Bile acids. XXXVII. Identification of the 3β isomers of allocholic and allochenodeoxycholic acids as metabolites of 5α-cholestanol in the rat

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Abstract Bile was collected for 18–24 days from adult male rats with cannulated bile ducts that had received intraperitoneally 0.8 mg of 5α-[4-^{14}C, 3α-^{3}H]cholestan-3β-ol. Bile from the first 2 days containing 14.2% of the administered ^{14}C and 3.3% of the ^{3}H was hydrolyzed, and the bile acids were separated by acetic acid partition chromatography. The previously unidentified metabolite more polar than cholic and allocholic acids was identified by isotopic dilution as 3β,7α,12α-trihydroxy-5α-cholanic acid and represented 3% of the biliary ^{14}C and 15% of the ^{3}H. Similarly, 3β,7α-dihydroxy-5α-cholanic acid was identified in fractions more polar than allochenodeoxycholic acid and represented 0.6% of the biliary ^{14}C and 8% of the ^{3}H. More polar fractions contained 4% of the ^{14}C and 31% of the ^{3}H in unidentified metabolites.

Supplementary key words bile fistula rat · cholestanol metabolism · isomeric allocholanic acid · 5α-cholanic acids · 5α-[4-^{14}C, 3α-^{3}H]cholestan-3β-ol · 3β-hydroxy allocholanic acids

In continuation of our studies on the identification of bile acids derived from cholestanol (1–4) and the mechanism of their formation, we have investigated the nature of a third unidentified metabolite reported by Karavolas and Elliott (2). Earlier studies have shown that allocholic acid (1, 2) is the major biliary acid obtained after intracardial administration of cholestanol to the rat with a cannulated bile duct and that allochenodeoxycholic acid is the principal dihydroxy acid formed from this sterol (3). This report establishes the identity of the 3β epimers of these allo acids as biliary metabolites of cholestanol in the rat. Preliminary reports of some of these studies have appeared (5, 6).

MATERIALS AND METHODS

Preparation of 5α-[4-^{14}C, 3α-^{3}H]cholestan-3β-ol

5α-Cholestan-3-one (76 mg, mp 132–133°C, prepared according to Bruce [7]) in 5 ml of cold methanol was reduced with 4 mg of sodium [^{3}H]borohydride (about 10 mCi). After dilution of the mixture and extraction with ether, the ether-soluble material was purified by chromatography on a column of silver nitrate–silicic acid (50 g, 25 cm × 18 mm) (3). Unchanged cholestanone appeared in fractions eluted with hexane–benzene 95:5; 8.2 mg of 5α-cholestan-3α-ol containing 19% of the recovered tritium was eluted with hexane–benzene 85:5. After elution with an additional 175 ml of the above solvent, 5α-cholestan-3β-ol was eluted with an additional 200 ml (30.7 mg containing 81% of the recovered ^{3}H). The desired product was acetylated and the acetate was purified by chromatography on neutral alumina, grade II. After alkaline hydrolysis of the acetate, chromatography of the sterol on a column of silver nitrate–silicic acid, and recrystallization from ethanol, the product showed no significant change in specific activity (1.25 × 10^{8} dpm/mg). Oxidation of an aliquot to 5α-cholestan-3-one and examination of the product showed that no more than

Abbreviations: Systematic nomenclature of the compounds referred to in the text by their trivial names is as follows: cholestanol, 5α-cholestan-3β-ol; cholestanone, 5α-cholestan-3-one; allocholic acid, 3α,7α,12α-trihydroxy-5α-cholan-24-oic acid; allochenodeoxycholic acid, 3α,7α-dihydroxy-5α-cholan-24-oic acid; all 5α-cholanic acids are 5α-cholan-24-oic acids.

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TABLE 1. Distribution of radioactivity in extracts of hydrolyzed bile after administration of 5α-[4-^{14}C, 3α-^{3}H]cholestan-3β-ol to rats A and B

Bile Sample	Bile Acid Fraction	Water Washes of BAF[a]	Non-saponifiable Fraction	Water Washes of NSF[a]	Hydrolyzed, Extracted Bile	Total Recovered
Rat A, days 1–2						
dpm ^{14}C $\times 10^{-5}$	8.12	0.40	0.83	0.08	0.45	9.88
% of biliary ^{14}C	78.1	3.8	8.0	0.8	4.3	95.0
dpm ^{3}H $\times 10^{-5}$	5.44	0.47	4.20	0.14	3.15	13.4
% of biliary ^{3}H	41.5	3.6	32.0	1.1	24.0	102.1
Rat A, days 3–4						
dpm ^{14}C $\times 10^{-5}$	6.33	0.96	0.66	0.08	0.84	8.87
% of biliary ^{14}C	71.4	10.8	7.4	0.9	9.5	98.4
dpm ^{3}H $\times 10^{-5}$	3.39	3.52	3.34	0.17	6.17	16.6
% of biliary ^{3}H	17.8	18.5	17.6	0.9	32.5	87.4
Rat A, days 5–6						
dpm ^{14}C $\times 10^{-5}$	2.54	0.80	0.76	0.04	0.60	4.74
% of biliary ^{14}C	38.1	12.0	11.4	0.6	9.0	71.2
dpm ^{3}H $\times 10^{-5}$	3.49	2.43	4.07	0.18	6.56	16.7
% of biliary ^{3}H	19.9	13.9	23.3	1.0	37.5	95.4
Rat B, days 1–2						
dpm ^{14}C $\times 10^{-5}$	3.00	0.17	0.28	0.07	0.16	3.68
% of biliary ^{14}C	71.4	4.0	6.6	1.7	3.7	87.4
dpm ^{3}H $\times 10^{-5}$	2.54	0.07	1.38	0.05	0.97	5.01
% of biliary ^{3}H	54.0	1.5	29.4	1.1	20.6	106.6

[a] BAF, bile acid fraction; NSF, nonsaponifiable fraction.

0.05% of the tritium in 5α-[3α-^{3}H]cholestan-3β-ol could be located at positions other than the C-3 position.

Doubly labeled cholestanol was prepared by combining 2.514 mg of 5α-[4-^{14}C]cholestan-3β-ol (sp act 2.02 $\times 10^7$ dpm/mg, purified by chromatography on silver nitrate–silicic acid [4]) with 2.127 mg of the above tritiated cholestanol. The product was chromatographed on the silver nitrate–silicic acid column (calculated specific activities: 5.73 $\times 10^7$ dpm ^{3}H/mg and 1.09 $\times 10^7$ dpm ^{14}C/mg; ^{3}H/^{14}C = 5.23). The tritiated component exhibited a small isotope effect and was eluted slightly later than the ^{14}C moiety (final sp act = 4.89 $\times 10^7$ dpm ^{3}H/mg, 0.905 $\times 10^7$ dpm ^{14}C/mg; ^{3}H/^{14}C = 5.40). A portion of this material was oxidized to the ketone; no tritium was detected in the 5α-cholestan-3-one.

Chromatography

Bile acids were separated by chromatography on an acetic acid partition column (8). The fractions have been designated according to the percentage of benzene in hexane; e.g., fraction 60-1 represents the first fraction of the eluent containing 60% benzene in hexane.

Other methods

Radioactivity was determined with a Packard Tri-Carb liquid scintillation spectrometer, model 3314, equipped with external standardization. Bray's solution (9), 15 ml, was used for the assay. Melting point determinations were taken on a Fisher-Johns apparatus and are reported as read.

Bile acids

Cholic acid was a generous gift of the Wilson Laboratories, Chicago, Ill. Allocholic and allochenodeoxycholic acids and their 3β isomers were prepared from methyl cholate and methyl chenodeoxycholate, respectively, by allomerization with Raney nickel (10, 11).

RESULTS

Preliminary experiments

These experiments were undertaken with the radioactive metabolites previously reported (3) in fraction 100-1 of the free bile acids obtained from bile collected during the first 2 days from a rat that had received daily subcutaneous injections of L-thyroxine at a level of 250 μg/kg/day for 26 days prior to cannulation of the bile duct and intraperitoneal administration of 0.38 mg (1.16 $\times 10^7$ dpm) of 5α-[4-^{14}C]cholestan-3β-ol. The residue (about 0.8 mg) from an aliquot of fraction 100-1 containing 4.26 $\times 10^4$ dpm was methylated with an excess of diazomethane. The product was dried in vacuo for 3 hr at 55°C and was diluted with 30.0 mg of synthetic methyl $3\beta,7\alpha,12\alpha$-trihydroxy-5α-cholanate, mp 200–201°C (12). The calculated specific activity of the mixture was 1.38 $\times 10^3$ dpm/mg; after two crystallizations from a mixture of acetone and hexane, the specific activities were 1.40 $\times 10^3$ dpm/mg and 1.39 $\times 10^3$ dpm/mg, respectively. Similar results were obtained with material from fractions 100-1 and 100-2 from the second rat (3). After four

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crystallizations of the diluted material from acetone–hexane and three crystallizations from benzene, the specific activity changed from 632 dpm/mg to 612 dpm/mg, suggesting the identity of this metabolite with 3β,7α,12α-trihydroxy-5α-cholanic acid.

Metabolism of [4-^{14}C, 3α-^{3}H]cholestanol

5α-[4-^{14}C, 3α-^{3}H]Cholestan-3β-ol (9.05 × 10^{6} dpm ^{14}C/mg, 4.89 × 10^{7} dpm ^{3}H/mg; ^{3}H/^{14}C = 5.40) was administered intraperitoneally immediately after surgery to two adult male rats (303 and 308 g, St. Louis University Colony) with cannulated bile ducts. Rat A received 0.81 mg and rat B received 0.82 mg; the solution for injection contained 1 mg of sterol dissolved in 0.1–0.2 ml of warm ethanol, 80–120 mg of Tween 80, and sufficient 0.9% sodium chloride to make a volume of 1 ml. Bile was collected for 24 and 18 days, respectively. Some fluid drained through the incisions of both animals, especially rat B, where the radioactivity could be detected with a laboratory monitor.

Bile from rat A from days 1 and 2, days 3 and 4, and days 5 and 6 was combined into three fractions containing 10.4, 9.08, and 6.06 × 10^{5} dpm of ^{14}C and 1.31, 1.90, and 1.75 × 10^{6} dpm of ^{3}H, respectively; each fraction was hydrolyzed with KOH according to the procedure of Mahowald et al. (13). Bile from rat B from days 1 and 2 containing 4.20 × 10^{5} dpm of ^{14}C and 4.70 × 10^{5} of ^{3}H was also combined, hydrolyzed, and fractionated. The distribution of radioactivity is shown in Table 1. The biliary acid fraction (137 mg) obtained from the bile of rat A from days 1 and 2 was chromatographed on an acetic acid partition column (Fig. 1). Only 4% of the chromatographed ^{3}H and 1% of the ^{14}C appeared in the region in which monohydroxy bile acids and fatty acids are eluted (fraction 0-1). Fractions 20-4 and 40-1, which correspond to 3α,7α-dihydroxy-5α-cholanic acid, contained 8% of the chromatographed ^{14}C and 9% of the ^{3}H; however, the tritium appeared to be a component of the next peak. Fractions 40-2 to 60-1 contained 48% of the chromatographed ^{3}H associated with 8% of the ^{14}C. Fractions 80-1 to 80-3, which correspond to 3α,7α,12α-trihydroxy-5α-cholanic acid, contained 72% of the ^{14}C and 9% of the ^{3}H. Fractions 80-4 to 100-2 contained 20% of the chromatographed ^{3}H and 7% of the ^{14}C. Similar distributions of radioactivity were obtained with the bile acid fractions from days 3 and 4 and from days 5 and 6.

Identification of metabolite in fractions 80-4 to 100-2

Fractions 80-4, 100-1, and 100-2 (Fig. 1), which originally contained 5.74 × 10^{4} dpm of ^{14}C and 1.08 × 10^{5} dpm of ^{3}H, were combined and the residue was methylated with diazomethane. The mass (3.3 mg) was diluted with 49.83 mg of methyl 3β,7α,12α-trihydroxy-

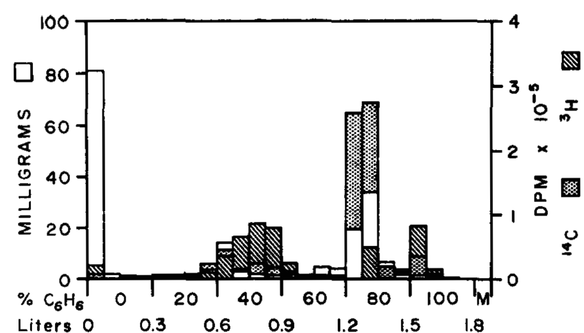

Fig. 1. Partition chromatography of the acidic fraction derived from bile from rat A in the first 2 days after administration of 5α-[4-^{14}C, 3α-^{3}H]cholestan-3β-ol. The height of the open bars *from the base line* denotes the amount of mass in the fractions; the heights of the stippled bars and hatched bars *from the base line* represent the amounts of ^{14}C and ^{3}H in the fractions, respectively. *M*, methanol.

5α-cholanate and chromatographed on an acetic acid partition column (Fig. 2); fractions 40-3 and 40-4 were combined and the residue was crystallized to constant specific activities (Table 2). On the basis of the final specific activities, methyl 3β,7α,12α-trihydroxy-5α-cholanate represents 2.43 × 10^{4} dpm of ^{14}C and 8.03 × 10^{4} dpm of ^{3}H in the diluted sample. Therefore, 3β,7α,12α-trihydroxy-5α-cholanic acid represents at least 3% of the ^{14}C and 15% of the ^{3}H in the biliary fraction recovered during the first 2 days after administration of 5α-[4-^{14}C, 3α-^{3}H]cholestan-3β-ol to rat A.

Identification of metabolites in fractions 20-4 to 60-1

Fractions 20-4 to 60-1 (Fig. 1), containing 1.32 × 10^{5} dpm of ^{14}C and 3.07 × 10^{5} dpm of ^{3}H, were combined and the residue was methylated with diazomethane. To the methylated material (22 mg) was added 49.80 mg of methyl 3α,7α-dihydroxy-5α-cholanate and 44.90 mg of methyl 3β,7α-dihydroxy-5α-cholanate. The diluted

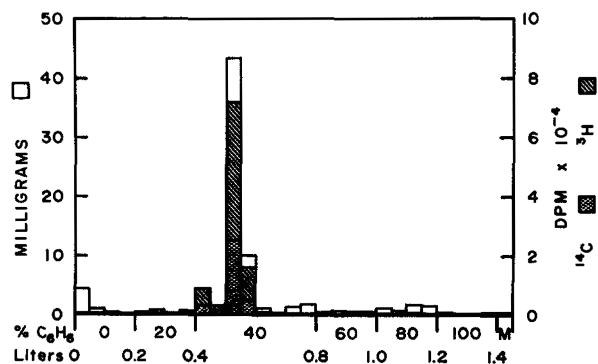

Fig. 2. Partition chromatography of the material from fractions 80-4 to 100-2 (Fig. 1) after methylation and isotopic dilution with methyl 3β,7α,12α-trihydroxy-5α-cholanate. *M*, methanol.

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TABLE 2. Isotopic dilution of the methylated metabolite from fractions 80-4, 100-1, and 100-2 (Fig. 1) with methyl 3β,7α,12α-trihydroxy-5α-cholanate

Crystallization No. and Solvent	Weight of Crystals	Weight in Mother Liquor	Specific Activities		$^3H/^{14}C$
			^{14}C	^3H	
	mg	*mg*	*dpm/mg*		
Calculated[a]	53.1		1048	1957	1.9
After partition chromatography (Fig. 2)					
Fractions 40-3 and 40-4	53.3		576	1650	2.9
1. Acetone	37.6	15.7	518	1620	3.1
2. Acetone	29.8	6.9	498	1620	3.2
3. Acetone	22.1	5.2	489	1590	3.3
4. Acetone	15.5	3.2	482	1630	3.4
5. Ethyl acetate	10.0	2.6	470	1600	3.4
6. Ethyl acetate	6.1	1.5	487	1610	3.3

[a] Methyl 3β,7α,12α-trihydroxy-5α-cholanate, 49.83 mg, was added to the combined methylated fractions that contained 3.3 mg, 5.57×10^4 dpm ^{14}C, and 1.04×10^5 dpm ^3H. If it is assumed that the amount of methyl 3β,7α,12α-trihydroxy-5α-cholanate in the fractions is negligible, then the calculated specific activities are 1118 dpm ^{14}C/mg and 2090 dpm ^3H/mg.

sample was chromatographed on an acetic acid partition column (Fig. 3). Fractions 10-1 and 10-2, which correspond to methyl 3α,7α-dihydroxy-5α-cholanate, were combined (58.6 mg) and the residue was crystallized to constant specific activity (Table 3). Since most of the 22 mg of the original combined fractions should consist of methyl chenodeoxycholate, which is difficult to crystallize (14), it would be expected to remain in the mother liquors. Accordingly, the first crop of crystals was slightly sticky, but successive crystallizations provided clean

TABLE 3. Crystallization of methyl 3α,7α-dihydroxy-5α-cholanate from the isotopic dilution of the methylated metabolites from fractions 20-4 to 60-1 (Fig. 1)

Crystallization No. and Solvent	Weight of Crystals	Weight in Mother Liquor	Melting Point of Crystals	Specific Activities	
				^{14}C	^3H
	mg	*mg*	°C	*dpm/mg* $\times 10^{-3}$	
Calculated[a]	49.8			1.01	0.15
1. Acetone–water	35.1	21.5	116–122	0.88	0.13
2. Acetone–water	22.5	11.5	119–124	0.90	0.11
3. Acetone–water	19.2	2.7	125–128	0.93	0.07
4. Acetone–water	14.3	2.7	127–129	0.92	0.07
5. Benzene–hexane	6.2	1.9	129–130	0.92	0.10

[a] Fractions 10-1 and 10-2 (Fig. 3) were combined for crystallization. The calculations are based on the assumption that the 58.6 mg present consists only of methyl chenodeoxycholate and methyl 3α,7α-dihydroxy-5α-cholanate (49.80 mg added in isotopic dilution) and that all the radioactivity in these fractions (5.02×10^4 dpm ^{14}C and 0.77×10^4 dpm ^3H) is methyl 3α,7α-dihydroxy-5α-cholanate.

crystals. The melting point of the final crop of crystals was 129–130°C (reported mp 125–126°C [3]). On the basis of the final specific activity, 3α,7α-dihydroxy-5α-cholanic acid represents at least 5.6% of the total ^{14}C in the biliary acid fraction.

Fractions 10-5 through 10-7 (Fig. 3), containing 33.8 mg, 1.10×10^4 dpm of ^{14}C and 4.72×10^4 dpm of ^3H, were combined and the residue was crystallized three times (Table 4). Partition chromatography of 5.4 mg of the third crop of crystals showed coincident elution of radioactivity and mass (Fig. 4). Although constant specific activities were not attained initially (perhaps due to low specific activities), final values of about 120 dpm of ^{14}C and 970 dpm of ^3H/mg were obtained. On the basis of these values, 3β,7α-dihydroxy-5α-cholanic acid represents 0.6% of the ^{14}C and 8% of the ^3H in the total bile acid fraction recovered during the first 2 days.

Fractions 15-1 to 20-1 (Fig. 3) contained 28 and 55% of the ^{14}C and ^3H, respectively, in the diluted sample, or 4 and 31% of the ^{14}C and ^3H, respectively, in the biliary acid fraction. The nature of these metabolites is under investigation.

DISCUSSION

These experiments were undertaken to elucidate the nature of a third unidentified acidic metabolite reported earlier after administration of 5α-[4-^{14}C]cholestanol to euthyroid and hyperthyroid rats (1, 2). Moreover, a detailed examination of the elution pattern of methyl allochenodeoxycholate obtained from the dihydroxy acid fractions (fractions 20-1 to 60-1) in these studies suggested the presence of an additional metabolite. To locate these metabolites more readily, 5α-[3β-^3H]cholestanol was added to the 5α-[4-^{14}C]cholestanol for these studies.

The unidentified metabolite more polar than allocholic acid (1, 3) appearing in fractions 80-4 to 100-2

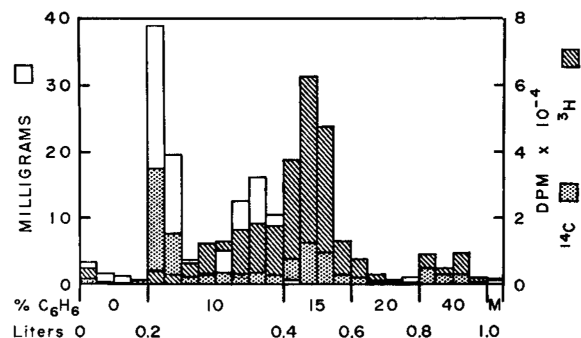

FIG. 3. Partition chromatography of the metabolites from fractions 20-4 to 60-1 (Fig. 1) after methylation and isotopic dilution with methyl 3α,7α- and 3β,7α-dihydroxy-5α-cholanates. *M*, methanol.

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TABLE 4. Crystallization of methyl 3β,7α-dihydroxy-5α-cholanate from isotopic dilution of the methylated metabolites from fractions 20-4 to 60-1 (Fig. 1)

Crystallization No. and Solvent	Weight of Crystals	Weight in Mother Liquor	Specific Activities ^{14}C	Specific Activities ^{3}H	$^{3}H/^{14}C$
	mg	*mg*	*dpm/mg*		
Calculated[a]	33.8		325	1400	4.3
1. Acetone	23.9	6.4	156	1010	6.5
2. Acetone	14.5	9.0	141	976	6.9
3. Acetone	7.9	4.3	126	937	7.4
After partition chromatography (Fig. 4)					
Fractions 10-4 to 10-7	5.6		122	972	8.0

[a] Fractions 10-5 to 10-7 (Fig. 3), which contained 33.8 mg, 1.10 × 10^4 dpm ^{14}C, and 4.72 × 10^4 dpm ^{3}H were combined and crystallized.

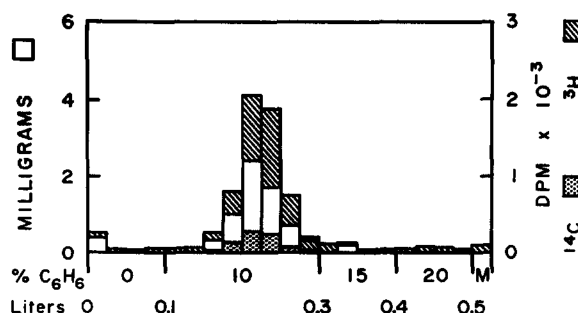

FIG. 4. Partition chromatography of methyl 3β,7α-dihydroxy-5α-cholanate obtained after three crystallizations (Table 4) of the material from fractions 10-5 to 10-7 (Fig. 3). *M*, methanol.

was obtained as 7 or 9%, respectively, of the chromatographed ^{14}C from hydrolyzed bile of the euthyroid or hyperthyroid rat. Results of the present experiment with the doubly labeled sterol show a recovery of 7% of the ^{14}C and 20% of the ^{3}H in these fractions. Identification of this metabolite as the 3β isomer of allocholic acid was facilitated by knowledge that the 3β epimers of the allo bile acids are eluted after their 3α epimers from the Celite column in acetic acid partition chromatography (11). Thus, 3β,7α,12α-trihydroxy-5α-cholanic acid, which is eluted in fractions 80-4 to 100-2 (4, 12), was identified by reverse isotopic dilution as the methyl ester before and after coincident elution of mass and isotopes from the column. Accordingly, these results confirm the identity of 3β,7α,12α-trihydroxy-5α-cholanic acid as a metabolite of cholestanol in the euthyroid and hyperthyroid rat (3).

The identification of two of the metabolites in fractions 20-4 to 60-1 was also facilitated by the doubly labeled sterol. From hydrolyzed bile of the hyperthyroid rat approximately two-thirds of the radioactive metabolites that appeared in fractions associated with dihydroxy bile acids was identified as allochenodeoxycholic acid. In the present experiments about one-third (36%) of the ^{14}C in these fractions was identified as allochenodeoxycholic acid via the methyl ester. Most of the chromatographed tritium (57%) appeared in these fractions; at least 8% of the recovered ^{3}H and 0.5% of the ^{14}C was associated with 3β,7α-dihydroxy-5α-cholanic acid. These results illustrate the separation of the methyl esters of these acids isomeric at position 3 by partition chromatography (3) and demonstrate an ability to remove methyl chenodeoxycholate from methyl allochenodeoxycholate by repeated crystallization (11). The small amount of 3β,7α-dihydroxy-5α-cholanic acid recovered with ^{14}C (0.5%) explains the absence of recognition and identification in earlier experiments in which only [4-^{14}C]cholestanol was used. Additional unidentified metabolites in the dihydroxy acid fractions contain the major amount of tritium.

The data of Table 1 reveal some aspects of the metabolism of this doubly labeled sterol. The recovery in dpm of ^{14}C and ^{3}H in the bile acid fractions for rat A [bile acid fraction/(total recovery − nonsaponifiable fraction)] was, respectively: 90 and 59%, days 1–2; 77 and 26%, days 3–4; 66 and 28%, days 5–6. The amount of ^{3}H retained in the hydrolyzed extracted bile [hydrolyzed bile/(total recovery − nonsaponifiable fraction)] was: 34%, days 1–2; 47%, days 3–4; 52%, days 5–6. In the conversion of the 3β-ol of the sterol to the predominant 3α-hydroxy allo bile acids, the 3α-^{3}H of the sterol has been removed. On the other hand, the $^{3}H/^{14}C$ ratio of the material in the neutral fraction is rather constant: 5.06, days 1–2; 5.06, days 3–4; 5.36, days 5–6. The material in this fraction from days 1–2 was identified as unchanged substrate by silver nitrate–silicic acid chromatography.

In an investigation of the conversion of cholestanol to allo bile acids by rat liver, Björkhem and Gustafsson (15) concluded that 7α-hydroxylation of cholestanol precedes epimerization of the 3β-hydroxyl group and that 12α-hydroxylation proceeded more efficiently with 5α-cholestane-3α,7α-diol than with 5α-cholestane-3β,7α-diol or 7α-hydroxy-5α-cholestan-3-one. Björkhem (16) has shown recently that the breaking of the C-H bond is not rate limiting in the microsomal 7α-hydroxylation of cholestanol or cholesterol, but is rate limiting in the conversion of cholest-5-ene-3β,7α-diol to 7α-hydroxycholest-4-en-3-one (17). In the latter case [4-^{14}C]cholest-5-ene-3β,7α-diol was oxidized to 7α-hydroxycholest-4-en-3-one about five times faster than [3α-^{3}H]cholest-5-en-3β,7α-diol. If such an isotope effect is operative with 5α-[4-^{14}C, 3α-^{3}H]cholestane-3β,7α-diol obtained from the administered sterol, more 5α-[3α-^{3}H]cholestane-3β,7α-diol would be available for the pathway to 3β,7α-dihydroxy-5α-cholanic acid than from the corresponding

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4-^{14}C diol, and hence the $^3H/^{14}C$ ratio in this dihydroxy acid should be higher than that of the substrate. Similarly, 5α-[3α-^{3}H]cholestane-3β,7α-diol should be less effectively 12α-hydroxylated than 7α-hydroxy-5α-[4-^{14}C]cholestan-3-one. 7α,12α-Dihydroxy-5α-[4-^{14}C]cholestan-3-one should be converted in part to the 3β-ol by the microsomal enzymes and thence to 3β,7α,12α-trihydroxy-5α-cholanic acid (18). Thus, this trihydroxy allo acid should have a lower $^3H/^{14}C$ ratio than the substrate. Experiments to test this hypothesis are in progress; a preliminary report regarding suitable substrates for 12α-hydroxylation in the 5α series has been made (19).

A portion of the material presented herein is taken from the dissertation submitted to the Graduate School of St. Louis University by Burton W. Noll in partial fulfillment of the requirements for the degree of Doctor of Philosophy, 1970.

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