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The Effect of Psyllium Hydrocolloid and Cholestyramine on Hepatic Bile Lipid Composition in Man

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The effects of a mucoid - psyllium hydrocolloid - and an anion exchange polymer-cholestryamine - on the total cholesterol, total phospholipid, total bile salt, cholate, chenodeoxycholate, and deoxycholate concentrations of hepatic bile were determined in six post-cholecystectomy patients. Bile was obtained by drainage through an indwelling T-tube, which was clamped except during bile collection. Psyllium hydrocolloid treatment (12 gm/day) for 6 to 29 days had little or no effect on the cholesterol or phospholipid concentration of hepatic bile, but increased the total bile salt pool by gradually increasing the concentration of deoxycholate. Cholestyramine treatment (12 gm/day) for 8 to 12 days had no significant effect on cholesterol, phospholipid or total bile salt concentrations. There was a significant increase in the tri- to di-hydroxy bile salt ratio due to decreases in chenodeoxycholate and deoxycholate concentrations. The ratio of taurine to glycine conjugates decreased because of reductions in concentrations of taurine conjugates and compensating increases in glycine conjugates. The influence of these changes on bile micelle stability and cholesterol solubility is discussed. It is concluded that the changes effected by psyllium hydrocolloid may result in more stable bile micelles and greater cholesterol solubility. No definite conclusions can be reached with respect to cholestyramine's effects.

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WORLD-WIDE epidemiological surveys have generated statistical evidence which suggests that there is a positive correlation between blood cholesterol concentration and the incidence of atherosclerotic heart disease. These findings have resulted in major efforts to develop hypocholesterolemic drugs which would presumably lessen the tendency to develop atherosclerosis. Two classes of effective drugs have resulted: (a) substances that inhibit or retard the rate of hepatic cholesterol synthesis, and (b) drugs that increase the rate of tissue cholesterol elimination via either the fecal neutral steroid or bile acid pathway. Substances of the latter type increase the rate of cholesterol elimination in certain species, including man, to such an extent that hepatic synthesis of new cholesterol molecules is unable to compensate for the loss, and a reduction of blood cholesterol concentration results.

Two types of drugs have been found that primarily increase tissue cholesterol elimination via the fecal bile acid pathway. They are the anion exchange polymers[1-4] and the hydrophilic mucoids.[5,6] Since these drugs greatly increase the rate of elimination of bile salts from their enterohepatic circulating pool, [1,5,6] it is possible that they also change the size and composition of this pool. Such changes could affect the solubility of cholesterol in bile, since this sterol is held in solution in mixed micelles containing definite ratios of cholesterol, phospholipids and bile salts.[7,8] The stability of bile micelles is of interest because changes in micelle composition, which affect cholesterol solubility, could trigger gallstone formation or dissolution.[8-11]

While it might seem reasonable to expect the increased rate of elimination of bile salts effected by the hydrophilic mucoids or anion exchange resins to alter biliary micellar lipid ratios, this may not be the case since the excretion of bile cholesterol, phospholipids and bile salts seems to be more or less interdependent. Thus, studies have shown that the rates of biliary cholesterol and phospholipid excretion depend on the concentration of bile salts in the enterohepatic circulation.[12-14] It is therefore apparent that the ratio of micellar components could remain constant in spite of a drop in biliary bile salt concentrations. Such interdependence, of course, does not preclude the possibility of changes in the composition of the biliary bile salt pool which could affect cholesterol solubility. In view of the uncertainty regarding these interrelationships, it was of interest to determine the effects of a hydrophilic mucoid and an anion exchange resin on the composition of bile lipids.

In the following study, we determined what changes, if any, administration of the anion exchange resin, cholestyramine, or the hydrophilic mucoid, psyllium hydrocolloid, induced in the ratio of cholesterol: phospholipids: bile salts and in the bile salt composition of human hepatic bile.

Protocol

The subjects of this experiment were six post-cholecystectomy patients with normal liver function. Short-arm, T-tube catheters were placed in the common bile duct, and were clamped continuously except during bile collections, which were begun at least three days prior to the start of the experimental control period.

When the patients were in a convalescent phase, they were placed on a standard 1500 calorie diet containing 77 gm protein, 138 gm carbohydrate and 70 gm fat. Starting with the second day on

Table I

EFFECT OF PSYLLIUM HYDROCOLLOID ON HEPATIC BILE LIPID COMPOSITION (All values in mmoles/liter bile)

Patient	Days on Control or treatment*	Cholesterol	Total Phospholipids	BILE ACIDS† Cholic acid	Chenodeoxycholic	Deoxycholic	Total B.A.	RATIO Cholesterol: Phospholipid: Total Bile Acid
1. (f)‡								
Control	6	2.65 ± 0.77	6.18 ± 1.96	10.88 ± 2.27	7.70 ± 2.14	1.54 ± 1.99	20.1 ± 3.8	1 : 2.35 : 7.60 ± 0.31 ± 2.60
Treated	6	4.81 ± 0.96	7.41 ± 1.32	9.28 ± 2.54	8.41 ± 2.49	5.26 ± 3.23	22.9 ±7.37	1 : 1.54 : 4.76 ± 0.44 ± 2.29
2. (f)								
Control	8	2.60 ± 0.46	3.70 ± 0.88	15.1 ± 4.3	19.6 ± 4.6	5.81 ± 3.07	40.5 ± 6.4	1 : 1.42 : 15.6 ± 0.37 ± 2.2
Treated	8	2.48 ± 0.33	4.29 ± 0.50	12.6 ± 3.4	19.1 ± 2.6	10.3 ± 4.5	42.0 ± 5.7	1 : 1.73 : 16.9 ± 0.19 ± 2.4
3. (m)								
Control	6	1.30 ± 0.35	7.17 ± 1.13	11.2 ± 2.80	14.3 ± 1.32	2.41 ± 0.16	27.9 ± 3.58	1 : 5.52 : 21.4 ± 0.80 ± 7.49
Treated**	29	1.35 ± 0.49	7.89 ± 1.57	11.0 ± 2.41	14.4 ± 3.40	10.9 ± 5.19	36.4 ± 7.46	1 : 5.85 : 27.0 ± 1.45 ± 7.08

*Bile collected in A.M. and P.M. on second day and alternate days of control or treatment period. Therefore number of collections is equal to number of days in these periods. Values given are averages for the collected samples ± the Standard Deviation. Subjects received 3 gm psyllium hydrocolloid four times a day during treatment period.

†Present in bile as glycine and taurine conjugated bile salts.

‡Sex

**Bile collections made on the 2nd, 4th, 6th, 8th, 13th, 15th, 20th, 22nd, 27th, and 29th days.

this diet, 10-20 ml bile collections were taken after breakfast and lunch every other day. Six to eight samples were collected from each patient during the control period. Following this period, 3 gm of psyllium hydrocolloid† was administered to three patients four times daily, before meals and at bed time. Four grams of cholestyramine‡ was given to the other three patients, three times daily. Bile collections were continued on alternate days during drug administration. An aliquot of bile was taken from one collection on each patient and cultured for bacteria.

Assay Methods

Bile was assayed for bile salts, phospholipids, and cholesterol. Following collection, duplicate samples of bile were dried by lyophilization, and the residues extracted with chloroform: methanol, 2:1 (v/v). Aliquots of these extracts, which contained the bile lipids, were used to determine total phospholipids[15] and conjugated bile salts. Second aliquots were used for determination of cholesterol and the free bile acids, which resulted from hydrolysis of conjugated bile salts. For these assays, the aliquots were evaporated to dryness, and then heated with 7 N sodium hydroxide at 15 lbs pressure (autoclave) to hydrolyze bile acid conjugates and cholesterol esters. The

†Generous gift of Fellows-Testagar Division of Fellows Medical Manufacturing Company.

‡Marketed by Merck Sharp & Dohme, Division of Merck & Company Inc., under the trade name of Cuemid.

Table II

EFFECT OF CHOLESTYRAMINE ON HEPATIC BILE LIPID COMPOSITION (All values in mmoles/liter bile)

Patient	Days on Control or Treatment*	Choles - terol	Total Phospho- lipids	BILE ACIDS† Cholic acid	Chenode- oxycholic	Deoxy- cholic	Total B.A.	RATIO Cholesterol: Phospholipid: Total Bile Acid	
4. (f)‡									
Control	6	3.62 ± 0.48	6.90 ± 1.17	35.2 ± 7.18	22.8 ± 4.98	0.0	58.0 ± 8.22	1 : 1.91 : ± 0.34	16.02 ± 3.16
Treated**	9	4.24 ± 0.83	6.81 ± 1.55	35.7 ± 5.64	10.3 ± 3.75	0.0	45.9 ± 7.90	1 : 1.61 : ± 0.41	10.82 ± 4.12
5. (f)									
Control	6	3.79 ± 0.55	6.37 ± 1.66	25.7 ± 7.69	16.1 ± 5.18	5.3 ± 4.42	47.1 ± 14.3	1 : 1.68 : ± 0.26	12.32 ± 5.35
Treated	8	3.44 ± 0.49	5.97 ± 1.31	29.4 ± 5.41	7.25 ± 1.77	0.0	36.7 ± 6.82	1 : 1.74 : ± 0.27	10.67 ± 3.84
6. (f)									
Control	6	2.17 ± 0.24	0.90 ± 0.26	6.98 ± 1.16	3.20 ± 1.17	1.94 ± 1.17	11.9 ± 3.05	1 : 0.39 : ± 0.09	5.16 ± 0.71
Treated	12	1.89 ± 0.56	0.35 ± 0.19	7.16 ± 1.18	1.45 ± 0.58	0.0	8.90 ± 2.31	1 : 0.18 : ± 0.07	4.97 ± 1.09

*Bile collected in A.M. and P.M. on second day and alternate days of control or treatment period. Therefore number of collections is equal to number of days in these periods. Values given are averages for the collected samples ± the Standard Deviation. Subjects received 4 gm cholestyramine three times daily during treatment period.

†Present in bile as glycine and taurine conjugated bile salts.

‡Sex

**Bile collections made on the 2nd, 4th, 6th, 8th, and 9th days.

alkaline hydrolysates were extracted with petroleum ether to recover the non-saponifiable material which contains cholesterol. Cholesterol was determined according to Sperry and Webb.[16]

The alkaline residues were acidified, and extracted with petroleum ether to remove fatty acids. The acidic residues were exhaustively extracted with ethyl ether to recover the "acidic fraction" containing free bile acids.

Free and conjugated bile acids were separated and determined as follows: Thin-layer chromatographic plates were prepared by coating 20 by 20 cm glass plates with uniform 200 μ layers of Silica Gel G. After air drying, the plates were activated for two hours at 100° C. Aliquots of extracts containing either free or conjugated bile acids, together with appropriate standard solutions, were spotted on the activated plates. Plates spotted with conjugated bile acids were developed with toluene:glacial acetic acid:water (7.5:12.5:1.0, v/v). To determine cholic acid, plates spotted with free bile acids were developed with ethyl acetate:glacial acetic acid (96:4, v/v). Other plates spotted with free bile acids were developed with ethyl acetate:2,2,4-trimethylpentane:glacial acetic acid (10:10:2, v/v) to assay chenodeoxycholic and/or deoxycholic acid. Following development, the plates were air dried and then sprayed to saturation with ethanolic phosphomolybdic acid (20%, wt/v). The sprayed plates were heated for 15 minutes at 100° C to effect color development. The concentrations of bile acids on the plates were then determined by scanning densitometry.[17,18]

TABLE III

EFFECT OF CHOLESTYRAMINE ON THE CONCENTRATION OF THE CONJUGATED BILE SALTS OF HEPATIC BILE (All values in mmoles/liter bile)

Patient	Days on Control or Treatment*	BILE SALTS				Total Bile Salt Conjugates
		Glyco-cholate	Tauro-cholate	Glycocheno-deoxycholate + Glycodeoxy-cholate	Taurocheno-deoxycholate + Taurodeoxy-cholate	
6.						
Control	6	3.18 ± 0.84	2.25 ± 1.14	3.79 ± 0.78	1.36 ± 0.50	10.58
Treated	12	4.35 ± 0.85	0.82 ± 0.35	3.04 ± 0.84	0.28 ± 0.37	8.49

*Bile collected in A.M. and P.M. on second day and alternate days of control or treatment period. Therefore number of collections is equal to number of days in these periods. Values given are averages for the collected samples ± the Standard Deviation. Subjects received 4 gm cholestyramine three times daily during treatment period.

Results

Tables I and II show concentrations of total cholesterol, total phospholipids, and the various bile salts, as well as the ratios of these lipids, in hepatic bile from six patients. The control data demonstrate that the composition of hepatic bile varied from patient to patient. However, for each individual patient's collections the variations in the lipid concentrations were relatively small, as shown by the standard deviations.

Data pertaining to the effects of psyllium hydrocolloid on the hepatic bile lipids of three human subjects are shown in Table I. In Patient No. 1, there was a significant increase ($P < .01$) in the bile cholesterol concentration. This was reflected by a change in the ratio of the bile lipids. Since, however, there was no increase in bile cholesterol concentrations in Patients 2 and 3, it may be that the change in Patient 1 was unrelated to psyllium hydrocolloid treatment.

No significant changes in total phospholipid, cholate or chenodeoxycholate concentrations were observed in the bile of any of the patients. In each case, however, the deoxycholate concentration increased significantly ($P < .01$). Since the per cent change was minor in the hepatic bile of Patients 1 and 2, this increase had only a small effect on the bile lipid ratio and the total bile acid concentration. However, in Patient 3, the percentage increase was larger and resulted in a significant ($P < .01$) change in the ratio of the bile lipids and a decided increase in the total bile salt concentration. Since Patient 3 was treated with psyllium hydrocolloid for a much longer period than Patients 1 an 2 and the deoxycholate concentration increased with treatment time in all three patients, long-term mucoid treatment could consistently result in an increased total biliary bile salt concentration and a changed bile salt composition. This possibility must be verified by further long-term experiments.

Table II shows the effects of cholestyramine on the bile lipid concentrations in three patients. There were no changes in the concentrations of bile cholesterol or cholate in any of the subjects treated with cholestyramine. There was a small but significant ($P <$

.01) decrease in the bile phospholipid in Patient 6; and deoxycholate was eliminated from the biliary bile salt pool in Patients 5 and 6. Cholestyramine treatment resulted in a decided decrease ($P < .001$) in the concentration of bile chenodeoxycholate in all three patients. As a result of the changes in bile salt composition, there was a tendency for the total bile salt concentration to decrease. Since deviations from patient to patient were rather large, it is difficult to assess the significance of this decrease. Nevertheless the trend is there, and confirms a similar trend found by other investigators.[19,20]

Table III presents data on the effects of cholestyramine on conjugated bile salt concentrations in hepatic bile of Patient 6. The data show that the resin increased the concentration of glycocholate, and decidedly decreased the concentrations of taurocholate, and taurochenodeoxycholate plus taurodeoxycholate. While one might have expected a compensating increase in the glycochenodeoxycholate plus glycodeoxycholate concentration, it must be remembered that cholestyramine treatment eliminated deoxycholate from the hepatic bile spectrum (Table II) of this patient.

Discussion

The increased concentration of deoxycholate in the hepatic bile of patients treated with psyllium hydrocolloid is of interest because it results in increases in the size of the total biliary bile salt pool and the ratio of total bile salts to cholesterol in hepatic bile. Increases such as these have been shown to enhance biliary micelle stability,[8-11] and to prevent gallstone formation and promote gallstone dissolution in experimental animals.[21,22] While this suggests that the increased deoxycholate concentration effected by psyllium hydrocolloid might result in less lithogenic bile, the relationship of bile micelle stability to deoxycholate (a dihydroxy bile salt) concentration has not been defined. It has, in fact, been theorized that micelles made up of cholestrol, lecithin and dihydroxy bile salts may be less stable than those made up with trihydroxy bile salts.[23,24] This theory is based on the concept that micelles containing the dihydroxy salts are larger and have less surface charge than those containing trihydroxy bile salts. On the other hand, increases in biliary chenodeoxycholate (also a dihydroxy bile salt) concentration have been shown to actually effect the dissolution of gallstones.[25,26]

The increased concentration of deoxycholate in the bile of psyllium hydrocolloid-treated subjects is difficult to explain. Deoxycholate is a secondary bile salt produced in the distal ileum and the large intestine by the bacterial 7α-dehydroxylation of cholate. It appears in bile following intestinal absorption, portal blood transport, and hepatic clearance and secretion. The fact that psyllium hydrocolloid increases the concentration of this anion in bile appears to argue against a bile acid-sequestering role for this drug, since one would expect a sequestrant to lower the deoxycholate concentration by interrupting absorption of this intestinally synthesized bile acid. Nevertheless it has been shown that the mucoid does increase bile acid excretion in man[6] and decreases the half-life of cholate in the bile acid pool of rats.[5] It would therefore seem that either psyllium hydrocolloid is an ineffective binding agent for deoxycholate or that there is an increase in deoxycholate production which exceeds the ability of the mucoid to remove it. Increased production of deoxycholate could result from a change in the bacterial population of

the distal ileum and large intestine. However, no studies have been made of the effects of psyllium hydrocolloid on intestinal bacteria.

While the data pertaining to the effects of cholestyramine on hepatic bile lipid concentrations were obtained on only three subjects treated for relatively short periods, the changes reported above have been observed by a number of investigators, [19,20,23,27-29] and merit a rather detailed discussion. Cholestyramine administration results in a large increase in the trihydroxy to dihydroxy bile salt ratio and a marked increase in the ratio of glycine to taurine conjugates in bile. The increase in the former ratio is due to decreases in the concentrations of chenodeoxycholate and deoxycholate anions.

The decrease in chenodeoxycholate could be effected by at least two different mechanisms. First, because dihydroxy bile salt anions have a higher affinity for cholestyramine than trihydroxy bile salt anions, [30,31] one would expect cholestyramine to remove chenodeoxycholate ions from the bile salt pool more rapidly than cholate ions. Since in human subjects treated with cholestyramine the synthesis of bile acids from cholesterol is rate-limiting, one would then expect an increase in the ratio of trihydroxy to dihydroxy bile salts in bile.

A second possibility is that cholestyramine may effect a change in the rate of bile acid synthesis at the enzyme level. Such a change could result in an increase in cholic acid synthesis at the expense of chenodeoxycholic acid synthesis.[32] While this is an attractive hypothesis, no change in the activity of the 12α-hydroxylase of cholest-4-en-7α-o1-3-one is seen in cholestryamin-treated rats.[33]

The decrease in deoxycholic acid concentration is more easily explained. As mentioned above, deoxycholate is a secondary bile salt produced by 7α-dehydroxylation of cholate anions by the intestinal flora. One would expect the concentration of this bile salt to drop precipitously in cholestyramine-treated subjects, since absorption of the intestinally synthesized bile salt would be retarded by resin. This effect of cholestyramine contrasts with the increased concentration of deoxycholic acid which appears during psyllium hydrocolloid treatment.

The increase in the glycine to taurine conjugate ratio probably stems from exhaustion of the limited hepatic taurine supply [34] and the fact that cholestyramine preferentially sequesters taurine conjugates.[31] The increased rate of hepatic bile acid synthesis effected by cholestyramine increases the need for the limited supply of taurine available for bile acid conjugation, and conjugation shifts to the more readily available glycine.

Although cholestyramine effects significant changes in the di- to trihydroxy bile salt and glycine to taurine conjugate ratios in bile, its effect on the ratio of cholesterol/phospholipids/bile salts, which is an important factor determining bile micelle stability, is uncertain. Our results, together with those of some others, [19,20] suggest a decrease in bile acid concentrations; however, other investigators find no change.[23,27] When the data obtained in one experiment[29] was plotted, using the triangular coordinate system of Small, Bourges and Dervichian,[7] there was no indication that cholestyramine treatment led to the formation of unstable biliary micelles. However, caution must be used in interpreting these results since the use of phase relationships in

a solution as complex as bile is not infallible and takes no account of the changes in bile acid anion composition that result from cholestyramine treatment.

The shift toward trihydroxy bile salts would appear to result in more stable micelles.[23,24] However, studies have shown that bile rich in chenodeoxycholate effects dissolution of gallstones.[25,26] The shift in the glycine to taurine conjugate ratio would appear to result in more stable bile micelles, since glycine conjugates appear to be more efficient cholesterol solubilizers than taurine conjugates.[20]

From the foregoing discussion, it is obvious that no definite conclusions as to the effects of cholestyramine on biliary cholesterol solubility can be made at this time. Since this resin is used in the long-term treatment of hypercholesterolemia, it is obviously important to devise experiments which will define more precisely the complex interrelationships involved in micelle stability, and to determine the actual effects of anion exchange polymers on their stability.

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