A Biochemical Abnormality in Cerebrotendinous Xanthomatosis

IMPAIRMENT OF BILE ACID BIOSYNTHESIS ASSOCIATED WITH INCOMPLETE DEGRADATION OF THE CHOLESTEROL SIDE CHAIN

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ABSTRACT Bile acid production in cerebrotendinous xanthomatosis (CTX) is subnormal, yet the activity of cholesterol 7α-hydroxylase, the rate-determining enzyme of bile acid synthesis, is elevated. To explain this discrepancy, bile acid precursors were sought in bile and feces of three CTX subjects. Over 10% of the total sterols excreted in bile and feces consisted of compounds more polar than cholesterol. Chromatographic analysis of the polar fractions in conjunction with gas-liquid chromatography (GLC)-mass spectrometry indicated two major constituents, 5β-cholestane-3α,7α,12α,25-tetrol and 5β-cholestane-3α,7α,12α,24ξ,25-pentol. After i.v. injection of [4-^{14}C]cholesterol both bile alcohols were radioactive proving that they were derived from cholesterol. The accumulation of alcohols hydroxylated at C-25 and C-24,25 suggests that decreased bile acid synthesis in CTX results from impaired oxidation of the cholesterol side chain. This finding and the virtual absence of intermediates hydroxylated at C-26 indicate that current views of the major pathway of bile acid synthesis may require revision.

INTRODUCTION

Cerebrotendinous xanthomatosis (CTX) [1] is a rare inherited lipid storage disease characterized by excessive accumulation of cholesterol and cholestanol in body tissues (1). We recently demonstrated that the increased deposition of sterols in the tissues is associated with increased rates of sterol synthesis (2, 3): cholesterol production rates calculated from specific activity decay curves and cholesterol turnover determined by balance techniques were substantially greater in two patients with CTX than in five controls (3). The agreement between isotope kinetic measurements and sterol balance in the control patients was excellent: the values differed by only 8%. In the subjects with CTX, cholesterol turnover calculated by isotope kinetics was 22% greater than that determined by the balance technique (3). It was further noted that in the CTX subjects bile acid formation was nearly 50% lower than in the controls (3) and that the concentration of chenodeoxycholic acid in bile was very low (1).

The present paper describes a metabolic defect in patients with CTX which explains, at least in part, some of the abnormalities of sterol and bile acid metabolism observed in this disease. The low values of cholesterol turnover, as determined by the sterol balance method, are apparently ascribable to an incomplete oxidation of the cholesterol side chain, since appreciable quantities of bile alcohols were detected in bile and feces of the CTX subjects. Two bile alcohols predominated in the polar sterol fractions, namely 5β-cholestane-3α,7α,12α,25-tetrol and 5β-cholestane-3α,7α,12α,24ξ,25-pentol; bile alcohols with a hydroxyl group in the 26-position were not detected. This suggests that some current concepts

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[1] *Abbreviations used in this paper:* CTX, cerebrotendinous xanthomatosis; RRT, relative retention time; TMS, trimethylsilyl.

TABLE I

Cholesterol and Bile Acid Turnover in Human Subjects Determined by Isotope Kinetic and Chromatographic Balance Methods

Patient	Production rates, *mg/day* A* Isotope kinetic method	Sterol balance method Neutral sterols	Bile acids	Total steroids	B§ Bile alcohols	Total (steroids + bile alcohols)
E. D. E.	1,085	788	136	924	153	1,077
J. C.	987	588	93	681	80	761
	1,036‡	688	115	803	117	919
Controls (5)	736‡±261	459±131	215±68	674±187	—‖	674±187

* Salen and Grundy (3).

‡ Average values (± indicates 1 SD).

§ Chromatographic balance plus bile alcohols.

‖ Not detectable by method employed in present study.

regarding the major pathway of cholic acid synthesis in man may require revision.

METHODS

Clinical

Studies were conducted in three subjects with CTX. Complete clinical descriptions and metabolic data have been published (1–3). The patients were maintained on formula diets containing 15% protein, 40% fat as cottonseed oil, and 45% glucose (4).

Steroid analyses

Complete stool collections were obtained from each patient and were combined into 4-day pools. The stools were mixed with water, homogenized, and stored at −20°C; aliquots were lyophilized, and the dried powder was used for steroid analyses. A single pool from each patient was analyzed for the purposes of the present study. Bile was collected by duodenal intubation after stimulation of the gallbladder with cholecystokinin (obtained from the late Professor Erik Jorpes, Karolinska Institutet, Stockholm, Sweden).

TABLE II

Fecal Excretion of Bile Alcohols in Patients with CTX

	Patient E. D. E.	J. C.	E. D. S.*
	mg/day	*mg/day*	*mg/day*
Bile alcohol			
5β-Cholestane-3α, 7α, 12α, 25-tetrol	42	60	186
5β-Cholestane-3α, 7α, 12α, 24ξ, 25-pentol	75	20	47
Unidentified‡	36	—	—
Total	153	80	233

* Treated with cholestyramine (10 g/day) for 24 days.

‡ Tentatively identified as 5β-cholestane-3α, 7α, 12α, 23ξ-tetrol.

Extraction of bile alcohols from bile and feces. A weighed aliquot of dried bile or feces was extracted with a mixture of 300 ml of ethanol and 2 ml of concentrated NH_4OH in a Sohxlet apparatus. An extraction period of 6 h was found to remove 98% of the extractable (labeled) material. The ethanol extract was evaporated on a 60°C water bath under nitrogen, leaving a residue which was suspended in 200 ml of water containing 1 ml concentrated NH_4OH. The neutral lipids (e.g., cholesterol, cholestanol, and sterol precursors (1) and the bile alcohols) were extracted from the aqueous suspension with three 100-ml portions of ethyl acetate. The ethyl acetate extracts were combined and washed with water to neutrality, and the ethyl acetate was then removed in a rotary evaporator.

Purification by reversed phase column-partition chromatography. To separate the bile alcohols from the biliary sterols, reversed phase column-partition chromatography was employed, using n-heptane-benzene, 5:45, vol/vol, as the stationary phase and water-methanol, 50:150, vol/vol, as the mobile phase. Glass columns (25 cm long; 1 cm ID) were packed with 5 g of hydrophobic Celite (Gaschrom Z, 100/120 mesh, Applied Science Labs, Inc., State College, Pa.) containing 7 ml of stationary phase. The sample was applied to the column mixed with 1 ml of stationary phase and 1 g of Celite. The bile alcohols were eluted from the column with 100 ml of mobile phase, while the less polar monohydroxy sterols remained on the column. The eluate was evaporated on a rotary evaporator with addition of benzene, and the residue was analyzed by thin-layer chromatography (TLC).

Separation of bile alcohols by preparative TLC. The bile alcohol fraction was dissolved in a small volume of ethyl acetate and was applied as a band to a 20 × 20-cm glass plate coated with a 1-mm thick layer of silica gel G (Analtech, Inc., Newark, Del.). The solvent system chloroform: acetone: methanol, 35:25:5, vol/vol, was used for development, and the bile alcohols were detected either with iodine or 2′,7′-dichlorofluorescein. Pertinent bands were scraped from the plate, and the bile alcohols were eluted from the silica gel with methanol.

Gas-liquid chromatography (GLC) of bile alcohols. The bile alcohols were quantitated as the trimethylsilyl (TMS)

derivatives, using 5α-cholestane as an internal standard and 5β-cholestane-3α,7α,12α,25-tetrol as external standard. An aliquot of a given bile alcohol was treated with 100 μl of Sil Prep (Applied Science Labs, Inc.) for 30 min at room temperature to form the TMS ether. Aliquots containing 2–10 μg of bile alcohol were injected into the GLC column. Mass measurements and retention times relative to 5α-cholestane were determined with a Packard model 7300 Gas-Chromatograph (Packard Instruments Co., Inc., Downers Grove, Ill.) employing flame ionization detectors and an electronic integrator-timer (model CRS-104, Infotronics Corp., Austin, Tex.). The TMS ethers were analyzed on either 4 ft × $\frac{1}{8}$-inch glass columns packed with 3% QF-1 on 80/100 mesh Gas-chrom Q or 6 ft × $\frac{1}{8}$-inch ID glass columns packed with 1% HiEff 8BP on 100/120 mesh Gas-chrom Q. The following operating conditions were employed: column temperature, 240°C; flash heater and detector temperature, 250°C; carrier gas (N_2) 40 ml/min.

Mass was calculated by comparison of a given bile alcohol peak area with that produced by authentic 5β-cholestane-3α,7α,12α,25-tetrol and by 5α-cholestane. Relative retention times were calculated by dividing the retention time of the bile alcohol by that of 5α-cholestane.

Mass spectrometry. Mass spectra of the bile alcohols were obtained with a Varian MAT-111 gas-chromatograph-mass spectrometer (Varian Associates, Palo Alto, Calif.). To obtain the mass spectra of individual bile alcohols, the samples were injected into a helical glass column, 6 ft × $\frac{1}{16}$ inch ID, packed with either 3% QF-1 or 3% SE-30 on 100/120 mesh Gas-chrom Q. The following operating conditions were employed: QF-1: column temperature, 250°C; flash heater and inlet line, 260°C; molecular separator, 290°C. SE-30: column temperature, 280°C; flash heater and inlet line, 290°C; molecular separator, 290°C. Helium flow was 15 ml/min, ion source pressure approximately 3×10^{-6} torr. Ion source temperature, current, and electron energy were about, 300°C, 270 μA, and 80 eV, respectively.

RESULTS

Recovery of bile alcohols from feces of CTX subjects. As stated in the introduction, there was a significant difference when cholesterol turnover in CTX patients was calculated by the isotope kinetic method or measured by the sterol balance technique (3). The results are summarized in Table I part A and show a 22% difference between the two methods in the CTX subjects, as compared with an 8% difference in the five controls. The large discrepancy in the two patients with CTX is undoubtedly due to the fact that on the average, these subjects excreted about 117 mg/day of bile alcohols which were not detected by the standard chromatographic balance method (3, 5). Since the bile alcohols are intermediates on the pathway between cholesterol and bile acids, they would be included when cholesterol turnover is calculated by the isotope kinetic method.

When the results of the sterol balance studies were corrected by adding the daily excretion of bile alcohols to that of neutral steroids and bile acids, the discrepancy between the isotope kinetic data and the sterol balance data was reduced from 22 to about 11% (Table I, part B). The remaining 10% difference is close to the overall experimental error and may also be due in part to the fact that only short-term specific activity data (6–9 wk) were analyzed in these subjects. Consequently, the isotope kinetic values may be overestimated 9–14% (6, 7). On the other hand, it must be emphasized that the bile alcohol analyses have not been corrected for incomplete recovery and are therefore minimum values.

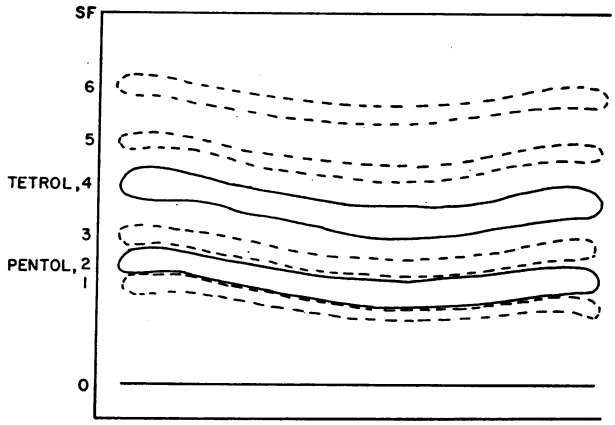

FIGURE 1 Preparative TLC of fecal bile alcohol fraction of patient with CTX. Layer thickness, 1 mm, silica gel G, solvent system chloroform: acetone: methanol, 35 : 25 : 5 vol/vol/vol, developed twice and sprayed with ammonium phosphomolybdate. 0, origin; band 2, 5β-cholestane-3α,7α,12α,24ξ,25-pentol; band 4, 5β-cholestane-3α,7α,12α,25-tetrol; SF, solvent front.

Table II illustrates the excretion (in milligrams per day) of the two major constituents of the fecal bile alcohol fraction, namely 5β-cholestane-3α,7α,12α,25-tetrol and 5β-cholestane-3α,7α,12α,24ξ,25-pentol. One of

TABLE III

R_f Values and Relative Retention Times of Bile Alcohols

Sterol	R_f*	RRT‡ 3% QF-1	RRT‡ 1% HiEff 8BP
Known 5β-cholestane-3α, 7α, 12α, 25-tetrol	0.27	3.01	1.19
Biosynthetic 5β-cholestane-3α, 7α, 12α, 25-tetrol	0.27	3.03	1.22
Biosynthetic 5β-cholestane-3α, 7α, 12α, 24ξ, 25-pentol	0.11	4.09	1.55

* 0.25-mm thick silica gel G plates, chloroform:acetone:methanol 35:25:5, vol/vol/vol.

‡ TMS ethers, relative to 5α-cholestane = 1. RRT of 5α-cholestane was 190 s on QF-1 column; 310 s on HiEff-8BP column. For operating conditions, see Methods.

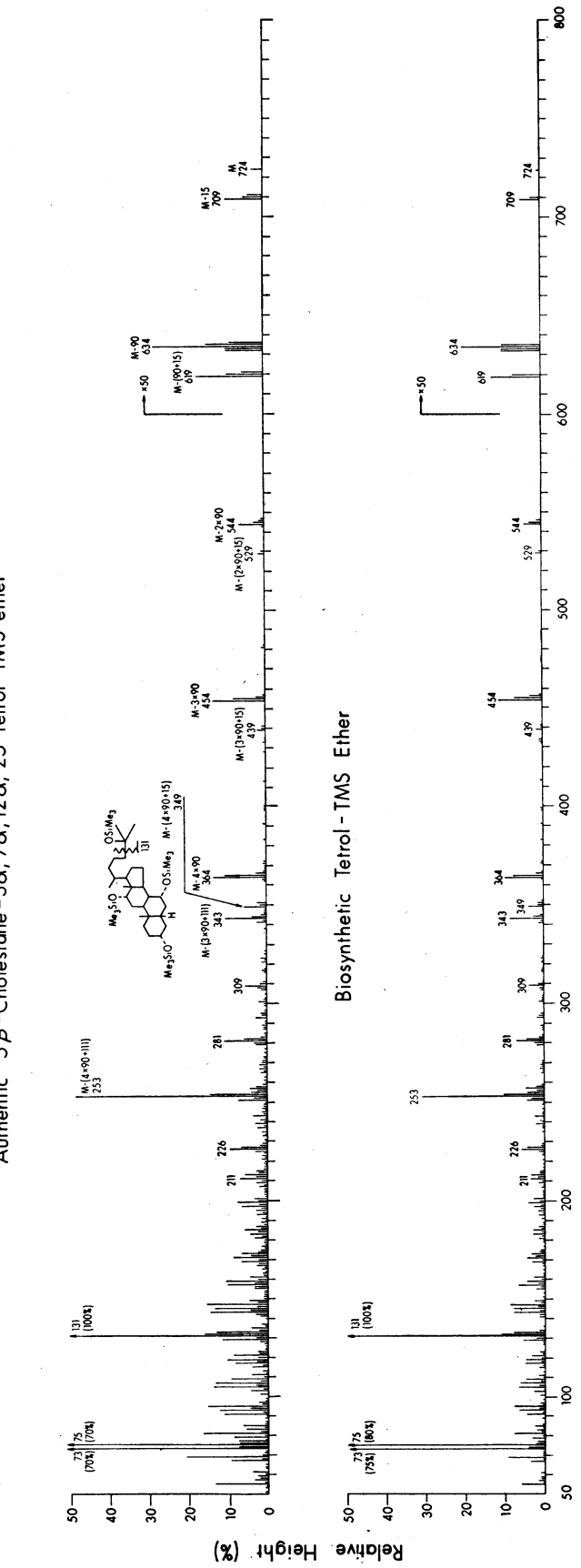

FIGURE 2 Mass spectrum of known 5β-cholestane-3α,7α,12α,25-tetrol (TMS ether) and of biosynthetic bile alcohol (TMS ether). The two spectra are identical in all important respects. For detailed discussion see Results section.

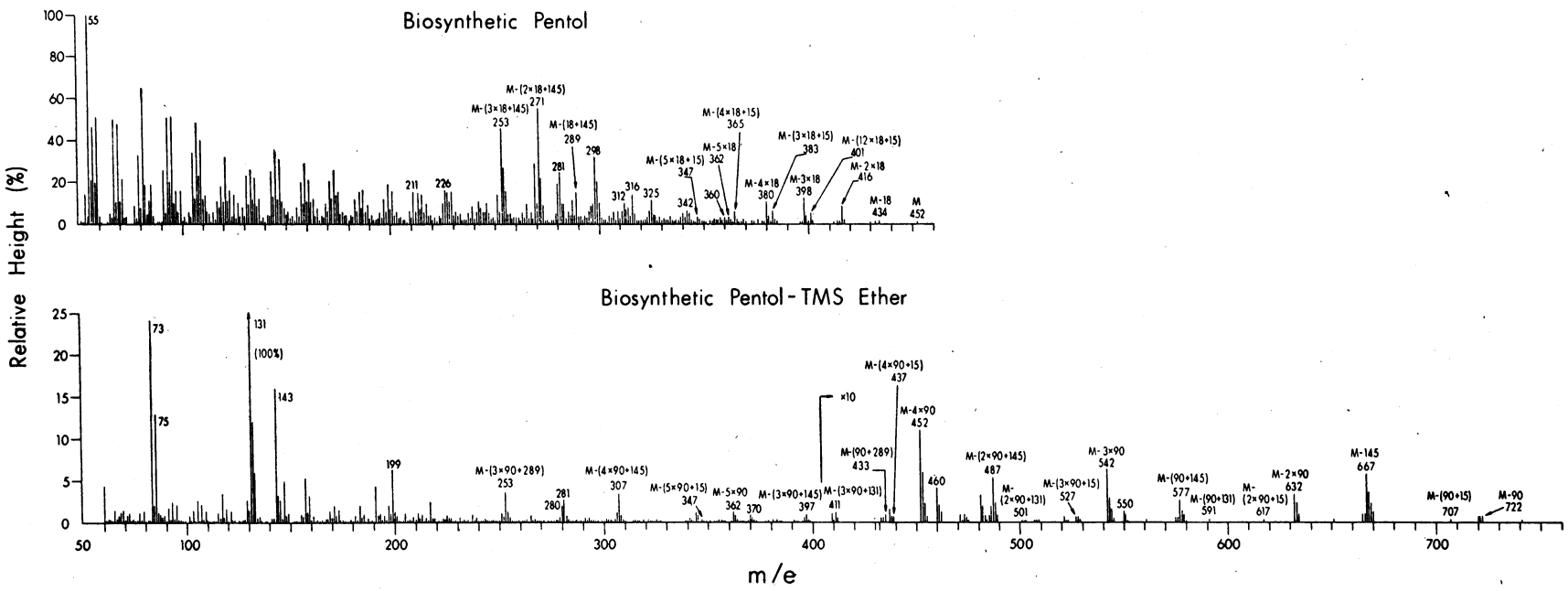

FIGURE 3 Mass spectra of biosynthetic bile alcohol and TMS derivative. The compound has been assigned the structure 5β-cholestane-3α,7α,12α,24ξ,25-pentol. For detailed description see Results section.

the patients (E. D. S.), who had been treated with the bile acid sequestrant cholestyramine for 24 days, had the greatest output of bile alcohols, 233 mg/day. In two of the three patients the cholestanetetrol was the predominant bile alcohol. One of the subjects (E. D. E.) excreted relatively more pentol and appreciable amounts of another bile alcohol, which was tentatively identified by mass-spectral data as 5β-cholestane-3α,7α,12α,23ξ-tetrol (8).

Identification of bile alcohols

5β-Cholestane-3α,7α,12α,25-tetrol. The bile alcohol fraction eluted from the partition column was separated into six distinct bands by preparative TLC, using multiple development (Fig. 1). The material in band 4 (Fig. 1) was removed from the plate and eluted with methanol. After evaporation of the methanol the residue was dissolved in ethyl acetate; crystals were obtained when a rather concentrated solution (10 mg/ml) was allowed to evaporate slowly during a 48-h period. After drying *in vacuo* at 60°C, the crystals were homogeneous when examined by analytical TLC and by GLC on two different columns (Table III).

Analysis of the TMS ether by GLC-mass spectrometry revealed the following fragmentation pattern (Fig. 2): the molecular ion (*M*) at *m/e* 724 and two series of peaks, one at *m/e* 634, 544, 454, 364, and a second at *m/e* 709, 619, 529, 439, and 349. The former series results from the consecutive loss of one, two, three, and four TMS groups plus a hydrogen atom. The base peak at *m/e* 131 arises from scission of the bond between carbons 24 and 25 (9). The mass spectrum indicates that we are dealing with a C_{27} bile alcohol, with hydroxyl groups at the 3α, 7α, 12α, and 25 positions. The mass spectrum of the biosynthetic bile alcohol was identical with that of known 5β-cholestane-3α,7α,12α,25-tetrol synthesized by the procedure of Pearlman (10) and with that published by Cronholm and Johansson (8). The synthetic reference compound and the biosynthetic material had identical IR spectra and identical R_f and relative retention time (RRT) values when examined by TLC and GLC (Table III). The biosynthetic cholestanetetrol melted at 189.5–191°C, the known reference compound (10) at 188–189°C; the mixed melting point was 187–189°C. These measurements conclusively establish the presence of 5β-cholestane-3α,7α,12α,25-tetrol in the feces of patients with CTX.

5β-Cholestane-3α,7α,12α,24ξ,25-pentol. Band 2 of the preparative TLC plate (Fig. 1) contained a bile alcohol that was more polar than 5β-cholestane-3α,7α,12α,25-tetrol. The material crystallized from ethyl acetate in colorless needles which, after drying at 60°C *in vacuo* for 48 h, melted at 207.5–209.5°C. The melting point reached a constant value of 210–211°C after three additional crystallizations from the same solvent. The substance was homogeneous when examined by TLC and GLC (Table III). The IR spectrum was identical in all important respects with that reported by Hoshita for 5β-cholestane-3α,7α,12α,24ξ,25-pentol (11). The compound synthesized by Hoshita melted at 191.5°C (11), suggesting that it is a mixture of two bile alcohols epimeric at C-24, while the cholestanepentol isolated by us (mp 210–211) represents a single epimer.

GLC-mass spectrometry of the biosynthetic material again indicated that we were dealing with a pentahydroxy C_{27} bile alcohol. After GLC on 3% SE-30 the mass spectrum of the underivatized substance (Fig. 3) showed a molecular ion (*M*) at *m/e* 452 and a prominent peak at *m/e* 253, suggesting a 5β-cholestanepentol with three hydroxyl substituents on the nucleus (12) and two in the side chain. There was a series of fragment ions at *m/e* 434, 416, 398, 380, and 362 that arose from the consecutive loss of one to five molecules of water, typical of polyhydroxy sterols (13). The side chain with its two TMS groups yields a fragment ion with $m/e = 289$. Therefore the ion with $m/e = 253$ represents loss of the side chain plus three nuclear TMS groups. The mass spectrum of the TMS derivative showed no molecular ion (*M*). However, there was a base peak at *m/e* 131 that is in accord with a TMS ether at C-25 (9). The prominent peaks at *m/e* 73 and *m/e* 75 are typical of TMS ethers in general and are not helpful with regard to structural analysis (14). A major peak at *m/e* 143 indicates the presence of a TMS ether at C-24, since scission of the bond between carbons 23 and 24 and loss of a TMS-ether group results in a side chain fragment with *m/e* 143. A fragment with *m/e* 143 could conceivably arise from 5β-cholestane-3α,7α,12α,23ξ,25-pentol by scission between carbons 24 and 25, followed by loss of the C-24 methylene group. The fragment ions *m/e* 667, 577, 487, 397, and 307 would be consistent with a 23-hydroxylated pentol. Consequently, the possibility that the biosynthetic pentol is 5β-cholestane-3α,7α,12α,23ξ,25-pentol has not been completely excluded.

In the light of the data obtained so far we propose that the unknown bile alcohol is 5β-cholestane-3α,7α,12α,24ξ,25-pentol. (The mass spectra of 5β-cholestane-3α,7α,12α,24ξ,26-pentol, and 5β-cholestane-3α,7α,12α,25,26-pentol, generously supplied by Professor Hoshita, differed from that of our biosynthetic material, and no evidence of C-26 substituted bile alcohols was obtained).

Biosynthetic origin of 5β-cholestane-3α,7α,12α,25-tetrol. Patient J. C. received an intravenous injection of [4-^{14}C]cholesterol, and after a period of 4 wk cholesterol and 5β-cholestane-3α,7α,12α,25-tetrol were isolated from his bile. The specific activities of the isolated and purified compounds were: cholesterol, 675

dpm/mg; cholestanetetrol, 721 dpm/mg. The presence of radioactivity in the tetrol suggests that it was derived from cholesterol. More detailed kinetic studies, now in progress, indicate a product-precursor relationship between cholesterol and the tetrol. The higher specific activity of the tetrol found in the present study is likewise consistent with the concept that the bile alcohol was formed from cholesterol.

DISCUSSION

In previous studies several abnormalities of sterol and bile acid metabolism were reported in patients with CTX. These include elevated concentrations of cholesterol and cholestanol in tissues (1) and greatly increased rates of cholesterol and cholestanol synthesis (3). Additional proof of increased synthesis was the demonstration of cholesterol precursors (lanosterol, dihydrolanosterol, and Δ^7-cholestenol) in the bile (1) and the finding that hepatic 3-hydroxy-3-methylglutaryl-CoA (HMG-CoA) reductase activity was higher than in any other patient studied so far (15). In sharp contrast to the elevated rate of cholesterol biosynthesis was the observation that bile acid production as determined by the balance method was approximately 50% below normal (3). Paradoxically, hepatic microsomal cholesterol 7α-hydroxylase which catalyzes the rate-limiting step of bile acid synthesis from cholesterol was significantly higher than normal (16). The association of elevated HMG-CoA reductase and cholesterol 7α-hydroxylase activities in subjects with CTX indicates that their livers are capable of producing cholesterol and 7α-hydroxycholesterol in amounts which are more than adequate to sustain normal bile acid production. Since bile acid production in these patients is abnormally low, the biochemical defect in CTX must be located at an enzymatic site along the pathway leading from 7α-hydroxycholesterol to the primary bile acids, cholic and chenodeoxycholic acids. The detection of bile alcohols in the bile and the isolation of substantial amounts from the feces indicated a relative impairment in the degradation of the cholesterol side chain. The abnormally high activities of the rate-controlling enzymes of bile acid synthesis, HMG-CoA reductase and cholesterol 7α-hydroxylase can then be ascribed to a failure of the bile alcohols to exert negative feedback control on these enzymes either because the bile alcohols are not absorbed or because they lack the structural requirements to interact with the hepatic regulatory system.

In other words, patients with CTX synthesize insufficient quantities of bile acids to maintain the pool size required for normal feedback control of bile acid and cholesterol production. Apparently, many of the enzymes along the biosynthetic pathway leading from

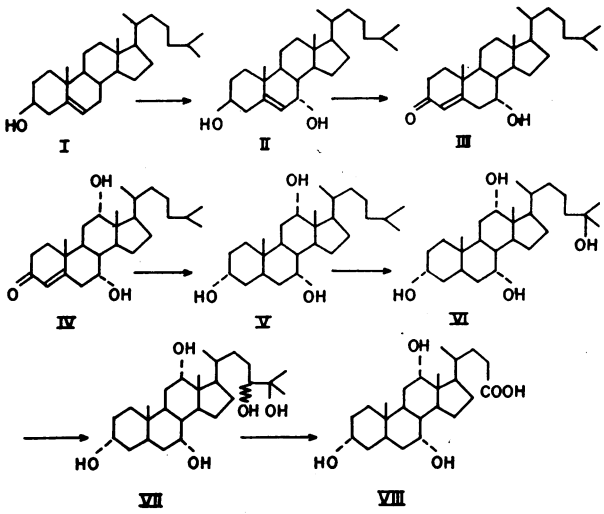

FIGURE 4 Hypothetical pathway of cholic acid biosynthesis via 25-hydroxylated intermediates. I, cholesterol; II, cholest-5-ene-3β,7α-diol; III, 7α-hydroxycholest-4-en-3-one; IV, 7α,12α-dihydroxycholest-4-en-3-one; V, 5β-cholestane-3α,7α,12α-triol; VI, 5β-cholestane-3α,7α,12α,25-tetrol; VII, 5β-cholestane-3α,7α,12α,24ξ,25-pentol; VIII, cholic acid.

acetyl-CoA to the 5β-cholanoic acids have abnormally elevated activities in this disease, thus accounting for the presence of cholesterol precursors in bile and perhaps also for the relative lack of biliary chenodeoxycholic acid.[2] It might be postulated that in CTX the activity of the enzyme that catalyzes the 12α-hydroxylation of 7α-hydroxycholest-4-en-3-one (III) to 7α,12α-dihydroxycholest-4-en-3-one (IV) (Fig. 4) is abnormally high. As a result there would be a relative reduction in the availability of 7α-hydroxycholest-4-en-3-one for transformation into 5β-cholestane-3α,7α-diol which is presumably on the major pathway of chenodeoxycholic acid biosynthesis (17).

The demonstration of substantial amounts (approximately 100 mg/day) of 5β-cholestane-3α,7α,12α,25-tetrol (VI) and 5β-cholestane-3α,7α,12α,24ξ,25-pentol (VII) (Fig. 4) in their feces suggests that patients with CTX have a relative lack of an enzyme that catalyzes the further transformation of the pentol to cholic acid. Apparently, the enzyme cholesterol 7α-hydroxylase is not rate-limiting for bile acid synthesis; it may be speculated that the enzyme in question is a dehydrogenase that catalyzes the transformation of the pentol to a 24-ketone, but we presently possess no experimental evidence concerning this point.

It is also conceivable that the basic genetic defect in CTX is a relative deficiency of an enzyme that catalyzes

[2] In three patients with CTX the percentage composition of biliary bile acids averaged: Cholic acid, 84%; chenodeoxycholic acid, 7%; deoxycholic acid, 3%; allocholic acid and unidentified bile acids, 6%.

the transformation of 5β-cholestane-3α,7α,12α-triol into 5β-cholestane-3α,7α,12α,26-tetrol. In that case, the triol would tend to accumulate in the liver cell, thus exposing it to the action of 23-, 24-, or 25-hydroxylases which normally are too inactive to compete effectively with the 26-hydroxylase.

In any case, the detection of 25-hydroxylated bile alcohols (and our failure to detect 26-hydroxylated intermediates) may have important implications concerning the biochemical pathway of bile acid synthesis. If the metabolic defect is ascribable to the relative deficiency of an enzyme or enzyme system catalyzing the further transformation of the pentol, then the accumulated bile alcohols represent normal biosynthetic intermediates. In that case, present concepts concerning the major pathway of cholic acid synthesis may require re-examination or modification. Largely on the basis of studies by Staple and his associates, side chain oxidation was thought to be catalyzed by mitochondrial enzymes and to proceed via 5β-cholestane-3α,7α,12α,26-tetrol and 3α,7α,12α-trihydroxy-5β-cholestanoic acid (17). Further metabolism of the C_{27} acid then proceeded via β-oxidation resulting in the formation of cholic acid and propionic acid. The present study suggests that there exists an alternate pathway of cholic acid biosynthesis involving the 25-hydroxylation of 5β-cholestane-3α,7α,12α-triol (Fig. 4), a pathway that excludes 5β-cholestanoic acid. There is evidence in the published literature in support of this hypothesis: Yamada (18) injected tritium-labeled 5β-cholestane-3α,7α,12α,25-tetrol into bile fistula rats and found that 30% of the biliary radioactivity was present in cholic acid. Since, following the injection of labeled 5β-cholestane-3α,7α,12α-triol 80% of the biliary radioactivity was in cholic acid, he concluded that the tetrol was probably not a normal intermediate.

Cronholm and Johansson incubated 5β-cholestane-3α,7α,12α-triol with rat liver microsomes and obtained a mixture of isomeric tetrols in which 5β-cholestane-3α,7α,12α,25-tetrol predominated (8). However, when injected into bile fistula rats the 26-hydroxylated tetrol (5β-cholestane-3α,7α,12α,26-tetrol) was converted to cholic acid more readily than the 25-hydroxylated isomer. At present it does not seem possible to rule out the existence of the 26-hydroxylation pathway. It seems likely that there exist two pathways of cholic acid synthesis: one, via the 26-hydroxylation of 5β-cholestanetriol by mitochondrial enzymes (17) the other, catalyzed by microsomal enzymes, involves the 25-hydroxylation of the triol, as illustrated in Fig. 4. This reaction sequence is analogous to the mechanism postulated for the cleavage of the cholesterol side chain by adrenal mitochondrial enzymes, yielding isocaproic aldehyde and pregnenolone (18). The pathway of cholic acid synthesis involving 25-hydroxylation would finally result in the formation of cholic acid and acetone (Fig. 4). Earlier work by Whitehouse, Staple, and Gurin (19) did demonstrate indeed the formation of labeled acetone from [26-^{14}C]cholesterol in vitro.

In the present paper, the abnormalities of sterol and bile acid metabolism in patients with CTX are ascribed, at least in part, to a lack of negative feedback regulation by bile acids on several hepatic microsomal enzymes, namely HMG-CoA reductase, cholesterol 7α-hydroxylase, and 7α-hydroxycholest-4-en-3-one 12α-hydroxylase. However, these findings offer neither a simple explanation for the abnormally low serum cholesterol concentrations found in CTX patients, nor for the accumulation of cholesterol and cholestanol in brain and other tissues, nor for the development of the clinical symptoms (1).

CTX may perhaps be added to a growing list of metabolic disorders in which a relative deficiency of an enzyme or enzyme system leads to the accumulation of biosynthetic intermediates. Clearly, the identification of such intermediates can be helpful in gaining a more detailed knowledge of normal metabolic pathways.

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