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Research Article

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Effect of Oral Chenodeoxycholic Acid on Bile Acid Kinetics and Biliary Lipid Composition in Women with Cholelithiasis

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ABSTRACT Bile acid kinetics and biliary lipid composition were characterized in six women with gallstones before and after 6 mo of oral therapy with chenodeoxycholic acid, an agent that induces dissolution of cholesterol gallstones in man. Over a dosage range of 1–4 g/day, absorption varied from 0.8 to 2.3 g/day. The chenodeoxycholic acid pool expanded two- to sixfold, and bile became composed predominantly (> 90%) of chenodeoxycholic acid conjugated chiefly with glycine. Cholic acid and deoxycholic acid pools decreased markedly, so that the total bile acid pool expanded much less, about twofold on the average. Cholic acid synthesis decreased in five of the six patients, consistent with negative feedback inhibition of cholic acid synthesis by chenodeoxycholic acid. In four patients whose bile was above or close to saturation with cholesterol, the bile became unsaturated; in two patients, whose bile was unsaturated, it remained so. In five patients with radiolucent gallstones, chenodeoxycholic acid therapy was continued after completion of kinetic and composition measurements; the stones decreased in size or dissolved entirely during the subsequent 6 to 18 mo. Similar measurements of bile acid kinetics and biliary lipid composition were made before

Presented in part at the meeting of the American Association for the Study of Liver Diseases, Chicago, Nov. 3 and 4, 1971, and published in abstract form (1). A preliminary report describing changes in gallstone size during chenodeoxycholic acid therapy has been published (2).

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INTRODUCTION

Cholesterol is transported in bile in a mixed bile acid-lecithin micelle, and the formation of bile that is supersaturated with cholesterol is considered a prerequisite for cholesterol cholelithiasis (3). In many patients with cholesterol gallstones, the fasting gallbladder bile is saturated or supersaturated with cholesterol (4, 5) if bile composition is treated as a model system composed solely of bile acids, lecithin, cholesterol, and water (6, 7).

We previously reported (8) that in patients with cholesterol cholelithiasis ingestion of chenodeoxycholic acid, one of the primary bile acids in man, caused the fasting duodenal bile to become unsaturated in cholesterol. In these bile samples, cholesterol crystals disappeared, and chenodeoxycholic acid became the predominant bile acid.

Because of these striking changes in biliary lipid and bile acid composition, we undertook the present study to define the influence of chenodeoxycholic acid on bile acid kinetics and on biliary lipid composition in women with cholelithiasis. During this study, stone dissolution was noted in certain patients; this aspect of the study has been reported elsewhere (2).

METHODS

Study groups. Seven women with gallstones and six healthy women (control group) were studied. The control group was studied twice, about 6 mo apart, to provide information on the variation of bile acid kinetics. The gallstone group was studied before and after 6 mo of treatment with chenodeoxycholic acid; one patient (patient 7) did not complete the study. It was assumed that variation among these patients would not be greater than that among

the control group. Chenodeoxycholic acid was not administered to control subjects because no information is available on the toxicity of chenodeoxylic acid in healthy persons.

All of the women were no longer able to bear children at the time of the study. The two groups did not differ significantly in age, height, and parity, but the patients with gallstones were about 20% heavier ($P < 0.05$) than the healthy controls. No consistent or significant changes in body weight occurred in either group during the study.

Of the women with gallstones, six had radiolucent, asymptomatic gallstones, and one had a concentrically calcified gallstone. All were otherwise healthy, and all had roentgenologically functioning gallbladders. Group characteristics were (mean \pm SE): age, 53.9 ± 2.4 yr; height 161 ± 2.3 cm; weight, 73.5 ± 5.6 kg.

The six healthy women had roentgenologically functioning gallbladders and no gallstones. Group characteristics were (mean \pm SE): age, 53.5 ± 3.2 yr; height, 163 ± 3.7 cm; weight, 60.1 ± 5.2 kg.

Protocol. The subjects were admitted to the Clinical Research Center for all studies. Each received a standard 2-day repeating diet (calculated from an age, height, and weight nomogram). For the gallstone patients, the diet averaged 22 kcal/kg body weight (range, 16–34 kcal/kg); for the healthy control subjects, the diet averaged 24 kcal/kg (range, 15–29 kcal/kg). The diet contained 40% of calories as fat, 40% as carbohydrate, and 20% as protein. Before breakfast on the morning after admission, an indwelling double-lumen nasoduodenal tube was passed, and the distal tip was positioned fluoroscopically in the third part of the duodenum. ^{14}C -labeled cholic acid (10 μCi) and ^3H -labeled chenodeoxycholic acid (60 μCi) were then administered intravenously. Before breakfast on the next morning, 3.0 ml of duodenal bile was collected after gallbladder stimulation by the intravenous infusion of 40 Ivy U of cholecystokinin. Four more collections were made on successive days. This represented the removal of approximately 0.2 mmol of bile acid or 3–5% of the bile acid pool/day for 5 days. Each bile sample was added to 20 ml of 95% ethanol and was stored at 5°C until all five samples were collected.

Patients with gallstones then took chenodeoxycholic acid (0.75–4.5 g/day in 250-mg gelatin capsules) (Weddel Pharmaceuticals, London) for 6 mo, after which they were rehospitalized and the entire procedure for the determination of bile acid kinetics was repeated. Dosage was individualized, and each patient took the maximal tolerable dose (the limiting side-effect was diarrhea). The dosage remained constant for each patient throughout the study. Patient 7 dropped out of the study.

Determination of bile acid kinetics. An isotope dilution procedure based on that of Lindstedt (9) was used. [2,4- ^3H]Chenodeoxycholic acid was synthesized (10), and [24- ^{14}C]cholic acid was purchased (New England Nuclear, Boston, Mass.). Both labeled bile acids were greater than 98% pure by gas-liquid chromatography (GLC) (11), thin-layer chromatography (TLC) (12), and zonal scanning (13).

The samples (3 ml of bile in 20 ml of 95% ethanol) were heated to precipitate protein and then filtered (Whatman no. 43, 12.5 cm, ashless) into large extraction tubes. 13 ml of distilled water was added, and the pH was brought up to 7.0 (if lower) with 1 M sodium bicarbonate. After three 40-ml extractions with equal volumes of petroleum ether, the ethanol-water phase was dried under a stream

of air. This residue was transferred into nickel bombs (Parr Instrument Co., Moline, Ill.) with 8 ml of alkaline ethanol (50% ethanol-2 N NaOH, 1:1, vol/vol) and heated for 4 h at 115°C to effect deconjugation. The samples were then cooled and acidified (to methyl orange) with 10 N HCl, and the bile acids were extracted three times with 3 vol of diethyl ether. The ether extract was dried and then methylated with freshly prepared ethereal diazomethane.

Because the ^{14}C in the total samples was present as both cholic and deoxycholic acids, the dihydroxy and trihydroxy methyl esters were separated by chromatography on columns containing 6 g of Woelm neutral aluminum oxide (grade IV) (Waters Associates, Inc., Framingham, Mass.). The samples were placed on the column in acetone-toluene, 3:1 (vol/vol), and the dihydroxy bile acids were eluted with 120 ml of the same solvent. Cholic acid was subsequently eluted with 120 ml of acetone-methanol, 92:8 (vol/vol).

Trifluoroacetates were prepared from a measured portion of each eluate to determine the mass of individual bile acids (mg/ml) by GLC (F & M 402 with flame ionization (Hewlett-Packard Co., Avondale Div., Avondale, Pa.); 1.2-m "U" columns, 3 mm ID; packed with 3% QF 1-coated Gas Chrom S) (14). To obtain a standard curve for each bile acid, pure reference bile acids in a range of known concentrations were included with each day's GLC run. $3\alpha,12\alpha$ -Dihydroxy-7-keto- 5β -cholanoic acid was used as an internal standard; 2.0 μg was added to each unknown sample and to each reference standard. To correct for errors of injection and detector response, the integrated areas obtained for each sample were corrected by the ratio of area determined for the internal standard in that sample (mean area per amount of internal standard injected for all samples). The corrected areas were then applied to the standard curve for that bile acid to obtain the mass present in the sample.

Another measured portion of each eluate was counted (dpm/ml) for ^{14}C and ^3H by liquid scintillation spectroscopy using external standardization to compensate for quenching (14 ml of Redisolve VI; Beckman LS-250 Beckman Instruments, Inc., Electronic Instruments Div., Schiller Park, Ill.).

Biliary bile acid composition. Biliary bile acid composition was determined by GLC of the trifluoroacetates (14) or acetates (11, 15) of methyl esters. To determine the concentration of sulfated lithocholic acid conjugates in bile (16), samples from patients 1, 4, and 6, taken during chenodeoxycholic acid treatment, were solvolyzed in 4 N HCl-ethyl acetate-diethyl ether, 1:2:4 (vol/vol), as described by Burstein and Lieberman (17). Samples were then extracted, saponified, and esterified with methanol (see below). With these conditions solvolysis of tetradecyl sulfate was complete as assessed by TLC with a solvent system for conjugated bile acids (12). A second sample of bile was saponified and esterified without prior hydrolysis. One portion of the methyl ester fraction was derivatized to trimethylsilyl ethers and chromatographed on a HiEff-8BP column (18); with this derivative and column, lithocholic acid has a retention time greater than that of any of the common bile acids. A second portion was acetylated and chromatographed on a cyanosilicone column (15); with this derivative and column, lithocholic acid has a retention time less than that of any of the common bile acids.

Glycine/taurine ratios were determined by mass analysis of bile acid after TLC separation by Gregg's solvent

system (19). The mobility of the individual conjugated bile acid classes was estimated from that of standards run on either side of the bile samples. The areas containing glycine-conjugated bile acids or taurine-conjugated bile acids were scraped into centrifuge tubes, and the bile acids were eluted by four washes (two with chloroform-methanol [2:1] and two with 95% ethanol). The washes were combined and dried in a rotary evaporator, the residue was dissolved in a small volume of methanol, and the mass of bile acid present was determined by an automated steroid dehydrogenase procedure (4).

Bile acid excretion. Daily stool samples were collected in preweighed paint cans and kept frozen until homogenates were made with distilled water. Aliquots of the homogenates were pooled, and samples (5.0 g) were taken for analysis of bile acid composition as described previously (20). In one patient, all urine was distilled to remove ^3H as $[^3\text{H}]\text{H}_2\text{O}$, and the residue was then combusted to $[^3\text{H}]\text{H}_2\text{O}$ by an oxygen flask technique (Packard sample oxidizer, Packard Instrument Co., Inc., Downers Grove, Ill.).

Biliary lipid composition. On the last morning of the study, a larger sample of bile (20 ml) was collected and filtered (Millipore Corp., Bedford, Mass.) at 37°C for measurement of the concentrations of bile acids, lecithin, and cholesterol in micellar solution (8) as well as biliary bile acid composition (14, 15).

Calculation and expression of results. The specific activities of $[^3\text{H}]$ chenodeoxycholic acid and $[^4\text{C}]$ cholic acid were determined for each sample of bile, and the natural logarithms were plotted (ordinate) against time (abscissa) to permit calculations of pool size, turnover rate, and synthesis rate, as described previously (21). For patients ingesting chenodeoxycholic acid, the decline of the specific activity decay curve reflects input from both endogenous synthesis and exogenous sources (oral dosage). Repeated determination of specific activity on the same bile samples had a coefficient of variation of 3%; the correlation coefficient for the specific activity decay curves was 0.97 ± 0.01 (mean \pm SE).

Deoxycholic acid and lithocholic acid pool sizes were calculated from the measured chenodeoxycholic acid and cholic acid pool sizes and the bile acid composition of bile (by GLC); the validity of this calculation had been previously established (19). The monohydroxy fraction increased considerably after solvolysis, indicating that monohydroxy-lithocholic acid sulfates were an important constituent of biliary bile acids, composing up to 30% of total biliary bile acids in fasting bile (16). We have not included these data in our calculation of biliary bile acid composition because it is not known whether the bile acid sulfate content of bile remains constant throughout the day and because the functional significance of bile acid sulfates in bile is unknown.

Since bile lipids vary in molecular weight from 387 (cholesterol) to 790 (lecithin), all bile lipid data have been expressed as micromoles in addition to the conventional units of weight. Furthermore, because the subjects varied considerably in weight but pool size correlated well ($r = 0.85$) with body weight, all data are expressed as $\mu\text{moles per kilogram of body weight}$.

Lipid composition of bile was expressed in mole fraction of bile acid, lecithin, and cholesterol, under the assumption that these are the only lipids present in bile. Changes in bile composition have been expressed as a saturation index, as suggested by Metzger, Heymsfield, and Grundy (22). Here, the value of 1.0 indicates saturation with cholesterol,

and values above and below 1.0 indicate supersaturation and unsaturation, respectively. Values were calculated from polynomial equations (23) describing the cholesterol solubility line proposed by Admirand and Small (4), as well as from the more recent line described independently by Hegardt and Dam (24) and Holzach, Marsh, Olszewski, and Holan (25).

Statistical analysis. Since the groups were small, differences were examined by the Wilcoxon rank sum and signed rank tests.

RESULTS

Effect of chenodeoxycholic acid on bile acid kinetics. Typical specific activity decay curves are shown in Fig. 1. The decreased value for the specific activity of the intercept at time 0 for chenodeoxycholic acid indicates expansion of the chenodeoxycholic acid pool (Table I and Fig. 2). The cholic acid pool decreased markedly. The pool size of deoxycholic acid, calculated from the biliary bile acid composition, also decreased markedly. Lithocholic acid composed a very small fraction of biliary bile acids before treatment and did not change in a consistent direction after chenodeoxycholic acid treatment, but sulfated lithocholic acid increased considerably in bile (see below). Because of these changes in pool size, biliary bile acids became composed of predominantly chenodeoxycholic acid (Table II).

Total bile acid pool size increased in five of the six patients ($P < 0.05$); the increase was about twofold on the average. The change in total bile acid pool size represents the algebraic sum of the increase in the chenodeoxycholic acid pool and the decrease in the pools of cholic and deoxycholic acids (Fig. 2). In patient 3, in whom the total bile acid pool actually decreased after chenodeoxycholic acid therapy, the magnitude of the increase in the chenodeoxycholic acid pool (32 μmol) was more than offset by the decreases in the cholic acid pool (20 μmol) and the deoxycholic acid pool (28 μmol).

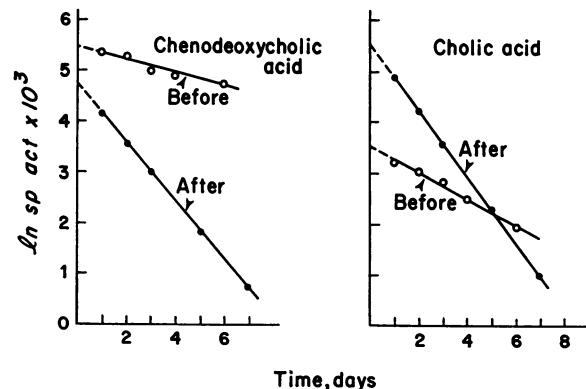


FIGURE 1 Specific activity decay curves for chenodeoxycholic acid (left) and cholic acid (right) before and after chenodeoxycholic acid treatment in patient with gallstones.

TABLE I
Effect of Chenodeoxycholic Acid Treatment on Bile Acid Kinetics in Patients with Gallstones

Study	Parameters*	Patients							Mean \pm SE	P‡
		1	2	3	4	5	6	7		
Chenodeoxycholic acid										
Before Rx	Pool, $\mu\text{mol}/\text{kg}$	12.5	5.9	20.4	23.9	5.9	17.8	5.1	13.1 \pm 2.9	
	Pool, mg	301	142	582	598	204	471	202	357 \pm 72	
	FTR, day^{-1}	0.39	0.39	0.13	0.11	0.42	0.17	0.29	0.27 \pm 0.05	
	Syn, $\mu\text{mol}/\text{kg}/\text{day}$	4.88	2.30	2.65	2.63	2.48	3.03	1.48	2.78 \pm 0.39	
	Syn, mg/day	117	55	76	66	86	80	58	76.9 \pm 8.0	
After Rx	Pool, $\mu\text{mol}/\text{kg}$	54.3	62.7	52.0	158.4	39.2	115.6	—	80.4 \pm 19.0	<0.05
	Pool, mg	1,210	1,417	1,483	3,788	1,337	2,924	—	2,027 \pm 437	
	FTR, day^{-1}	0.76	0.67	0.57	0.61	0.65	0.29	—	0.59 \pm 0.07	<0.05
	Syn, $\mu\text{mol}/\text{kg}/\text{day}$	41.3	42.0	29.6	96.6	25.5	32.9	—	44.6 \pm 10.7	<0.05
	Syn, mg/day	916	952	845	2,311	874	816	—	1,119 \pm 239	
Cholic acid										
Before Rx	Pool, $\mu\text{mol}/\text{kg}$	17.4	4.4	23.5	34.7	11.7	29.9	9.3	18.7 \pm 4.2	
	Pool, mg	436	111	698	903	425	767	379	531 \pm 103	
	FTR, day^{-1}	0.47	0.71	0.26	0.12	0.68	0.24	0.43	0.42 \pm 0.08	
	Syn, $\mu\text{mol}/\text{kg}/\text{day}$	8.2	3.1	6.1	4.2	8.0	6.7	4.0	5.8 \pm 0.8	
	Syn, mg/day	205	79	181	108	289	184	164	173 \pm 26	
After Rx	Pool, $\mu\text{mol}/\text{kg}$	2.0	2.7	3.4	2.9	2.9	3.9	—	3.0 \pm 0.3	<0.05
	Pool, mg	49	68	102	76	106	100	—	84 \pm 9	
	FTR, day^{-1}	0.99	1.54	0.65	0.81	0.62	0.41	—	0.84 \pm 0.16	<0.05
	Syn, $\mu\text{mol}/\text{kg}/\text{day}$	2.0	4.2	2.2	2.3	1.8	1.6	—	2.4 \pm 0.4	<0.05
	Syn, mg/day	49	104	66	62	66	44	—	65.2 \pm 8.6	
Deoxycholic acid										
Before Rx	Pool, $\mu\text{mol}/\text{kg}$	10.4	5.4	27.8	6.9	11.7	14.0	5.4	11.7 \pm 3.0	
	Pool, mg	252	130	792	172	408	370	206	333 \pm 86	
After Rx	Pool, $\mu\text{mol}/\text{kg}$	0	0	0	0	0.8	0	—	0.13 \pm 0.13	<0.05
	Pool, mg	0	0	0	0	26	0	—	4.3 \pm 4.3	
Total										
Before Rx	Pool, $\mu\text{mol}/\text{kg}$	40.3	16.3	71.5	65.8	30.0	61.0	20.3	43.6 \pm 8.5	
	Pool, mg	989	397	2,080	1,673	1,063	1,642	803	1,235 \pm 221	
After Rx	Pool, $\mu\text{mol}/\text{kg}$	57.5	66.3	57.3	158.5	44.5	117.3	—	83.6 \pm 18.2	
	Pool, mg	1,307	1,530	1,663	3,863	1,548	3,024	—	2,156 \pm 424	

* FTR, fractional turnover rate. Syn, synthesis calculated from specific activity decay curve.

† For change after treatment.

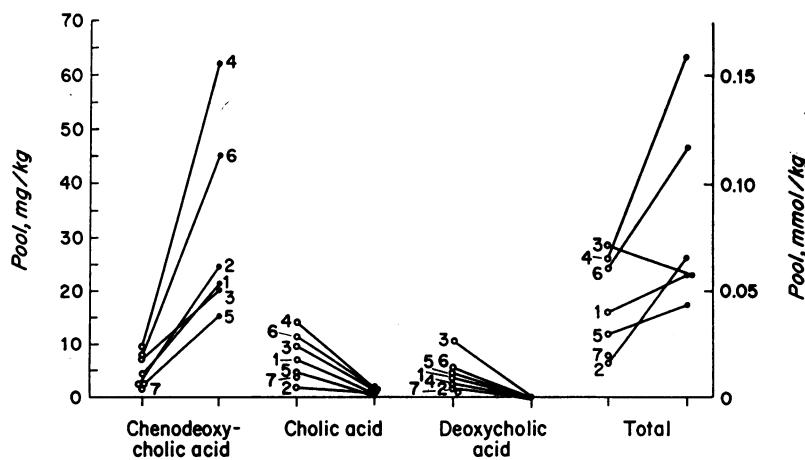


FIGURE 2 Effect of chenodeoxycholic acid treatment on bile acid pools in women with gallstones. (Modified from Danzinger, R. 1972. Oral chenodeoxycholic acid: favourable effect on bile acid pool and bile composition with dissolution of gallstones in women with cholelithiasis. In Bile Acids in Human Diseases. P. Back and W. Gerok, editors. Friedrich-Karl Schattauer-Verlag, Stuttgart. 167. By permission.)

TABLE II
Effect of Chenodeoxycholic Acid Treatment on Biliary Bile Acid Composition in Patients with Gallstones

Patient	Time	Molar composition			
		Chenodeoxycholic	Cholic	Deoxycholic	Lithocholic*
	mo	%			
1	0	33.7	41.1	25.2	<0.5
	6	95.8	<0.5	<0.5	4.2
	12	93.7	<0.5	3.2	3.0
	18	96.0	<0.5	0.6	3.4
2	0	32.1	31.6	32.1	4.2
	6	96.8	<0.5	<0.5	3.2
	12	91.6	<0.5	<0.5	8.4
3	0	32.8	29.0	38.2	<0.5
	6	95.5	<0.5	<0.5	4.7
4	0	44.8	44.8	10.4	<0.5
	6	99.0	<0.5	<0.5	<0.5
	Cessation of drug	38.7	55.8	5.5	<0.5
5	0	31.5	28.0	38.0	2.5
	6	90.9	2.3	1.7	5.1
6	0	35.4	40.0	22.7	1.9
	6	99.0	<0.5	<0.5	<0.5

* Based on GLC after alkaline saponification, methylation, and acetylation. After chenodeoxycholic treatment, the majority of the monohydroxy fraction appeared to be present as sulfates, as judged by GLC after solvolysis. Therefore, the lithocholic acid fraction values shown here are too low by a factor ranging from 1 to 10.

Cholic acid synthesis rates, which did not differ significantly from those of the control group before treatment, became significantly lower in five of the six patients (Fig. 3). The experimental design provided no information on the effect of oral chenodeoxycholic acid on endogenous chenodeoxycholic acid synthesis.

Monohydroxy bile acids in bile. By GLC, the monohydroxy fraction of biliary bile acids increased in all five patients whose bile acids were solvolyzed before alkaline saponification. Based on the increase, the fraction of monohydroxy bile acids present in bile as sulfates was between 50 and 90%, so that free and sulfated lithocholic acid content composed 10–30% of biliary bile acids in fasting bile.

Urinary water contained ^3H that was considered to arise from bacterial removal of ^3H (26), but no ^3H was found in urinary solids in the one patient examined.

Absorption of chenodeoxycholic acid. Because the patients were ingesting chenodeoxycholic acid while bile acid kinetics were measured, the decrease of the chenodeoxycholic acid specific activity curve was caused by absorption of unlabeled (exogenous) chenodeoxycholic acid and possibly also by continuing syn-

thesis of (endogenous) chenodeoxycholic acid. The maximal absorption of ingested chenodeoxycholic acid was calculated by assuming that the input of chenodeoxycholic acid represented solely ingested chenodeoxycholic acid (endogenous synthesis totally repressed) (Table III). Minimal absorption was calculated by assuming that endogenous synthesis remained unchanged. Absorption varied from 26 to 84% and averaged 60%; its efficiency appeared to decrease with increasing dose (Fig. 4). The chenodeoxycholic acid pool appeared to expand in direct proportion to the amount of chenodeoxycholic acid absorbed (Fig. 5).

Bile acid conjugation. The fraction of bile acids conjugated with glycine (glycine/taurine ratio) increased in all patients ingesting chenodeoxycholic acid (Table IV). Before treatment, bile acids were conjugated about equally with glycine and taurine. After treatment, glycine conjugation represented four-fifths of the bile acids.

Bile acid excretion. The dose of chenodeoxycholic acid was individualized to an amount just below that which produced diarrhea, so fecal weight during the treatment period reflected the dose-response characteristics of each patient. Two patients had a considerable increase in fecal weight during the treatment period. The others showed no change although patient 1 had diarrhea followed by constipation during the hospitalization period. Before treatment, fecal weights of both groups were similar.

Fecal bile acid composition before and after chenodeoxycholic acid treatment is given in Table V. During

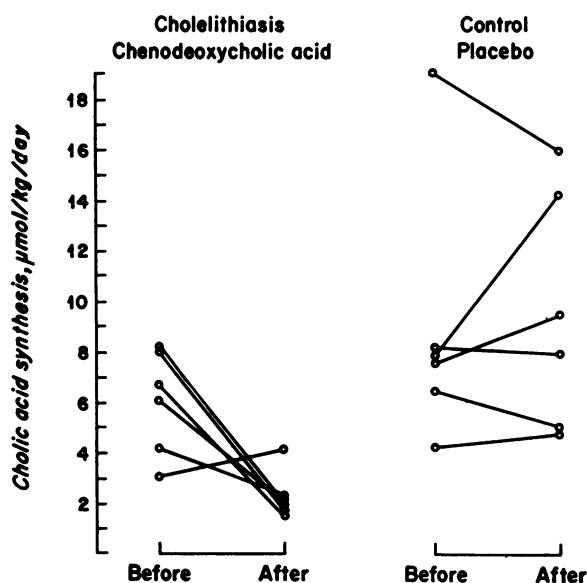


FIGURE 3 Cholic acid synthesis before and after chenodeoxycholic acid therapy in gallstone patients (left) and before and after placebo in control subjects (right).

TABLE III
Absorption of Ingested Chenodeoxycholic Acid and Change in Gallstones

Patient	Amount ingested/day		Input/day*		Absorption†		Gallstones
	mg	$\mu\text{mol}/\text{kg}$	mg	$\mu\text{mol}/\text{kg}$	Minimal %	Maximal %	
1	1,500	67.2	920	41.2	54	61	Initial mean diam, 9 mm; 3 mm at 18 mo; dissolved at 2 yr.
2	1,500	61.8	1,020	42.0	64	68	Initial mean diam, 4 mm; 3 mm at 18 mo.
3	1,000	35.2	840	29.6	76	84	Initial mean diam, 15 mm; 7 mm at 6 mo; dissolved at 14 mo.
4	4,000	167.4	2,310	96.6	56	58	Initial mean diam, 5 mm; dissolved at 6 mo.
5	1,500	44.1	870	25.5	52	58	Initial mean diam, 17 mm; no change at 6 mo; smaller at 12 mo.
6	3,000	118.5	860	32.9	26	29	Single calculus with calcified rings; no change at 1 yr; elective cholecystectomy

* Input from exogenous (ingested) and endogenous (synthesized from cholesterol) chenodeoxycholic acid.

† Absorption percentage calculated for no change in endogenous synthesis (minimal absorption) and for complete repression of endogenous synthesis (maximal absorption).

the control period, fecal bile acid composition probably was not different from that of healthy persons, although the proportion of $3\alpha,12\beta$ -dihydroxy- 5β -cholanoic acid seems higher than published values. After chenodeoxycholic acid treatment, fecal bile acids became composed predominantly of chenodeoxycholic acid or lithocholic acid or both, reflecting the large dose of chenodeoxycholic acid as well as the decreased cholic acid synthesis (Fig. 6).

The two patients on the highest dosages of chenodeoxycholic acid (patient 4, 4,000 mg/day; patient 7, 3,000 mg/day) had the least dehydroxylation and the greatest amount of diarrhea based on fecal weight. Patient 1, whose data are not shown, had diarrhea (daily fecal weights of 240 and 260 g/day) during the first 2 days of her second hospitalization period. Fecal bile acids collected at this time were composed pre-

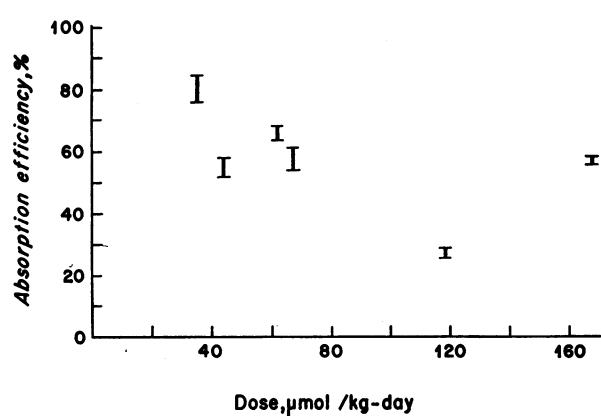


FIGURE 4 Absorption efficiency of chenodeoxycholic acid in relation to dose for patients with gallstones. The vertical lines indicate minimal and maximal efficiency when calculated as described in Table III.

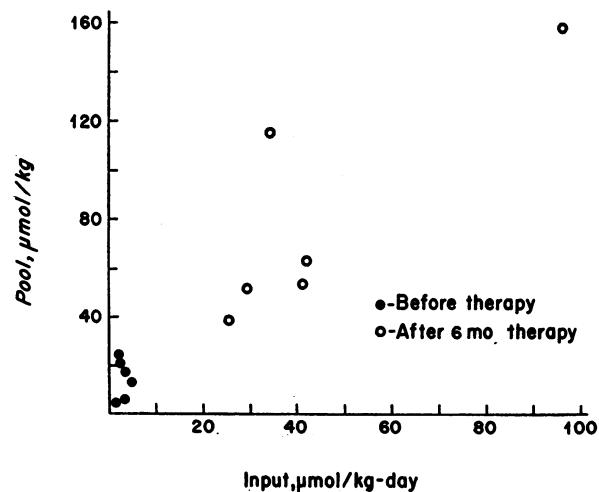


FIGURE 5 Chenodeoxycholic acid pool size in relation to input of chenodeoxycholic acid.

TABLE IV
Effect of Chenodeoxycholic Acid Treatment on Conjugation of Bile Acids in Patients with Gallstones

Patient	Glycine/taurine		N* gly	
	Before	After	Before	After
1	1.22	5.93	0.55	0.86
2	2.18	4.79	0.69	0.83
3	1.35	6.30	0.57	0.86
4	0.73	17.80	0.42	0.95
5	0.90	3.00	0.47	0.75
6	0.80	4.48	0.44	0.82
Mean \pm SE	1.20 \pm 0.22	7.05 \pm 2.20	0.52 \pm 0.04	0.84 \pm 0.03
	<i>P</i> < 0.01			

* Mole fraction of biliary bile acids conjugated with glycine.

dominantly (90 and 86%) of chenodeoxycholic acid. Her diarrhea ceased, and she had no stool until the final day of hospitalization; in this sample, lithocholic acid was the predominant (91%) bile acid, and chenodeoxycholic acid composed only 5% of the fecal bile acids.

Bile acid kinetics in control group. Bile acid pool sizes and synthesis rates were unchanged at the second study, 6 mo later (Table VI).

Effect of chenodeoxycholic acid on biliary lipids. By Hegardt and Dam's (24) criterion, two patients (2 and 5) had bile that was markedly supersaturated with cholesterol, and two others (1 and 3) had bile with a composition just beyond the micellar zone (Table VII). The mole fraction of cholesterol decreased in all four of these patients. Two patients (4 and 6) had bile unsaturated with cholesterol; in these, there was no change in the mole fraction of cholesterol. Changes in biliary lipid composition are shown in Fig. 7 by triangular coordinates and by the saturation index.

Biliary lipids in control group. Bile was close to saturation in all six normal controls (Table VIII); biliary lipids were unchanged 6 mo later.

DISCUSSION

Chenodeoxycholic acid treatment and total bile acid pools. Chenodeoxycholic acid administration increased the chenodeoxycholic acid pool an average of sixfold, and the increase in size was positively correlated with the amount of chenodeoxycholic acid absorbed. Despite the high input of chenodeoxycholic acid, the fractional turnover rate of the markedly expanded chenodeoxycholic acid pool increased little, indicating efficient intestinal conservation.

The total bile acid pool expanded less (about two-fold) because of the decrease in the pools of deoxycholic acid and cholic acid. After chenodeoxycholic acid treatment, four of the six patients had bile acid pool sizes similar to those present in healthy controls but different in composition, being composed chiefly of

TABLE V
Fecal Bile Acids before and after Chenodeoxycholic Acid Treatment*

	Patients				
	2	3	4	5	6
% composition					
Before treatment					
Lithocholic	26.7	28.3	13.8	35.2	11.4
Chenodeoxycholic	4.4	3.9	12.4	4.6	15.0
Deoxycholic	47.9	50.5	55.0	39.8	44.6
Cholic	Tr	Tr	18.9	Tr	15.5
Other secondary	21.2	17.4	Tr	20.5	13.8
Total chenodeoxycholic	31.1	32.2	26.2	39.8	26.4
Total cholic	69.1	67.9	73.9	60.3	73.9
Mean fecal wt, g/day	87	39	66	107	—
After treatment					
Lithocholic	21.8	53.5	4.8	87.5	12.8
Chenodeoxycholic	70.7	36.4	93.8	8.4	84.7
Deoxycholic	1.3	6.1	Tr	3.6	Tr
Cholic	Tr	Tr	Tr	Tr	Tr
Other secondary	6.3	4.0	1.5	0.7	2.6
Total chenodeoxycholic	92.5	89.9	98.6	95.9	97.5
Total cholic	7.6	10.1	1.5	4.3	2.6
Mean fecal wt, g/day	226	100	423	114	438

* For all but patient 3, feces from the last 3 days were pooled for analysis. Patient 3 had only three stools during entire second study period and these were pooled. Major constituent of the "other secondary" fraction had the retention time of $3\alpha,12\beta$ -dihydroxy- 5β -cholanic acid, a bacterial derivative of cholic acid previously identified in human stool. In the calculation, it has been assumed that all unidentified peaks were secondary bile acids derived from cholic acid.

Tr, trace.

chenodeoxycholic acid. In the remaining two, the total bile acid pool was much larger than that in any of the controls.

The fraction of administered chenodeoxycholic acid absorbed appeared to decrease with increasing dose. Whether this was caused by incomplete dissolution in the small intestine or by a limitation in the absorption capacity of the small intestine or both is unclear. Chenodeoxycholic acid is insoluble at the pH of gastric contents during digestion (27, 28) but is presumed to dissolve in the small intestine and to be absorbed by passive nonionic diffusion as well as active ileal transport (29, 30).

Cholic acid and deoxycholic acid pools. Chenodeoxycholic acid administration suppressed cholic acid synthesis in five of six patients. Since cholic acid administration to patients with gallstones causes chenodeoxycholic acid to disappear from the biliary bile acids (8), it seems likely that each of the primary bile acids suppresses the synthesis of the other in patients with cholesterol cholelithiasis and presumably in healthy persons (31, 32). As yet, there is no strong evidence that either primary bile acid suppresses its own synthesis in man.

The greater decrease in cholic acid pool size than in synthesis is explained by the increased fractional

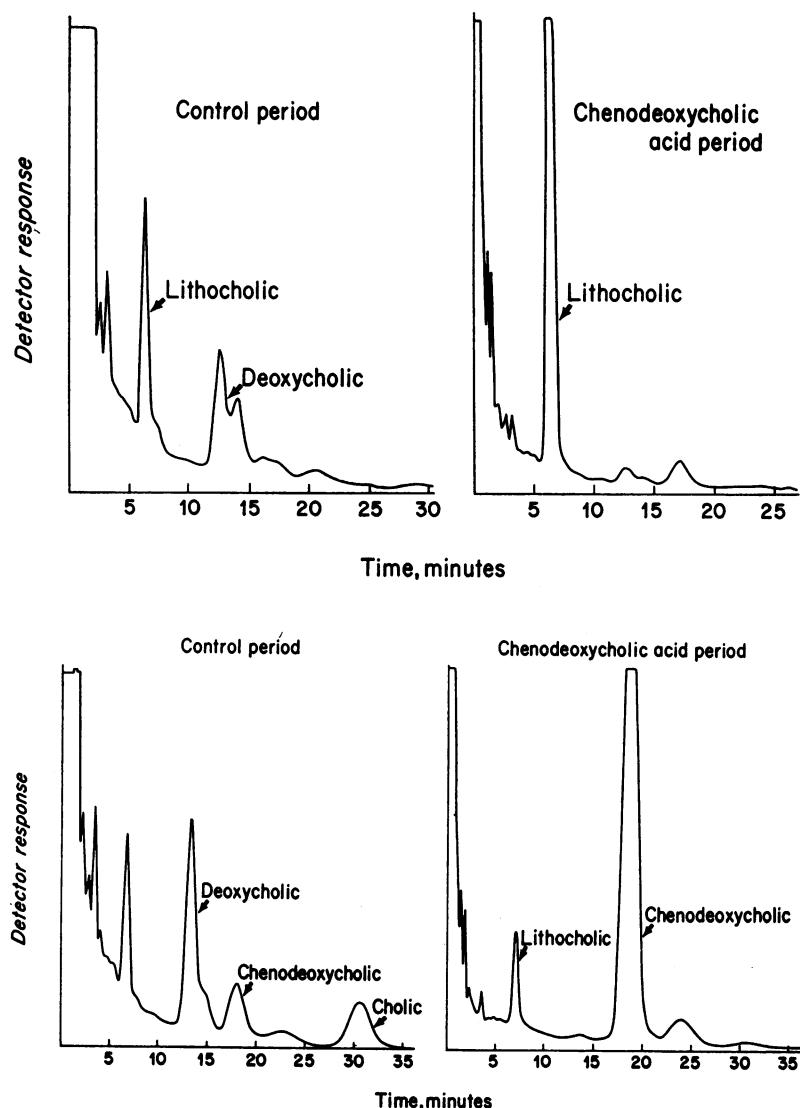


FIGURE 6 GLC of fecal bile acids before (left) and after (right) chenodeoxycholic acid therapy. *Above*, patient 5. *Below*, patient 4.

turnover rate, which also means decreased intestinal conservation. This in turn is perhaps explained by chenodeoxycholylglycine acting as a competitive inhibitor of cholylglycine transport in the terminal ileum (33). Thus, the combination of decreased synthesis and decreased efficiency of intestinal absorption results in a marked decrease in the absolute size of the cholic acid pool.

The decrease in deoxycholic acid pool presumably is related to two factors. First, decreased cholic acid synthesis results in less cholic acid entering the colon in the steady state. Second, the fractional turnover rate would be increased if deoxycholic acid behaved similarly to the other bile acids during chenodeoxycholic acid therapy. Thus, the transformation in biliary bile

acid composition induced by chenodeoxycholic acid is related to a marked increase in the input of chenodeoxycholic acid together with a decrease in the input of cholic and deoxycholic acids. In addition, efficient intestinal absorption conserves the greatly expanded chenodeoxycholyl pool.

Lithocholic acid pools. Our data suggest a considerable increase in the sulfated monohydroxy fraction of biliary bile acids. Such bile acids are not present in measurable concentrations in healthy persons, and in our patients they are presumed to originate from the monohydroxy bile acids formed in the distal intestine. Characterization of lithocholic acid metabolism in patients receiving chenodeoxycholic acid appears indicated.

TABLE VI
Bile Acid Kinetics in Healthy Controls

Study	Parameters	Patients						Mean \pm SE
		1	2	3	4	5	6	
Chenodeoxycholic acid								
Period 1	Pool, $\mu\text{mol}/\text{kg}$	39.5	23.9	26.7	27.3	23.7	17.8	26.5 \pm 2.9
	Pool, mg	1,021	735	410	594	563	430	625.5 \pm 92.7
	FTR, day^{-1}	0.07	0.22	0.32	0.15	0.16	0.21	0.19 \pm 0.03
	Syn, $\mu\text{mol}/\text{kg}/\text{day}$	2.76	5.26	8.54	4.10	3.79	3.74	4.7 \pm 0.8
	Syn, mg/day	72	162	131	89	90	90	105.7 \pm 13.8
Period 2	Pool, $\mu\text{mol}/\text{kg}$	38.0	16.6	25.0	17.8	35.7	23.0	26.0 \pm 3.7
	Pool, mg	971	527	386	386	839	547	609.3 \pm 100.0
	FTR, day^{-1}	0.09	0.27	0.37	0.22	0.19	0.22	0.23 \pm 0.04
	Syn, $\mu\text{mol}/\text{kg}/\text{day}$	3.42	4.48	9.25	3.92	6.78	5.26	5.5 \pm 0.9
	Syn, mg/day	88	142	142	86	155	118	121.8 \pm 12.0
Cholic acid								
Period 1	Pool, $\mu\text{mol}/\text{kg}$	20.8	20.5	14.9	24.0	25.2	37.7	23.8 \pm 3.1
	Pool, mg	560	657	239	544	623	946	594.8 \pm 92.8
	FTR, day^{-1}	0.21	0.40	0.53	0.27	0.30	0.52	0.37 \pm 0.05
	Syn, $\mu\text{mol}/\text{kg}/\text{day}$	4.37	8.20	7.90	6.48	7.56	19.6	9.0 \pm 2.2
	Syn, mg/day	118	263	127	147	187	492	222.3 \pm 58.1
Period 2	Pool, $\mu\text{mol}/\text{kg}$	27.1	18.6	20.8	17.1	23.7	27.6	22.5 \pm 1.8
	Pool, mg	722	615	337	389	581	684	554.7 \pm 64.3
	FTR, day^{-1}	0.18	0.43	0.69	0.30	0.40	0.58	0.43 \pm 0.08
	Syn, $\mu\text{mol}/\text{kg}/\text{day}$	4.88	8.00	14.35	5.13	9.48	16.0	9.64 \pm 1.90
	Syn, mg/day	130	265	234	117	229	400	229.2 \pm 42.0
Deoxycholic acid								
Period 1	Pool, $\mu\text{mol}/\text{kg}$	6.4	11.7	22.2	39.7	12.2	11.2	17.2 \pm 5.0
	Pool, mg	165	360	340	866	290	270	381.8 \pm 100.8
Period 2	Pool, $\mu\text{mol}/\text{kg}$	9.7	13.8	19.6	22.2	6.9	6.4	13.1 \pm 2.7
	Pool, mg	250	438	301	483	163	154	298.2 \pm 56.3
Total								
Period 1	Pool, $\mu\text{mol}/\text{kg}$	69.8	57.3	64.5	91.5	62.5	68.3	69.0 \pm 4.8
	Pool, mg	1,839	1,791	1,009	2,031	1,513	1,676	1,643.2 \pm 145.0
Period 2	Pool, $\mu\text{mol}/\text{kg}$	74.5	50.0	66.3	57.8	68.0	57.0	62.3 \pm 3.6
	Pool, mg	1,942	1,618	1,045	1,282	1,634	1,381	1,483.7 \pm 128.4

Lithocholic acid formation and diarrhea. Only slight conversion of chenodeoxycholic acid to lithocholic acid occurred in the two patients ingesting large doses of chenodeoxycholic acid. In these patients, the amount of bile acids passing into the colon was similar to that in patients with ileal resection and bile acid diarrhea, in which decreased dehydroxylation has also been reported (15, 34-36). Thus, in both groups of patients, there is an increased proportion of chenodeoxycholic acid compared to that present in health. Since chenodeoxycholic acid has been shown to induce secretion of water and electrolytes by the human colon (37), the diarrhea observed in the patients who ingested large amounts of chenodeoxycholic acid may be a bile acid diarrhea, similar to that occurring in patients with ileal resection. It seems unlikely that decreased

7α -dehydroxylation can be explained by diarrhea alone because patients with large ileal resections and diarrhea do not have decreased 7α -dehydroxylation (15, 34).

Our preliminary findings, that a majority of the monohydroxy bile acids present in the bile of patients ingesting chenodeoxycholic acid are sulfated, indicate that the amount of lithocholic acid absorption occurring in these patients is unknown. However, in patients ingesting chenodeoxycholic acid for prolonged periods, liver function has remained normal except for slight and transient increases of serum transaminase levels (38).

Because the amount of chenodeoxycholic acid excreted should be approximately equal to the amount ingested, the daily rate of 7α -dehydroxylation can be calculated to be about 1,300 mg/day in patients 1 and 5.

TABLE VII
Effect of Chenodeoxycholic Acid Treatment on Biliary Lipids and Cholesterol Saturation in Patients with Gallstones

Patient	Time	Molar composition			Saturation index‡		
		BA	Le	Ch*	A&S	H&D; H	
1	mo		%				
	0	74.1	17.5	8.4	0.87	1.49	
	7	76.7	18.7	4.7	0.48	0.82	
	13	77.6	17.7	4.7	0.49	0.85	
2	18	75.9	19.3	4.8	0.49	0.81	
	0	65.9	16.5	17.6	1.81	3.01	
	6	65.9	26.7	7.3	0.73	0.96	
3	12	61.5	35.0	3.5	0.37	0.40	
	0	69.4	21.3	9.3	0.93	1.42	
	6	70.6	23.8	5.6	0.56	0.81	
4	0	71.3	23.4	5.3	0.53	0.78	
	6	75.1	21.1	3.8	0.38	0.61	
5	0	59.5	27.6	12.9	1.31	1.60	
	6	66.9	24.6	8.6	0.85	1.19	
6	0	76.8	19.4	3.9	0.40	0.66	
	6	69.9	24.4	5.6	0.56	0.80	
	11	71.2	24.5	4.4	0.44	0.63	
	0	60.5	28.3	11.1	1.13	1.37	
Mean±SE§		66.8±5.9	22.4±5.0	10.8±4.2	0.98±0.21	1.49±0.34	
		6	71.0±4.8	23.0±3.1	6.0±2.0	0.59±0.07	0.87±0.07

* For change with treatment, $P = 0.05$ for patients with radiolucent gallstones. Patient 6 (radiopaque gallstones), whose bile was most unsaturated during the control period, showed a small increase in saturation after 6 mo of chenodeoxycholic acid therapy. If her data are included, the decrease in the mole fraction of cholesterol associated with chenodeoxycholic acid therapy loses statistical significance in the Wilcoxon signed rank test.

† A&S, based on Admirand and Small (4); H&D; H, based on Hegardt and Dam (24), and Holzbach et al. (25).

§ For patients 1 through 6.

TABLE VIII
Biliary Lipids and Cholesterol Saturation in Healthy Controls

Patient	Time	Molar composition			Saturation index*	
		BA	Le	Ch	A&S	H&D; H
1	mo		%			
	0	71.5	21.5	6.9	0.69	1.06
2	6	70.6	20.8	8.7	0.87	1.35
	0	67.9	23.4	8.7	0.87	1.24
3	6	66.9	24.8	8.4	0.83	1.15
	0	77.1	18.1	4.8	0.50	0.85
4	6	76.1	17.8	5.5	0.57	0.98
	0	67.0	25.1	7.9	0.79	1.08
5	6	72.1	20.3	7.7	0.78	1.23
	0	70.8	21.8	7.3	0.73	1.11
6	6	74.2	19.7	6.1	0.62	1.01
	0	68.6	24.1	7.2	0.72	1.02
Mean±SE	6	70.8	22.1	7.1	0.71	1.07
	0	70.5±3.7	22.3±2.5	7.1±1.3	0.71±0.05	1.06±0.19
	6	71.8±3.2	20.9±2.4	7.2±1.3	0.73±0.04	1.13±0.06

* A&S, based on Admirand and Small (4); H&D; H, based on Hegardt and Dam (24) and Holzbach et al. (25).

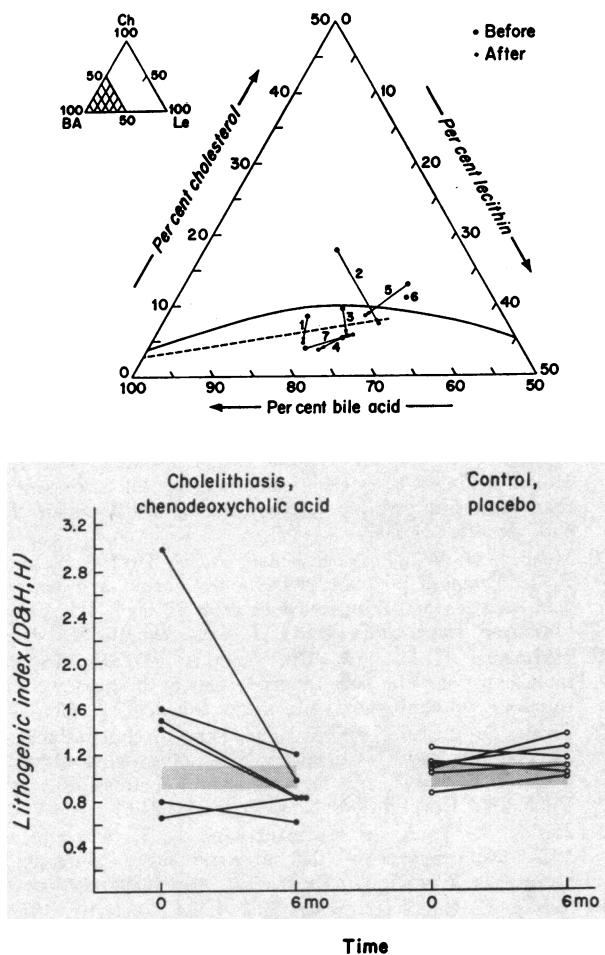


FIGURE 7 Effect of chenodeoxycholic acid treatment on biliary lipid composition. *Above*, molar composition plotted on triangular coordinates. Solid curve indicates limit of the micellar zone defined by Admirand and Small (4); broken line shows limit defined by Hegardt and Dam (24) and Holzbach et al. (25). *Below*, saturation index, using the values for cholesterol solubility proposed by Hegardt and Dam (24) and Holzbach et al. (25).

Although this rate of bacterial 7α -dehydroxylation is about three times that occurring in health, it is still less than half that observed in patients with ileal resection, in whom bile acid synthesis rates may increase as high as 4 g/day and most of which may be 7α -dehydroxylated in the colon (15, 34).

Bile acid conjugation. The increase in the proportion of bile acids conjugated with glycine is most readily explained by the hypothesis that the size of the readily exchangeable taurine pool determines the amount of bile acids conjugated with taurine (39). When the requirement for bile acid conjugation increases, glycine conjugation increases because of an

abundant source of glycine precursors whereas taurine conjugation remains constant.

Relationship of size and bile acid composition of bile acid pool to bile lithogenicity. The extensive studies by Vlahcevic, Bell, Buhac, Farrar, and Swell (40) have shown unequivocally that a majority of Caucasian men with cholesterol gallstones have decreased bile acid pools. The present study, as well as our previous study (2), indicates that chenodeoxycholic acid expands the bile acid pool, renders bile unsaturated in cholesterol, and induces gallstone dissolution.

It seems unlikely to us that expansion of the total bile acid pool per se will markedly decrease the lithogenicity of bile in patients with cholesterol cholelithiasis. First, in patient 3, the total bile acid pool actually diminished; yet when chenodeoxycholic acid became the predominant bile acid, the bile became unsaturated and the stones dissolved. Second, based on observations in one patient (unpublished) and observations in rats (41), cholic acid administration expands the bile acid pool. Yet when cholic acid is administered to patients with cholesterol gallstones, bile does not become unsaturated (8), and cholesterol gallstones apparently do not dissolve (38). Together, these observations suggest that chenodeoxycholic acid administration has a specific effect on bile lithogenicity. Recent studies (42) of biliary lipid secretion in man using a perfusion technique suggest that chenodeoxycholic acid therapy decreases cholesterol secretion in bile relative to that of lecithin and bile acid.

Constancy of bile acid kinetics. We elected to study a group of healthy controls before and after 6 mo of no medication, because the study with chenodeoxycholic acid was not randomized. The finding that bile composition, pool size, and turnover rate were the same at a second observation 6 mo later in the healthy control group extends our previous observation that such indices of bile acid metabolism remained constant during 4 mo in four subjects with cholesterol gallstones receiving a placebo (8).

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