Metabolism of potential precursors of chenodeoxycholic acid in cerebrotendinous xanthomatosis (CTX)

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Abstract In patients with cerebrotendinous xanthomatosis (CTX), diminished cholic acid production is associated with incomplete oxidation of the cholesterol side chain and the excretion of C_{25}-hydroxy bile alcohols. The aims of this investigation were *1)* to provide quantitative information on the pool size and production rate of chenodeoxycholic acid by the isotope dilution technique; and *2)* to investigate the possible existence of a block in chenodeoxycholic acid synthesis and explain the absence of chenodeoxycholic acid precursors in CTX.

After the injection of [24-^{14}C]chenodeoxycholic acid, measurements of chenodeoxycholic acid pool size and production rate in a CTX subject were, respectively, 1/20 and 1/6 as great as controls. Further, three potential precursors of chenodeoxycholic acid, namely [G-^{3}H]7α-hydroxy-4-cholesten-3-one, [G-^{3}H]5β-cholestane-3α,7α,25-triol, and [G-^{3}H]5β-cholestane-3α,7α,26-triol, were administered to the CTX and control subjects and the specific activity curves of [G-^{3}H]cholic acid and [G-^{3}H]chenodeoxycholic acid were constructed and compared. In the control subjects, the two bile acids decayed exponentially, but in the CTX patient maximum specific activities were abnormally delayed, indicating the hindered transformation of precursor into bile acid. These results show that chenodeoxycholic acid synthesis is small in CTX and that the conversion of 7α-hydroxy-4-cholesten-3-one, 5β-cholestane-3α,7α,25-triol, and 5β-cholestane-3α,7α,26-triol to both chenodeoxycholic acid and cholic acid were similarly impaired.

Supplementary key words cholic acid · pool size · bile acid precursors · bile alcohols

Although the major clinical manifestations (tendon and cerebral xanthomas) in cerebrotendinous xanthomatosis (CTX) result from the deposition of cholesterol and cholestanol, the inherited biochemical abnormality in this disease appears to be defective bile acid synthesis (1, 2). Specifically, the daily formation of bile acids as measured by the steroid balance technique in CTX subjects is less than 50% of normal (100 mg/day) (3). Biliary bile acid composition is also abnormal with cholic acid accounting for 77–85% of the bile acids while the proportion of chenodeoxycholic acid makes up less than 10% of the total (3, 4). In addition, CTX subjects excrete substantial quantities of bile alcohols which have been identified as 5β-cholestane-3α,7α,12α,25-tetrol, 5β-cholestane-3α,7α,12α,24α,25-pentol, and 5β-cholestane-3α,7α,12α,23ξ,25-pentol (1, 4). In recent studies we have demonstrated that the 5β-cholestane-3α,7α,12α,25-tetrol is on a pathway leading to cholic acid via 5β-cholestane-3α,7α,12α,24β,25-pentol (5). Since these bile alcohols contain hydroxyl groups at C-12 and the isooctyl side chain of cholesterol, we postulated that the cholic acid synthetic pathway was partially blocked beyond the formation of 5β-cholestane-3α,7α,12α,25-tetrol. However, several key questions about the specific location of the enzymatic defect emerged, including whether the chenodeoxycholic acid synthetic pathway was similarly impaired. According to current views, side-chain cleavage of 5β-cholestane-3α,7α,12α-triol to cholic acid involves a C-26 hydroxylated intermediate, namely 5β-cholestane-3α,7α,12α,26-tetrol which is then further oxidized to 3α,7α,12α-trihydroxy-5β-cholestan-26-oic acid (6). Insertion of a hydroxy group at C-24 gives 3α,7α,12α,24ξ-tetrahydroxy-5β-cholestan-26-oic acid and cleavage of the terminal three carbons yields cholic acid and propionic acid (6). However, the accumulation of C-25 hydroxy bile alcohols in CTX suggested that side-chain cleavage in cholic acid synthesis might proceed via this alternate pathway. This premise was suggested by the demonstration that virtually all the cholic acid formed in two CTX subjects was derived from 5β-cholestane-3α,7α,12α,25-tetrol

Abbreviations: CTX, cerebrotendinous xanthomatosis; GLC, gas–liquid chromatography; TLC, thin-layer chromatography.

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(5). However, this finding did not rule out the possibility that the metabolic defect in this disease was an impaired 26-hydroxylation of 5β-cholestane-3α,7α,12α-triol. As a consequence the C-25 hydroxy pathway might be activated to provide cholic acid. Further, although a deficiency of chenodeoxycholic acid exists in the bile of CTX subjects (7), there was no detectable accumulation of bile alcohols lacking 12α-hydroxy groups, e.g., 5β-cholestane-3α,7α,25-triol, 5β-cholestane-3α,7α,24ξ,25-tetrol, or 5β-cholestane-3α,7α,26-triol (1), which would suggest a similar impairment of the chenodeoxycholic acid pathway. Therefore, to examine whether side-chain cleavage for chenodeoxycholic acid synthesis in CTX is abnormal and to explore the metabolism of a C-26 hydroxy bile alcohol in this disease, 7α-hydroxy-4-cholesten-3-one (a precursor common to cholic acid and chenodeoxycholic acid) and 5β-cholestane-3α,7α,26-triol (the expected precursor of chenodeoxycholic acid) were administered to three control subjects and two CTX patients. The role of 5β-cholestane-3α,7α,25-triol as a potential precursor of chenodeoxycholic acid was also considered. In addition, quantitative measurements of chenodeoxycholic acid pool size and production rate were made by the isotope dilution technique. Our results showed that chenodeoxycholic acid synthesis was defective in CTX subjects, and that this abnormality was associated with an impaired oxidation of both the 25- and 26-hydroxylated cholestanetriols.

METHODS

Clinical

Studies were conducted in five patients who were hospitalized at the East Orange Veterans Administration Hospital. Pertinent clinical information is listed in **Table 1.** Patient J.C. is a 38-year-old man with CTX who exhibits Achilles' tendon xanthomas and mild pulmonary insufficiency. Patient E.D.E. is a 50-year-old woman who suffers from moderately severe neurologic dysfunction (spinal cord paresis) and bilateral Achilles' tendon xanthomas, and is more severely affected than patient J.C. Detailed clinical descriptions and metabolic studies have appeared elsewhere (3, 7). Patient R.P. is a 52-year-old man with cholelithiasis and Type IV hyperlipoproteinemia. Patient A.S. is a 55-year-old man who suffers from angina pectoris but has normal plasma lipid levels. Patient J.S. is a 57-year-old man with Type IV hyperprebetalipoproteinemia. The patients were fed regular hospital diets. The caloric intake was adjusted to maintain constant body weight throughout each study.

Radioactive compounds

The [G-^{3}H]7α-hydroxy-4-cholesten-3-one was a gift from Professor Henry Danielsson. The material was prepared from [G-^{3}H]chenodeoxycholic acid by the method of Danielsson (8). The specific activity was 44 μCi/mg and the radioactive purity was greater than

TABLE 1. Clinical data

Patient	Diagnosis	Clinical	Plasma Lipids		Radioactive Compound and Dose (μCi) Administered
			TC[a]	TG[b]	
			mg/100 ml		
J.C.[c]	CTX	Achilles' tendon xanthomas, mild pulmonary insufficiency	156	58	[G-^{3}H]7α-hydroxy-4-cholesten-3-one (4.5 μCi)
E.D.E.	CTX	Achilles' tendon xanthomas, spinal cord paresis, spastic gait	144	87	5β-[G-^{3}H]cholestane-3α,7α,26-triol (3 μCi) + 5β-[24-^{14}C]cholestane-3α,7α,25-triol (1 μCi)
R.P.	Type IV Hyperlipoproteinemia	Gallstones	206	421	[G-^{3}H]7α-hydroxy-4-cholesten-3-one (4.5 μCi)
A.S.	Normolipidemia	Angina pectoris	235	100	5β-[G-^{3}H]cholestane-3α,7α,26-triol (3 μCi)
J.S.	Type IV Hyperlipoproteinemia	Abdominal angina	350	1300	5β-[G-^{3}H]cholestane-3α,7α,26-triol (3 μCi)

[a] TC, total cholesterol.
[b] TG, triglycerides.
[c] This patient subsequently received [2,4-^{3}H]cholic acid (25 μCi) + [24-^{14}C]chenodeoxycholic acid (6.7 μCi) to determine bile acid kinetics (see Table 2).

98% when tested by TLC on silica gel H in the system chloroform–acetone–methanol 7:5:0.3 (v/v/v).

5β-[G-^{3}H]Cholestane-3α,7α,26-triol was prepared from [G-^{3}H]chenodeoxycholic acid according to the method described by Berséus and Danielsson (9) and Danielsson (10). The radioactive purity of the product was greater than 95% determined by TLC on silica gel H in the system of chloroform–acetone–methanol 7:5:0.3 (v/v/v). The specific activity of this material was 2 μCi/mg. 5β-[24-^{14}C]Cholestane-3α,7α,25-triol was prepared as described previously (11). The specific activity of this material was 1 μCi/mg. All bile alcohols were dissolved in ethanol and dispersed in 150 ml of 0.9% NaCl solution which was infused intravenously.

Isolation of cholic acid and chenodeoxycholic acid from bile and feces

The bile acids present in the intestinal bile or feces of the CTX and control subjects were isolated according to the method of Salen, et al. (5). Briefly, a weighed specimen of dried bile or lyophilized feces was extracted with ethanol NH_4OH in a Soxhlet apparatus. The ethanol was evaporated and the mixture resuspended in aqueous ammonium hydroxide. The neutral lipids were extracted from the aqueous suspension by solvent partition with ethyl acetate. 5β-Cholestane-3α,7α,12α,25-tetrol was isolated by column chromatography and purified to constant specific activity by TLC (5).

To isolate the bile acids, sufficient NaOH was added to make the solution 2 N and the aqueous phase was boiled to remove NH_3. The mixture was heated at 120°C in an autoclave for 3 hr, cooled in an ice bath and acidified to pH 2 by the addition of 6 N HCl. The free bile acids were extracted three times with two volumes of peroxide-free ethyl ether. The ethyl ether phases containing the free bile acids were pooled, washed with water, and evaporated in a rotary evaporator. The bile acids were applied to 20 × 20-cm TLC plates (silica gel G, 0.25 mm thick) along with reference standards of cholic acid and chenodeoxycholic acid. The plates were developed in glacial acetic acid–diisopropyl ether–isooctane 25:25:50 (v/v/v). Cholic acid (R_f 0.12), deoxycholic acid (R_f 0.35), and chenodeoxycholic acid (R_f 0.40) were eluted with methanol after visualizing the bands with I_2 vapor. The solvent was evaporated and the individual bile acids were redissolved in 5 ml of methanol containing 0.25 ml of conc. H_2SO_4 and allowed to stand for 16 hr to form the methyl esters. Five ml of water was then added and methyl cholate and methyl chenodeoxycholate were extracted with three 20-ml portions of ethyl ether–benzene 2:1 (v/v). The benzene–ethyl ether layers were collected and washed to neutrality, first with aqueous saturated $NaHCO_3$ and then with water, and evaporated. The residue was dissolved in methanol containing 5α-cholestane as an internal standard. Aliquots were taken for radioactivity assay and for GLC.

GLC

Methyl cholate and methyl chenodeoxycholate were quantitated as trimethylsilyl derivatives using 5α-cholestane as an internal standard and authentic methyl cholate and methyl chenodeoxycholate as external standards. Aliquots that contained 2–10 μg of methyl cholate or methyl chenodeoxycholate were injected into 6-ft glass columns packed with 3% QF-1 on Gas Chrom Q (100–120 mesh, Applied Science Labs, Inc., State College, PA). The instrument was a Hewlett-Packard Model 7610A (Hewlett-Packard, Co., Palo Alto, CA) equipped with a flame ionization detector and electronic integrator. The following operating conditions were employed: column temperature, 230°C; detector, 250°C; and flash heater, 250°C.

Radioactivity measurements

Radioactivity was measured in a Beckman Model LS-200B liquid scintillation spectrometer (Beckman Instruments, Inc., Fullerton, CA). Appropriate corrections were made for quench and background. The counting efficiency for tritium was 51%.

Experimental design

A) To test the conversion of [G-^{3}H]7α-hydroxy-4-cholesten-3-one into primary bile acids and determine whether both the cholic acid and chenodeoxycholic acid synthetic pathways are impaired in CTX, 4.5 μCi of this precursor was injected intravenously into patients R.P. (control) and J.C. (CTX). Bile specimens were obtained daily during the following week via a duodenal tube. Bile flow was stimulated with cholecystokinin (75 I.U.). Cholic acid and chenodeoxycholic acid were isolated from these specimens and the specific activity of each bile acid was determined and plotted against time.

B) To compare the conversion of 26- and 25-hydroxy bile alcohols into both cholic acid and chenodeoxycholic acid in CTX, 3 μCi of 5β-[G-^{3}H]cholestane-3α,7α,26-triol and 1 μCi of 5β-[24-^{14}C]cholestane-3α,7α,25-triol were given simultaneously to patient E.D.E. (CTX). Cholic acid and chenodeoxycholic acid were isolated daily from specimens of feces and the respective specific activities of the bile acids were plotted against time.

C) The pool size and production rate of cholic acid and chenodeoxycholic acid were determined in subjects J.C. (CTX), A.S., and J.S. (control subjects). Subject J.C. received 25 μCi of [2,4-^{5}H]cholic acid and 6.7 μCi of [24-^{24}C]chenodeoxycholic acid. Subjects

A.S. and J.S. received 10 μCi each of [24-^{14}C]cholic acid and [24-^{14}C]chenodeoxycholic acid. Bile samples were obtained daily over the next week after pulse labeling and radioactive cholic acid and chenodeoxycholic acid were isolated and the specific activity was measured and plotted semilogarithmically. Calculations of pool size and production rates were made by the isotope dilution technique according to Lindstedt (12).

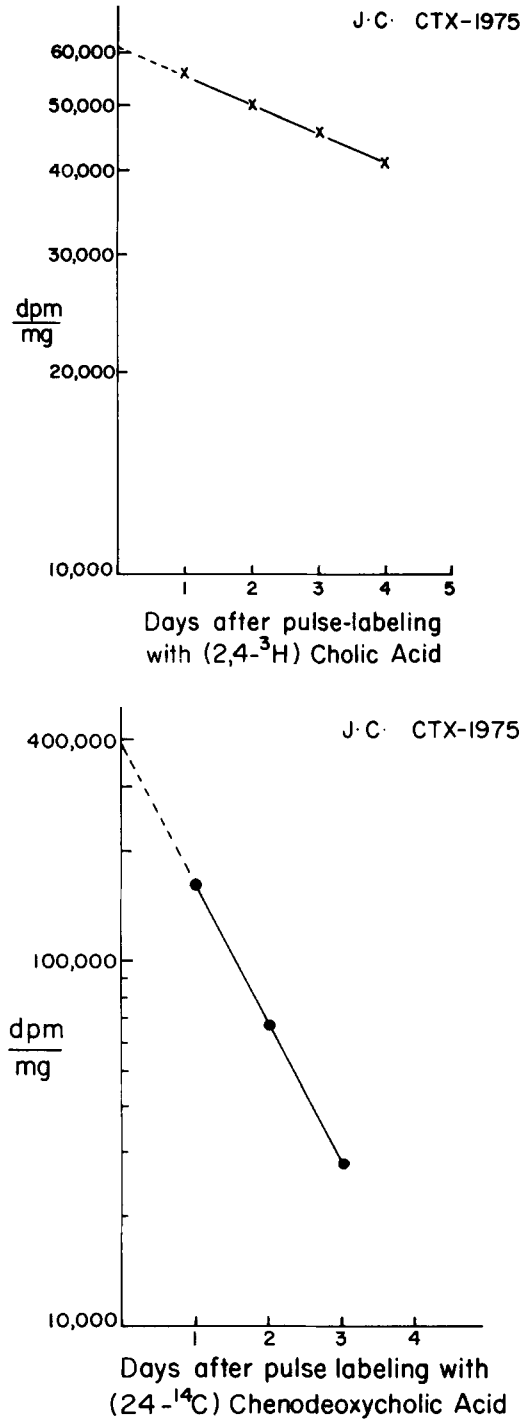

Fig. 1. Specific activities of biliary cholic acid (*a*) and chenodeoxycholic acid (*b*) of patient J.C. (CTX) after pulse labeling with 25 μCi of [2,4-^{3}H]cholic acid and 6.7 μCi of [24-^{14}C]chenodeoxycholic acid.

TABLE 2. Bile acid pool size and production rates[a]

Patient[b]	Cholic Acid		Chenodeoxycholic Acid	
	Pool Size	Production Rate	Pool Size	Production Rate
	mg	*mg/day*	*mg*	*mg/day*
J.C.	902	84	37	32
A.S.	870	240	830	205
J.S.	1200	360	790	190

[a] Calculated by the isotope dilution technique of Lindstedt (13). For details see Experimental Design section C.
[b] See Table 1.

RESULTS

Measurement of bile acid pool size and turnover by isotope dilution technique

After the intravenous administration of tracer doses of [2,4-^{3}H]cholic acid and [24-^{14}C]chenodeoxycholic acid to subject J.C. (CTX) and control subjects A.S. and J.S., the specific activities of both primary bile acids were measured in specimens of bile over the ensuing week. **Fig. 1**, *a* and *b* illustrate the decay curves for cholic acid and chenodeoxycholic acid, respectively, in subject J.C. (CTX), and show that both bile acid curves decayed linearly. This indicates that cholic acid and chenodeoxycholic acid are distributed as a single pool within the enterohepatic circulation. However, the cholic acid curve (Fig. 1*a*) decayed more slowly than the chenodeoxycholic acid curve (Fig. 1*b*) ($t_{1/2}$ for cholic acid was 7.5 days and for chenodeoxycholic acid was 0.8 days). Mathematical analysis of the curves, according to the method of Lindstedt (12), gives values for cholic acid and chenodeoxycholic acid pool sizes of 902 mg[2] and 37 mg respectively (**Table 2**). The pool size of cholic acid was 20 times that of chenodeoxycholic acid, which is in sharp contrast to the findings in normal subjects A.S. and J.S. in whom the pool sizes of the primary bile acids were similar in magnitude (Table 2). This table also illustrates the daily production rates of both bile acids. In the CTX subject the synthesis of cholic acid was 84 mg/day and synthesis of chenodeoxycholic acid was 32 mg/day. Both values are considerably below those found in the

[2] The use of [2,4-^{3}H]cholic acid may result in as much as a 20% error in the estimation of the bile acid pool size and production rate (13). Therefore, maximum values are reported for cholic acid pool size and production rate in this subject.

TABLE 3. Mass and radioactivity of biliary bile acids derived from [G-^{3}H]7α-hydroxy-4-cholesten-3-one[a]

Patient	Days After Pulse Labeling	Cholic Acid mg	Cholic Acid dpm	Cholic Acid Specific Activity dpm/mg	Chenodeoxycholic Acid mg	Chenodeoxycholic Acid dpm	Chenodeoxycholic Acid Specific Activity dpm/mg
R.P. (Control)	1	3.1	9,300	3,000	3.6	15,000	4,170
	2	3.0	5,700	1,900	2.0	7,200	3,600
	3	7.9	9,200	1,165	8.5	21,400	2,520
	4	3.4	800	235	4.3	4,200	977
	5	2.9	400	138	5.0	3,200	640
	6	1.8	200	111	2.0	1,000	500
J.C. (CTX)	1	17.8	14,700	826	1.5	1,700	1,133
	2	3.5	6,000	1,714	0.3	1,000	3,333
	4	7.7	4,660	605			
	5	10.8	1,600	148	0.2	200	1,000
	7	20.0	1,500	75	2.8	100	36

[a] Patients were pulse labeled by intravenous injection with 4.5 μCi of [G-^{3}H]7α-hydroxy-4-cholesten-3-one. Mass and radioactivity of cholic acid and chenodeoxycholic acid were determined by a combination of TLC, GLC, and liquid scintillation counting. See Methods section and (5).

two control subjects and in subjects with and without gallstones, studied by Vlahcevic, et al. (14). Although the cholic acid pool in the CTX subject was comparable in size to the value found in the two control subjects and that reported in the literature (14), the total bile acid pool was low because of the deficiency of chenodeoxycholic acid and the virtual absence of deoxycholic acid.

Conversion of [G-^{3}H]7α-hydroxy-4-cholesten-3-one into bile acids

In patients J.C. (CTX) and R.P. (control) cholic acid and chenodeoxycholic acid were isolated from specimens of bile after equal doses (4.5 μCi) of [G-^{3}H]7α-hydroxy-4-cholesten-3-one were administered, and mass and radioactivity of the bile acids were determined. The results are presented in **Table 3** and **Fig. 2,** *a* and *b*. As expected, one day after intravenous pulse labeling both primary bile acids were labeled with tritium which indicated that [G-^{3}H]7α-hydroxy-4-cholesten-3-one had been transformed into cholic acid and chenodeoxycholic acid in the control and CTX subjects. When the bile acid specific activities were plotted against time, maximum values for cholic acid and chenodeoxycholic acid were noted one day after pulse labeling in control subject R.P. and the curves decayed exponentially thereafter (Fig. 2*a*). In contrast, the maximum specific activity values for cholic acid and chenodeoxycholic acid in patient J.C. (CTX) were not reached until the second day after which the curves for both bile acids declined (Fig. 2*b*). This finding confirms that, in the CTX subjects, the production of the primary bile acids is abnormally slow.

Transformation of 5β-[24-^{14}C]cholestane-3α,7α,25-triol to bile acid in CTX

After the administration of 5β-[24-^{14}C]cholestane-3α,7α,25-triol to patient E.D.E., cholic acid, chenodeoxycholic acid, and 5β-cholestane-3α,7α,12α,25-tetrol isolated from feces were radioactive (**Table 4**)[3]. It was possible to measure the specific activity of 5β-cholestane-3α,7α,12α,25-tetrol, but not of 5β-cholestane-3α,7α,25-triol, since only traces of the latter compound were present. Presumably, the injected triol was 12α-hydroxylated and thereby converted to 5β-cholestane-3α,7α,12α,25-tetrol. Because of the block in the cholic acid pathway beyond 5β-cholestane-3α,7α,12α,25-tetrol, part of the newly formed tetrol was excreted unchanged along with its product, cholic acid (5). The specific activities of the primary bile acids and of 5β-cholestane-3α,7α,12α,25-tetrol are plotted against time as shown in **Fig. 3**[3]. Both bile acids reached maximum specific activity 7 days after pulse labeling. Further, the specific activity curve of 5β-cholestane-3α,7α,12α,25-tetrol intersected with that of cholic acid at the maximum of the latter, suggesting that the cholic acid was largely derived from this intermediate (5).

Transformation of 5β-[G-^{3}H]cholestane-3α,7α,26-triol into bile acid in CTX

After the intravenous administration of 5β-[G-^{3}H]cholestane-3α,7α,26-triol to a CTX subject (E.D.E.), cholic acid and chenodeoxycholic acid were isolated daily from feces.[3] The results are given in **Table 5** and

[3] In separate experiments in E.D.E., we have demonstrated that the specific activities of bile acids in the feces equal the specific activities of bile acid in the bile one day earlier.

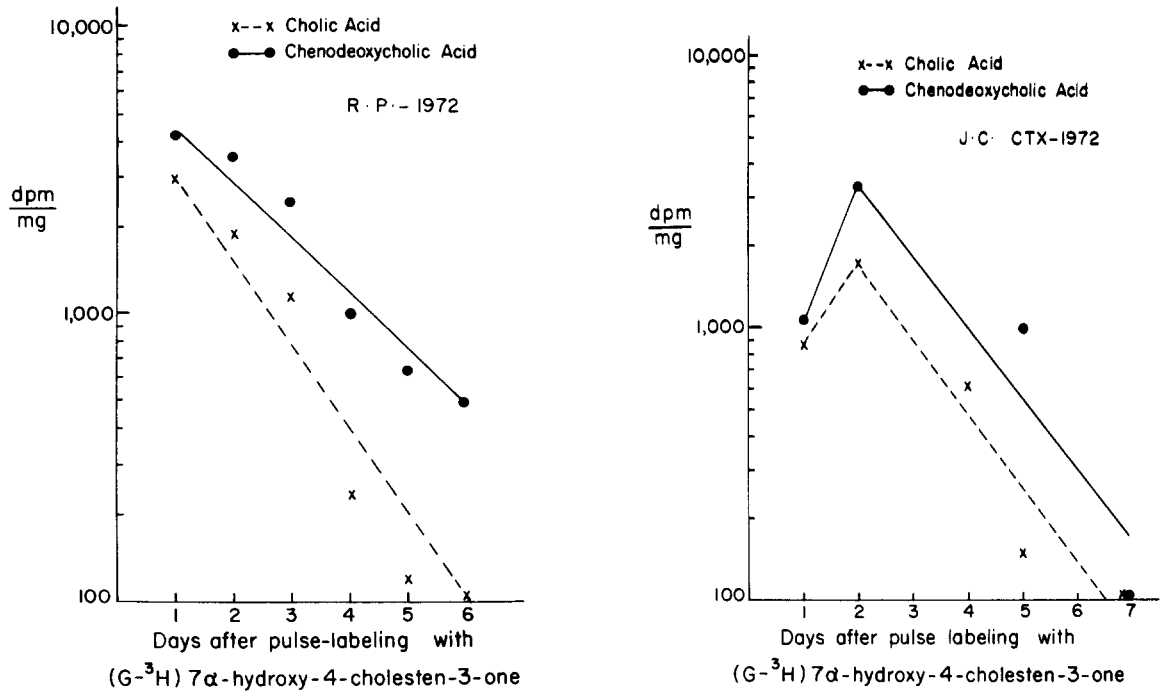

Fig. 2. Specific radioactivities of biliary cholic acid and chenodeoxycholic acid vs. time after intravenous pulse labeling with 4.5 μCi of [G-^{3}H]7α-hydroxy-4-cholesten-3-one. Control patient R.P.; CTX patient J.C.

Fig. 4. In this subject, both primary bile acids were labeled with tritium. The specific activity vs. time curves of chenodeoxycholic acid and cholic acid were similar to those obtained after administration of [24-^{14}C] cholestane-3α,7α,25-triol; maximum specific activity values of both bile acids in feces were not reached until 7 days after pulse labeling (Fig. 4).

Transformation of 5β-[G-^{3}H]cholestane-3α,7α,26-triol to bile acid in normal subjects

In the two normal subjects (J.S. and A.S.) the specific activities of both biliary cholic acid and chenodeoxycholic acid were highest on the first day after pulse labeling with 5β-[G-^{3}H]cholestane-3α,7α,26-triol (**Fig. 5, Table 6**). It was noteworthy that the initial specific activities of chenodeoxycholic acid were about eight times higher than those of cholic acid, although the pool of chenodeoxycholic acid almost equaled that of cholic acid in A.S. (830 mg vs. 870 mg) and was only 30% smaller in J.S. (790 mg vs. 1,200 mg). This finding suggested that 5β-cholestane-3α,7α,26-triol was converted preferentially to chenodeoxycholic acid. Deoxycholic acid isolated from the bile of the subjects was also labeled and the specific activity curves formed a

TABLE 4. Mass and radioactivity in fecal bile acid derived from 5β-[24-^{14}C]cholestane-3α,7α,25-triol[a]

Patient	Days After Pulse Labeling	5β-Cholestane-3α,7α,12α,25-tetrol		Cholic Acid		Chenodeoxycholic Acid	
		mg	dpm	mg	dpm	mg	dpm
E.D.E. (CTX)	1	0.11	60	11.5	150	1.27	80
	2	0.41	1,300	4.05	70	0.12	50
	3	0.12	2,000	1.42	120	0.38	410
	4	0.38	2,800	2.07	380	0.25	530
	5	0.49	900	1.46	410	0.49	1,990
	7	0.64	160	1.00	450	0.51	3,650
	9	1.09	160	1.30	420	0.26	1,000
	11	1.30	170	1.62	230	1.01	1,800

[a] Patient was pulse labeled by intravenous injection of 1 μCi of 5β-[24-^{14}C]cholestane-3α,7α,25-triol. Mass and radioactivity of 5β-cholestane-3α,7α,12α,25-tetrol, cholic acid, and chenodeoxycholic acid were determined by a combination of TLC, GLC, and liquid scintillation counting. See Methods section and (5).

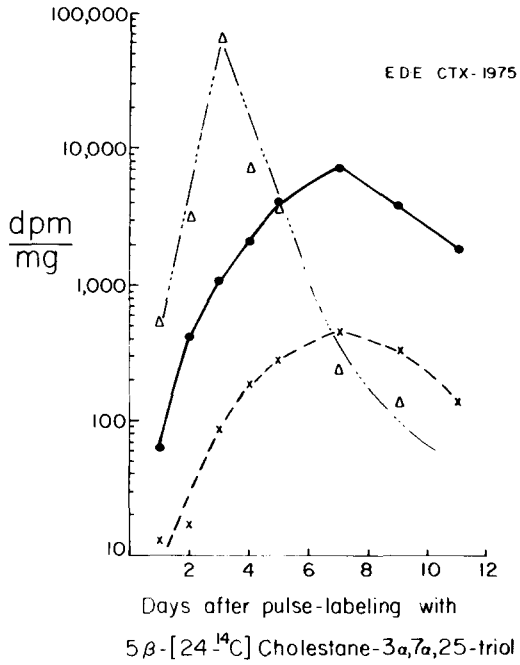

Fig. 3. Specific radioactivities of fecal 5β-cholestane-3α,7α,12α, 25-tetrol (Δ – – —— – – Δ), cholic acid (x – – – x), and chenodeoxycholic acid (● —— ●) plotted against time, after intravenous pulse labeling with 1 μCi of 5β-[24-^{14}C]cholestane-3α,7α,25-triol. CTX patient E.D.E.

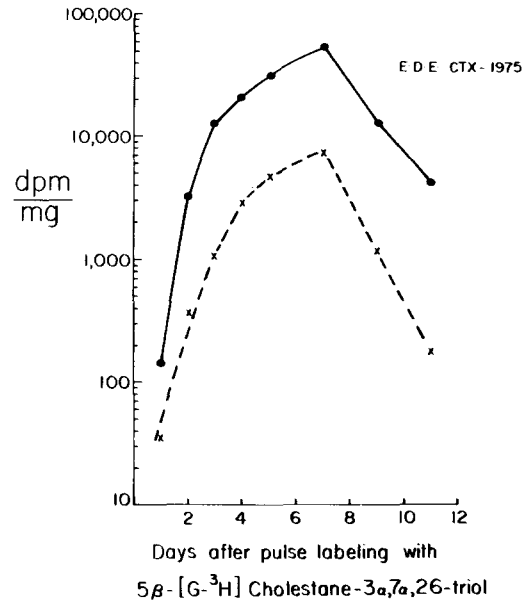

Fig. 4. Specific radioactivities of fecal cholic acid (x – – – x), and chenodeoxycholic acid (● —— ●) plotted against time, after intravenous pulse labeling with 3 μCi of 5β-[G-^{3}H]cholestane-3α,7α,26-triol. CTX patient E.D.E.

precursor–product relationship with those of cholic acid as expected.

DISCUSSION

The aims of this investigation were to examine whether chenodeoxycholic acid production was abnormally low and could be accounted for by a block in the synthetic pathway in CTX. Although initial studies showed that there was a marked deficiency of chenodeoxycholic acid in the bile of these subjects (7), the existence of a specific block in the bile acid synthetic pathway could not be predicted by the isolations of chenodeoxycholic acid precursors as was possible in the case of cholic acid intermediates (1, 2, 4, 5). However, bile acid production in patient J.C. (CTX) as measured by the isotope dilution technique was extremely low when compared with control values (Table 2) and similar data derived for patients with and without gallstones reported by Vlahcevic, et al. (14). Further, total bile acid synthesis in this subject measured 116 mg/day, which agrees with measurements of bile acid production as determined by the independent sterol balance method (93 mg/day) (3). The similarity of these results lends credence to our hypothesis that an inherited biochemical defect in CTX is abnormal bile acid synthesis. Despite the subnormal production of both primary bile acids, the cholic acid pool size was normal as compared with that of control subjects. However, the chenodeoxycholic acid pool was quite small. Apparently the preservation of the cholic acid pool was accomplished by a reduced fractional turnover rate and a larger synthesis of cholic acid than chenodeoxycholic acid. Because of the low rate of chenodeoxycholic acid synthesis, the experiments with the radioactive precursors [G-^{3}H]7α-hydroxy-4-cholesten-3-one, [24-^{14}C]5β-cholestane-3α,7α,25-triol, and [G-^{3}H]5β-cholestane-3α,7α,26-triol were performed to gain information on the location of the chenodeoxycholic acid synthetic defect. Further, these

TABLE 5. Mass and radioactivity in fecal bile acids derived from 5β-[G-^{3}H]cholestane-3α,7α,26-triol[a]

Patient	Days After Pulse Labeling	Cholic Acid		Chenodeoxycholic acid	
		mg	dpm	mg	dpm
E.D.E. (CTX)	1	11.5	400	1.27	190
	2	4.07	1,560	0.12	390
	3	1.42	1,500	0.39	4,900
	4	2.07	5,900	0.25	5,800
	5	1.46	6,900	0.49	14,700
	7	1.00	7,300	0.51	26,300
	9	1.30	1,000	0.26	3,400
	11	1.62	300	1.01	4,400

[a] Patients were pulse labeled by intravenous injection of 3 μCi of 5β-[G-^{3}H]cholestane-3α,7α,26-triol. Mass and radioactivity of cholic acid and chenodeoxycholic acid were determined by a combination of TLC, GLC, and liquid scintillation counting. See Methods section and (5).

experiments were designed to compare the rate of formation of chenodeoxycholic acid with cholic acid in the CTX subject.

When [G-^{3}H]7α-hydroxy-4-cholesten-3-one was injected intravenously, tritium was found in the cholic acid and chenodeoxycholic acid isolated from the bile of control and CTX subjects (Table 3). In the control subject, the specific activities of cholic acid and chenodeoxycholic acid were highest one day after pulse labeling and then decayed exponentially, although at different turnover rates (Fig. 2*a*). This indicates that [G-^{3}H]7α-hydroxy-4-cholesten-3-one was transformed rapidly to both primary bile acids. Further, when the decay curves were extrapolated back to t = 0, both lines intersected at about the same value of specific activity (5,600 dpm/mg). This suggests that both primary bile acids were synthesized from a common hepatic pool of 7α-hydroxy-4-cholesten-3-one. These findings are in accord with the work of Björkem et al. in rats (8) and Hanson, Klein, and Williams in man (15), who showed that 7α-hydroxy-4-cholesten-3-one was a direct precursor of cholic acid and chenodeoxycholic acid.

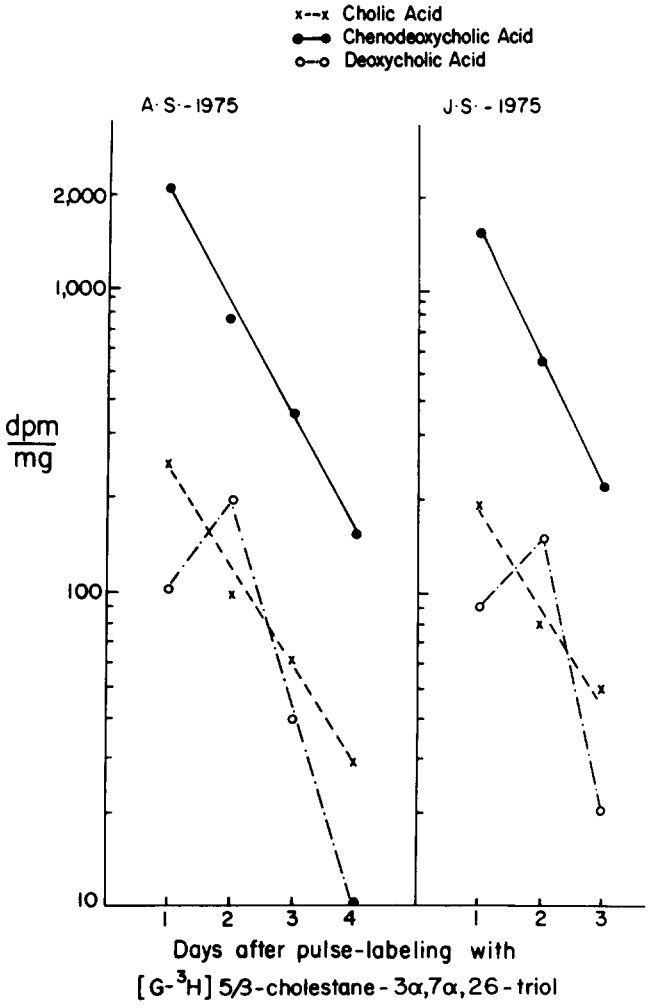

Fig. 5. Specific radioactivities of biliary cholic acid (x – – – x), chenodeoxycholic acid (● —— ●), and deoxycholic acid (○ –·– ○) plotted against time, after intravenous pulse labeling with 3 μCi of 5β-[G-^{3}H]cholestane-3α,7α,26-triol in subjects A.S. and J.S.

TABLE 6. Mass and radioactivity in biliary bile acids derived from 5β-[G-^{3}H]cholestane-3α,7α,26-triol[a]

Patient	Days After Pulse Labeling	Cholic Acid		Chenodeoxycholic acid	
		mg	dpm	mg	dpm
A.S. (Normal)	1	4.00	1,020	0.61	1,280
	2	24.3	2,400	0.19	160
	3	10.8	680	0.29	120
	4	6.40	180	0.50	80
J.S. (Normal)	1	4.70	840	0.85	1,400
	2	9.00	720	0.55	340
	3	15.1	760	0.90	210

[a] Patients were pulse labeled by intravenous injection of 3 μCi of 5β-[G-^{3}H]cholestane-3α,7α,26-triol. Mass and radioactivity of cholic acid and chenodeoxycholic acid were determined by a combination of TLC, GLC, and liquid scintillation counting. See Methods section and (5).

In contrast, in the CTX patient, maximum specific activities of cholic acid and chenodeoxycholic acid were not reached until the second day after intravenous pulse labeling with [G-^{3}H]7α-hydroxy-4-cholesten-3-one (Fig. 2*b*). Apparently, the formation of both primary bile acids from this precursor is impaired equally in CTX, although only precursors of cholic acid could be detected in bile and feces of these patients. Our failure to detect measurable quantities of chenodeoxycholic acid precursors may be ascribed to the fact that such intermediates can be rapidly 12α-hydroxylated (16) and either enter the cholic acid pathway or are excreted as 12α-hydroxy bile alcohols.

To consider this possibility and gain more precise information on the defect in side-chain oxidation, two potential precursors of chenodeoxycholic acid were given. This experiment demonstrated that 5β-cholestane-3α,7α,25-triol is converted to chenodeoxycholic acid in the CTX patient (Fig. 3). However, a portion of the injected triol was 12α-hydroxylated and detected as 5β-cholestane-3α,7α,12α,25-tetrol. It has been established that the further transformation of this tetrol to cholic acid is abnormal in CTX (5). Therefore, because the specific activity–time curves of chenodeoxycholic acid and cholic acid in the feces were very similar, it can be presumed that the biosynthesis of chenodeoxycholic acid from 5β-cholestane-3α,7α,25-triol is likewise impaired in CTX.

5β-Cholestane-3α,7α,26-triol was administered also to the CTX subject. There was a similar delay in reaching maximum specific radioactivity values (on day 7) for cholic acid and chenodeoxycholic acid as had been

observed in the case of 5β-cholestane-3α,7α,25-triol. Therefore, because the transformation of 5β-cholestane-3α,7α,25-triol to cholic and chenodeoxycholic acids was known to be abnormally slow in CTX (Fig. 3), it can be assumed that the conversion of 5β-cholestane-3α,7α,26-triol to both primary bile acids is also impaired.

When 5β-cholestane-3α,7α,26-triol was administered to normal individuals, the bile alcohol appeared to be preferentially converted to chenodeoxycholic acid (Fig. 5).[3] However, label from this precursor was also found in cholic acid and its bacterial metabolite, deoxycholic acid. This indicated that a portion of the administered 5β-[G-^{3}H]cholestane-3α,7α,26-triol was 12α-hydroxylated and thus transformed into cholic acid. The fact that the maximum specific activity was noted one day after intravenous pulse labeling indicates that the bile alcohol was rapidly taken up and converted to chenodeoxycholic acid and cholic acid in the normal subjects. The conversion of 5β-cholestane-3α,7α,26-triol to both chenodeoxycholic acid and cholic acid has also been reported in the bile fistula rat by Berséus and Danielsson (9). Their results showed that 85% of the radioactive 5β-cholestane-3α,7α,26-triol was converted to chenodeoxycholic acid, but that the remaining 15% appeared as cholic acid. These data are similar to our findings in the normal subjects. Further, when the decay curves for both bile acids were extrapolated to t = 0, they did not intersect, even though the pool sizes of the primary bile acids were nearly equal. This suggests, but does not prove, that 5β-cholestane-3α,7α,26-triol is a major precursor of chenodeoxycholic acid, but probably is not on the major pathway of cholic acid biosynthesis.

In summary, the results of these experiments suggest that chenodeoxycholic acid synthesis like cholic acid synthesis is defective in CTX. Further, it was shown that the abnormality affects the conversion of both 25- and 26-hydroxy intermediates.

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