Bile acids. XXXVIII. Conversion of 5α-cholestane-3β,7α-diol to allo bile acids by the rat

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Abstract 5α-[4-^{14}C, 3α-^{3}H]Cholestane-3β,7α-diol was prepared from individual samples of 5α-[3α-^{3}H]cholestane-3β,7α-diol and 5α-[4-^{14}C]cholestane-3β,7α-diol, each derived from 3β-acetoxycholest-5-en-7-one. Bile was collected for 11 days from adult male rats, with cannulated bile ducts, that had received intraperitoneally 0.90–0.92 mg of the doubly labeled diol. Bile from the first 10 hr, containing 63% of the administered ^{14}C and 6% of the ^{3}H, was hydrolyzed, and the bile acids were separated by acetic acid partition chromatography. Allochenodeoxycholic and allocholic acids contained at least 20.6% and 48.6%, respectively, of the ^{14}C retained in the biliary acids. Small amounts of ^{14}C (2.5% and 1.9%, respectively) were present in the 3β isomers of these acids, but the tritium content totaled more than half of that found in the bile acid fraction. No evidence was obtained for presence of the extensive quantities of the allomuricholates.

Supplementary key words 3β-hydroxy allo bile acids · allocholic acid · allochenodeoxycholic acid · allomuricholates · 5α-[4-^{14}C]cholestane-3β,7α-diol · 5α-[3α-^{3}H]cholestane-3β,7α-diol

PREVIOUS STUDIES from this laboratory have shown that 5α-cholestanol is metabolized in the rat and Mongolian gerbil principally to allocholic acid (1–3). Allochenodeoxycholic acid has been identified as a major biliary metabolite of this sterol in the hyperthyroid and in the normal rat (4, 5). Recently, the identification of the 3β isomers of these allo acids as metabolites of 5α-cholestanol in the rat has been reported (5). To explore further the mechanisms involved in the conversion of cholestanol to these allo bile acids, this paper presents studies on the formation of allo acids from 5α-[4-^{14}C, 3α-^{3}H]cholestane-3β,7α-diol. A preliminary report of some of these results has been made (6).

METHODS AND MATERIALS

Bile acids were separated by acetic acid partition chromatography (7). The fractions have been designated according to the percentage of benzene in hexane; e.g., fraction 20-4 represents the fourth fraction of the eluent containing 20% benzene in hexane. PLC and TLC were carried out as described (4) on plates coated with silica gel H or G (Brinkmann Instruments, Inc.), respectively; Adsorbosil-1 (Applied Science Laboratories, Inc.) and Supelcosil 12B (Supelco, Inc.) were also used for TLC.

Radioactivity was determined by Bray's solution as described previously (5). Elemental analyses were performed by Galbraith Laboratories, Inc., Knoxville, Tenn. Molecular weights and mass spectra of nonradioactive derivatives were obtained by mass spectrometry (4). Infrared spectra and melting points were determined as reported (4, 5). Rotations were determined with a Rudolph polarimeter, model 200-S, in 1% chloroform. Allo bile acids were obtained from available methyl cholanates (8).

5α-[3α-^{3}H]Cholestane-3β,7α-diol

5α-Cholestane-3β,7α-diol diacetate (mp 141–142°C; reported mp 138–139°C) (9) was prepared from 3β-acetoxycholest-5-en-7-one according to Wintersteiner

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

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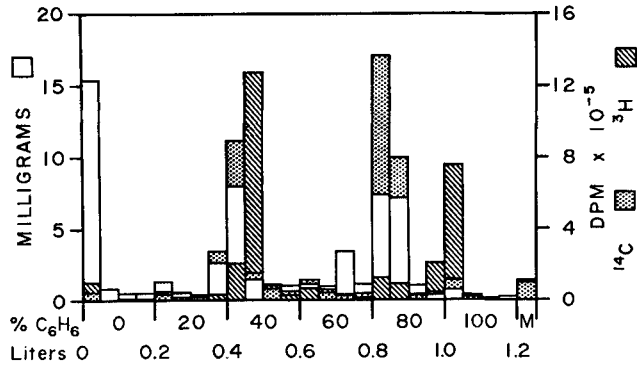

FIG. 1. Partition chromatography of the acidic fraction derived from bile obtained from rat C in the first 10 hr after the administration of 5α-[4-^{14}C, 3α-^{3}H]cholestane-3β,7α-diol. The heights of the open bars from the base line denote the amounts of mass in the fractions; the heights of the stippled bars and of the hatched bars from the base line represent the amounts of ^{14}C or ^{3}H in these fractions, respectively. *M* represents methanol wash.

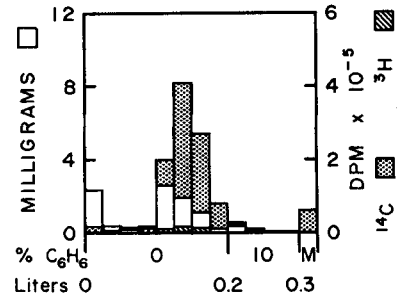

FIG. 2. Partition chromatography of the combined, methylated material from fraction 20-4 (Fig. 1) and fractions 20-4 through 20-7 after rechromatography of fraction 40-1 of Fig. 1. Bar heights and fraction *M* are defined in legend to Fig. 1.

and Moore (9). Partial hydrolysis of the diacetate (1.007 g) in 4% hot methanolic KOH provided a mixture of products from which 5α-cholestane-3β,7α-diol-7-monoacetate (768 mg, 83%) was separated by PLC in benzene–ethyl acetate 2:3. After crystallization from methanol, an analytical sample of the 7-monoacetate was obtained, mp 143–144°C.

Analysis: $C_{29}H_{50}O_3$; calculated: C, 77.97; H, 11.28
found: C, 78.20; H, 11.42

Oxidation of the monoacetate (300 mg) overnight with chromic anhydride and purification of the product by PLC in benzene–ethyl acetate 4:1 provided 230 mg of a colorless oil that showed a single spot (R_F 0.5) on TLC (benzene–ethyl acetate 4:1), intermediate in polarity between the starting material (R_F 0.2) and 3β-acetoxy-5α-cholestan-7-one (R_F 0.8). The oil was crystallized at −20°C with some difficulty from 2 ml of methanol containing one drop of water to give 162 mg of small needles, mp 86–107°C. After drying in vacuo, an analytical sample of 7α-acetoxy-5α-cholestan-3-one was obtained, mp 126–127°C.

Analysis: $C_{29}H_{48}O_3$; calculated: C, 78.33; H, 10.88
found: C, 78.58; H, 11.03

7α-Acetoxy-5α-cholestan-3-one (53 mg) was reduced with sodium [^{3}H]borohydride (20 mCi). After alkaline hydrolysis, purification of the product by PLC in 2,2,4-trimethylpentane–ethyl acetate 2:3 provided 19.8 mg of 5α-[3α-^{3}H]cholestane-3β,7α-diol (R_F 0.50) and a small amount (0.3%) of 5α-[3β-^{3}H]cholestane-3α,7α-diol (R_F 0.55). Crystallization from methanol provided 12.5 mg of the desired 3β,7α-diol (sp act 3.36 × 10^8 dpm/mg); 99.3% of the chromatographed ^{3}H appeared in a single spot (R_F 0.50) in 2,2,4-trimethylpentane–propan-2-ol–acetic acid 60:20:0.5.

5α-[4-^{14}C]Cholestane-3β,7α-diol

[4-^{14}C]Cholesterol (3.38 mg, 0.5 mCi, New England Nuclear Corp.) was diluted with 7.88 mg of nonradioactive cholesterol and purified by chromatography on a column of silver nitrate–silicic acid (4). The product was acetylated with acetic anhydride and oxidized with chromic anhydride to 3β-acetoxy-[4-^{14}C]cholest-5-en-7-one (R_F 0.50). Reduction as previously described provided 5α-[4-^{14}C]cholestane-3β,7α-diol 3-monoacetate after purification by PLC. Alkaline hydrolysis and purification of the products by PLC in benzene–ethyl acetate 2:3 provided 5α-[4-^{14}C]cholestane-3β,7α-diol (3.15 mg, 2.11 × 10^7 dpm/mg); 99.4% of the chromatographed ^{14}C appeared in a single spot (R_F 0.52) in 2,2,4-trimethylpentane–propan-2-ol–acetic acid 60:20:0.5.

5α-[4-^{14}C, 3α-^{3}H]Cholestane-3β,7α-diol

The radioactive diol used in the metabolic experiments was prepared by combination of 3.00 mg of 5α-[4-^{14}C]-cholestane-3β,7α-diol (2.11 × 10^7 dpm/mg), 1.77 mg of 5α-[3α-^{3}H]cholestane-3β,7α-diol (3.36 × 10^8 dpm/mg), and 1.24 mg of unlabeled diol; 6.01 mg of the doubly labeled material (calculated sp act, 1.05 × 10^7 dpm/mg of ^{14}C and 0.99 × 10^8 dpm of ^{3}H; ^{3}H/^{14}C = 9.4) was crystallized from methanol to provide 5 mg of material with specific activities of 1.08 × 10^7 dpm/mg of ^{14}C and 1.00 × 10^8 dpm of ^{3}H.

RESULTS

Metabolism of 5α-[4-^{14}C, 3α-^{3}H]cholestane-3β,7α-diol

5α-[4-^{14}C, 3α-^{3}H]Cholestane-3β,7α-diol was administered intraperitoneally to two adult male rats with bile fistulas immediately after surgery while the animals were still anesthetized. Rat C (365 g) received 0.90 mg (9.5 × 10^6 dpm of ^{14}C, 9.0 × 10^7 dpm of ^{3}H) and rat D (381 g) received 0.92 mg (9.7 × 10^6 dpm of ^{14}C, 9.2 × 10^7 dpm of ^{3}H). The solutions for injection contained 1 mg of sterol dissolved in 0.1 ml of warm ethanol, 80–120 mg of Tween

TABLE 1. Isotopic dilution of the methylated metabolite from fractions 20-4 and 40-1 (Fig. 1) with methyl allochenodeoxycholate

Crystallization No. and Solvent	Weight of Crystals	Specific Activity	
		^{14}C	^{3}H
	mg	*dpm/mg* × 10^{-4}	
Calculated value[a]	65.5	1.41	0.00
1. Acetone–water	48.1	1.58	0.05
2. Acetone–water	39.6	1.59	0.06
3. Methanol–water	26.0	1.59	0.05

[a] Authentic methyl allochenodeoxycholate, 57.53 mg, was added to 9.27 × 10^5 dpm of ^{14}C associated with 8.0 mg of mass. If it is assumed that the amount of methyl allochenodeoxycholate in the fractions is negligible, the calculated specific activity is 1.61 × 10^4 dpm of ^{14}C/mg.

TABLE 2. Isotopic dilution of the methylated metabolite from fractions 40-2 and 40-1 (Fig. 1) with methyl 3β,7α-dihydroxy-5α-cholanate

Crystallization No. and Solvent	Weight of Crystals	Specific Activity			
		dpm/mg		dpm/mmole	
		^{14}C × 10^{-3}	^{3}H × 10^{-4}	^{14}C × 10^{-6}	^{3}H × 10^{-7}
	mg				
Calculated value[a]	34.36	6.26	4.19	2.54	1.70
Methyl ester					
1. Acetone–hexane	27.9	3.92	3.97	1.59	1.62
2. Acetone–hexane	25.1	3.88	4.00	1.58	1.62
Free acid after hydrolysis and chromatography					
3. Acetone	8.2	3.50	3.94	1.37	1.54
Methyl ester					
4. Acetone–hexane	5.3	3.37	3.84	1.37	1.56
5. Acetone–hexane	4.8	3.55	3.93	1.44	1.60

[a] Methyl 3β,7α-dihydroxy-5α-cholanate, 31.86 mg, was added to combined methylated fractions that contained 2.5 mg, 2.15 × 10^5 dpm of ^{14}C, and 1.44 × 10^6 dpm of ^{3}H, and the mixture was chromatographed before crystallization.

80, and sufficient physiological saline to make a volume of 1 ml. Some radioactivity exuded from the abdomen at the site of incision of rat D as detected by a laboratory monitor. Bile samples were collected 10 hr after administration of radioactivity and at 12-hr intervals thereafter until day 5, after which 24-hr samples were taken through day 11.

Bile from rat C in the first 10 hr containing 5.96 × 10^6 dpm of ^{14}C (63% of the injected ^{14}C) and 5.37 × 10^6 dpm of ^{3}H (6% of the injected ^{3}H) was hydrolyzed (5) and fractionated with the following distribution of radioactivity: acidic fraction (84% ^{14}C and 60% ^{3}H); nonsaponifiable fraction (0.6% ^{14}C and 0.7% ^{3}H); water wash of the latter fraction (0.2% ^{14}C and ^{3}H, respectively); hydrolyzed, extracted bile (4.7% ^{14}C and 26.6% ^{3}H). The acidic material (55.6 mg; 4.44 × 10^6 dpm of ^{14}C, 3.28 × 10^6 dpm of ^{3}H) was chromatographed on an acetic acid partition column (Fig. 1). Only 3% of the chromatographed ^{3}H and 1% of ^{14}C appeared in the region in which monohydroxy bile acids are eluted (fraction 0-1). Fractions 20-4 and 40-1, which correspond to allochenodeoxycholic acid, contained 26% of the chromatographed ^{14}C and 7.2% of the ^{3}H; fractions 40-2 and 40-3, which correspond to 3β,7α-dihydroxy-5α-cholanic acid, contained 47% of the ^{3}H and 5% of the ^{14}C; fractions 80-1 and 80-2, corresponding to allocholic acid, contained 49% of the ^{14}C and 6% of the ^{3}H; and fractions 80-4 to 100-2, corresponding to 3β,7α,12α-trihydroxy-5α-cholanic acid, contained 29% of the ^{3}H and 3% of the ^{14}C.

Identification of metabolites in fractions 20-4 and 40-2

Since the material in fraction 40-1 (Fig. 1; 7.9 mg, 8.71 × 10^5 dpm of ^{14}C, 2.00 × 10^5 dpm of ^{3}H) probably contained some of the tritiated metabolites in fractions 40-2 and 40-3, the residue of fraction 40-1 was rechromatographed and eight fractions instead of the usual four were collected with 20% benzene in hexane. Material from fractions 20-4 through 20-7 was combined with residue from fraction 20-4 (Fig. 1) and methylated with diazomethane (11.1 mg; 1.04 × 10^6 dpm of ^{14}C, 0.08 × 10^6 dpm of ^{3}H; $^{3}H/^{14}C$ = 0.08), and the ester was rechromatographed (Fig. 2). Fractions 0-5 through 10-1 (Fig. 2) were combined (8.0 mg), diluted with methyl allochenodeoxycholate (57.53 mg), and crystallized successively from different solvents to constant specific activity (1.59 × 10^4 dpm/mg ^{14}C and 0.05 × 10^4 dpm/mg ^{3}H) (Table 1). Assuming no measurable mass associated with the radioactivity, the calculated specific activity for ^{14}C agrees with the value found. Accordingly, allochenodeoxycholic acid represents 80% of the ^{14}C in fractions 20-4 and 40-1 (Fig. 1) or 20.6% of the ^{14}C and 0.85% of the ^{3}H in the biliary acid fraction.

Fractions 40-2 (Fig. 1) and 20-8 to 40-2 from the rechromatography of fraction 40-1 above were combined; the residue was methylated (2.5 mg; 2.15 × 10^5 dpm of ^{14}C, 1.44 × 10^6 dpm of ^{3}H; $^{3}H/^{14}C$ = 6.7), diluted with authentic methyl 3β,7α-dihydroxy-5α-cholanate (31.86 mg), and chromatographed. The column was eluted with 0, 10, and 15% benzene in hexane. Mass and radioactivity were eluted coincidentally in fractions 10-4 to 15-3. The material in these fractions was combined, crystallized twice (Table 2), and hydrolyzed, and the free acid was chromatographed. Mass and radioactivity were eluted coincidentally in fractions 40-1 to 60-1. After crystallization of the acid from acetone, the material was remethylated, and the ester was crystallized twice from acetone–hexane. The data in Table 2 show a significant drop in specific activity of the ^{14}C compared with the tritium before constant specific activities were achieved. Based on final specific activities of 3.55 × 10^3 dpm/mg ^{14}C and 3.93 × 10^4 dpm/mg ^{3}H ($^{3}H/^{14}C$ =

TABLE 3. Isotopic dilution of the methylated metabolite from fractions 80-1 and 80-2 (Fig. 1) with methyl allocholate

Crystallization No. and Solvent	Weight of Crystals	Specific Activity
	mg	*dpm/mg* × 10^{-4}
Calculated value[a]	58.08	1.97
1. Acetone–hexane	35.1	2.01
2. Acetone–hexane	27.4	2.00
3. Ethyl acetate–benzene	24.8	1.99
After PLC and partition chromatography[b]		
4. Acetone	20.0	2.14
5. Acetone	13.2	2.12
6. Ethyl acetate	9.6	2.13
7. Ethyl acetate	8.0	2.13

[a] An aliquot (1/2) of the combined fractions 80-1 and 80-2 (Fig. 1), which contained 7.7 mg, 1.14 × 10^6 dpm of ^{14}C, and 5 × 10^4 dpm of ^3H was methylated, added to 50.38 mg of methyl allocholate, and chromatographed before crystallization. If all the radioactivity is in methyl allocholate and the mass originally present in the fractions will be removed from added mass, the specific activity will be 2.26 × 10^4 dpm.

[b] The third crystals and all the mother liquors were recombined and subjected to repeated PLC in solvent system S6 (10) to remove methyl cholate. A sample, 35.7 mg, nearly pure by TLC, was subjected to partition chromatography before further crystallization.

11.0), 3β,7α-dihydroxy-5α-cholanic acid accounts for 2.5% of the ^{14}C and 36.9% of the tritium recovered in the biliary acid fraction.

Investigation of the metabolite in fractions 80-1 and 80-2

An aliquot (7.7 mg) of fractions 80-1 and 80-2 was methylated with diazomethane, the residue was added to 50.38 mg of methyl allocholate, and the mixture was chromatographed. Fractions 40-3 to 60-1, which contained at least 96% of the chromatographed ^{14}C and 93% of the chromatographed mass, were combined, and the residue was crystallized from two different solvents with no significant change in specific activity (Table 3). Since methyl allocholate and methyl cholate cocrystallize (8), the third crop of crystals and the residue from the mother liquors were combined and subjected to PLC in cyclohexane–ethyl acetate–acetic acid 7:23:3 (10) to remove methyl cholate (R_F 0.41). A sample of 35.7 mg corresponding to methyl allocholate (R_F 0.32) and free from methyl cholate was then purified by partition chromatography, and the residue from fractions 40-3 to 60-1 was combined and crystallized twice from acetone and twice from ethyl acetate (Table 3) to provide material with a constant specific activity of 2.13 × 10^4 dpm/mg. Thus, at least 2.16 × 10^6 dpm of ^{14}C was in allocholic acid and represented 48.6% of the ^{14}C in the biliary acid fraction. Tritium was not detected in samples from the last five crystallizations.

Investigation of the metabolite in fractions 80-4 and 100-1

Fractions 80-4 and 100-1 (Fig. 1), which contained 1 mg, 1.5 × 10^5 dpm of ^{14}C, and 9.56 × 10^5 dpm of ^3H, were combined, the residue was added to 42.20 mg of authentic 3β,7α,12α-trihydroxy-5α-cholanic acid, and the mixture was chromatographed. Due to a technical error a portion of the material was lost, but the residue from fractions 100-1 to 100-4 was combined and crystallized from acetone (Table 4). The acid was treated with diazomethane, and the material was rechromatographed. The residues in fractions 60-1 through 60-4 (32.2 mg, 6.26 × 10^4 dpm of ^{14}C, 5.44 × 10^5 dpm of ^3H; $^3H/^{14}C$ = 8.7) were combined and crystallized three times with little change in specific activities (Table 4). Thus,

TABLE 4. Isotopic dilution of the metabolites from fractions 80-4 and 100-1 (Fig. 1) with 3β,7α,12α-trihydroxy-5α-cholanic acid

Crystallization No. and Solvent	Weight of Crystals	Specific Activity dpm/mg ^{14}C × 10^{-3}	Specific Activity dpm/mg ^3H × 10^{-4}	Specific Activity dpm/mmole ^{14}C × 10^{-5}	Specific Activity dpm/mmole ^3H × 10^{-6}	$^3H/^{14}C$
	mg					
Calculated value[a]	43.2	3.52	2.25	14.9	9.49	6.4
Free acid	41.5	2.22	1.63	9.07	6.66	7.3
1. Acetone	34.4	2.01	1.66	8.21	6.78	8.3
After methylation and chromatography of the ester	32.2	1.94	1.69	8.22	7.14	8.7
2. Acetone–hexane	24.1	1.98	1.63	8.37	6.89	8.2
3. Acetone–hexane	18.8	2.00	1.65	8.43	7.02	8.3
4. Ethyl acetate–benzene	5.4	1.93	1.60	8.16	6.76	8.3

[a] 3β,7α,12α-Trihydroxy-5α-cholanic acid, 42.20 mg, was added to the combined material from fractions 80-4 and 100-1 (Fig. 1), which contained 1 mg, 1.52 × 10^6 dpm of ^{14}C, and 9.73 × 10^6 dpm of ^3H, and the mixture was chromatographed before crystallization.

3β,7α,12α-trihydroxy-5α-cholanic acid represents at least 1.9% of the ^{14}C and 21.8% of the tritium in the biliary acid fraction.

DISCUSSION

The metabolism of 5α-cholestane-3β,7α-diol was studied in the rat with a cannulated bile duct in order to provide additional information on a mechanism of formation of allo bile acids. 5α-[4-^{14}C, 3α-^{3}H]Cholestane-3β,7α-diol was prepared to facilitate comparison with doubly labeled cholestanol (5), to amplify differences in their metabolism, and to shed light on metabolic pathways not readily seen with singly labeled substrates. That the diol is metabolized more rapidly than cholestanol is apparent from the quantities of isotopes excreted in bile within 10 hr. After intraperitoneal injection of the doubly labeled diol, 63% of the injected ^{14}C and 6% of the ^{3}H were recovered in bile within 10 hr; within 22 hr the values were 84% and 8.9% for ^{14}C and ^{3}H, respectively (rat C). The values for rat D are somewhat lower because of loss of substrate through the site of incision (28.6% ^{14}C and 2% ^{3}H in 10 hr and 39.6% ^{14}C and 3.8% ^{3}H in 22 hr). Upon alkaline hydrolysis of the bile from the first 10 hr (rat C), 84% of the biliary ^{14}C and 60% of the ^{3}H remained in the acid fraction, whereas only 0.6% and 0.7% of ^{14}C and ^{3}H, respectively, were retained in the nonsaponifiable fraction. As previously reported (5), after intraperitoneal administration of comparable amounts of doubly labeled cholestanol, rat A excreted only 14.2% of injected ^{14}C and 3.9% of ^{3}H in 2 days and 25.8% ^{14}C and 9.4% ^{3}H in 4 days; values for rat B were somewhat lower due to loss of substrate at the incision. After alkaline hydrolysis of bile from rat A (2 days), 78% of the biliary ^{14}C and 41.5% of the ^{3}H were retained in the acid fraction, but 8% of the ^{14}C and 32% of the ^{3}H were found in the nonsaponifiable fraction. Thus, considerably less neutral material remained in the nonsaponifiable fraction from the diol after only 10 hr (rat C) compared with that from cholestanol after 2 days (rat A). Alternately, more biliary ^{14}C and ^{3}H remained in the acid fraction derived from the diol than the stanol.

Analysis of the bile acid fraction showed that the diol provided more allochenodeoxycholic acid (20.6%) than cholestanol (5.6%), but less allocholic acid (48.6%) than the stanol (about 70%) (1, 5). Significantly more 3β,7α-dihydroxy-5α-cholanic acid was formed from the diol (2.5% as ^{14}C) compared with cholestanol (0.6% as ^{14}C). The ratio of ^{3}H to ^{14}C in 3β,7α-dihydroxy-5α-cholanic acid (11.0) is higher than that of the administered diol (9.4), while that of 3β,7α,12α-trihydroxy-5α-cholanic acid (8.3) is slightly lower than the substrate. These observations coincide with the predictions made earlier (5), based on an isotope effect of the 3α-^{3}H of the diol, relative to the observation of Björkhem (11), who reported a fivefold difference in rate of microsomal oxidation of [4-^{14}C]cholest-5-en-3β,7α-diol compared with [3α-^{3}H]-cholest-5-en-3β,7α-diol. To establish that this isotope effect is real with cholestane-3β,7α-diol, the rates of dehydrogenation of the ^{3}H and the ^{14}C components of the substrate should be measured independently.

Since this study was completed, Björkhem and Gustafsson (12) have proposed that allochenodeoxycholic and allocholic acids may be derived from 5α-cholestanol via 7α-hydroxylation to 5α-cholestane-3β,7α-diol, dehydrogenation to 7α-hydroxy-5α-cholestan-3-one, and microsomal reduction to the key intermediate, 5α-cholestane-3α,7α-diol, a pathway consistent with our results. However, they found only 11% of the administered dose of 5α-[6β-^{3}H]cholestane-3β,7α-diol excreted in the first 24 hr in bile. Administration of the sterol as an emulsion stabilized with serum albumin may contribute to this striking difference in rate of excretion of biliary metabolites.

Finally, it should be noted that fractions containing 4% ^{14}C and 31% ^{3}H as unidentified metabolites from doubly labeled cholestanol that were more polar than allochenodeoxycholic acid (5) were not found as metabolites of the doubly labeled diol. Although chenodeoxycholic acid is metabolized to α- and β-muricholic acids in the rat (13), allochenodeoxycholic acid is not similarly converted to appreciable amounts of allomuricholic acids (14). No evidence for the presence of extensive amounts of allomuricholates (15) was found among the metabolites of 5α-cholestane-3β,7α-diol, demonstrating a significant difference in the metabolism by the rat of the 3,7-dihydroxy-5α-cholestane nucleus as compared with the 5β epimers (16).

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