, and 1.04% taurine in combination with 0.0, 0.07, 0.56, 1.12, and 1.68% cholestyramine in a 4 × 5 factorial experiment.

Individual fecal bile salt excretion rates during the second week of the 2-week test period were determined as in Expt. 1.

Results. Expt. 1. Addition of 0.5 and 1.0% taurine to diets containing 0, 2, or 4 cholestyramine generally increased fecal bile salt excretion by about 50% (Table II). Rats fed 2% cholestyramine with dietary taurine excreted more bile salts than rats fed 4% cholestyramine without supplementary taurine. Factorial analysis of fecal bile salt excretion data (16) revealed a significant ($p<.025$) cholestyramine × taurine interaction.

Liver taurine levels in rats fed cholestyramine were about one-half those in the control rats. Dietary taurine, with or without cholestyramine, increased liver taurine levels 2- to 10-fold, but had no effect on growth, food or water consumption, or organ weights.

Expt. 2. Dietary taurine increased fecal bile salt excretion by as much as 80% in hamsters fed cholestyramine. Excretion rates during the forth week of the experiment are shown in Table II. Essentially the same values were obtained for the second and third weeks. The percentage of gallbladder bile salts conjugated with taurine also was increased by dietary taurine.

Expt. 3. Lower levels of cholestyramine were used in this experiment than were used in Expts. 1 and 2. Fecal bile salt excretion was increased slightly at the 0.35% dietary taurine level and was increased further at the 1.04% dietary taurine level (Table III). Analysis of variance of this 4 × 5 factorial experiment showed a significant taurine × cholestyramine interaction ($p<.05$). A series of 2 × 2 factorial analyses revealed a significant interaction ($p<.05$) between the 1.04% level of taurine and the 0.56, 1.12, and 1.68% resin levels. When compared over all levels of resin, 0.35% taurine significantly ($p<.05$) increased bile salt excretion when compared with controls receiving no dietary taurine.

Discussion. Two or 4% dietary cholestyramine resulted in a 6- to 8-fold increase in fecal bile salt excretion in normocholesterolemic rats. Addition of dietary taurine to this regimen resulted in a further increase of 44–56% in fecal bile salt excretion.

The bile salt excretion rate of 21 mg/100 g of body wt/day in rats fed 4% cholestyramine without taurine is similar to bile salt secretion rates in rats with cannulated bile ducts (17, 18). Under these conditions, bile salt synthesis appears to be limited by the amount of hepatic taurine available for conjugation, since rats fed 2 or 4% cholestyramine plus taurine excreted considerably more than 21 mg of bile salt/100 g of body wt/day. This postulation is supported by the observation of Brauser and Hermann (19) that taurine increased the synthesis rate of taurocholate from cholesterol 3- to 4-fold in perfused rat livers.

The beneficial effect of dietary taurine on cholestyramine efficiency is particularly interesting since the rat has a relatively high capacity for hepatic taurine synthesis (20) and normally conjugates bile salts predominantly with taurine (21). However, since taurine-conjugated bile salts are preferentially bound to anion exchange resins [(7), Footnote 1], the increase in fecal bile salt excretion associated with cholestyramine administration may decrease liver taurine

[4] Obtained from Engle's Laboratory Animals, Farmersburg, IN.

TABLE II. Fecal Bile Salt Excretion, Liver Taurine, and Bile Composition of Rats and Hamsters Receiving Taurine or Cholestyramine or Both.

Dietary treatment; (g/100 g of diet)		Expt.: 1 Rat		2 Hamster	
Taurine	Cholestyramine	Fourth week fecal bile salts (mg/100 g of body wt/day)	Liver taurine (mg/g of wet tissue)	Fourth week fecal bile salts (mg/100 g of body wt/day)	% Taurine conjugated bile salts in gallbladder bile
0.0	0.0	2.8 ± 0.7[a]	0.26[b]	0.66[b]	69 ± 7[a]
0.5	0.0	3.1 ± 0.7	0.54	0.59	76 ± 10
1.0	0.0	4.1 ± 0.9	1.01	0.60	88 ± 11
0.0	2.0	17.6 ± 3.9	0.11	3.6	75 ± 8
0.5	2.0	26.1 ± 4.7	0.84	6.0	92 ± 7
1.0	2.0	25.4 ± 5.5	1.06	4.9	96 ± 3
0.0	4.0	21.4 ± 5.1	0.13	3.2	76 ± 9
0.5	4.0	30.8 ± 9.0	0.67	5.3	92 ± 8
1.0	4.0	33.5 ± 9.0	1.12	5.8	95 ± 4

[a] Mean ± 1 SD.

[b] Mean of duplicate analyses on group-pooled samples.

TABLE III. Fecal Bile Salt Excretion in Rats Receiving Taurine or Cholestyramine or Both (Expt. 3).

Cholestyramine (g/100 g of diet)	Taurine (g/100 g of diet)			
	0.00	0.035	0.35	1.04
0.00	2.3 ± 0.6[a]	2.6 ± 0.9	2.9 ± 1.0	3.1 ± 1.0
0.07	3.1 ± 0.9	3.0 ± 0.6	3.5 ± 0.9	4.0 ± 1.1
0.56	8.3 ± 1.7	8.6 ± 2.2	10.0 ± 2.0	11.2 ± 1.9
1.12	13.6 ± 2.6	12.8 ± 4.0	14.6 ± 2.3	17.7 ± 3.7
1.68	15.4 ± 3.2	15.1 ± 4.3	16.8 ± 4.9	22.3 ± 7.1

[a] Fecal bile salts (mg/100 g of body wt/day); mean ± 1 SD.

levels. In accord with this hypothesis, increased fecal taurine excretion and a decrease in the proportion of taurine-conjugated bile salts in bile have been reported in humans receiving cholestyramine (8, 9).

Plasma cholesterol levels were not affected by dietary treatment in this experiment even though fecal bile salt excretion was increased up to 12-fold. This is probably due to the ability of the rat to compensate for increased cholesterol catabolism via bile salt loss by increasing hepatic cholesterol synthesis 12- to 15-fold (6).

In hamsters, dietary taurine increased fecal bile salt excretion by as much as 80% and increased the proportion of gallbladder bile salts conjugated with taurine. Oral administration of taurine has a similar effect on bile salt composition of gallbladder bile in humans (10, 11) and in rats fed diets containing cholesterol and sodium cholate.[1]

Dietary taurine may enhance fecal bile salt excretion in rats and hamsters fed cholestyramine by either or both of two mechanisms. First, dietary taurine may increase the ability of the liver to oxidize cholesterol by providing what might otherwise be a limiting factor in the rate of bile salt synthesis and conjugation (19). Second, taurine enables the liver to synthesize a relatively higher proportion of taurine-conjugated bile salts (10, 11), which have a greater affinity for cholestyramine in the gut than the corresponding glycine conjugates. Intestinal instillation studies in our laboratory indicate that cholestyramine retains taurine conjugates in the rat ileum to a greater extent than the corresponding glycine conjugates.[1]

In Expt. 3, fecal bile salt excretion patterns in response to cholestyramine and taurine were similar to those observed in the first two experiments. Fecal bile salt excretion was not affected by 0.035% taurine at any level of cholestyramine. Although the bile salt excretion was increased with 0.35% taurine, the response was less marked than at 1.04% taurine. These results are not surprising since the rat has a relatively high capacity for hepatic taurine synthesis (20) and can probably synthesize sufficient taurine from dietary methionine and cysteine to partially mask the effect of dietary taurine at the levels of cholestyramine fed. In contrast, the capacity of human liver for taurine synthesis is thought to be lower than that of rat liver (20).

The results of these experiments indicate that dietary taurine increases fecal bile salt excretion in animals fed cholestyramine. Taurine administration may be a feasible means of increasing fecal bile salt excretion at a given level of cholestyramine intake, or of maintaining a given bile salt excretion rate with lower levels of the resin.

Summary. When fed with 2 or 4% cholestyramine, 0.5 and 1.0% dietary taurine increased fecal bile salt excretion by 44 to 56% in rats and by as much as 80% in hamsters. Taurine also increased bile salt excretion 30–44% in rats fed 0.07–1.68% cholestyramine. Dietary taurine increased the percentage of gallbladder bile salts conjugated with taurine in hamsters fed cholestyramine. These findings are consistent with *in vitro* findings that cholestyramine has a greater affinity for taurine-conjugated bile salts than for the corresponding glycine conjugates, and may provide a feasible means of improving the *in*

vivo efficiency of the resin.

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