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The Effect of Sodium Benzoate and Taurocholic Acid Feeding on Human Bile Composition[1, 2] (38592)

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Although the precise nature of cholesterol gall stone formation has not been elucidated the precipitation of cholesterol from a supersaturated solution in bile has been felt to play an integral role. The description of the cholesterol solubilizing effect of bile salts and phospholipids in micellar formation has led to treatment directed toward increasing the bile salt pool. Stones do not form in all biles supersaturated with cholesterol however and simple precipitation does not explain the aggregation necessary to produce gall stones.

Since stones rarely form naturally in species conjugating bile acids primarily with taurine (1) we have been investigating some of the factors involved in altering bile salt conjugation in the liver. In previous work we demonstrated dissolution of lithocholic acid stones in rats by altering the bile salt conjugation in protein depleted animals (2). A change to glycine conjugation leads to conditions favorable for the formation of these stones.

In humans, glycine conjugation predominates over taurine conjugation in a ratio of 3 or 4:1. This is probably due to a borderline availability of taurine or its precursors since it can be markedly altered by prolonged taurine feeding (3). Under these conditions the ratio may be reversed.

This report involves an investigation of the use of glycine depletion to effect a reversal of conjugation patterns in bile.

Quick (4) introduced the use of sodium benzoate feeding as a test of liver function. Investigations subsequently demonstrated that sodium benzoate is converted to hippuric acid by conjugation with glycine (5). With a 4–6 g dose the glycine stores may be quickly depleted since 94% of the benzoate is conjugated with glycine in preference to glucuronic acid. In our work a dose of 6 g of sodium benzoate was given over several days and then combined with 3 g of taurocholic acid to see if the glycine/taurine (G/T) conjugation ratio could be reversed under acute conditions.

Methods. Four patients were evaluated. All patients had cholecystectomy for cholesterol gall stones and common bile duct exploration with the insertion of a Baldwin T-tube. Six days were allowed for stabilization prior to testing, at which time the patients were all eating a regular diet. Sodium benzoate was given in a dose of 2 g three times a day for 2 or 3 days. In three patients taurocholic acid 1.0 g three times a day was added for the next 3 days. Bile was sampled daily and at varying intervals complete occlusion of the bile duct by inflation of the distal balloon allowed complete collections for measurement of excretion rates. Obstruction was never carried out for more than 2 h in any one day to avoid the effects of complete interruption of the enterohepatic circulation.

Biles were analyzed for cholesterol, phospholipids, total bile acids, taurine and glycine conjugates of cholic acid and chenodeoxycholic acid. Cholesterol was measured by the method of Anderson and Key (6). Phospholipids were calculated by multiplying the phosphorus level (7) by 25. Total bile acids were determined by modification of the methods of Talalay (8) and Small and Rapo (9) using enzymatic digestion. Specific bile acids and glycine and taurine conjugates were measured by thin layer chromatography using solvent systems of: Proprionic acid, isoamylacetate, water, and *N*-propanol

[1] This research was approved by the Committee on Human Experimentation of the Downstate Medical Center and informed consent was obtained from each patient involved.

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(15:200:5:10) or acetic acid, carbon tetrachloride, di-isopropyl ether, isoamylacetate, propanol and benzene (0.5:2:3:4:1:1) (10–12).

Results. No significant changes were noted in serum alkaline phosphatase, bilirubin or GOT levels during the experiment. Cultures of the bile were not done but no patient demonstrated a febrile course.

Composition of the Bile (Table I). A summary of the conjugation patterns of the patients showed that the benzoate was incapable of significantly lowering the glycine conjugates in the bile. Taurocholic acid significantly increased the concentration of taurine conjugates and markedly altered the G/T ratio. Since this summary includes collections of biles after varying amounts of the drug the individual data is summarized in Tables II–V. The only consistent results were the increase in taurine conjugates after feeding taurocholic acid. Total bile acids were

TABLE I. CONCENTRATION OF BILE SALT CONJUGATES IN HUMAN BILE AFTER TAUROCHOLIC ACID FEEDING

	Taurine conjugates ($\mu M\%$ ± SE)	Glycine conjugates ($\mu M\%$ ± SE)	G/T (± SE)
Baseline	296 ± 31	1135 ± 237	3.8 ± 0.5
Na benzoate	248 ± 20	1011 ± 104	4.1 ± 0.3
Na benzoate + Taurocholic acid	945 ± 125	1076 ± 84	1.5 ± 0.2

TABLE II. BILE COMPOSITION AFTER SODIUM BENZOATE AND TAUROCHOLIC ACID FEEDING.

Patient No. 1

Day	Medication	Total Bile acids	Lecithin	Cholesterol	Total cholates	Total chenodeoxy cholates	Taurine conj.	Glycine conj.	G/T
					μM/cc				
0	Baseline	23.16	11.83	5.35	17.79	5.37	3.66	19.50	5.3
1	Sodium benzoate	21.53	12.89	6.98	16.22	5.31	3.75	17.78	4.7
2	Sodium benzoate	22.26	11.59	6.85	16.95	5.25	4.47	17.79	4.0
3	Sodium benz. + Taurocholic acid	32.33	14.64	6.85	23.49	8.84	16.02	16.31	1.0

TABLE III. BILE COMPOSITION AFTER SODIUM BENZOATE AND TAUROCHOLIC ACID FEEDING

Patient No. 2

Day	Medication	Total bile acids	Lecithin	Cholesterol	Total cholates	Total chenodeoxy cholates	Taurine conj.	Glycine conj.	G/T
					μM/cc				
0	Baseline	10.44	4.36	4.86	8.49	1.95	3.51	6.93	2.0
1	Sodium benzoate	7.70	4.67	5.02	5.63	2.07	1.88	5.82	3.1
2	Sodium benzoate	7.47	4.47	4.89	5.55	1.92	1.50	5.97	4.0
3	Sodium benzoate	10.41	4.73	3.29	7.68	2.73	1.38	9.03	6.5
4	Sodium benz. + Taurocholic acid	26.16			21.43	5.18	10.58	15.58	1.5
5	Sodium benz. + Taurocholic acid	21.78			17.49	4.29	9.51	12.27	1.3

TABLE IV. BILE COMPOSITION AFTER SODIUM BENZOATE AND TAUROCHOLIC ACID FEEDING.

Patient No. 3									
Day	Medication	Total bile acids	Lecithin	Cholesterol	Total cholates	Total chenodeoxy cholates	Taurine conj.	Glycine conj.	G/T
					μM/cc				
0	Baseline	11.10	4.66	5.72	8.19	2.91	3.12	7.98	2.6
1	Sodium benzoate	12.31	6.10	6.70	9.85	2.46	2.98	9.33	3.1
2	Sodium benzoate	17.21	7.72	5.79	13.69	3.52	2.77	14.44	5.2
3	Sodium benz. + Taurocholic acid	15.76	7.92	8.30	12.57	3.19	3.16	12.60	4.0
4	Sodium benz. + Taurocholic acid	14.74	7.25	8.30	10.43	4.31	7.63	7.11	0.9
5	Sodium benz. + Taurocholic acid	18.69	7.09	6.70	14.14	4.55	8.75	9.94	1.1

TABLE V. BILE COMPOSITION AFTER SODIUM BENZOATE AND TAUROCHOLIC ACID FEEDING

Patient No. 4									
Day	Medication	Total bile acids	Lecithin	Cholesterol	Total cholates	Total chenodeoxy cholates	Taurine conj.	Glycine conj	G/T
					μM/cc				
0	Baseline	10.23	4.0	.78	8.16	2.05	1.96	8.27	4.2
1	Sodium benzoate	7.45	2.01	1.01	5.87	1.58	1.72	5.73	3.3
2	Sodium benzoate	10.09	3.01	1.89	8.30	1.79	1.89	8.20	4.3
3	Sodium benzoate	7.60	2.35	1.47	6.20	1.40	1.47	6.13	4.2
4	Sodium benz. + Taurocholic acid	12.57	5.04	3.44	9.68	2.89	5.04	7.53	1.5
5	Sodium benz. + Taurocholic acid	21.17	7.32	3.83	16.98	4.19	10.37	10.80	1.0

significantly increased ($P < 0.01$) in all patients with a marked fall in G/T ($P < 0.05$). A proportional increase in biliary phospholipids and cholesterol was also demonstrated. There was no consistent change in the ratio of cholate to chenodeoxycholate concentration in any of the patients.

Excretion data. Figures 1–4 demonstrate the rates of excretion of total bile acids, taurine and glycine conjugates during the experiment. Three of four patients demonstrated a slight fall in bile acid excretion which returned to normal due to an increase in taurine conjugated bile acid excretion. Before taurocholic acid feeding was started the per cent change in excretion of bile acids acids was −48%, −30%, +68%, −28%. Although there was a slight increase in glycine conjugated bile acid excretion, the rise in total bile acid excretion was predominantly due to an increase in excretion of taurine conjugates. Of interest as can be seen from Table VI both taurocholate and chenodeoxytaurocholate excretion increased in all patients fed taurocholic acid.

Discussion. We have previously demonstrated that short term feeding of taurocholic acid will increase the excretion of taurine conjugated and total bile acids in humans (2). Complete reversal of G/T is possible

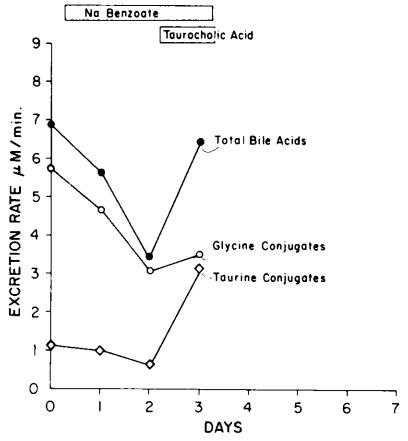

FIG. 1. Bile acid excretion rates after feeding benzoate alone with taurocholic acid.

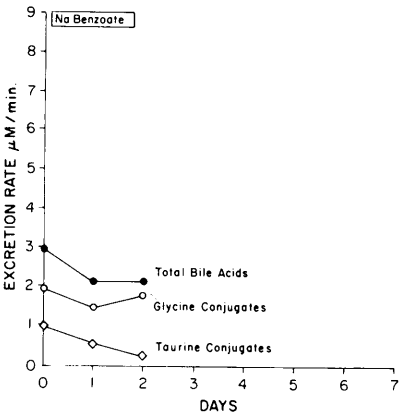

FIG. 2. Bile acid excretion rates after feeding benzoate.

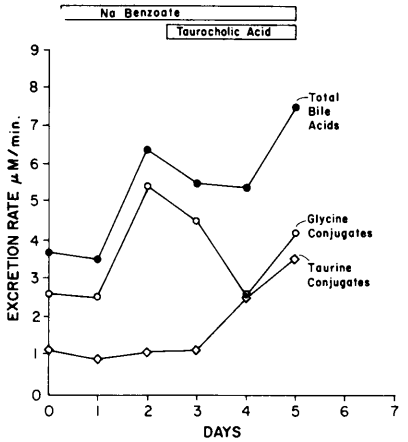

FIG. 3. Bile acid excretion rates after feeding ben zoate alone and with taurocholic acid.

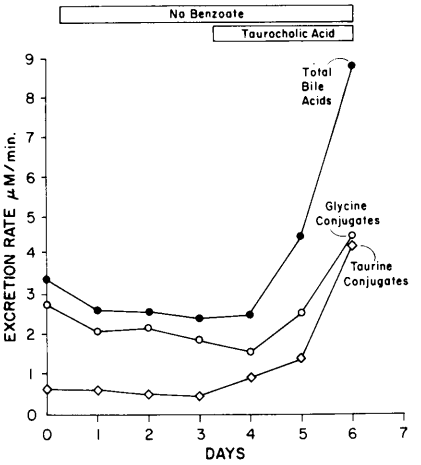

FIG. 4. Bile acid excretion rates after feeding benzoate alone with taurocholic acid.

with long term administration of taurine (3). The implication of this work is that the usual G/T ratio of 3 or 4:1 consistently found in humans is the result of a relatively low taurine pool available for conjugation of bile acids and not due to preferential conjugation with glycine. In our work the total bile acid excretion is increased by taurocholic acid feeding and therefore this compound may represent a preferred means of altering bile composition without the side effects usually produced by chenodeoxycholic acid. Of interest in this regard is the fact that both cholate and chenodeoxycholate excretion is increased (Table VI). More detailed work is now necessary to determine if longer term feeding of this compound will result in the same increase in bile salt concentration and can be useful for the dissolution of gall stones *in vivo*.

In order to test the hypothesis that a short period of glycine depletion can lead to preferential conjugation with taurine these patients were fed sodium benzoate alone and then in combination with taurocholic acid. The dose of 6 grams a day was chosen on the basis of older work which demonstrated 94% conjugation of the benzoate with glycine (13) and conversion to hippuric acid. The rate limiting factor in the conversion of benzoate to hippuric acid is the availability of glycine and therefore it appeared likely that glycine diverted to the formation of hippuric acid

TABLE VI. EXCRETION OF SPECIFIC BILE ACIDS WITH TAUROCHOLIC ACID FEEDING.

	Excretion rate μM/min											
	TC[a]			TCD[a]			GC[a]			GCD[a]		
Patients	1	2	3	1	2	3	1	2	3	1	2	3
Before taurocholic acid	0.7	0.7	0.5	0.4	0.4	0.2	4.6	2.1	2.2	1.2	0.6	0.5
After taurocholic acid	2.2	1.9	4.0	1.0	1.0	0.5	2.5	1.8	3.1	0.8	0.7	0.6

[a] TC—Taurocholate; TCD—Chenodeoxytaurocholate; GC—Glycocholate; GCD—Chenodeoxyglycocholate.

may not be available for conjugation of bile salts. Investigations into the site of action of conjugation have revealed that conjugation of cholic acid requires the presence of liver microsomes and supernatant fractions while the conjugation of benzoic acid requires the additional presence of mitochondria (14) indicating that similar mechanisms are not utilized.

This was borne out in our patients who failed to demonstrate a significant drop in glycine conjugated bile salt output despite feeding sodium benzoate. When taurocholic acid was added to the regimen conversion of the G/T was no greater than we have previously demonstrated with taurocholic acid alone. It therefore appears that sodium benzoate as used in this experiment is not able to significantly reduce the output of glycine conjugated bile salts in bile.

Summary. Four patients were fed sodium benzoate after stabilization following common bile duct exploration. Bile collections revealed no change in the output of glycine conjugated bile acids. Three patients had taurocholic acid added to the regimen after 3 days and demonstrated a significant increase in total and taurine conjugated bile acid output with marked reduction of the G/T ratio. These latter changes are similar to those produced by taurocholic acid feeding alone and therefore no benefit of the combination of drugs on bile salt excretion or conjugation ratio was demonstrated.

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