Metabolism of 5β-cholestane-3α,7α,12α,26-tetrol and 5β-cholestane-3α,7α,12α,25-tetrol into cholic acid in normal human subjects

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Abstract Side chain oxidation and cleavage of precursors in cholic acid synthesis is thought to involve initial hydroxylation at either position 25 or 26 of the side chain. Therefore, the conversion of 5β-cholestane-3α,7α,12α,26-tetrol and 5β-cholestane-3α,7α,12α,25-tetrol into cholic acid was studied in normal subjects after single intravenous injections of these labeled alcohols. Eighty-six percent and 82% of 5β-cholestane-3α,7α,12α,26-tetrol was converted into cholic acid in two subjects, respectively. However, only 14 and 16% of the injected 5β-cholestane-3α,7α,12α,25-tetrol was converted into cholic acid in two subjects, respectively. Thus, this study indicates that 5β-cholestane-3α,7α,12α,25-tetrol is an inefficient substrate for cholic acid biosynthesis in man and that the major route of cholic acid synthesis probably involves the 26-hydroxylated intermediate.—**Hanson, R. F., A. B. Staples, and G. C. Williams.** Metabolism of 5β-cholestane-3α,7α,12α,26-tetrol and 5β-cholestane-3α,7α,12α,25-tetrol into cholic acid in normal human subjects. *J. Lipid Res.* 1979. **20:** 489–493.

Supplementary key words bile acid synthesis · alligator bile

Cholic acid is synthesized in the liver from cholesterol through a series of steps that start with changes in the ring system followed by oxidation and shortening of the side chain (1). The initial point of oxidation of the side chain is thought to occur at either position 26 or 25 (see **Fig. 1**). Previous studies have shown that 3α,7α,12α-trihydroxy-5β-cholestan-26-oic acid, a precursor in the 26-oxidation pathway (See Fig. 1), is present in human bile (2) and is rapidly and efficiently converted into cholic acid when administered to man (3, 4). Similarly, 5β-cholestane-3α,7α,12α,25-tetrol is also present in human bile (5) and is metabolized into cholic acid in normal subjects, although there is no information available on the efficiency of this reaction (5). Therefore, the present study was carried out to compare the conversion of intravenously administered 5β-cholestane-3α,7α,12α,26-tetrol and 5β-cholestane-3α,7α,12α,25-tetrol into cholic acid in normal human subjects.

METHODS

Reference and labeled compounds

5β-Cholestane-3α,7α,12α,26-tetrol. This compound was prepared by reducing 3α,7α,12α-trihydroxy-5β-cholestan-26-oic acid (THCA) according to the method described by Kazuno, Takako, and Hoshita (6). THCA was isolated from the bile of *Alligator mississippiensis* and labeled with tritium according to methods described previously (7). Briefly, [^{3}H]methyl THCA (sp act 24.4 μCi/mol) was refluxed with $LiAlH_4$ in ether for 2 hr, cooled, and extracted with 1 M H_2SO_4. The ether phase was extracted with ethyl acetate which was discarded. The residue in the ether phase was chromatographed by TLC using chloroform–acetone–methanol 70:25:15 (v/v). Over 90% of the radioactivity was in a band distinct from THCA and THCA methyl ester. Mass spectroscopy of this compound using an AEI MS-30 instrument (AEI Scientific Apparatus, Manchester, England) at 70 eV with an ion source temperature of 200°C showed a M^+ ion of 436, $M^+ - H_2O$ of 418, $M^+ - 2H_2O$ of 400, and $M^+ - 3H_2O$ of 382. [^{3}H]-5β-Cholestane-3α,7α,12α,26-tetrol prepared as described above was greater than 98% pure on TLC.

5β-Cholestane-3α,7α,12α,25-tetrol. The method described by Dayal et al. (8) was used to prepare 5β-cholestane-3α,7α,12α-25-tetrol. The 3-keto derivative was obtained after oxidation with silver carbonate–Celite (9) and this compound was labeled by exchange labeling with tritiated water on alumina (10). The labeled product was reduced with $NaBH_4$ (10) and purified by TLC using benzene–acetone–1,4-dioxane 10:12.5:7.5 (v/v) as developing solvent. The radioactivity corresponding to the 5β-cholestane-3α,7α,12α,

Abbreviations: THCA, 3α,7α,12α-trihydroxy-5β-cholestan-26-oic acid; TLC, thin-layer chromatography.

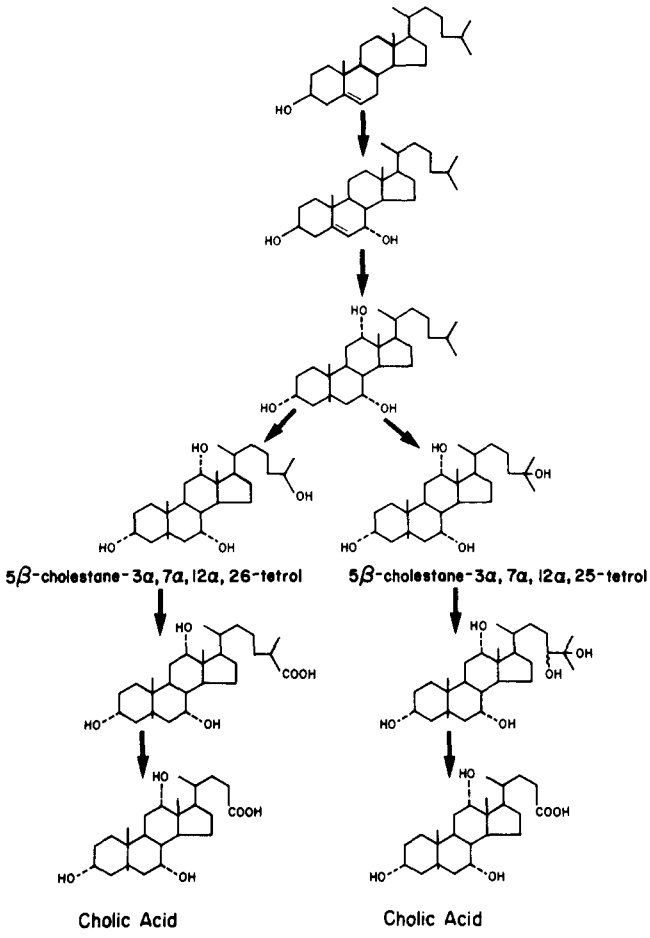

Fig. 1. Proposed pathway of bile acid synthesis involving either 5β-cholestane-3α,7α,12α,25-tetrol or 5β-cholestane-3α,7α,12α,26-tetrol.

25-tetrol control was scraped off and eluted with methanol. The R_f values of the 3α and 3β epimers were 0.46 and 0.55, respectively. The purity of the 3α,7α, 12α,25-tetrol product was assayed by TLC using the following solvent systems: iso-octane–ethyl acetate–acetic acid 5:5:1 (v/v); amyl acetate–water–propionic acid–isopropanol 4:1:3:2 (v/v); chloroform–acetone–methanol 7:2.5:1.5 (v/v); benzene–acetone 6:4 (v/v). In each system used the labeled 5β-cholestane-3α,7α, 12α,25-tetrol was at least 98% pure. The specific activity was 39.8 μCi/μmol. Electron impact mass spectroscopy using the same instrument and conditions described above gave peaks corresponding to $M^+ - H_2O$ of 418, $M^+ - 2H_2O$ of 400, $M^+ - 3H_2O$ of 382, and $M^+ - 4H_2O$ of 364. Chemical ionization mass spectroscopy using a Finnigan 4000 instrument (Finnigan Company, Sunnyvale, CA) with a source temperature of 170°C confirmed the molecular weight of 5β-cholestane-3α,7α,12α,25-tetrol.

[^{14}C]Cholic acid (3α,7α,12α-trihydroxy-5β-cholan-24-oic acid) was purchased from New England Nuclear Corp., Boston, MA. Thin-layer chromatography showed the radioactivity to be at least 99% pure.

Experimental design

The experimental design of this study is similar to that described by us previously for the study of 3α,7α,12α-trihydroxy-5β-cholestan-26-oic acid in normal human subjects (4). Briefly, this method involves the comparison of the areas under the specific activity decay curve of [^{3}H]cholic acid (formed from the precursor being studied) to the area under the specific activity decay curve of [^{14}C]cholic acid. The specific activities are expressed as percent of the injected amount/μmol of cholic acid. If there is complete and rapid conversion of the precursor into cholic acid, the areas under the specific activity decay curves of [^{3}H]- and [^{14}C]cholic acids will be identical. However, if a smaller percentage of the proposed precursor is converted into cholic acid, the area under the [^{3}H]-cholic acid curve will be decreased to the same percentage.

The four male subjects ranging in age from 24 to 36 years swallowed a polyvinyl tube at 8 AM on the first day of the experiment; the tip of the tube was positioned in the duodenum. Each subject was in good health, had had a normal physical examination, and had given informed consent. A liquid formula diet containing 40% of the diet as fat (11) was infused over the next 6–7 hr to contract the gallbladder and mix the labeled cholic acid in the cholic acid pool. Two hours after starting the infusion, 8 μCi of ^{3}H-labeled tetrol and 8 μCi of [^{14}C]cholic acid dissolved in 2 ml of sterile absolute ethanol were injected intravenously. Mixed bile samples (2–3 ml) were collected at hourly intervals for the next 4–5 hr. Additional bile samples were obtained after gallbladder contraction at 24, 48, 72, and 96 hr after the injection.

Analytical methods and calculations

The specific activities of [^{14}C]- and [^{3}H]cholic acid in the bile samples and the areas under the specific activity decay curves were measured as described previously (4, 12).

RESULTS

5β-Cholestane-3α,7α,12α,26-tetrol

Fig. 2 shows that the injected [^{3}H]-5β-cholestane-3α,7α,12α,26-tetrol was rapidly and efficiently metabolized into cholic acid. The specific activity of [^{3}H]-cholic acid peaked in the bile within the first hour after the injection. Therefore, the metabolism of intravenously administered 5β-cholestane-3α,7α,12α,26-

tetrol into cholic acid followed by conjugation and biliary secretion was just as rapid as the biliary secretion of [^{14}C]cholic acid, which required only prior conjugation before secretion, indicating that the injected [^{3}H]-5β-cholestane-3α,7α,12α,26-tetrol was rapidly metabolized into cholic acid.

The slopes of the two decay curves are nearly identical in each subject and the areas under the [^{3}H]-cholic acid specific activity decay curves are within 86 and 82% of the area under the [^{14}C]cholic acid specific activity decay curves, respectively (see **Table 1**). Thus, 86 and 82%, respectively, of the injected 5β-cholestane-3α,7α,12α,26-tetrol was metabolized into cholic acid in the two subjects.

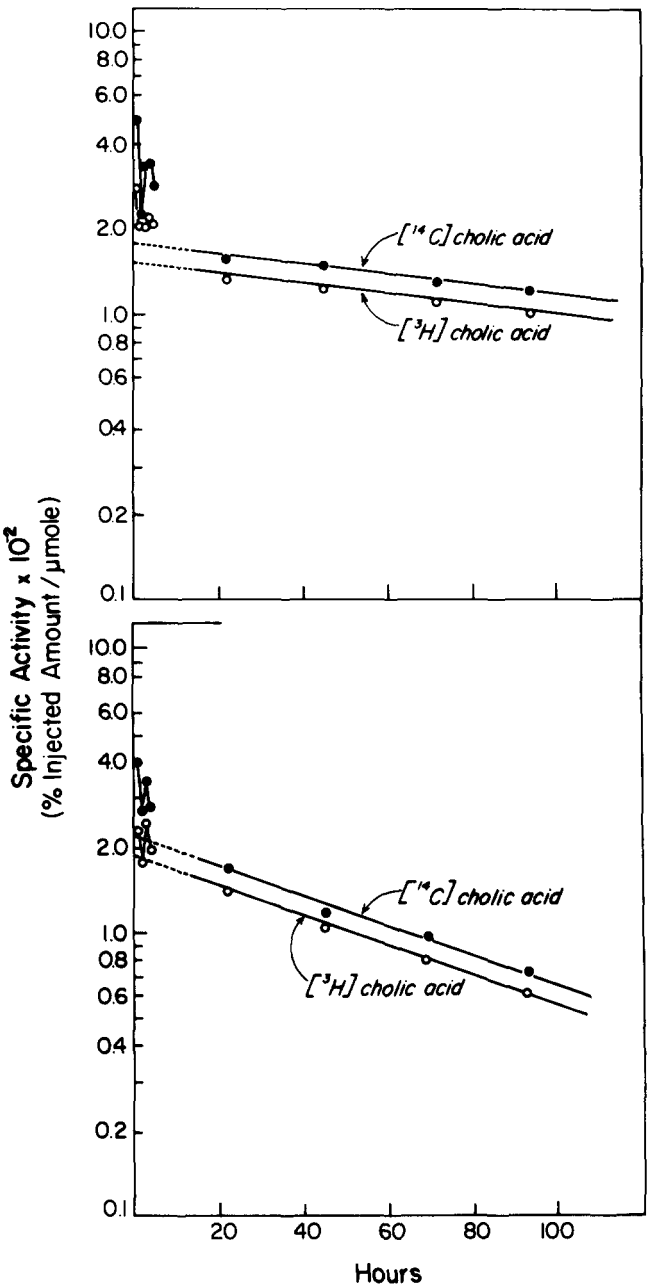

Fig. 2. Specific activity decay curves of [^{14}C]- and [^{3}H]cholic acid after the intravenous administration of [^{3}H]-5β-cholestane-3α,7α,12α,26-tetrol and [^{14}C]cholic acid.

TABLE 1. Kinetic data for [^{3}H]- and [^{14}C]cholic acid

Subject	[^{14}C]Cholic Acid Area × 10^{-2} [a]	Slope [b]	[^{3}H]Cholic Acid Area × 10^{-2} [a]	Slope [b]
[^{3}H]-5β-Cholestane-3α,7α,12α,26-tetrol				
1	4.18	0.504	3.59	0.499
2	11.20	0.154	9.20	0.160
[^{3}H]-5β-Cholestane-3α,7α,12α,25-tetrol				
1	8.56	0.347	1.16	0.335
2	10.70	0.450	1.72	0.470

[a] Percent injected amount/µmol·day.
[b] Day^{-1}.

5β-Cholestane-3α,7α,12α,25-tetrol

Fig. 3 shows the specific activities of [^{3}H]cholic acid formed after an injection of 5β-cholestane-3α,7α,12α,25-tetrol. The area under the ^{3}H specific activity decay curves were only 14 and 16%, respectively, of the area under the [^{14}C]cholic acid specific activity decay curve in the two subjects. The small triangular area above the data points and below the extrapolated line during the first 4 hr was subtracted to give the final area shown in Table 1. Therefore, in these two subjects only 14 and 16% of the injected [^{3}H]-5β-cholestane-3α,7α,12α,25-tetrol was metabolized into cholic acid (See Table 1). The slopes of the ^{3}H specific activity decay curves were nearly identical to the slopes of the ^{14}C specific activity decay curves (See Table 1). The radioactivity excreted into the bile that was not cholic acid was not further identified.

DISCUSSION

This study was designed to compare the metabolism of 5β-cholestane-3α,7α,12α,26-tetrol and 5β-cholestane-3α,7α,12α,25-tetrol into cholic acid in normal human subjects with intact enterohepatic circulation. The results indicate that the human liver is much more efficient in converting 5β-cholestane-3α,7α,12α,26-tetrol into cholic acid than 5β-cholestane-3α,7α,12α,25-tetrol. Based on this information, the natural pathway of side chain oxidation of 5β-cholestane-3α,7α,12α-triol involves 5β-cholestane-3α,7α,12α,26-tetrol and not 5β-cholestane-3α,7α,12α,25-tetrol (See Fig. 1).

Fig. 2 shows that shortly after the injection of the [^{14}C]cholic acid and [^{3}H]5β-cholestane-3α,7α,12α,26-

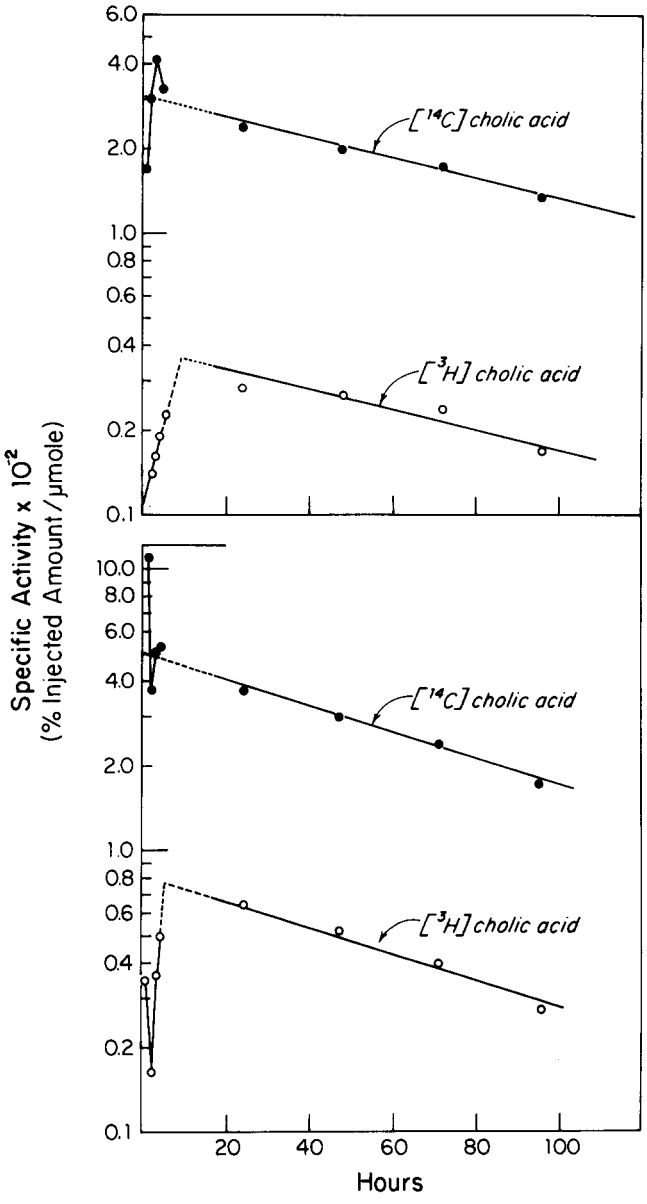

Fig. 3. Specific activity decay curves of [^{14}C]- and [^{3}H]cholic acid after the intravenous administration of [^{3}H]-5β-cholestane-3α,7α, 12α,25-tetrol and [^{14}C]cholic acid.

tetrol the specific activities were higher than expected based on the extrapolation of the specific activity decay curves from 24 hr back to the time of injection. This is the distribution phase of the decay curve and probably results from inadequate mixing of the labeled cholic acid with the pool of cholic acid. After the injection of [^{3}H]5β-cholestane-3α,7α,12α,25-tetrol the specific activities of [^{3}H]cholic acid rose more slowly than did the [^{14}C]cholic acid (See Fig. 3). The most likely explanation for the delayed peak of [^{3}H]cholic acid is a reduced rate of metabolism of 5β-cholestane-3α,7α, 12α,25-tetrol into cholic acid compared to the rate of metabolism of 5β-cholestane-3α,7α,12α,26-tetrol.

In a previous study, Schwartz et al. (13) investigated the metabolism of labeled 5β-cholestane-3α,7α,25-triol and labeled 5β-cholestane-3α,7α,26-triol in bile fistula patients. Between 60 and 75% of 5β-cholestane-3α,7α,26-triol was converted into chenodeoxycholic acid and cholic acid while less than 10 and 20% of 5β-cholestane-3α,7α,25-triol was converted into chenodeoxycholic acid and cholic acid. Thus, both 5β-cholestane-3α,7α,25-triol and 5β-cholestane-3α,7α, 12α,25-tetrol are poorly converted into bile acids. In the previous study, between 48 and 84% of the administered label of 5β-cholestane-3α,7α,25-triol was recovered in the bile, indicating that the inefficient conversion of this compound was not due to poor uptake by the liver (13). In the present study, we have no information on total biliary excretion of the radioactivity after an injection of 5β-cholestane-3α,7α,12α, 25-tetrol. However, based on the earlier work that 5β-cholestane-3α,7α,25-triol is removed from the blood by the liver (13), reduced hepatic uptake of 5β-cholestane-3α,7α,12α,25-tetrol is probably not the cause for the reduced conversion of this latter compound into cholic acid. Thus, it appears that neither 5β-cholestane-3α,7α,25-triol nor 5β-cholestane-3α,7α, 12α,25-tetrol are natural substrates for bile acid synthesis, making the 25α-hydroxylation pathway for bile acid synthesis unlikely.

The hydroxyl at C-26 of the 5β-cholestane-3α,7α, 12α,26-tetrol results in an asymmetry at C-25. In the present study, this compound was synthesized from THCA isolated from alligator bile. Previous work by Haslewood and Bridgwater (14, 15) showed that THCA from this species is the 25*R*-isomer. Carey and Haslewood (2) crystallized THCA from human bile and found that this material did not depress the melting point of THCA isolated from alligator bile (2). Therefore, 25*R*-5β-cholestane-3α,7α,12α,26-tetrol was used in the present study and this is probably the natural configuration of this intermediate in man.

This study was supported by grant AM15077, and by a grant, RR-400, from the General Clinical Research Centers Program of the Division of Research Resources, NIH.

Manuscript received 4 August 1978; accepted 15 November 1978.

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