Sterol synthesis: studies of the metabolism of 14α-methyl-5α-cholest-7-en-3β-ol

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Abstract [3α-^{3}H]14α-Methyl-5α-cholest-7-en-3β-ol has been prepared by chemical synthesis. The metabolism of this compound has been studied in the 10,000 *g* supernatant fraction of liver homogenates of female rats. Efficient conversion to cholesterol was observed. Other labeled compounds recovered after incubation of [3α-^{3}H]14α-methyl-5α-cholest-7-en-3β-ol with the enzyme preparations include the unreacted substrate, 5α-cholesta-7,14-dien-3β-ol, 5α-cholesta-8,14-dien-3β-ol, cholesta-5,7-dien-3β-ol, 5α-cholest-8(14)-en-3β-ol, 5α-cholest-8-en-3β-ol, and 5α-cholest-7-en-3β-ol. In addition, significant amounts of incubated radioactivity were recovered in steryl esters. The steroidal components of these esters were found to contain labeled 14α-methyl-5α-cholest-7-en-3β-ol, 5α-cholesta-8,14-dien-3β-ol, 5α-cholesta-7,14-dien-3β-ol, 5α-cholest-8-en-3β-ol, 5α-cholest-7-en-3β-ol, and cholesterol.—**Chan, J. T., T. E. Spike, S. T. Trowbridge, and G. J. Schroepfer, Jr.** Sterol synthesis: studies of the metabolism of 14α-methyl-5α-cholest-7-en-3β-ol. *J. Lipid Res.* 1979. **20:** 1007–1019.

Supplementary key words sterol metabolism · biosynthesis of cholesterol

The enzymatic conversion of lanosterol (4α,4β,14α-trimethyl-cholesta-8,24-dien-3β-ol)[2] to cholesterol involves three general processes: reduction of the Δ^{24}-double bond, "shift" of the nuclear double bond from the Δ^8-position in lanosterol to the Δ^5-position in cholesterol, and removal of the three "extra" methyl groups at carbon atoms 4 and 14 of lanosterol. Until recently, available evidence suggested that the removal of the three extra methyl groups proceeded by initial removal of the 14α-methyl group. This suggestion rested on the fact that none of the sterols isolated from animal tissues (1, 2) were assigned structures corresponding to 4,14-dimethyl-cholestenols or 14α-methyl-cholestenols. However, the reported isolations of a number of 4α,14α-dimethyl-cholestenols and 14α-methyl-sterols from a variety of plant sources and yeast mutants (3–9) and the isolation of 4α,14α-dimethyl-cholest-7-en-3β-ol and 4α,14α-dimethyl-cholest-8-en-3β-ol from feces and meconium of newborn infants (10) suggest that removal of the three extra methyl groups of lanosterol can be initiated by removal of either the 4α-methyl function (11–13 and references cited therein) or the 14α-methyl group. In addition, Knight, Klein, and Szczepanik (14) have demonstrated the convertibility of 14α-methyl-cholest-7-en-3β-ol to cholesterol in rat liver homogenate preparations. Martin, Huntoon, and Schroepfer (15) have demonstrated the convertibility of another 14α-methyl sterol (14α-methyl-cholest-7-en-3β,15β-diol) (15, 16) to cholesterol in rat liver homogenates.

The purpose of the present study was to investigate in more detail the metabolism of 14α-methyl-cholest-7-en-3β-ol. Preliminary accounts of portions of this work have been presented (17–20).

EXPERIMENTAL PROCEDURES AND RESULTS

General procedures

Melting points were recorded in sealed, evacuated capillary tubes using a Thomas Hoover melting point apparatus and are uncorrected. NMR spectra were recorded in $CDCl_3$ on a Varian HR-220 spectrometer using tetramethylsilane as an internal standard. Infrared spectra were recorded on a Perkin-Elmer Model 521 grating spectrophotometer using KBr pellets. Mass spectra were recorded on a Varian MAT CH-5 mass spectrometer under the following general operating conditions: ionization energy, 70 eV; accelerating voltage, 3 Kv; and emission current, 100 μA. Molecular ions were confirmed by low electron voltage scans at 8–12 eV. Samples were introduced via the direct probe method. Radioactivity was measured in a Beckman LS-250 liquid scintillation

Abbreviations: TLC, thin-layer chromatography; GLC, gas–liquid chromatography; NMR, nuclear magnetic resonance.

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[2] The configuration of the hydrogen at carbon atom 5 in the various sterols mentioned in this report is α. The designation of the configuration as 5α has been omitted throughout the text to conserve space.

spectrometer as described previously (21). GLC analyses were made on columns of 3% QF-1 or 1% SE-30 on Gas Chrom Q (100–120 mesh) using a Hewlett-Packard Model 402 instrument or a Barber-Colman model 5000 instrument. Analyses of the radioactivity of the effluents from GLC columns were carried out as described previously (21). Unless stated otherwise, analytical TLC was carried out on plates of silica gel G (E. Merck, Darmstadt). Preparative TLC was carried out on plates of silica gel $PF_{254+366}$ (Brinkmann Instruments, Inc., Westbury, NY). Analyses of the radioactivity on thin-layer chromatoplates were carried out either as described elsewhere (21) or using a Packard radiochromatogram scanner. Colorimetric analyses of sterols and steryl acetates were carried out using the Liebermann-Burchard reagent described by Abell et al. (22) with readings of absorbance at times of maximum color development for the individual compounds in question (21, 23–26). Alumina–Super Cel–$AgNO_3$ columns and silica gel G–Super Cel–$AgNO_3$ columns were prepared as previously described (26, 27).

Chromatography on silicic acid–Super Cel 2:1 columns, using 10% ether in benzene as the eluting solvent, was employed to separate sterol esters, sterols, and polar sterols. Chromatography on silica gel G–Super Cel–$AgNO_3$ columns, employing hexane–benzene 7:3 as the eluting solvent, was employed to separate the acetates of monounsaturated sterols from those of diunsaturated sterols and also to separate the various diunsaturated steryl acetates from each other. Unless stated otherwise, alumina–Super Cel–$AgNO_3$ columns, using benzene–hexane 90:10 as the eluting solvent, were used to separate the acetate derivatives of the various monounsaturated sterols from one another. All columns were 100 cm in length and 1 cm in diameter. Radioactive cholesterol was purified by way of the dibromide by a modification (28) of the method of Fieser (29).

Female rats of the Sprague-Dawley strain weighing between 100 and 150 g were used in this study. Unless noted otherwise, the excised livers were homogenized in 0.1 M potassium phosphate buffer (pH 7.4; 2.5 ml per g of liver) using a loose fitting Teflon-on-glass homogenizer. Unbroken cells and nuclei were removed by centrifugation at 500 *g* for 15 min. The resulting supernatant fraction was recentrifuged at 10,000 *g* for 30 min. The resulting supernatant was used as a source of enzyme in this study.

Materials

Sodium borotritide was purchased from New England Nuclear Corporation. The preparation of 3β-acetoxy-cholest-8-ene, 3β-acetoxy-cholest-8(14)-ene, 3β-acetoxy-cholest-7-ene, 3β-acetoxy-cholest-5-ene, 3β-acetoxy-cholesta-8,14-diene, 3β-acetoxy-cholesta-7,14-diene, and 3β-acetoxy-cholesta-5,7-diene has been described previously (21, 23–26). 3β-Benzoyloxy-14α-methyl-cholest-7-en-15-one was prepared as described previously (15, 16). [1,2-^{3}H]Cholesterol (137 mCi per mg) was purchased from New England Nuclear Corporation and purified by chromatography on a 2:1 silicic acid–Super Cel column (50 cm × 1 cm) using benzene as the eluting solvent. The purified [^{3}H]cholesterol showed a single radioactive component upon radio-TLC (solvent systems: $CHCl_3$ and 35% ethyl acetate in $CHCl_3$) and, after dilution with authentic unlabeled cholesterol, was purified by way of the dibromide without significant change in specific activity.

14α-Methyl-cholest-7-en-3β-ol

Sodium (0.2 g) was dissolved in diethylene glycol (20 ml; freshly distilled) and the resulting solution was heated to 180°C under a nitrogen atmosphere. Hydrazine (previously dried by refluxing with an equal weight of KOH overnight) was distilled into the solution until the glycol began to reflux at 180°C. After cooling the resulting solution to 80°C, 3β-benzoyloxy-14α-methyl-cholest-8(14)-en-15-one (400 mg) was added and the temperature of the resulting mixture was again raised to reflux with vigorous stirring under a nitrogen atmosphere. After refluxing for 24 hr under these conditions, the hydrazine was allowed to distill from the solution until the temperature reached 210°C. The mixture was then heated under reflux at 210°C for 25 hr and then cooled to room temperature. Water (100 ml) was added and the resulting mixture was extracted four times with ether (150-ml portions). The combined ether extract was washed with a saturated solution of NaCl (100 ml), dried over anhydrous Na_2SO_4, and evaporated to dryness. The residue (400 mg) was subjected to preparative TLC using benzene–ethyl acetate 2:1 as the developing solvent. Material with an R_f of ~0.5 was scraped from the plate and eluted with $CHCl_3$, yielding 250 mg of 14α-methyl-cholest-7-en-3β-ol[3] (81% yield). Recrystallization from $CHCl_3$–methanol gave long white needles melting at 128–129°C

[3] Repeated synthetic runs gave variable amounts (trace to 30%) of an impurity which was inseparable from 14α-methyl-cholest-7-en-3β-ol as the free alcohol or as the acetate ester on plates of silica gel H or of silica gel $PF_{254+366}$ or on columns of silica gel or alumina or either of these supports impregnated with silver nitrate. The unknown compound was eluted prior to 14α-methyl-cholest-7-en-3β-ol on gas–liquid chromatography on a 3% QF-1 column. Combined gas–liquid chromatography–mass spectrometry indicated a molecular weight of 398.

(literature: 128–130°C (14)). The compound gave a single component on TLC on a silica gel H plate (solvent, benzene–ethyl acetate 2:1). On GLC analysis (3% QF-1; 220°C) the retention time (relative to cholestane) was 3.67. The NMR spectrum had an absorption at 5.20 ppm due to the C(7)-olefinic proton and a complex multiplet due to the 3α-proton at 3.60 ppm. The absence of aromatic protons confirmed the absence of benzoyl function. The infrared spectrum was compatible with the assigned structure and showed no absorption due to a carbonyl function. The low-resolution mass spectrum showed a molecular ion at *m/z* 400. Analysis of the low-resolution mass spectra indicated the following prominent ions in the high mass region of the spectra, their relative intensities, and their probable mode of origin: *m/z* 400 (28%; M); *m/z* 385 (100%; M − CH_3); *m/z* 367 (54%; M − CH_3 − H_2O); *m/z* 287 (4%; M − alkyl side chain); *m/z* 273 (11%); *m/z* 269 (3%; M − H_2O − alkyl side chain); *m/z* 259 (22%); *m/z* 255 (4%); and *m/z* 245 (21%).

14α-Methyl-cholest-7-en-3β-ol, upon treatment with the Liebermann-Burchard reagent under the conditions described by Abell et al. (22) yields a yellow color with an absorbance maximum at 460 nm. The time course of color development showed that maximum absorbance at 460 nm was achieved approximately 10 min after the addition of the reagent and that the absorbance at this wave length was stable for approximately 30 min.

14α-Methyl-cholest-7-en-3-one

14α-Methyl-cholest-7-en-3β-ol (130 mg) was dissolved in dry pyridine (1 ml) and cooled to 4°C. A solution of chromium trioxide (130 mg) in dry pyridine (7 ml), previously chilled to 4°C, was added and the reaction mixture was allowed to stand for 65 hr at 4°C. Cold water (200 ml) was added and the resulting mixture was extracted five times with ether (200-ml portions). The combined extract was washed twice with a saturated NaCl solution, dried over anhydrous Na_2SO_4, and evaporated to dryness. The residue was subjected to preparative TLC using benzene–ethyl acetate 2:1 as the developing solvent.

Unreacted starting material (40 mg) was recovered from material with an R_f of ~0.95 and crystallized from $CHCl_3$–methanol in the form of long white needles melting at 146–147°C (literature: 147–148°C (14)). GLC analysis (3% QF-1; 218°C) gave a retention time (relative to cholestane) of 7.0 and indicated a purity in excess of 97%. Mass spectral analysis showed a molecular ion at *m/z* 398 and a base peak at *m/z* 383 (M − CH_3). The NMR spectrum showed the absence of a carbinol proton absorption and retention of the C(7)-olefinic proton resonance. Infrared spectroscopy confirmed the presence of the ketone function by the absorbance at 1720 cm^{-1}.

[3α-^{3}H]14α-Methyl-cholest-7-en-3β-ol

To 14α-methyl-cholest-7-en-3-one (100 mg) in ethanol (40 ml) was added sodium borotritide (10.5 mg; 70 mCi). After 3 hr at room temperature, water (100 ml) was added and the resulting mixture was extracted three times with ether (200-ml portions). The combined ether extracts were washed with a saturated solution of NaCl, dried over anhydrous Na_2SO_4, and evaporated to dryness. The residue was dissolved in a minimal amount of benzene and subjected to preparative TLC. After development of the plate with benzene–ethyl acetate 2:1, three bands were noted which corresponded to the unreacted ketone (13.9 mg), the 3α-alcohol (11.9 mg), and the 3β-alcohol (34 mg). The [3α-^{3}H]14α-methyl-cholest-7-en-3β-ol had a specific activity of 1.08×10^8 cpm per mg. This material showed a single labeled component with the same mobility as authentic 14α-methyl-cholest-7-en-3β-ol on *1*) radio-TLC analysis in three solvent systems (benzene, chloroform–acetone 95:5, and benzene–ethyl acetate 2:1, *2*) radio-GLC analysis (3% QF-1 and 1% SE-30), and *3*) column chromatographic analysis on an alumina–Super Cel–$AgNO_3$ column ($CHCl_3$–acetone 98:2) (**Fig. 1**). The labeled compound also showed a single radioactive component when subjected to chromatography on a silicic acid–Super Cel column using benzene as the eluting solvent (**Fig. 2**).

Incubations of [3α-^{3}H]14α-Methyl-cholest-7-en-3β-ol with rat liver homogenate preparations

Experiments I, II, and III

The purposes of these experiments were to evaluate in detail the metabolism of labeled 14α-methyl-cholest-7-en-3β-ol upon incubation with the 10,000 *g* supernatant fraction of rat liver homogenates and to attempt to identify each of the metabolites formed from the labeled substrate.

[3α-^{3}H]14α-Methyl-cholest-7-en-3β-ol (111 μg; 1.2×10^7 cpm) in propylene glycol (0.2 ml) was incubated under aerobic conditions for 3 hr at 37°C with a 10,000 *g* supernatant fraction (20 ml) of a rat liver homogenate preparation. The enzyme preparation was fortified with NAD (1 mM), NADP (1 mM), glucose-6-phosphate (3 mM), nicotinamide (30 mM), and $MgCl_2$ (5 mM). At the end of the incubation period 15% ethanolic KOH (20 ml) was added. The resulting mixture was heated for one hr on a steam bath and, after cooling to room temperature, was

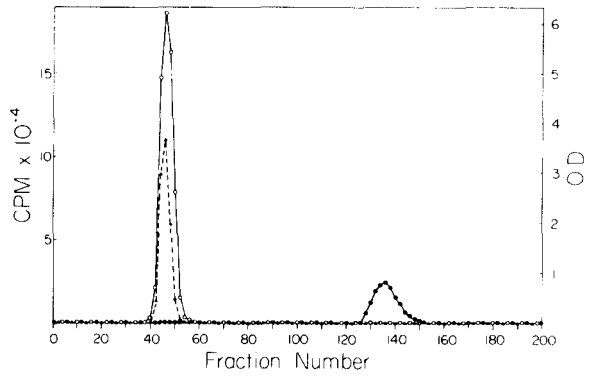

Fig. 1. Alumina–Super Cel–silver nitrate column (100 cm × 1 cm) chromatography of [3α-^{3}H]14α-methyl-cholest-7-en-3β-ol. Chloroform–acetone 98:2 was used as the eluting solvent (flow rate 0.09 ml per min). ▲– – –▲, Radioactivity; ○——○, authentic unlabeled 14α-methyl-cholest-7-en-3β-ol, determined colorimetrically; ●——●, authentic unlabeled cholesterol, determined colorimetrically.

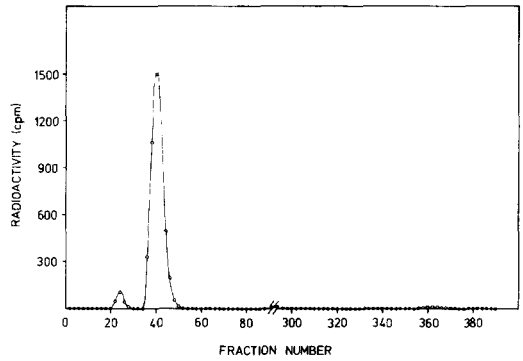

Fig. 3. Silicic acid–Super Cel column (100 cm × 1 cm) chromatography of labeled material recovered after incubation of [3α-^{3}H]-14α-methyl-cholest-7-en-3β-ol with a 10,000 *g* supernatant fraction of a rat liver homogenate preparation (Experiment I). The eluting solvent was 10% ether in benzene (flow rate, 0.13 ml per min). ○——○, Radioactivity.

extracted twice with petroleum ether (40-ml portions) and twice with ether (50-ml portions). The combined extracts were dried over anhydrous Na_2SO_4 and the solvent was evaporated under nitrogen. The residue was dissolved in benzene and applied to a silicic acid–Super Cel column. Fractions 2.6 ml in volume (20 min per fraction) were collected.

The resulting chromatogram is shown in **Fig. 3.** Three distinct peaks of radioactivity were noted. The contents of fractions 20–29 contained 2.8×10^5 cpm and showed the same chromatographic mobility as cholesteryl palmitate on radio-TLC analysis (**Fig. 4**). This material was designated as "unhydrolyzed steryl esters" (vide infra). The contents of fractions 340–384, corresponding to a broad but discrete peak of radioactivity, contained 9.8×10^4 cpm and was designated as "polar sterols".

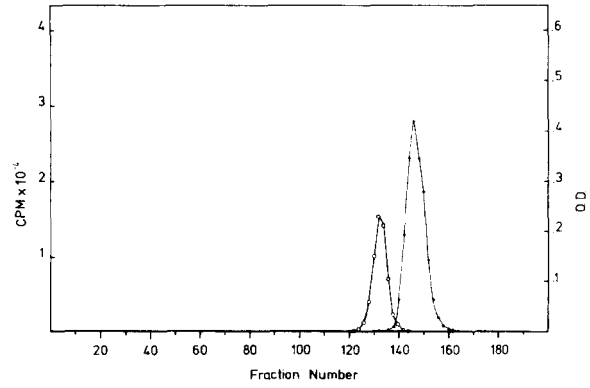

Fig. 2. Silicic acid–Super Cel column (100 cm × 1 cm) chromatography of [3α-^{3}H]14α-methyl-cholest-7-en-3β-ol. The eluting solvent was benzene (flow rate, 2.0 ml per min). ●——●, Radioactivity; ○——○, authentic unlabeled cholesterol determined colorimetrically.

The bulk (96.3%) of the radioactivity was eluted as a sharp peak centered at fraction 40. This material (fractions 30–70) contained 9.7×10^6 cpm and was designated as "free monohydroxysterols". This material was acetylated with acetic anhydride and pyridine as described previously (21) and the resulting acetates were applied to a silica gel G–Super Cel–$AgNO_3$ column along with unlabeled 3β-acetoxy-cholesta-5,7-diene, 3β-acetoxy-cholesta-7,14-diene, and 3β-acetoxy-cholesta-8,14-diene. Fractions 3.5 ml in volume (20 min per fraction) were collected. The resulting chromatogram is shown in **Fig. 5.**

The material that eluted as the first peak (center at fraction 41) corresponded in mobility to that of authentic 3β-acetoxy-14α-methyl-cholest-7-ene upon radio-TLC analysis. The bulk (5.8×10^6 cpm) of the radioactivity was eluted in fractions 45–61. This material was pooled for further analysis. Small amounts of radioactivity were eluted in fractions 117–130, 131–139, and 140–180, corresponding in

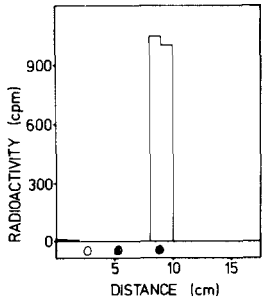

Fig. 4. Thin-layer radiochromatographic anaylsis of contents of fractions 20–29 (from silicic acid–Super Cel column chromatography; Fig. 3) on silica gel G plates (developing solvent, 10% ether in hexane). ○, Unlabeled authentic cholesterol; ⊘, unlabeled authentic cholesteryl acetate; ⊗, unlabeled authentic cholesteryl palmitate.

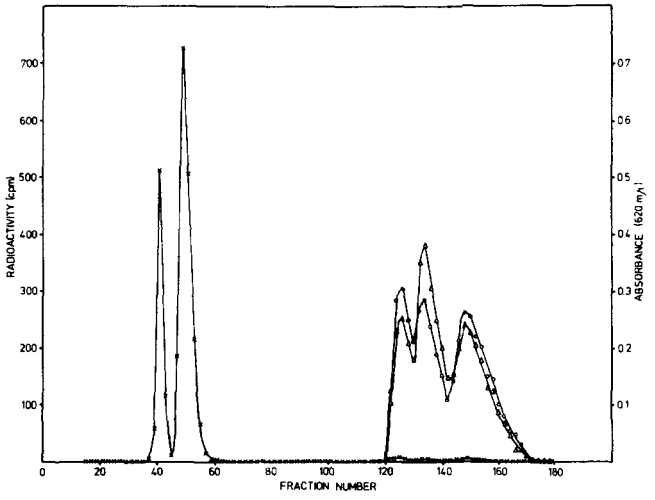

Fig. 5. Silica gel G–Super Cel–silver nitrate column (100 cm × 1 cm) chromatography of acetylated material corresponding to the "free monohydroxy-sterols" (fractions 30–70 of Fig. 3) derived from incubation of [3α-^{3}H]14α-methyl-cholest-7-en-3β-ol with a 10,000 *g* supernatant fraction of liver (Experiment I) along with the 3β-acetate derivatives of authentic unlabeled cholesta-7,14-dien-3β-ol, cholesta-8,14-dien-3β-ol, and cholesta-5,7-dien-3β-ol. × —— ×, Radioactivity; ○ —— ○, absorbance at 620 nm 1.25 min after addition of Liebermann-Burchard color reagent; △ —— △, absorbance at 620 nm 7 min after addition of Liebermann-Burchard color reagent. The three mass peaks in their order of elution are 3β-acetoxy-cholesta-7,14-diene, 3β-acetoxy-cholesta-8,14-diene, and 3β-acetoxy-cholesta-5,7-diene.

chromatographic mobility to those of 3β-acetoxy-cholesta-7,14-diene, 3β-acetoxy-cholesta-8,14-diene, and 3β-acetoxy-cholesta-5,7-diene, respectively. The labeled material corresponding to the 7,14-diene showed the same chromatographic mobility on radio-GLC as did authentic unlabeled 3β-acetoxy-cholesta-7,14-diene.

Another portion of the labeled material corresponding chromatographically to the 7,14-diene was diluted with unlabeled 3β-acetoxy-cholesta-7,14-diene (~25 mg) and subjected to repeated crystallization. After one crystallization from methanol, a second recrystallization did not result in a change in specific activity (**Table 1**). The labeled material corresponding to the mobility of the $\Delta^{8,14}$ diene was diluted with unlabeled 3β-acetoxy-cholesta-8,14-diene (~25 mg) and subjected to repeated crystallization from methanol. The specific activity was unchanged after the first crystallization (Table 1). The labeled material corresponding to the 5,7-diene showed the same chromatographic behavior on radio-GLC as did authentic unlabeled 3β-acetoxy-cholesta-5,7-diene. Another portion of the labeled material corresponding chromatographically to the 5,7-diene was diluted with unlabeled 3β-acetoxy-cholest-5,7-diene (~50 mg) and subjected to repeated crystallization from methanol. Little or no change in specific activity was observed (Table 1).

The labeled material corresponding to the contents of fractions 45–61 from the silica gel G–Super Cel–$AgNO_3$ column was applied to an alumina–Super Cel–$AgNO_3$ column along with authentic, unlabeled 3β-acetoxy-cholest-8(14)-ene (4.7 mg), 3β-acetoxy-cholest-8-ene (3.5 mg), 3β-acetoxy-cholest-7-ene (3.9 mg), and cholesteryl acetate (4.7 mg). Fractions 4.2 ml in volume (20 min per fraction) were collected. The resulting chromatogram is shown in **Fig. 6.** Approximately 0.7% of the radioactivity recovered from the column was associated chromatographically with 3β-acetoxy-cholest-8(14)-ene. This material showed the same chromatographic mobility on radio-GLC as did authentic unlabeled 3β-acetoxy-cholest-8(14)-ene. It is important to note that complete separation of 3β-acetoxy-cholest-8(14)-ene, and 3β-acetoxy-14α-methyl-cholest-7-ene was easily achieved in this chromatographic system. The retention times (relative to cholestane) for the two compounds were 2.49 and 2.93, respectively. Another portion of the material was diluted with unlabeled 3β-acetoxy-cholest-8(14)-ene (~25 mg) and subjected to analysis by cocrystallization. After one recrystallization from methanol, a second crystallization did not result in a significant change in specific activity (Table 1).

Approximately 0.1% of the radioactivity recovered

TABLE 1. Cocrystallization of ^{3}H-labeled steryl acetates with authentic carriers

Steryl Acetate	Specific Activity		
	Initial	After One Crystallization	After Two Crystallizations
		cpm/mg	
3β-Acetoxy-cholesta-7,14-diene	2,870	1,850	1,860
3β-Acetoxy-cholesta-8,14-diene	1,360	578	572
3β-Acetoxy-cholesta-5,7-diene	2,980	2,860	2,910
3β-Acetoxy-cholest-8(14)-ene	936	843	845
3β-Acetoxy-cholest-7-ene	326	213	226
3β-Acetoxy-cholest-5-ene	35,000	35,100	35,100

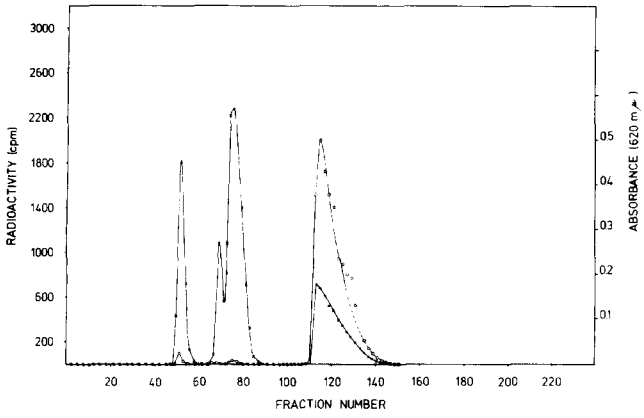

Fig. 6. Alumina–Super Cel–silver nitrate column (100 cm × 1 cm) chromatographic analysis of the radioactive material corresponding to the contents of fractions 45–61 of the silica gel G–Super Cel–silver nitrate column chromatography (Fig. 5) along with the 3β-acetate derivatives of authentic unlabeled cholest-8(14)-en-3β-ol, cholest-8-en-3β-ol, cholest-7-en-3β-ol, and cholesterol. ○—○, Radioactivity; ×—×, steryl acetate, determined colorimetrically.

from the alumina–Super Cel–$AgNO_3$ column had the same chromatographic mobility as 3β-acetoxy-cholest-8-ene. Insufficient material was available for further analysis. Approximately 0.5% of the radioactivity recovered from the alumina–Super Cel–$AgNO_3$ column was associated chromatographically with 3β-acetoxy-cholest-7-ene. A portion of this labeled material was subjected to radio-GLC analysis along with authentic 3β-acetoxy-cholest-7-ene. The resulting chromatogram indicated that the bulk of this material had the same chromatographic mobility as that of the authentic Δ^7-steryl acetate. Another portion of the labeled material corresponding to the mobility of the Δ^7-steryl acetate was diluted with authentic 3β-acetoxy-cholest-7-ene (~50 mg) and subjected to repeated crystallization. After one crystallization from methanol, a second crystallization did not result in a significant change in specific activity.

Most (98.7%) of the radioactivity eluted from the alumina–Super Cel–$AgNO_3$ column had the same chromatographic mobility as cholesteryl acetate. This labeled material also showed the same chromatographic behavior on radio-GLC as cholesteryl acetate and cocrystallized with unlabeled cholesteryl acetate (Table 1). Moreover, purification of the labeled cholesterol (obtained from the acetate derivative) by way of the dibromide indicated no significant change in specific activity. The specific activities before and after this purification procedure were 35,900 cpm per mg and 35,800 cpm per mg, respectively.

Two additional independent incubations were carried out in which the products of the incubations were analyzed in the same manner as in the case described above. The results of the three experiments are summarized in **Table 2.**

Experiment IV

The purpose of this experiment was to determine the distribution of radioactivity in the free sterol and steryl ester fraction upon incubation of labeled 14α-methyl-cholest-7-en-3β-ol with the 10,000 *g* supernatant fraction of a rat liver homogenate preparation. In addition, we sought the identification of each of the labeled sterols found in the free and esterified fractions.

This incubation was identical to those described above except that a larger amount of substrate (278 μg; 3.0×10^7 cpm) in propylene glycol (0.5 ml) was added. At the end of the incubation the mixture was extracted with $CHCl_3$–methanol 2:1. The resulting lower phase (containing 100% of the incubated radioactivity) was dried over anhydrous $MgSO_4$ and applied to a silicic acid–Super Cel column in benzene (0.5 ml). Fractions 2.6 ml in volume (20 min per fraction) were collected. The resulting chromatogram is shown in **Fig. 7.** The contents of fractions 18–30 contained 3.1×10^6 cpm, and were designated as the "steryl ester fraction". The contents of fractions 31–66 (20.9×10^6 cpm) were designated as the "free sterol fraction". The contents of fractions 278–351 contained 0.5×10^6 cpm and were designated as the "polar sterol fraction".

A portion of the steryl ester fraction was subjected to radio-TLC analysis along with unlabeled cholesterol, cholesteryl acetate, and cholesteryl palmitate. Most of the radioactivity had the same general mobility as cholesteryl palmitate. Another portion of the steryl ester fraction was heated under reflux with 15% KOH in 90% ethanol for one hr. Analysis by radio–TLC indicated that the saponification was essentially

TABLE 2. Metabolism of [3α-^{3}H]14α-methyl-cholest-7-en-3β-ol

	% Total Recovered Radioactivity		
	I	II	III
Unhydrolyzed steryl esters	2.8	4.3	4.7
Polar sterols	0.9	0.4	0.8
Monohydroxysterols	96.3	95.3	94.4
Unreacted substrate	17.9	30.6	42.2
Cholesta-7,14-dien-3β-ol	4.8	(3.2)[a]	2.0
Cholesta-8,14-dien-3β-ol	2.3	(3.2)[a]	0.8
Cholesta-5,7-dien-3β-ol	9.7	(3.2)[a]	5.0
Cholest-8(14)-en-3β-ol	0.4	0.3	0.8
Cholest-8-en-3β-ol	0.05	0.3	0.2
Cholest-7-en-3β-ol	0.3	0.3	4.9
Cholest-5-en-3β-ol	61.3	60.6	38.5

[a] These three sterols were not resolved from one another in this experiment.

complete. The resulting free sterols (derived from the steryl ester fraction) were acetylated with acetic anhydride in pyridine and the resulting labeled acetates were subjected to chromatography on a silica gel G–Super Cel–$AgNO_3$ column along with 3β-acetoxy-cholesta-7,14-dien-3β-ol, 3β-acetoxy-cholesta-8,14-dien-3β-ol, and 3β-acetoxy-cholesta-5,7-diene. Fractions 3.9 ml in volume (20 min per fraction) were collected. Five major radioactive peaks were noted. The least polar component (fractions 54–60), corresponding to the mobility of 3β-acetoxy-14α-methyl-cholest-7-ene in this chromatographic system, contained 1.5×10^5 cpm. The second component eluted from the silica gel G–Super Cel–$AgNO_3$ column (fractions 61–100), corresponding to the general mobility of C_{27} monounsaturated steryl acetates in this system, contained 1.74×10^6 cpm. A portion of this material was applied to an alumina–Super Cel–$AgNO_3$ column along with unlabeled 3β-acetoxy-cholest-8(14)-ene, 3β-acetoxy-cholest-8-ene, 3β-acetoxy-cholest-7-ene, and cholesteryl acetate. Fractions 2.2 ml in volume (20 min per fraction) were collected.

The bulk of the radioactivity (8.8×10^5 cpm) eluted from the column was associated chromatographically with cholesteryl acetate (center at fraction 199). Radio-GLC indicated that the labeled material had the same chromatographic mobility as that of authentic cholesteryl acetate. Cocrystallization experiments with authentic cholesteryl acetate indicated no significant change in specific activity (initial, 17,600 cpm per mg; after one crystallization from methanol, 17,700 cpm per mg; after two crystallizations, 17,700 cpm per mg). Moreover, there was no change in specific activity of the labeled cholesterol (obtained from the acetate derivative) upon purification by way of the dibromide. The specific activities before and after this procedure were 12,300 cpm per mg and 12,300 cpm per mg, respectively.

A significant portion of the radioactivity recovered from the alumina–Super Cel–$AgNO_3$ column (1.9×10^5 cpm) was eluted from the column as a discrete peak (center at fraction 75), clearly separated from the unlabeled standard of 3β-acetoxy-cholest-8(14)-ene. This material corresponds to the mobility of the acetate of 14α-methyl-cholest-7-en-3β-ol in this system and probably represents the unreacted substrate which was not completely removed upon chromatography on the silica gel G–Super Cel–$AgNO_3$ column. This material was not studied further.

Small amounts of radioactivity were eluted from the alumina–Super Cel–$AgNO_3$ column with mobilities corresponding to 3β-acetoxy-cholest-8-ene (fractions 107–113; 3.15×10^3 cpm) and 3β-acetoxy-cholest-7-ene (fractions 114–145; 1.1×10^4 cpm). The latter material showed the same mobility as authentic 3β-acetoxy-cholest-7-ene upon radio-GLC. Upon crystallization from methanol with unlabeled authentic 3β-acetoxy-cholest-7-ene, the specific activity fell from an initial value of 375 cpm per mg to 314 cpm per mg. A second crystallization did not result in a significant change in specific activity (321 cpm per mg). The third, fourth, and fifth labeled components eluted from the silica gel G–Super Cel–$AgNO_3$ column, correspond chromatographically with 3β-acetoxy-cholesta-7,14-diene, 3β-acetoxy-cholesta-8,14-diene, and 3β-acetoxy-cholest-5,7-diene, respectively.

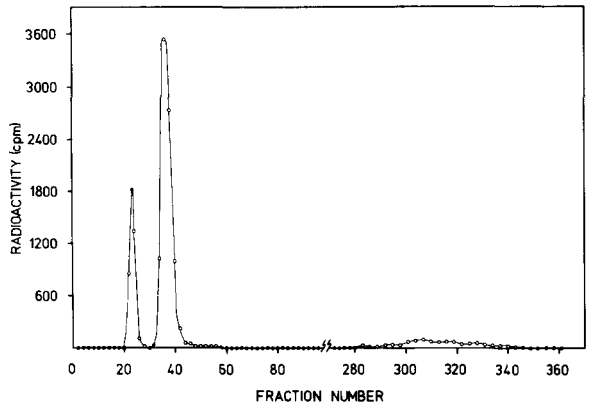

Fig. 7. Silicic acid–Super Cel column (100 cm × 1 cm) chromatography of labeled material recovered after incubation of [3α-^{3}H]14α-methyl-cholest-7-en-3β-ol with a 10,000 *g* supernatant fraction of a rat liver homogenate preparation (Experiment IV). The eluting solvent was 10% ether in benzene (flow rate, 0.13 ml per min). ○ —— ○, Radioactivity.

A portion of the labeled material from fractions 31–66 from the initial silicic acid–Super Cel column (corresponding to the mobility of unesterified sterols in this system) was analyzed by radio-TLC (solvent, 10% ether in hexane). The radioactive material had the same mobility as cholesterol in this system. Another portion of this material was acetylated with acetic anhydride in pyridine and the resulting labeled acetates were applied to a silica gel G–Super Cel–$AgNO_3$ column. Fractions 4.8 ml in volume (20 min per fraction) were collected. The resulting chromatogram indicated three major radioactive components: fractions 32–48 (1.3×10^6 cpm; corresponding to the mobility of the acetate of 14α-methyl-cholest-7-en-3β-ol), fractions 49–84 (6.1×10^6 cpm; corresponding to the mobility of C_{27} monounsaturated steryl acetates), and fractions 140–222 (3.5×10^5 cpm; corresponding to the mobility of C_{27} diunsaturated steryl acetates). The first and third components were not studied further. The material corresponding to fractions 49–84 was applied to an alumina–Super Cel–$AgNO_3$ column along with

TABLE 3. Metabolism of [3α-^{3}H]14α-methyl cholest-7-en-3β-ol

	% Total Recovered Radioactivity
Sterol Esters	12.6
14α-Methyl-cholest-7-en-3β-ol	2.18
Cholest-8-en-3β-ol	0.03
Cholest-7-en-3β-ol	0.09
Cholest-5-en-3β-ol	6.80
Cholesta-7,14-dien-3β-ol	0.04
Cholesta-8,14-dien-3β-ol	0.17
Cholesta-5,7-dien-3β-ol	3.42
Free Sterols	85.3
14α-Methyl-cholest-7-en-3β-ol	14.7
Cholest-7-en-3β-ol	0.3
Cholest-5-en-3β-ol	66.5
Dienes	3.8
Polar Sterols	2.0

authentic samples of 3β-acetoxy-cholest-8(14)-ene, 3β-acetoxy-cholest-8-ene, 3β-acetoxy-cholest-7-ene, and 3β-acetoxy-cholest-5-ene. Fractions 2.3 ml in volume (20 min per fraction) were collected. Three discrete radioactive components were noted.

The bulk (3.4×10^6 cpm) of the radioactivity was eluted in fractions 126–171 which corresponded to the mobility of cholesteryl acetate. Cocrystallization of the material with authentic cholesteryl acetate did not result in a significant change in specific activity (initial, 40,300 cpm per mg; after one crystallization from methanol, 41,000 cpm per mg; after two crystallizations, 40,600 cpm per mg). Moreover, purification by way of the dibromide did not result in a significant change in specific activity. The values before and after this purification procedure were 21,800 cpm per mg and 21,900 cpm per mg, respectively.

A small (1.8×10^4 cpm) but significant portion of the radioactivity recovered from the alumina–Super Cel–$AgNO_3$ column was eluted in fractions 45–78. This material, which was eluted immediately prior to 3β-acetoxy-cholest-8(14)-ene, corresponded to the mobility of 3β-acetoxy-14α-methyl-cholest-7-ene in this system. Another small (1.3×10^4 cpm) portion of the radioactivity recovered from the column was eluted in fractions 76–103 (corresponding to the mobility of the standard of 3β-acetoxy-cholest-7-ene). This material was mixed with authentic 3β-acetoxy-cholest-7-ene (specific activity, 445 cpm per mg). After one recrystallization from methanol, the specific activity fell to 386 cpm per mg. The specific activity after a second crystallization was 372 cpm per mg.

The combined results of the experiment are summarized in **Table 3.** A significant fraction of the recovered radioactivity was found in the steryl ester fraction. The percentage of the individual sterols found in the ester fraction was similar to the percentage of the same sterols found in the free sterol fraction.

Experiment V

The purposes of this experiment were to determine the extent of formation of labeled steryl esters upon incubation of labeled 14α-methyl-cholest-7-en-3β-ol with the 10,000 *g* supernatant fraction of a rat liver homogenate and to demonstrate the enzymatic nature of the formation of the steryl esters.

[3α-^{3}H]14α-Methyl-cholest-7-en-3β-ol (56 μg; 6×10^6 cpm) in propylene glycol (0.1 ml) was incubated in duplicate with 20-ml portions of the 10,000 *g* supernatant fraction of a rat liver homogenate as described above. In addition, the substrate was incubated with the enzyme preparation which had been heated at 100°C for 20 min prior to the addition of substrate. At the end of the incubation period, the boiled enzyme control incubation mixture and one of the duplicate enzyme incubation mixtures were extracted four times with two volumes of petroleum ether and three times with two volumes of ether. In each case, the combined organic extracts were washed three times with water and dried over anhydrous $MgSO_4$. The third incubation mixture was heated under reflux for 3 hr with an equal volume of 15% ethanolic KOH. The resulting mixture was extracted with petroleum ether and ether as in the cases of the other incubations. Each of the organic extracts was subjected to silicic acid–Super Gel column chromatography as described above.

In the case of the boiled enzyme control, virtually all of the recovered radioactivity was found in the free sterol fraction (**Table 4**). In the case of the incubation mixture that had been saponified for 3 hr under the conditions described above, virtually all of the radioactivity was similarly found in the free sterol fraction. Analysis of the incubation mixture that had not been saponified indicated that approximately ⅓ of the recovered radioactivity was in the sterol ester fraction. After saponification of this fraction with 15% ethanolic KOH for 3 hr and workup as described above, over 99% of the radioactivity in this fraction showed the same mobility on radio-TLC analyses (solvent systems, $CHCl_3$ and 10% ether in hexane) as did cholesterol.

Experiment VI

The purposes of this experiment were to confirm and to extend the findings obtained in the above experiments and to determine the extent of esterification of added labeled cholesterol by the 10,000 *g* supernatant fraction of a rat liver homogenate preparation.

TABLE 4. Metabolism of [3α-^{3}H]14α-methyl-cholest-7-en-3β-ol and [1,2-^{3}H]cholesterol

	% Incubated Radioactivity Recovered in Organic extracts	% Recovered Radioactivity		
		Ester	Free Mono-hydroxysterols	Polar
Experiment V				
Substrate [3α-^{3}H]14α-methyl-cholest-7-en-3β-ol				
Boiled enzyme	62	1.6	98.4	
No saponification	87	32.6	67.4	
Saponified	103	0.6	99.4	
Experiment VI				
Substrate [3α-^{3}H]14α-methyl-cholest-7-en-3β-ol				
Boiled enzyme	90	0	98.5	1.5
No saponification	92	23.7	71.8	4.4
Substrate [1,2-^{3}H]cholesterol				
No saponification	93	36.2	63.8	

[3α-^{3}H]14α-Methyl-cholest-7-en-3β-ol (111 μg; 1.2 × 10^7 cpm) in propylene glycol (0.2 ml) was incubated with a 10,000 *g* supernatant fraction of a rat liver homogenate preparation as described previously. A similar incubation was made with an enzyme preparation that had been heated at 100°C for 20 min prior to the addition of substrate. [1,2-^{3}H]Cholesterol (33 μg; 3 × 10^6 cpm) in propylene glycol (0.2 ml) was similarly incubated with the enzyme preparation. After a 3 hr incubation at 37°C, each of the incubation mixtures was extracted with a 2:1 mixture of $CHCl_3$ and methanol. The organic extracts were washed with water and dried over anhydrous Na_2SO_4. Recoveries of incubated radioactivity in the organic extract are listed in Table 4. The labeled material from each incubation was subjected to silicic acid–Super Cel column chromatography as described above. Significant percentages of the incubated radioactivity of [3α-^{3}H]14α-methyl-cholest-7-en-3β-ol and [1,2-^{3}H]cholesterol were recovered in the sterol ester fraction (Table 4).

DISCUSSION

14α-Methyl-5α-cholest-7-en-3β-ol has been prepared by a modification of the method of Knight et al. (14) and fully characterized. Of considerable interest is the colorimetric behavior of this Δ^7-sterol with the Liebermann-Burchard reagent. While 5α-cholest-7-en-3β-ol and 4α-methyl-5α-cholest-7-en-3β-ol react quickly to give maximal absorbance at 620 nm about 1.5 min after the addition of the reagent, 14α-methyl-5α-cholest-7-en-3β-ol gives a yellow color with maximum absorbance at 460 nm approximately 10 min after the addition of the reagent. The pronounced effect of the 14α-methyl group on the color response with the Liebermann-Buchard reagent is also illustrated by the finding that lanosterol (4,4,14α-trimethyl-cholesta-8,24-dien-3β-ol) also gives a yellow color (λ_{max} = 460 nm) upon treatment with the Liebermann-Burchard reagent (30).

The chromatographic properties of 14α-methyl-cholest-7-en-3β-ol and its derivatives are also of interest. While 4α-methyl-5α-cholest-7-en-3β-ol (30) elutes prior to cholesterol upon silicic acid–Super Cel column chromatography, 14α-methyl-cholest-7-en-3β-ol is much more polar in this chromatographic system and, in fact, is eluted after cholesterol in this system (Fig. 2). On alumina–Super Cel–silver nitrate column chromatography, 14α-methyl-5α-cholest-7-en-3β-ol shows a low polarity and is eluted considerably before cholesterol (Fig. 2). The same relationship holds for 3β-acetoxy-14α-methyl-5α-cholest-7-ene relative to cholesteryl acetate.

The 14α-methyl-5α-cholest-7-en-3β-ol was oxidized to the corresponding 3-ketone. Reduction of the latter compound with sodium borotritide gave [3α-^{3}H]14α-methyl-5α-cholest-7-en-3β-ol which was used as a substrate in metabolic studies.

Knight et al. (14) have previously reported on the results of studies of the metabolism of [2,4-^{3}H]14α-methyl-5α-cholest-7-en-3β-ol. This substrate was incubated with a 700 *g* supernatant fraction of a rat liver homogenate preparation fortified with ATP and NAD. A 7% conversion of the substrate to cholesterol was reported. The labeled cholesterol was characterized by digitonide formation, dibromide formation, cocrystallization of the regenerated free sterol, and cochromatography of the acetylated sterol with cholesteryl acetate. The formation of other sterols from the labeled substrate was not reported. The development of improved column chromatographic methods for the separation of po-

tential sterol intermediates in the overall conversion of the compound in question to cholesterol (2, 15, 23–26) has permitted a detailed investigation of this matter.

In the present study [3α-^{3}H]14α-methyl-5α-cholest-7-en-3β-ol was incubated with the 10,000 *g* supernatant fraction of homogenates prepared from the livers of female rats. The 10,000 *g* supernatant preparations were fortified with NAD, NADP, glucose-6-phosphate, nicotinamide, and $MgCl_2$. Enzymatic conversion of the labeled substrate to cholesterol was much higher than that reported previously. In four independent studies, 61.3, 60.6, 38.5, and 72.3% of the radioactivity recovered after incubation was associated with cholesterol (or a fatty acid ester of cholesterol). Other labeled sterols recovered after incubation were the unreacted substrate, cholesta-7,14-dien-3β-ol, cholesta-8,14-dien-3β-ol, cholesta-5,7-dien-3β-ol, cholest-8(14)-en-3β-ol, cholest-8-en-3β-ol, and cholest-7-en-3β-ol.

In initial studies (Experiments I, II, and III) incomplete saponification of the incubation mixtures indicated the presence of labeled material with the properties of steryl esters. Further studies (Experiments IV, V, and VI), in which the incubation mixtures were not initially subjected to saponification, indicated that substantial amounts of the incubated radioactivity were recovered in the steryl ester fraction. In three separate incubations, the percentages of the radioactivity recovered in the steryl ester fraction were 12.6, 32.6, and 23.7. Of considerable interest is the finding that the radioactivity in the steryl ester fraction yielded, upon saponification, labeled material corresponding to the incubated substrate, cholest-8-en-3β-ol, cholest-7-en-3β-ol, cholesta-8,14-dien-3β-ol, cholesta-7,14-dien-3β-ol, cholesta-5,7-dien-3β-ol, and cholesterol. Brady and Gaylor (31) have reported results which are particularly relevant to the matter of esterification of added sterols upon incubation with the 10,000 *g* supernatant fraction of rat liver homogenate preparations. The recovery of significant amounts of labeled material with the chromatographic properties of steryl esters was observed upon incubation of [30-^{14}C]4α-methyl-cholest-7-en-3β-ol and [3α-^{3}H]cholest-7-en-3β-ol. Considerably lower amounts of labeled material corresponding chromatographically to steryl esters was recovered after incubation of [30,31-^{14}C]4,4-dimethyl-cholest-7-en-3β-ol and [24,25-^{3}H]-4,4,14α-trimethyl-cholest-7-en-3β-ol with the 10,000 *g* supernatant liver preparations.

The nature of the labeled sterol component of the steryl esters derived from the various substrates was not studied except in the case of the [30-^{14}C]4α-methyl-cholest-7-en-3β-ol. In this case saponification of the resulting steryl ester yielded labeled material which had the same mobility on thin-layer chromatographic analysis as 4α-methyl-cholest-7-en-3β-ol. In a separate experiment, aerobic incubation of the steryl ester fraction formed from [30-^{14}C]4α-methyl-cholest-7-en-3β-ol with microsomes in the presence of NAD gave no labeled carbon dioxide. In the present experiments it is noteworthy that the labeled steryl esters formed from [3α-^{3}H]14α-methyl-cholest-7-en-3β-ol were comprised of a number of sterols in addition to that of the incubated substrate and included the following demethylated products: cholest-8-en-3β-ol, cholest-7-en-3β-ol, cholesta-8,14-dien-3β-ol, cholesta-7,14-dien-3β-ol, cholesta-5,7-dien-3β-ol, and cholesterol. This finding has implications as to the complexity of the general metabolism involved in sterol biosynthesis and to the possible control mechanisms that are operative.

The precise mechanisms involved in the enzymatic removal of the 14α-methyl group (carbon atom 32) of cholesterol precursors have not been conclusively established. On the basis of analogy with the reactions involved in the enzymatic removal of carbon atoms 30 and 31 of cholesterol precursors and the enzymatic oxidative demethylation of other organic substrates (12), the first reaction at carbon atom 32 can be assumed to be an oxygen-dependent hydroxylation to yield the corresponding 14α-hydroxymethyl sterol. Gibbons and Mitropoulos (32) have suggested that cytochrome P-450 might be involved in one or more of the reactions involved in the overall removal of the 14α-methyl group of cholesterol precursors. The results of subsequent studies have also suggested that the enzymatic removal of the 14α-methyl group of sterol precursors in yeast involves cytochrome P-450 (33–36). In the present study we recovered small but significant amounts of radioactive polar material upon incubation of 14α-methyl-cholest-7-en-3β-ol with rat liver homogenate preparations. The general chromatographic mobility of this material was compatible with that of 14α-hydroxymethyl-cholest-7-en-3β-ol but insufficient material was available for the conclusive establishment of structure.

Mitropoulos, Gibbons, and Reeves (37) have demonstrated that, upon incubation of [32-^{14}C]4,4,14α-trimethyl-cholest-8-en-3β-ol with rat liver microsomes, the label of carbon atom 32 was recovered as formic acid. This finding represented an extension of studies by Alexander et al. (38) on the metabolism of 14α-hydroxymethyl sterols by rat liver microsomes. In these studies the recovery of carbon atom 32 as formic acid was observed, a finding subsequently confirmed in this laboratory (18–20) and

extended by Akhtar et al. (39, 40). The latter workers have proposed that the initial product of the dealkylation of lanost-8-en-3β,32-diol is 4,4-dimethyl-cholesta-8,14-dien-3β-ol and that the initial product of the dealkylation of lanost-7-en-3β,32-diol is 4,4-dimethyl-cholesta-7,14-dien-3β-ol (39, 40). The possible role of $\Delta^{8,14}$ and/or $\Delta^{7,14}$ sterols in this process was initially suggested by the results of experiments indicating the stereospecific loss of one of the hydrogen atoms at carbon atom 15 of lanosterol upon enzymatic formation of cholesterol (41–43). In the C_{27} series we have shown that cholesta-8,14-dien-3β-ol and cholesta-7,14-dien-3β-ol serve as efficient precursors of cholesterol (25, 26, 44) in rat liver homogenate preparations. In these studies the enzymatic conversion of the $\Delta^{8,14}$ sterol to cholest-8(14)-en-3β-ol, cholest-8-en-3β-ol, cholest-7-en-3β-ol, and cholesterol and the enzymatic conversion of the $\Delta^{7,14}$ sterol to cholest-8(14)-en-3β-ol, cholest-7-en-3β-ol, and cholesterol were documented. While no conversion of the $\Delta^{8,14}$ sterol to the $\Delta^{7,14}$ sterol could be found upon incubation with rat liver microsomes (26), efficient conversion of the $\Delta^{7,14}$ sterol to the $\Delta^{8,14}$ sterol has been observed (45).

The overall enzymatic conversion of cholest-8-en-3β-ol to cholesterol proceeds via the successive intermediates, cholest-7-en-3β-ol and cholesta-5,7-dien-3β-ol (1, 2, 23, 46, 47). 5α-Cholest-8(14)-en-3β-ol has been isolated from rat skin and has been shown to be efficiently converted to cholesterol upon incubation with rat liver homogenate preparations (24, 48). The formation of cholesta-8,14-dien-3β-ol and cholest-7-en-3β-ol from the $\Delta^{8(14)}$ sterol has also been demonstrated (24, 49). Precise mechanisms involved in the formation of cholest-8(14)-en-3β-ol are not clear. As noted above, the enzymatic formation of the $\Delta^{8(14)}$ sterol from cholesta-8,14-dien-3β-ol and cholesta-7,14-dien-3β-ol has been established. The possibility exists that the $\Delta^{8(14)}$ sterol can also be formed as an alternative primary product of the enzymatic reactions involved in the removal of carbon atom 32 of 14α-methyl sterol precursors of cholesterol (50).

In the present study the following labeled sterols were recovered and identified after incubation of [3α-^{3}H]14α-methyl-cholest-7-en-3β-ol with rat liver homogenates: cholesta-8,14-dien-3β-ol, cholesta-7,14-dien-3β-ol, cholest-8(14)-en-3β-ol, cholest-8-en-3β-ol, cholest-7-en-3β-ol, and cholesta-5,7-dien-3β-ol. As noted above, all of the above sterols can be considered as potential intermediates in the overall enzymatic conversion of 14α-methyl-cholest-7-en-3β-ol to cholesterol. The enzymatic formation of the $\Delta^{8(14)}$ sterol from the former sterol is especially noteworthy. Also noteworthy is the formation of labeled cholesta-8,14-dien-3β-ol and cholesta-7,14-dien-3β-ol from the 14α-methyl-Δ^7-sterol. If the suggestion (39) that the enzymatic dealkylation of carbon atom 32 of 14α-hydroxymethyl-Δ^7-sterols yields the $\Delta^{7,14}$ sterol but not the $\Delta^{8,14}$ sterol is correct, the observed formation of labeled cholesta-8,14-dien-3β-ol from the 14α-methyl-Δ^7-sterol may represent subsequent formation of the $\Delta^{8,14}$ sterol from the $\Delta^{7,14}$ sterol as noted previously.

Further studies relative to the mechanisms involved in the enzymatic removal of carbon atom 32 of sterol precursors of cholesterol are in progress. These studies have received added impetus by our recent finding that 14α-hydroxy-methyl sterols are potent inhibitors of cholesterol biosynthesis and represent potential regulators of sterol synthesis (51).

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