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The formation of ursodeoxycholic acid, the 7 beta-hydroxy epimer of chenodeoxycholic acid, was investigated in three subjects with cerebrotendinous xanthomatosis and in four subjects with gallstones. Total biliary bile acid composition was analyzed by gas-liquid chromatography before and after 4 months of treatment with 0.75 g/day of chenodeoxycholic acid. Individual bile acids were identified by mass spectrometry. Before treatment, bile from cerebrotendinous xanthomatosis (CTX) subjects contained cholic acid, 85%; chenodeoxycholic acid, 7%; deoxycholic acid, 3%; allocholic acid, 3%; and unidentified steroids, 2%; while bile from gallstone subjects contained cholic acid, 45%; chenodeoxycholic acid, 43%; deoxycholic acid, 11%, and lithocholic acid, 1%. In all subjects, 4 months of chenodeoxycholic acid therapy increased the proportion of this bile acid to approximately 80% and decreased cholic acid to 3% of the total biliary bile acids, the remaining 17% of bile acids were identified as ursodeoxycholic acid. After the intravenous injection of [3H]chenodeoxycholic acid, the specific activity of biliary ursodeoxycholic acid exceeded the specific activity of chenodeoxycholic acid, and the resulting specific activity decay curves suggested precursor-product relationships. When [3H]7-ketolithocholic acid was administrated to another patient treated with chenodeoxycholic acid, radioactivity was detected in both the ursodeoxycholic acid and chenodeoxycholic acid fractions. These results indicate that substantial amounts of ursodeoxycholic acid are formed in patients treated with chenodeoxycholic acid. The ursodeoxycholic acid was synthesized […]

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Increased Formation of Ursodeoxycholic Acid in Patients Treated with Chenodeoxycholic Acid

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ABSTRACT The formation of ursodeoxycholic acid, the 7β-hydroxy epimer of chenodeoxycholic acid, was investigated in three subjects with cerebrotendinous xanthomatosis and in four subjects with gallstones. Total biliary bile acid composition was analyzed by gas-liquid chromatography before and after 4 months of treatment with 0.75 g/day of chenodeoxycholic acid. Individual bile acids were identified by mass spectrometry. Before treatment, bile from cerebrotendinous xanthomatosis (CTX) subjects contained cholic acid, 85%; chenodeoxycholic acid, 7%; deoxycholic acid, 3%; and unidentified steroids, 2%; while bile from gallstone subjects contained cholic acid, 45%; chenodeoxycholic acid, 43%; deoxycholic acid, 11%, and lithocholic acid, 1%. In all subjects, 4 months of chenodeoxycholic acid therapy increased the proportion of this bile acid to approximately 80% and decreased cholic acid to 3% of the total biliary bile acids, the remaining 17% of bile acids were identified as ursodeoxycholic acid. After the intravenous injection of [3H]chenodeoxycholic acid, the specific activity of biliary ursodeoxycholic acid exceeded the specific activity of chenodeoxycholic acid, and the resulting specific activity decay curves suggested precursor-product relationships. When [3H]-7-ketolithocholic acid was administrated to another patient treated with chenodeoxycholic acid, radioactivity was detected in both the ursodeoxycholic acid and chenodeoxycholic acid fractions. These results indicate that substantial amounts of ursodeoxycholic acid are formed in patients treated with chenodeoxycholic acid. The ursodeoxycholic acid was synthesized from chenodeoxycholic acid presumably via 7-ketolithocholic acid.

INTRODUCTION

Although cholic acid,1 chenodeoxycholic acid, and deoxycholic acid are the predominant bile acids in human bile (1), small amounts of other bile acids including ursodeoxycholic acid are frequently detected (2). This bile acid is the 7β-epimer of chenodeoxycholic acid (Fig. 1) and under normal conditions represents less than 1% of the total biliary bile acids. Little is known about the metabolism and biosynthesis of this 7β-hydroxy bile acid, although several reports have indicated that chenodeoxycholic acid is a precursor (3–5).

Recently, large quantities of the primary bile acid, chenodeoxycholic acid, have been administered to patients with gallstones (6, 7) and the rare inherited lipidosis, cerebrotendinous xanthomatosis (CTX)2 (8). In the patients with gallstones progressive diminution in size and eventual dissolution of the stones have been

1 The following systematic names are given to sterols and bile acids referred to by trivial names: cholesterol, cholest-5-en 3β-ol; 7α-hydroxy-cholesterol, cholest-5-ene-3β, 7α-diol; cholic acid 3α, 7α, 12α-trihydroxy 5β-cholanoic acid; chenodeoxycholic acid, 3α, 7α-dihydroxy-5β-cholanoic acid; deoxycholic acid, 3α, 12α-dihydroxy-5β-cholanoic acid; lithocholic acid, 3α-hydroxy-5β-cholanoic acid; 7-ketolithocholic acid, 3α-hydroxy-7-keto-5β-cholanoic acid; allocholic acid, 3α, 7α, 12α-trihydroxy-5α-cholanoic acid.

2 Abbreviations used in this paper: CTX, cerebrotendinous xanthomatosis; GLC, gas-liquid chromatography; RRT, relative retention time; SGOT, serum transaminase; TLC, thin-layer chromatography.
described, while in the CTX subjects, marked reduction in cholesterol and cholestanol synthesis rates were noted. During the course of these studies, striking alterations in biliary bile acid composition were observed which included the virtual disappearance of cholic acid from the bile (6). This report describes the changes in biliary bile acid composition in seven individuals treated with chenodeoxycholic acid for 4 months. In addition to the effect on cholic acid metabolism, increased amounts of a new bile acid were detected. This bile acid was identified as ursodeoxycholic acid, and was derived from chenodeoxycholic acid probably via a keto-bile acid intermediate.

METHODS

Clinical. Studies were conducted in seven patients hospitalized at the Manhattan Veterans Administration Hospital. Three subjects (E. D. E., E. D. S., and J. C.) suffered from the rare inherited lipidosis, cerebrotendinous xanthomatosis. Complete clinical and biochemical descriptions of these patients have appeared elsewhere (9-11). Four subjects (T. S., R. P., L. R., and K. S.) had radiolucent gallstones demonstrated by oral cholecystography. No patient was acutely ill during the course of these studies, and clinical laboratory tests including a complete blood count, urinalysis, fasting blood sugar, blood urea nitrogen, serum bilirubin, serum transaminase (SGOT), serum alkaline phosphatase, and plasma prothrombin time were normal at the beginning of the study and did not change during chenodeoxycholic acid treatment. Liver biopsies were performed after 4 months of bile acid treatment and were interpreted as normal. The patients ate regular diets that were devoid of foods containing large amounts of cholesterol. Caloric intakes were adjusted to maintain constant weight during these studies.

Bile acid analysis. The percent composition of individual bile acids in specimens of duodenal bile was determined according to the method described for fecal bile acids by Grundy, Ahrens, and Miettinen (12). Samples of bile were aspirated from the duodenum via a Rehfs tube (David, Inc., Providence, R. I.) that was positioned under fluoroscopic guidance. Cholecystokinin (obtained from the late Professor Erik Jorpes, Karolinska Institute, Stockholm, Sweden) was injected intravenously to facilitate gallbladder bile flow.

2 ml (200) of bile were refluxed for 1 h with 20 ml of N ethanolic NaOH. After the neutral sterols were extracted with hexane, the ethanol was evaporated from the mixture and 15 ml of 2 N NaOH were added. Further saponification for 3 h at 15 psi was carried out to deconjugate the bile acids. After acidification to pH 2 with concentrated HCl and the addition of 20 ml of methanol, the free bile acids were extracted with 3-40-ml portions of chloroform. The solvent was evaporated and the bile acid methyl esters were formed by the addition of 5% HCl in dry methanol (w/w). The methyl esters of the trihydroxy-, dihydroxy-, and monohydroxycholanoic acids were isolated as a group by TLC on 20 x 20 cm glass plates coated with 0.5 mm thick layers of Silica Gel H prerin in methanol and activated at 110°C for 1 h before use. The plates were first developed with benzene to separate the fatty acids and then with trimethylpentane: isopropanol: acetic acid, 75: 25: 1; solvent migration was stopped beneath the fatty acid band. The total bile acid mixture was eluted with acetone in a vacuum aspirator (13) after spraying lightly with BileSpra (Supelco, Inc., Bellafonte, Pa.) and visualizing the bile acid area under long wave ultraviolet illumination. A measured amount (280 μg) of 5α-cholestanol was added as an internal standard, and the solvent was evaporated. The bile acid methyl esters were converted to their respective TMS-ether derivatives by the addition of 100 μl of Sil Prep (Applied Science Labs, Inc., State College, Pa.). Samples containing 2 to 10 μg of total bile acids were analyzed by GLC.

Quantitative analysis of the bile acid methyl ester TMS-ether derivatives was performed on a Packard Model 7300 gas-chromatograph. (Packard Instruments, Downers Grove, Ill.) fitted with a flame ionization detector. Peak areas were determined with an electronic integrator, Model CRS-104 (Infotronics, Inc., Austin, Texas). The TMS-ethers of the bile acid methyl esters were separated on 6-foot glass U columns packed with 1% Hi Eff 8BP on Gas Chrom Q (100/120 mesh, Applied Science Labs, Inc.) maintained at 230°C with a nitrogen carrier gas flow of 40 cm3/min. Under these conditions, the columns offered about 3000 theoretical plates for the TMS-ether of methyl chenodeoxycholate. The respective RRT values (compared with 5α-cholestanol) for the TMS ether derivatives of the following methylated bile acids were: allocholic acid, 1.60; cholic acid, 2.00; deoxycholic acid, 2.95; chenodeoxycholic acid, 3.15; lithocholic acid, 4.15; ursodeoxycholic acid, 4.45; and 7-ketolithocholic acid, 6.65. The individual bile acids were quantitated by relating their peak area to the peak area produced by a known amount of 5α-cholestanol and were expressed as the percent of the total mixture.

For radioactivity assay of pure methyl ursodeoxycholate and pure methyl chenodeoxycholate the aforementioned procedure was modified slightly. After the bile acid methyl esters were formed, an aliquot was applied to 20 x 20 cm glass plates coated with Silica Gel H. The plate was developed with chloroform: acetone: methanol, 70:20:5. Ref-
Table I
Effect of CDCA on Biliary Bile Acid Compositions

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* Gallstones.  
‡ 0.75 g of CDCA administered each day.

The following doses of chenodeoxycholic acid were administered to the patients: J. C., 22 μCi; E. D. E., 7 μCi; and E. D. S., 7 μCi. The radioactive bile acids dissolved in 1 ml of ethanol was added to 120 ml of 0.9% NaCl solution. The saline dispersion was immediately infused intravenously.

The [H]7-ketolithocholic acid was prepared from [H]-chenodeoxycholic acid by the method of Samuelsson (15). The bile acid was purified by column partition chromatography on celite. Methyl [H]-ketolithocholic was eluted with 40% benzene in hexane. After hydrolysis and recrystallization from acetone: water, 50: 50, the final specific activity of the free bile acid was 6 μCi/mmole. Less than 0.1% of the radioactivity migrated with chenoxycholic acid when examined by TLC. About 5 mg were packed into a gelatin capsule and fed as a single dose to patient T. S.

Experimental Design. (a) Total bile acid composition was determined by GLC on specimens of duodenal bile obtained from seven subjects before and after 4 months of treatment with chenodeoxycholic acid.* 0.75 g/day. Further identification of the bile acid composition was made by GLC-mass spectrometry.

(b) In patients E. D. E., E. D. S., and J. C., the transformation of [H]-chenodeoxycholic acid into ursodeoxycholic acid was examined. After the intravenous injection of [H]-chenodeoxycholic acid, specific activity decay curves for both chenodeoxycholic acid and ursodeoxycholic acid were constructed and precursor-product relationship was sought.

(c) To assess the importance of 7-ketolithocholic acid as a possible intermediate in the formation of ursodeoxycholic acid, a radioactive dose of this keto bile acid was administered to patient T. S. during treatment with chenodeoxycholic acid. The specific activity decay curves of ursodeoxy-

The chenodeoxycholic acid was purchased from Weddel Pharmaceuticals, London, England, Batch 2064. The material was 98% chenodeoxycholic acid and contained 1% lithocholic acid and 1% 3α,7α-dihydroxy-12-keto-5β-cholanolic acid as determined by GLC; no ursodeoxycholic acid was detected. Under the conditions of operation; 10 ng was the minimum amount of material measurable at the detector. Since a 10 μg sample was analyzed and no ursodeoxycholic acid was found, less than 0.1% ursodeoxycholic acid was present in our sample. Therefore, the maximum daily intake of exogenous ursodeoxycholic acid was less than 0.75 mg/day.
cholic acid and chenodeoxycholic acid were compared over the ensuing week.

RESULTS

Biliary bile acid composition. In Table I are listed the percentage composition of bile acids present in the bile of three CTX subjects and four subjects with gallstones. Before therapy, the bile from the CTX individu-
als contained exceedingly small amounts of the dihydroxy bile acids, deoxycholic acid and chenodeoxycholic acid, and correspondingly larger proportions of cholic acid. Small quantities of allocholic acid (the 5α-isomer of cholic acid) and several unidentified polar steroids also were present. In contrast, the bile from the four gallstone subjects contained almost equal proportions of the primary bile acids, cholic acid and chenodeoxy-

![Figure 2](A and B) Gas-chromatograms of TMS-ether derivatives of biliary bile acid methyl esters before and after treatment with chenodeoxycholic acid. Marked alterations in bile acid composition were observed after treatment including a substantial increase in the quantity of ursodeoxycholic acid in the bile.

The Formation of Ursodeoxycholic Acid in Man 615
cholic acid (36% and 37%, respectively), and substantial quantities of the secondary bile acid deoxycholic acid. Trace amounts of the secondary bile acid lithocholic acid also were found. After 4 months of treatment, the proportion of chenodeoxycholic acid increased to over 80% in six of seven subjects, while cholic acid declined to less than 10% of the total bile acids. In the gallstone subjects, the secondary bile acid, deoxycholic acid, disappeared from the bile, while in the CTX subjects both alchoholic acids and the polar steroids were eliminated. However, all subjects showed increased amounts (3-37% of the total bile acids) of a new bile acid that was conclusively identified (see below) as ursodeoxycholic acid. In subject T. S., a small amount (2%) of ursodeoxycholic acid was detected before chenodeoxycholic acid therapy, but the proportion increased to 37% of the total biliary bile acids after treatment (Figs. 2 A and B).

It is noteworthy that in five of six subjects lithocholic acid the 7α-dehydroxylated bacterial metabolite of chenodeoxycholic acid did not increase in the bile of the subjects despite the large intake of chenodeoxycholic acid. Perhaps lithocholic acid was not formed or was not absorbed from the colon because of the diarrhea associated with feeding chenodeoxycholic acid (6). Another explanation for the absence of lithocholic acid from the bile is the possibility of sulfate formation. Lithocholic acid sulfate is quite polar and unfortunately, if present, would not be detected by our analytical procedure.

Identification of ursodeoxycholic acid. As illustrated in Fig. 2 B, the TMS-ether derivative of methyl ursodeoxycholate and methyl chenodeoxycholate were easily distinguished by GLC. Furthermore, the two bile acid methyl esters could be separated by TLC (see Methods). When samples of known methyl ursodeoxycholate were cochromatographed with the biosynthetic product (peak V, Fig. 2 B), identical Rf and RRT values were observed.

Positive identification of peak V, Fig. 2 B was established by GLC-mass spectrometry. Fig. 3 illustrates the mass spectra for the TMS-ether derivatives of known methyl chenodeoxycholate, known methyl ursodeoxycholate, and peak V. Although methyl chenodeoxycholate and methyl ursodeoxycholate share the same chemical formula, striking differences were observed in their fragmentation patterns. For the TMS ether of methyl chenodeoxycholate, no molecular ion (M) was detected at m/e 550, and the largest fragment was at m/e 370 which corresponds to M − 2 × (OSi(CH₃)₃H) or 550 − 2×90. In contrast the mass spectrum of the TMS ether derivative of known methyl ursodeoxycholate shows a molecular ion (M) at m/e 550 and lines at m/e, 535.

![Mass spectra of the TMS-ether derivatives of known methyl ursodeoxycholate, known methyl chenodeoxycholate, and methyl ester of peak V (Fig. 2 B).](image)
FIGURE 4 Daily change in biliary bile acid composition in J. C. Immediately after initiation of bile acid therapy, the proportion of chenodeoxycholic acid (CDCA) increased and cholic acid (CA) decreased. However, ursodeoxycholic acid (UDCA) was not detected in the bile until the fifth day.

The effect of chenodeoxycholic acid on the sequential changes in biliary bile acid composition. Fig. 4 illustrates the daily changes in bile acid composition that were produced by the administration of chenodeoxycholic acid to patient J. C. (CTX). After 1 day of therapy, chenodeoxycholic acid increased to 40% of the biliary bile acids, then rose to over 80% of the total bile acids by the third day. In contrast, the proportion of cholic acid declined to less than 10% of the total bile acids by day 5. Ursodeoxycholic acid did not appear in the bile until the fifth day and then increased to 7% of the total bile acids by day 7. Since only very small amounts (<0.1%) of ursodeoxycholic acid could be present in the administered chenodeoxycholic acid, these

FIGURE 5 Specific activity-time curves of methyl chenodeoxycholic and methyl ursodeoxycholate after the intravenous administration of a tracer dose of \[^3\text{H}\]chenodeoxycholic acid. In each patient, the specific activity or ursodeoxycholic acid exceeded the specific activity of chenodeoxycholic acid.

The Formation of Ursodeoxycholic Acid in Man
findings confirm the endogenous origin of this 7β-hydroxy bile acid. Also if chenodeoxycholic acid had been contaminated with a sizable amount of ursodeoxycholic acid, the 7β-hydroxy bile acid should have been detected in the bile before the fifth day.

**Transformation of [H]chenodeoxycholic acid into [H]ursodeoxycholic acid.** After the intravenous administration of [H]chenodeoxycholic acid to three subjects with CTX who were receiving chenodeoxycholic acid, the specific activities of ursodeoxycholic acid and chenodeoxycholic acid were measured for the next 3–4 days. The results are presented in Fig. 5. As expected, the specific activity of chenodeoxycholic acid decayed linearly in all three subjects. In contrast, the specific activity of ursodeoxycholic acid in E. D. S. on day 1 was $3$ lower than that of chenodeoxycholic acid, and then rose to exceed the specific activity of chenodeoxycholic acid. In J. C., the specific activity of ursodeoxycholic acid was greater than chenodeoxycholic acid by the second day and then decayed in a parallel manner. In E. D. E., the specific activity of ursodeoxycholic acid rose and became greater than chenodeoxycholic acid on the third day. The demonstration of $^4$H radioactivity in the ursodeoxycholic acid fractions of these subjects plus the appearance of the specific activity-time curves suggest that ursodeoxycholic acid was derived from chenodeoxycholic acid.

**The transformation of $^4$H7-ketolithocholic acid into chenodeoxycholic acid and ursodeoxycholic acid.** To further delineate the suspected ursodeoxycholic acid biosynthetic pathway (Fig. 6), a dose of $^4$H7-ketolithocholic acid was given to patient T. S. during her treatment with chenodeoxycholic acid. The specific activities of chenodeoxycholic acid and ursodeoxycholic acid were measured over the next week. The results are presented in Fig. 7. I day after oral pulse-labeling, tritium radioactivity was detected in both the dihydroxy bile acids and indicated that 7-ketolithocholic acid was converted into chenodeoxycholic acid and ursodeoxycholic acid. Since the specific activities of both bile acids were almost identical on day 1, the 7-keto bile acid probably was reduced equally into its respective 7α- and 7β-hydroxy
derivatives. This interpretation seems valid because the proportion of ursodeoxycholic acid in the bile at this time was almost as large as chenodeoxycholic (37% vs. 49%) and suggests that the pool of ursodeoxycholic acid was about the same size as the pool of chenodeoxycholic acid. Although this experiment does not conclusively prove that 7-ketolithocholic acid is an obligatory precursor of ursodeoxycholic acid, our findings support such a possibility.

**DISCUSSION**

The results of this investigation demonstrate the striking changes in bile acid metabolism produced by the administration of chenodeoxycholic acid to gallstone and CTX subjects. After 4 months of therapy, cholic acid was virtually eliminated from the bile and was replaced by chenodeoxycholic acid. In the gallstone subjects, deoxycholic acid was no longer detected. This change probably reflects the absence of cholic acid from the enterohepatic circulation and, as a consequence, a decreased availability of this bile acid as substrate for bacterial 7α-dehydroxylation. In the CTX subjects, the small amounts of allocholic acid and polar steroids were also reduced below the limits of detectability. These differences apparently result from the feedback inhibition of chenodeoxycholic acid on cholic acid production. The major rate-determining reactions governing bile acid synthesis are summarized in Fig. 8. Both cholic acid and chenodeoxycholic acid are derived from a common intermediate, 7α-hydroxycholesterol-4-en-3-one, which is formed from 7α-hydroxycholesterol (16). The keto steroid is then 12α-hydroxylated to form 7α,12α-dihydroxycholesterol-4-en-3-one the first totally committed precursor of cholic acid biosynthesis. Therefore, cholic acid synthesis would be suppressed by either the inhibition of the 12α-hydroxylation reaction (step II) or the inhibition of the 7α-hydroxylation of cholesterol (step I). In the former case, only cholic acid production would be affected, while in the latter instance both cholic acid and chenodeoxycholic acid formation would be diminished.

The second major alteration that was noted in bile acid composition during chenodeoxycholic acid therapy was the appearance of increased amounts of ursodeoxycholic acid in the bile. This bile acid is the 7β-hydroxy epimer of chenodeoxycholic acid and accounted for 3-37% of the circulating bile acid pool. Since virtually no ursodeoxycholic acid was present in the administered chenodeoxycholic acid, we conclude that this bile acid was formed endogenously. This fact was confirmed in the CTX subjects by the demonstration of radioactivity in ursodeoxycholic acid after the intravenous injection of a tracer of [3H]chenodeoxycholic acid. Furthermore, the specific activity of ursodeoxycholic acid rose to exceed the specific activity of chenodeoxycholic acid suggesting...
a precursor product relationship. These results are compatible with the previous studies of Samuelsson in rats (4) and of Hellström and Sjövall in man (3) that radioactive chenodeoxycholic acid was converted into ursodeoxycholic acid. Although the precise biochemical pathway has not been elucidated, there is evidence that 7-ketocholesteric acid is an intermediate. This premise was supported by the observation of Mahowald et al. (5) and Samuelsson (15) who showed that radioactivity 7-ketocholesteric acid was converted into both ursodeoxycholic and 3α, 6β, 7β-trihydroxy-5β-cholanoic acid (β-muricholic acid) in rats. Since the enterohepatic circulation in these animals was interrupted, the reduction of the 7-keto group was presumably a hepatic process rather than carried out by bacteria in the intestine. In the present study, 7-ketolithocholic acid was not detected in bile and feces of the chenodeoxycholic acid-treated patients. However, Haslewood, Murphy, and Richardson have isolated a strain of E. coli from the intestinal contents of a patient with bacterial overgrowth that has specific 7α-hydroxy dehydrogenase activity (18). This finding suggests that bacteria are capable of producing 7-ketolithocholic acid from chenocholesteric acid. Therefore, although a tracer dose of radioactive 7-ketolithocholic acid was converted into ursodeoxycholic acid, neither its formation nor its role as a precursor of ursodeoxycholic acid have been definitely established. An alternative explanation for the formation of ursodeoxycholic acid is that the 7α-hydroxyl group of chenocholesteric acid could be directly epimerized in the liver. This mechanism might control the hepatic content of chenocholesteric acid and may be analogous to the formation of α and β-muricholic acids from chenocholesteric acid in the rat (4, 17, 19). Furthermore, since 7-ketolithocholic acid was also reduced to chenocholesteric acid, it is possible that ursodeoxycholic acid was derived indirectly from 7-ketolithocholic acid via chenocholesteric acid. Also we have not ruled out the possibility that ursodeoxycholic acid was produced by bacteria in the intestine.

A major advance of this study was the conclusive identification of ursodeoxycholic acid by the combination of TLC, GLC, and mass spectrometry. These steps greatly minimized the possibility that the radioactivity found in the ursodeoxycholic acid fraction represented contamination with chenocholesteric acid.

A number of important questions are suggested by our results. For example, what is the clinical significance of ursodeoxycholic acid in man? Is it possible that this bile acid is responsible for the dissolution of cholesterol gallstone (6, 7) and the control of sterol synthesis in CTX (8)? Alternatively, the formation of ursodeoxycholic acid may represent a detoxification mechanism to control the hepatic concentration of chenocholesteric acid. Finally, can ursodeoxycholic acid produce hepatic toxicity and can significant amounts of this bile acid be detected in patients with intestinal or liver disease where alterations in bile acid composition have been described (20)? It is of interest that Gleich and Hofmann described a patient with chronic diarrhea in whom 13% of the biliary bile acids were tentatively identified as ursodeoxycholic acid (21). Although there is scant documentation of the presence of ursodeoxycholic acid in human bile, the increasing use of chenocholesteric acid and demonstration of its convertability into ursodeoxycholic acid mandates a fuller investigation of the metabolism of these bile acids in man.

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REFERENCES


