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Research Article

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Effect of Cholestyramine on Bile Acid Metabolism in Normal Man

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ABSTRACT The effect of cholestyramine administration on the enterohepatic circulation of bile acids was studied in eight normal volunteers. In six subjects the metabolism of sodium taurocholate- ^{14}C was determined after its intravenous injection before and during the 6th wk of cholestyramine administration, 16 g/day. In two subjects, the metabolism of cholic acid- ^{14}C was observed before and during the 2nd wk of cholestyramine, 16 g/day. Bile acid sequestration resulted in a more rapid disappearance of the injected primary bile acid and its metabolic products. The composition of fasting bile acids was promptly altered by cholestyramine to predominantly glycine-conjugated trihydroxy bile acid. In four subjects, unconjugated bile acid- ^{14}C was administered during cholestyramine administration; the relative proportion of glycine-conjugated bile acid- ^{14}C before enterohepatic circulation was similar to the relative proportion of unlabeled glycine-conjugated bile acid present in duodenal contents after an overnight fast, indicating that a hepatic mechanism was responsible for the elevated ratios of glycine- to taurine-conjugated bile acid (G:T ratios) observed. The relative proportions of both dihydroxy bile acids, chenodeoxycholic and deoxycholic, were significantly reduced. Steatorrhea did not occur, and the total bile acid pool size determined after an overnight fast was unaltered by cholestyramine. These findings suggest that in normal man bile acid sequestered from the enterohepatic circulation by cholestyramine is replaced by an increase in hepatic synthesis primarily via the pathway leading to production of glycocholic acid.

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INTRODUCTION

The capacity to maintain adequate concentrations of conjugated bile acid in the biliary tree and the intestinal lumen is a prerequisite for efficient micellar solubilization of cholesterol in bile and digestion and absorption of lipids from the intestine (1, 2). In normal man, the total bile acid pool is maintained by enterohepatic recirculation primarily via ileal absorption (3, 4). Lesser contributions are provided by colonic absorption of unconjugated bacterial metabolic products of bile acids (5, 6) and *de novo* hepatic synthesis, the latter replacing small daily fecal losses (7). Interruption of bile acid enterohepatic circulation has been demonstrated in patients with ileal disorders and clinical observations in such patients relate the sequelae of steatorrhea (4, 8, 9) and an increased incidence of gallstones (10, 11) to abnormalities in bile acid metabolism. When ileal function is insufficient, enterohepatic recirculation of conjugated bile acid is greatly diminished; maintenance of total bile acid content depends primarily on the varying contributions of enhanced hepatic synthesis and intestinal reabsorption by passive diffusion. Furthermore, an excessive amount of bile acid may reach the colonic lumen in such patients and is considered one of the etiologic factors in the watery diarrhea frequently observed (12).

The sequestering agent, cholestyramine, partially interrupts bile acid enterohepatic circulation with subsequent enhanced hepatic synthesis of bile acid from cholesterol (13, 14, 15). The purpose of this study was to examine the effect of cholestyramine on bile acid reabsorption and synthesis in normal man, and compare these effects with the altered physiology observed in the presence of ileal disorders. These observations on cholestyramine-induced alterations of bile acid metabolism in normal man may also be applicable in explaining the mechanism of its beneficial effects in reducing the diarrhea in some patients with bile acid malabsorption due to ileal resection.

METHODS

Subjects

Seven male and three female volunteers, ages 21–30 yr, served as subjects. All were hospital personnel in good health with a dietary intake of approximately 2500 cal containing 70–80 g of fat restricted to three meals per day throughout the study. Base line serum cholesterol values ranged from 164–259 mg/100 ml.

Study procedure

In each of six subjects (three male and three female), after an overnight fast, 5.0 μ Ci of sodium taurocholate-24- 14 C was dissolved in 25 ml of sterile saline, passed through a 0.45 μ Millipore filter, and administered intravenously. Intubation of the duodenum was fluoroscopically controlled and sampling obtained by aspiration during a 20 min intravenous infusion of cholecystokinin (0.75 Ivy dog units/kg, Cecekin, Vitrum Chemical Co., Stockholm, Sweden), dissolved in 100 ml of normal saline (4, 8). Samples of well-mixed fasting duodenal contents, always less than 5% of the total aspirate (40–100 ml) were obtained serially at 3, 24, 48, and 72 hr after injection of the isotope. After completion of the base line sampling, cholestyramine (Questran, Mead Johnson Laboratories, Evansville, Ind.) was administered for 6 wk in a total daily dose of 16 g, 4 g with each meal and at bedtime. Cholecystokinin-stimulated fasting duodenal contents were sampled at weekly intervals. During the 6th wk of cholestyramine administration, each subject again received an intravenous dose of 5.0 μ Ci of sodium taurocholate-24- 14 C and serial sampling of fasting duodenal contents was performed in a manner identical to the base line study described above. The drug was then discontinued and 1 wk later a final sample of fasting duodenal bile was obtained.

Four additional male subjects were studied in a similar manner except that the isotopically labeled bile acid employed was cholic acid-24- 14 C (three subjects) or chenodeoxycholic acid-24- 14 C (one subject). Measurements in these subjects were obtained during the 2nd wk of cholestyramine administration 16 g/day.

Preparation and analysis of duodenal samples. Duodenal samples were refrigerated until prepared for thin-layer chromatography by protein precipitation and extraction with ethanol. Separation of the conjugated bile acids was performed using the solvent system isoamyl acetate-propionic acid-*n*-propanol-water (40:30:20:10, v/v) (16). Using appropriate standards, the glycine and taurine conjugates of cholic acid were readily identified. The glycine and taurine conjugates of the dihydroxy bile acids, were also identified in this system. No attempt was made to separate the conjugates of deoxycholic acid from the corresponding conjugates of chenodeoxycholic acid which had similar R_f values. Radioactivity in these fractions arising from bacterial modification of sodium taurocholate-24- 14 C would be expected to be principally conjugates of deoxycholate-24- 14 C. Unconjugated bile acids were not present in any of the samples in identifiable amounts.

Measurement of taurocholate- 14 C specific activity and disappearance rate. After thin-layer chromatographic identification, the specific activity counts per minute per milligram (cpm/mg) of taurocholate was determined using the modified Pettenkoffer reaction (17), and the 14 C-radioactivity measured in samples of eluted fractions (4, 8). In two subjects studied after administration of cholic acid- 14 C, both

glycocholate and taurocholate were eluted and their specific activities determined in an identical manner.

Measurement of total 14 C radioactivity, composition, and disappearance rate. A sample of each duodenal aspirate obtained serially after the injection of sodium taurocholate-24- 14 C or cholic acid-24- 14 C was subjected to thin-layer chromatography and the 14 C radioactivity present in the taurocholate and glycocholate fractions as well as the fractions corresponding to their bacterial products was determined as previously described (6). The relative amounts of 14 C radioactivity appearing in the glycocholate, taurocholate, glycodeoxycholate, and taurodeoxycholate fractions after bacterial modification of the injected label were expressed as a percentage of the total radioactivity recovered from the entire chromatogram. The total 14 C radioactivity from each chromatogram was normalized for dilutional differences in the original fasting aspirates by determining the total cholate concentration colorimetrically in eluates from a duplicate chromatogram. These serial measurements estimate the recirculation of the injected label as well as its recirculating radioactive bacterial metabolites (total 14 C cpm/mg cholate).

Measurement of the ratio of glycine- to taurine-conjugated bile acid (G:T ratio).¹ In all samples, duplicate 0.5 ml portions of the eluates containing the glycocholate and taurocholate fractions were measured for trihydroxy bile acid content (17). The relative conjugation of cholic acid with glycine and taurine was determined before administration of cholestyramine, at weekly intervals during the study period, and 1 wk after discontinuing the drug. In four subjects unconjugated 14 C-labeled bile acid was administered during cholestyramine administration (see study procedure); continuous aspiration of fasting duodenal contents was accomplished during the 3 hr period after injection and subsequent stimulation of bile flow with cholecystokinin. Relative hepatic conjugation was determined by measurement of the 14 C radioactivity partitioned between the glycine and taurine fractions of thin-layer chromatograms. This value (14 C-G:T ratio) determined on bile acid which had not undergone enterohepatic circulation was then compared with the G:T ratio of total cholate in each aspirate which was representative of the enterohepatically circulating bile acid pool.

Measurement of the relative composition of total bile acids by gas-liquid chromatography. Glycine and taurine conjugates in each duodenal sample were separated by thin-layer chromatography and eluted in ethanol. Eluates were prepared for gas-liquid chromatography by alkaline hydrolysis and subsequent conversion to their methyl ester trifluoroacetates. The purification procedure and analysis on a triple component column utilized the methods of Okishio, Nair, and Gordon (18), modified by the use of chemical rather than enzymatic hydrolysis. Peak areas of individual bile acids identified by gas-liquid chromatography were compared to external standards of the corresponding bile acids prepared on an equal weight basis, to correct for column losses and allow determination of the relative masses of individual bile acids in each sample.

Chemicals. Sodium taurocholate-24- 14 C was obtained from Tracerlab Company, Waltham, Mass. Cholic acid- 14 C was obtained from New England Nuclear Corporation, Boston, Mass. and chenodeoxycholic acid- 14 C from Mallinckrodt Nuclear, Orlando, Fla. Thin-layer chromatographic analysis demonstrated greater than 95% of the radioactivity recoverable in the appropriate fractions. Bile acid standards were

¹Abbreviation used in this paper: G:T ratio, ratio of glycine- to taurine-conjugated bile acid.

TABLE I
Conjugated Bile Acid Specific Activities and Recirculation*

Subject	Sex	Bile acid- ¹⁴ C injected		Duodenal bile acid			Specific activity	TC	Total ¹⁴ C
				Day 1	Day 2	Day 3			
		<i>cpm × 10⁶</i>		<i>cpm/mg × 10³</i>			<i>at t₀†</i>	<i>t₁ days</i>	<i>t₂ days</i>
Base line study									
		<i>TC-¹⁴C</i>							
B. A.	F	3.80	TC	3.87	3.41	2.80	4.60	4.3	4.5
M. C.	F	4.38	TC	2.80	1.30	0.50	6.84	0.8	1.1
C. H.	F	4.25	TC	6.65	3.52	1.88	12.49	1.1	2.3
L. J.	M	5.09	TC	4.41	2.96	1.79	7.04	1.5	2.9
T. E.	M	4.72	TC	7.61	2.36	0.58	28.65	0.5	0.9
B. M.	M	4.55	TC	10.90	2.88	0.89	37.15	0.6	1.1
		<i>C-¹⁴C</i>							
C. D.	M	6.76	TC	4.76	3.58	2.56	6.54	2.2	6.9
			GC	4.24	3.21	2.14	6.10		
W. L.	M	6.74	TC	3.73	2.19	1.32	6.24	1.4	3.4
			GC	5.38	2.88	1.39	10.77		
Cholestyramine‡									
		<i>TC-¹⁴C</i>							
B. A.	F	5.09	TC	4.70	2.17	0.78	12.03	0.8	0.9
M. C.	F	4.62	TC	3.48	0.73	0.18	14.90	0.5	0.5
C. H.	F	4.39	TC	8.47	2.01	0.68	28.18	0.5	0.6
L. J.	M	4.83	TC				8.67	0.5¶	0.5¶
T. E.	M	4.50	TC				24.40	0.5¶	0.8¶
B. M.	M	4.06	TC				20.01	0.3¶	0.5¶
		<i>C-¹⁴C</i>							
C. D.	M	7.63	TC	0.82	0.25	0.08	2.70	0.6	0.7
			GC	0.79	0.33	0.11	2.25		
W. L.	M	7.54	GC	0.94	0.19	0.05	4.07	0.5**	0.5¶

* TC, taurocholate; GC, glycocholate; C, cholic acid.

† Calculated from regression line, all correlation coefficients were above 0.99.

‡ Taurocholate-¹⁴C administered during 6th wk of cholestyramine at dose of 16 g daily, cholic acid-¹⁴C administered during 2nd wk of cholestyramine, 16 g daily.

|| Determined from duodenal aspirate 3 hr after administration of isotope, radioactivity absent in 48- and 72-hr samples.

¶ Estimated from two valid points only, no measurable radioactivity after 48 hr.

** Glycocholate t₁.

obtained from Maybridge Chemical Company, Cornwall, England.

Calculations. The exchangeable taurocholate pool was estimated using standard isotope dilution techniques (19). The applicability of this method to the study of human bile acids has been previously discussed (4, 8, 20, 21). Specific activity data were subjected to statistical analysis to obtain regression lines. The exchangeable taurocholate (TC) pool size was determined from the amount of administered ¹⁴C activity and the extrapolated bile acid specific activity at zero time. The rate constant (*k*) determined from the regression line was used to calculate the half-life (*t*₁ = 0.693/*k*). The total conjugated bile acid pool and its components were calculated using the estimated TC pool, the G:T ratio, and the relative proportions of cholic per cent (C), chenodeoxycholic (per cent CD), deoxycholic (per cent DC) determined on a weight basis by gas-liquid chromatography. The calculations are summarized as follows:

glycocholate (GC) mg = TC mg

$$\times G:T \text{ ratio} \times \frac{\text{mol wt GC}}{\text{mol wt TC}},$$

total C mg = TC mg + GC mg,

$$\text{total CD mg} = \text{C mg} \times \frac{\text{per cent CD}}{\text{per cent C}},$$

$$\text{total DC mg} = \text{C mg} \times \frac{\text{per cent DC}}{\text{per cent C}}.$$

RESULTS

Measurement of taurocholate-¹⁴C specific activity and disappearance rate. The total dose of administered ¹⁴C

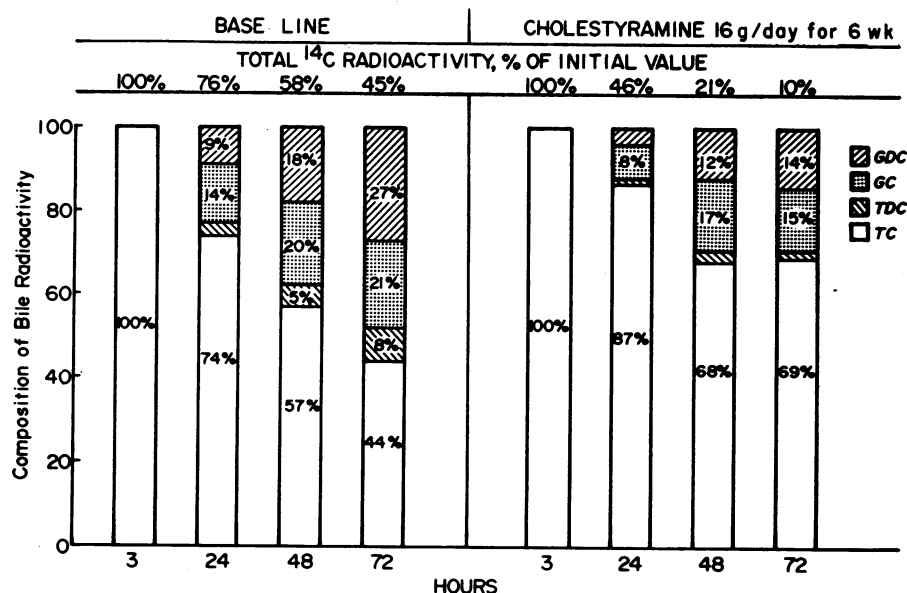


FIGURE 1 Composition of ^{14}C radioactivity in duodenal fluid obtained after intravenous injection of sodium taurocholate- $^{24}\text{-}^{14}\text{C}$; average values in six normal subjects before and during the 6th wk of cholestyramine administration 16 g/day. Data are expressed in terms of percentage of ^{14}C radioactivity contributed by each bile acid fraction to the total recoverable ^{14}C radioactivity at 3, 24, 48, and 72 hr. The total recoverable ^{14}C radioactivity expressed as a percentage of the initial 3 hr value is shown at the top of each column. GDC, glycodeoxycholate; GC, glycocholate; TDC, taurodeoxycholate; TC, taurocholate.

radioactivity, sequential bile acid specific activities, estimated half-time ($t_{1/2}$) and specific activity at zero time (t_0) before and during cholestyramine administration are shown in Table I. In three subjects, identified in Table I, the rapid disappearance of ^{14}C radioactivity during cholestyramine prevented serial specific activity determinations and the specific activity at t_0 represents the value obtained in duodenal aspirates taken 3 hr after injection of the label. These values assume adequate mixing with the total bile acid pool but can not be statistically validated. Base line taurocholate $t_{1/2}$ averaged 1.6 ± 1.2 days (Table I). As expected, the administration of cholestyramine resulted in a more rapid disappearance of the injected label, the taurocholate $t_{1/2}$ was shortened to 0.5 ± 0.1 days. Rate constants for taurocholate- ^{14}C in the base line period averaged 0.685 ± 0.390 and during cholestyramine average values were 1.309 ± 0.322 ($P < 0.02$).

Measurement of total ^{14}C radioactivity, composition, and disappearance rate. Total ^{14}C radioactivity $t_{1/2}$, representing recirculation of the injected label and its bacterial products is also shown in Table I. During cholestyramine, the total ^{14}C radioactivity $t_{1/2}$ averaged 0.6 ± 0.2 days, a significant reduction from base line values of 2.9 ± 1.9 days ($P < 0.02$). Average values for the relative chemical composition of total ^{14}C radioactivity before and during cholestyramine are depicted in Fig. 1.

Radioactivity was always present as the injected labeled bile acid in 3 hr samples. During the base line period, metabolic products accounted for 26, 43, and 56% of the total radioactivity in the 24-, 48-, and 72-hr samples, respectively; values similar to those reported previously in normal subjects (6). Conjugates of deoxycholate- ^{14}C comprised an increasing increment of total radioactivity (Fig. 1). During cholestyramine administration, the relative proportion of metabolic products was decreased to 13, 32, and 31% of the total radioactivity at 24, 48, and 72 hr, respectively, most evident as a reduction in deoxycholate conjugates.

Measurements of G:T ratio. Relative conjugation of cholic acid with glycine and taurine during the base line period, at weekly intervals during cholestyramine administration, and 1 wk after the drug was discontinued are shown in Table II A. Base line and wk 6 values represent the mean G:T ratio for each subject during the 4 consecutive days of aspiration of fasting duodenal content utilized for the isotopic studies described above (study procedure). All other values represent means of duplicate analysis of single fasting specimens obtained at the identified time interval (Table II A). The G:T ratio was significantly elevated as early as 1 wk after administration of cholestyramine and remained elevated until the drug was discontinued (Table II A). 1 wk after withdrawal

TABLE II A
Relative Conjugation of Cholate with Glycine and Taurine

Subject	Base line 1 wk pre-drug	G:T ratio (cholestyramine 16 g/day)						Recovery 1 wk post- drug
		wk 1	wk 2	wk 3	wk 4	wk 5	wk 6	
B. A.	1.3	11.4	9.3	9.5	6.2	11.5	10.2	2.9
M. C.	0.8	3.1	2.5	2.2	2.3	2.7	3.4	1.1
C. H.	2.3	7.5	12.7	9.8	6.1	11.8	15.6	3.8
L. J.	2.4	8.5	6.0	7.1	5.2	10.8	6.3	3.0
T. E.	3.7	9.8	7.8	11.8	7.5	10.4	12.2	2.1
B. M.	5.2	12.2	8.9	10.0	10.9	10.5	10.7	3.2
Mean	2.6	8.8	7.9	8.4	6.4	9.6	9.1	2.7
SD	±1.5	±3.0	±3.1	±3.1	±2.6	±3.1	±3.8	±0.9
P*		<0.01	<0.02	<0.02	<0.05	<0.01	<0.02	>0.5

TABLE II B
Relative Composition of Total Bile Acids†

Subject	Base line			Cholestyramine‡			Recovery		
	C	CD	DC	C	CD	DC	C	CD	DC
		%			%			%	
B. A.	38	44	18	82	16	2	53	39	8
M. C.	52	25	23	87	13	0	61	20	19
C. H.	47	33	20	86	14	0	44	37	19
L. J.	41	35	24	82	18	0	45	35	20
T. E.	37	44	19	80	20	0	32	42	26
B. M.	50	32	18	89	10	1	38	40	22
C. D.¶	40	40	20	90	10	0		n. d.**	
W. L.¶	38	51	11	83	17	0		n. d.	
Mean	43	38	19	85	15	0	45.5	35.5	19

* Comparison of difference between means of paired data, base line vs. each serial measurement.

† C, cholate; CD, chenodeoxycholate; DC, deoxycholate, expressed as per cent of total milligrams per sample.

Measurements obtained during the 6th wk of cholestyramine administration, 16 g daily.

|| Measurements obtained 1 wk after cholestyramine discontinued.

¶ Studied before and during the 2nd wk of cholestyramine administration, 16 g daily.

** n. d., not done.

of cholestyramine, the G:T ratio of each subject had returned toward the base line level. To further elucidate the mechanism responsible for the observed preferential conjugation with glycine, duodenal aspiration was accomplished immediately after intravenous administration of unconjugated bile acid-¹⁴C in four subjects receiving cholestyramine (see Methods). Relative hepatic conjugation of labeled bile acid which had not undergone enterohepatic circulation ((G:T)¹⁴C ratio 4.7, 12.5, 11.0, and 7.0) closely approximated the G:T ratio of the enterohepatically circulating bile acid pool in each subject (G:T ratio of total cholate 6.6, 12.6, 11.0, and 7.0, respectively).

Measurement of the relative composition of total bile acid. Relative composition of the total fasting bile

acids determined in each subject before, during the 6th wk of cholestyramine and 1 wk after the drug was discontinued is shown in Table II B. Lithocholic acid represented less than 5% of the total bile acids recovered in each instance and is not included in the calculations. In accord with previous reports of the fasting composition of normal human bile (22), base line cholate averaged 43%, chenodeoxycholate 38%, and deoxycholate 19% of the total. The ratio of dihydroxy to trihydroxy bile acid averaged 1.3. Relative composition was markedly altered by cholestyramine; during the 6th wk, cholate represented the major component averaging 85% of the total (Table II B). Relative proportions of chenodeoxycholate and deoxycholate decreased to mean values of 15% and <1%, respec-

TABLE III
Total Conjugated Bile Acid Content before and during Cholestyramine

Subject	Taurocholate		Glycocholate		Cholate		Chenodeoxycholate		Deoxycholate		Total	
	*	†	*	†	*	†	*	†	*	†	*	†
	<i>g</i>		<i>g</i>		<i>g</i>		<i>g</i>		<i>g</i>		<i>g</i>	
B. A.	0.83	0.42	0.98	3.93	1.80	4.35	2.09	0.85	0.85	0.10	4.73	5.31
M. C.	0.64	0.31	0.47	0.96	1.11	1.27	0.53	0.19	0.49	—	2.13	1.46
C. H.	0.34	0.16	0.71	2.22	1.05	2.37	0.74	0.39	0.45	—	2.24	2.76
L. J.	0.72	0.56	1.59	3.18	2.31	3.73	1.98	0.82	1.35	—	5.64	4.55
T. E.	0.17	0.18	0.56	2.04	0.73	2.13	0.86	0.55	0.37	—	1.96	2.68
B. M.	0.12	0.20	0.57	1.97	0.70	2.17	0.45	0.24	0.25	0.02	1.39	2.44
C. D.‡	0.30	0.50	0.78	2.74	1.09	3.24	1.09	0.36	0.51	—	2.69	3.60
W. L.‡	0.16	0.14	0.53	1.72	0.69	1.86	0.92	0.39	0.19	—	1.80	2.25
Mean	0.41	0.31	0.78	2.34	1.19	2.64	1.08	0.47	0.56	0.02	2.82	3.13
SD	±0.26	±0.16	±0.35	±0.86	±0.55	±0.97	±0.58	±0.23	±0.35	±0.03	±1.43	±1.19
P	n.s.		<0.01		<0.02		<0.05		<0.01		n.s.	

* Base line study.

† Measurements obtained during 6th wk of cholestyramine administration, 16 g daily.

‡ Measurements obtained during 2nd wk of cholestyramine administration, 16 g daily after intravenous injection of cholic acid-¹⁴C.

|| Comparison of difference between means of paired data; n.s., not significant.

tively; average dihydroxy to trihydroxy ratio was 0.18. Analysis of relative composition performed at weekly intervals revealed that these alterations were present 1 wk after beginning cholestyramine and were maintained throughout the study. 1 wk after cholestyramine was discontinued, bile acid relative composition had returned to base line values (Table II B).

Measurement of total bile acid content. The exchangeable taurocholate pool determined from the amount of administered ¹⁴C activity and the extrapolated bile acid specific activity at *t*₀ (Table I) before and during the 6th wk of cholestyramine administration is shown in Table III. The estimated taurocholate pool, the G:T ratio, and the relative composition of total bile acid were used to calculate the component and total bile acids pools (see Methods and Table III). It is evident that cholestyramine administration markedly altered the composition of fasting bile acids in these normal subjects without causing a significant change in total bile acid content, 2.82±1.43 g before and 3.13±1.19 g during cholestyramine. The alteration in composition involved primarily an increase in the relative amount of trihydroxy bile acid, thus, total conjugated cholate increased from base line mean of 1.19±0.55 g to a mean of 2.64±0.97 g during cholestyramine. It is apparent from Table III that an increase in glycocholate content accounted for the enhanced cholate pool size. Concomitantly chenodeoxycholate content decreased an average of twofold. Most striking was the reduction in deoxycholate content, a bacterial product of cholate, which during cholestyramine, represented less than 5% of the total bile acid in all subjects.

DISCUSSION

These observations demonstrate prompt and sustained alterations of bile acid composition and metabolism by the administration of cholestyramine in normal man. They confirm in normal individuals, similar alterations during bile acid sequestration in humans under less physiologic conditions (23–26). The bile acid binding capacity of cholestyramine has been adequately documented both by *in vitro* studies (27), as well as determinations *in vivo* of increased fecal bile acid loss and increased cholesterol turnover during its administration (13, 15, 28). The present observations demonstrate that cholestyramine administration decreased the enterohepatic circulation of the primary bile acid taurocholate (Table I) as well as recirculation of its major bacterial product, deoxycholate (Table III). The precise mechanism responsible for the virtual absence of secondary bile acids is not known but may involve both an inability of bacterial enzymes to degrade cholic acid bound to cholestyramine and/or binding of secondary bile acids to the resin after their formation, preventing reabsorption. Regardless of mechanism, cholestyramine administration to normal man resulted in a significant reduction in the secondary bile acid pool, particularly in the total content of deoxycholate (Table III).

The G:T ratio in normal man approximates 3:1 (22), whereas this value is elevated in patients with malabsorption of bile acids due to ileal disorders to levels averaging greater than 10:1 (29). The present study demonstrates that chemical interruption of enterohepatic circulation of bile acids also results in a rapid and sustained elevation of the G:T ratio (Table II

A). Similar findings in obese subjects have been reported by Wood, Shioda, Estrich, and Splitter (23). Fasting duodenal bile collected continuously after the injection of unconjugated ^{14}C -labeled bile acid, and therefore, before enterohepatic circulation, revealed marked similarity between the G:T ratio of newly conjugated bile acid- ^{14}C and the G:T ratio of the total cholate content representative of the circulating bile acid pool. On the basis of similar studies in patients with ileal disorders (29), we have suggested that the elevation in G:T ratio observed is primarily the result of increased hepatic synthesis of conjugated bile acids and a lack of taurine available to the hepatic parenchymal cells leading to preferential conjugation with glycine (30). Others have emphasized the possible contribution of accumulation of glycine conjugates via their greater capacity for transport by passive mechanisms in the absence of ileal function (31–32). The present study clearly demonstrates that deviation of the G:T ratio occurs when the enterohepatic circulation of bile acid is diminished and adds chemical sequestration to the previously described mechanisms in man, ileal disease (29, 31–32), and bile fistula (33). During cholestyramine administration, as in bile fistula, such elevations occur despite minimal or absent passive reabsorption evidenced in this study by the virtual disappearance of deoxycholate from the enterohepatic space. These observations further support the role of the hepatic conjugating system in the development of elevated G:T ratios. It seems likely that the observed preferential glycine conjugation is a result of increased turnover of bile acid through the conjugating system with subsequent overutilization of available taurine.

The rapid turnover of the primary trihydroxy bile acid, taurocholate (Table I), and the virtual disappearance of its dihydroxy metabolic product, deoxycholate (Table II A), during the administration of cholestyramine is in keeping with previous reports documenting increased fecal bile acid excretion and cholesterol turnover under the influence of this agent (13, 15, 28). Nevertheless, malabsorption of lipid is minimal in normal subjects receiving cholestyramine in a dose of 16 g/day (34). Despite the considerable interruption of bile acid enterohepatic circulation during cholestyramine administration noted in this study (Table I), daily fecal fat excretion remained normal ranging from 1.4 to 6.7 g. Furthermore, the present data demonstrate that the fasting bile acid pool is maintained at its control levels during cholestyramine administration (Table III). It thus appears that normal subjects compensate for the increased fecal bile acid loss by increasing hepatic synthesis of new bile acid to a degree sufficient to maintain lipid absorption. The altered composition of

total fasting bile acids during cholestyramine (Tables II A and B) provide data on the nature of the enhanced synthesis. Base line values obtained before cholestyramine were similar to previous reports of normal bile acid composition in man (22); cholate averaged 43% of the total bile acid with the combined dihydroxy components, chenodeoxycholate and deoxycholate being slightly greater, i.e., an average of 57% of the total. Within the 1st wk of cholestyramine administration, cholate represented approximately 85% of the total bile acid content (Table II B). It is apparent from the present data that the increased synthesis in normal man under the influence of cholestyramine administration occurs mainly via the production of glycocholate. These findings support previous observations in patients with T-tube drainage receiving cholestyramine in whom an initial rapid decrease in the concentration of both cholate and chenodeoxycholate was followed within 48 hr by an increase primarily in the concentration of cholate (25).

Interruption of the enterohepatic circulation of bile acids by cholestyramine or ileal resection results in increased bile acid turnover and synthesis and similar qualitative changes in the G:T ratio. An increased relative proportion of dihydroxy bile acids in fasting duodenal contents of patients with ileal disorders has been noted (32, 35). The use of cholestyramine in the treatment of watery diarrhea in some of these patients is presumably due to its capacity to bind dihydroxy bile acids (12). However, if bile acid metabolism is altered by cholestyramine in patients with ileal disorders in a manner similar to that reported here in normal subjects, increased hepatic synthesis and secretion of trihydroxy bile acid may be beneficial, since this configuration does not appear to effect colonic water absorption (36). Furthermore, data concerning the control of primary bile acid synthesis in man remains incomplete, the maximal capacity for synthesis in subjects with ileal disorders is unknown. If a mechanism of feedback inhibition exists in man, as recently shown in animals (37), it will be of interest to determine if relative degrees of inhibition exist according to the chemical configuration of specific bile acids. The production and maintenance of the secondary bile acid pool, in particular, deoxycholate, might contribute to such a mechanism. The relative increase in trihydroxy bile acids in the absence of increased steatorrhea in certain patients with ileal disorders during cholestyramine administration suggests a possible relationship between enhanced cholic acid synthesis and the relative quantity of dihydroxy bile acid in the enterohepatic space (35).

These studies have described the effect of cholestyramine on the enterohepatic circulation of bile acids in nor-

mal man and suggest the value of this model in future evaluation of the regulation of human bile acid synthesis. Clinical application of these findings to subjects with ileal disorders may provide a basis for future insight into the perplexing questions dealing with appropriate therapy for such patients who appear to have too little bile acid in the proximal enterohepatic space with tendencies toward gallstone formation and steatorrhea, and too much bile acid in the distal intestinal tract associated with watery diarrhea and the formation of renal stones (38).

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REFERENCES

- Hofmann, A. F., and D. M. Small. 1967. Detergent properties of bile salts: correlation with physiological function. *Ann. Rev. Med.* **18**: 333.
- Admirand, W. H., and D. M. Small. 1968. The physicochemical basis of cholesterol gallstone formation in man. *J. Clin. Invest.* **47**: 1043.
- Borgström, B., G. Lundh, and A. Hofmann. 1963. The site of absorption of conjugated bile salts in man. *Gastroenterology*. **45**: 229.
- Austad, W. I., L. Lack, and M. P. Tyor. 1967. Importance of bile acids and of an intact distal small intestine for fat absorption. *Gastroenterology*. **52**: 638.
- Samuel, P., G. M. Saypol, E. Meilman, E. H. Mosbach, and M. Chafizadeh. 1968. Absorption of bile acids from the large bowel in man. *J. Clin. Invest.* **47**: 2070.
- Garbutt, J. T., R. M. Wilkins, L. Lack, and M. P. Tyor. 1970. Bacterial modification of taurocholate during enterohepatic recirculation in normal man and patients with small intestinal disease. *Gastroenterology*. **59**: 553.
- Bergström, S. 1962. Metabolism of bile acids. *Fed. Proc.* **21** (Pt. 2): 28.
- Heaton, K. W., W. I. Austad, L. Lack, and M. P. Tyor. 1968. Enterohepatic circulation of C^{14} -labeled bile salts in disorders of the distal small bowel. *Gastroenterology*. **55**: 5.
- Hardison, W. G. M., and I. H. Rosenberg. 1967. Bile-salt deficiency in the steatorrhea following resection of the ileum and proximal colon. *N. Engl. J. Med.* **277**: 337.
- Heaton, K. W., and A. E. Read. 1969. Gallstones in patients with disorders of the terminal ileum and disturbed bile salt metabolism. *Br. Med. J.* **3**: 494.
- Cohen, S., M. Kaplan, L. Gottlieb, and J. Patterson. 1971. Liver disease and gallstones in regional enteritis. *Gastroenterology*. **60**: 237.
- Hofmann, A. F., and J. R. Poley. 1969. Cholestyramine treatment of diarrhea associated with ileal resection. *N. Engl. J. Med.* **281**: 397.
- Hashim, S. A., and T. B. Van Itallie. 1965. Cholestyramine resin therapy for hypercholesterolemia: clinical and metabolic studies. *J. Am. Med. Assoc.* **192**: 289.
- Zurier, R. B., S. A. Hashim, and T. B. Van Itallie. 1965. Effect of medium chain triglyceride on cholestyramine-induced steatorrhea in man. *Gastroenterology*. **49**: 490.
- Grundy, S. M., E. H. Ahrens, Jr., and G. Salen. 1971. Interruption of the enterohepatic circulation of bile acids in man: comparative effects of cholestyramine and ileal exclusion on cholesterol metabolism. *J. Lab. Clin. Med.* **78**: 94.
- Hofmann, A. F. 1962. Thin layer adsorption chromatography of free and conjugated bile acids on silicic acid. *J. Lipid Res.* **3**: 127.
- Irvin, J. L., C. G. Johnston, and J. Kopala. 1944. A photometric method for the determination of cholates in bile and blood. *J. Biol. Chem.* **153**: 439.
- Okishio, T., P. P. Nair, and M. Gordon. 1967. Studies on bile acids. The microquantitative separation of cellular bile acids by gas-liquid chromatography. *Biochem. J.* **102**: 654.
- Sterling, K. 1951. The turnover rate of serum albumin in a man as measured by I^{125} -tagged albumin. *J. Clin. Invest.* **30**: 1228.
- Lindstedt, S. 1957. The turnover of cholic acid in man. Bile acids and steroids 51. *Acta Physiol. Scand.* **40**: 1.
- Lindstedt, S., J. Avigan, D. S. Goodman, J. Sjövall, and D. Steinberg. 1965. The effect of dietary fat on the turnover of cholic acid and on the composition of the biliary bile acids in man. *J. Clin. Invest.* **44**: 1754.
- Sjövall, J. 1960. Bile acids in man under normal and pathological conditions. Bile acids and steroids 73. *Clin. Chim. Acta.* **5**: 33.
- Wood, P. D., R. Shioda, D. L. Estrich, and S. D. Splitter. 1972. Effect of cholestyramine on composition of duodenal bile in obese human subjects. *Metab. (Clin. Exp.)*. **21**: 107.
- van der Linden, W., and F. Nakayama. 1969. Change of bile composition in man after administration of cholestyramine (a gallstone dissolving agent in hamsters). *Acta Chir. Scand.* **135**: 433.
- Juul, A. H., and W. van der Linden. 1969. Short term reaction of biliary bile acids to cholestyramine medication. *Acta Physiol. Pharmacol. Neerl.* **15**: 469.
- Thistle, J. L., and L. J. Schoenfield. 1969. Induced alteration of bile composition in humans with cholelithiasis. *J. Lab. Clin. Med.* **74**: 1020. (Abstr.)
- Johns, W. H., and T. R. Bates. 1969. Quantification of the binding tendencies of cholestyramine I: effect of structure and added electrolytes on the binding of unconjugated and conjugated bile-salt anions. *J. Pharm. Sci.* **58**: 179.
- Moore, R. B., C. A. Crane, and I. D. Frantz, Jr. 1968. Effect of cholestyramine on the fecal excretion of intravenously administered cholesterol- $4^{14}C$ and its degradation products in a hypercholesterolemic patient. *J. Clin. Invest.* **47**: 1664.
- Garbutt, J. T., K. W. Heaton, L. Lack, and M. P. Tyor. 1969. Increased ratio of glycine to taurine conjugated bile salts in patients with ileal disorders. *Gastroenterology*. **56**: 711.
- Garbutt, J. T., L. Lack, and M. P. Tyor. 1971. Physiological basis of alterations in the relative conjugation of bile acids with glycine and taurine. *Am. J. Clin. Nutr.* **24**: 218.

31. McLeod, G. M., and H. S. Wiggins. 1969. Bile salts in small intestinal contents after ileal resection and in other malabsorption syndromes. *Lancet*. 1: 873.
32. Abaurre, R., S. G. Gordon, J. G. Mann, and F. Kern. 1969. Fasting bile salt pool size and composition after ileal resection. *Gastroenterology*. 57: 679.
33. Ekdhal, P. H., and J. Sjövall. 1958. On the conjugation and formation of bile acids in the human liver. I. On the excretion of bile acids by patients with postoperative choledochostomy drainage. Bile acids and steroids 61. *Acta Chir. Scand*. 114: 439.
34. Hashim, S. A., S. S. Bergen, Jr., and T. B. Van Itallie. 1961. Experimental steatorrhea induced in man by bile acid sequestrant. *Proc. Soc. Exp. Biol. Med.* 106: 173.
35. Garbutt, J. T., and P. Leff. 1971. Cholestyramine-induced alterations in hepatic synthesis of bile acids. *Clin. Res.* 19: 33. (Abstr.)
36. Mekhjian, H. S., S. F. Phillips, and A. F. Hofmann. 1971. Colonic secretion of water and electrolytes induced by bile acids: perfusion studies in man. *J. Clin. Invest.* 50: 1569.
37. Shefer, S., S. Hauser, I. Bekersky, and E. H. Mosbach. 1969. Feedback regulation of bile acid biosynthesis in the rat. *J. Lipid Res.* 10: 646.
38. Hofmann, A. F., P. J. Thomas, L. H. Smith, and J. T. McCall. 1970. Pathogenesis of secondary hyperoxaluria in patients with ileal resection and diarrhea. *Gastroenterology*. 58: 960. (Abstr.)