On the Metabolism of Desmosterol in Mouse Liver Homogenates
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The metabolism of $^{14}$C-desmosterol was studied in fortified preparations of mouse liver mitochondria. The polar products formed were chromatographically similar to those formed from $^{14}$C-cholesterol under the same conditions of incubation. One main enzymically formed product was isolated. This compound had chromatographic properties very similar to those of 26-hydroxycholesterol but was not identical with this compound. After hydrogenation the metabolite was shown to be identical with 5α-cholestan-3β,26-diol. It was suggested that the compound isolated is cholesta-5,24-diene-3β,26-diol.

Desmosterol (cholesta-5,24-diene-3β-ol) has been implicated as the immediate precursor of cholesterol in the biosynthesis of cholesterol, at least in the pathway with steroid intermediates carrying a side-chain double bond.¹ ² However, the quantitative importance, under physiological conditions, of a pathway having desmosterol as an intermediate has recently been questioned.³ Stokes et al.⁴ who were the first to isolate desmosterol and demonstrate its ready conversion to cholesterol, suggested that desmosterol might also be an intermediate in the conversion of cholesterol to bile acids.

In animals and man, treatment with the drug MER-29 (triphyenol) leads to an accumulation of desmosterol in blood and tissues due to inhibition of the enzyme that catalyzes the reduction of the side-chain double bond of desmosterol.⁴ Goodman et al.⁵ have studied the conversion of 2-$^{14}$C-mevalonic acid into sterols and bile acids in humans fed MER-29, and have obtained evidence indicating a direct conversion of desmosterol into bile acids without the intermediate formation of cholesterol under these conditions. The oxidation of desmosterol to carbon dioxide in mitochondrial preparations from normal rat liver has been studied by Kritchevsky and Staple ⁷ who found that the side-chain of desmosterol was oxidized more readily than that of cholesterol. These experiments did not exclude the possibility that the added desmosterol was transformed into cholesterol prior to oxidation. However,
experiments by Goodman et al.\textsuperscript{8} have demonstrated that the side-chain of desmosterol can be oxidized directly. Thus, the yield of labeled carbon dioxide in incubations of \textsuperscript{14}C-desmosterol with liver mitochondria from MER-29 treated mice was the same as that obtained from \textsuperscript{14}C-cholesterol incubated with liver mitochondria from normal mice. In addition, no cholesterol was formed from desmosterol in the incubations with liver mitochondria from MER-29 treated mice.

Work in Gurin's and in this laboratory on the mechanism of conversion of cholesterol to bile acids has led to the formulation of the likely sequence of reactions concerned with the side-chain oxidation. It has been postulated that in the formation of bile acids the cholesterol side-chain is oxidized by an $\omega$-oxidation followed by a "$\beta$"-oxidation with the release of propionic acid, probably as the coenzyme-A derivative (cf. Ref.\textsuperscript{9}). The initial reaction in the $\omega$-oxidation has been shown to be an hydroxylation at the C-26 position.\textsuperscript{10} It is not known with certainty at what stage of the nuclear transformations the C-26 hydroxylation occurs in cholic acid formation. Recent work by Mendelsohn and Staple\textsuperscript{11} indicates that 5\(\beta\)-cholestan-3\(\alpha\),7\(\alpha\),12\(\alpha\)-triol could be the substrate for the 26-hydroxylase in cholic acid formation.

The metabolism of cholesterol in cell-free liver preparations has been studied in some detail. The main enzymically formed product in mitochondrial systems has been identified as 26-hydroxycholesterol,\textsuperscript{12,13} whereas in microsomal systems 5\(\beta\)-cholestan-3\(\alpha\),7\(\alpha\),12\(\alpha\)-triol is the major metabolite.\textsuperscript{11} As desmosterol is efficiently converted to bile acids \textit{in vivo}, it was of interest to examine the metabolism of desmosterol in liver homogenates in an effort to obtain information on the mechanism of conversion of desmosterol to bile acids.

**Experimental**

\textit{Labeled compounds and reference compounds.} The \textsuperscript{14}C labeled desmosterol used in this investigation was a generous gift from Dr. U. Gloor, Hoffmann-La Roche Research Laboratories, Basle, Switzerland. It had been isolated from livers of MER-29 treated rats previously injected with \textsuperscript{3}H\textsuperscript{-} mevalonic acid. Cholesterol-4,\textsuperscript{14}C was purchased from the Radiochemical Centre, Amersham, England. Cholesterol-3\(\beta\),5\(\beta\),6\(\beta\)-triol was prepared according to Fieser and Rajagopalan\textsuperscript{14} and 26-hydroxycholesterol was a generous gift from the late Dr. E. Mossettig.

\textit{Incubation procedures.} White male mice of the Danish State Serum Institute strain, weighing about 25 g, were used. Liver homogenates, 33 \% (liver wet weight/volume), were prepared in 0.25 M sucrose containing 3.6 mg of nicotinamide/ml with a teflon-glass Potter-Elvehjem homogenizer. After centrifugation of the homogenate for 10 min at 800 \texttimes g the mitochondrial fraction was isolated by centrifugation of the 800 \texttimes g supernatant fluid for 15 min at 10 000 \texttimes g. The mitochondrial fraction was washed twice with the homogenizing medium and then resuspended in a small amount of homogenizing medium with a loose-fitting pestle for 20 sec. The 10 000 \texttimes g supernatant fluid was centrifuged for 45 min at 100 000 \texttimes g. "Boiled juice" was prepared by heating the 100 000 \texttimes g supernatant tffuid for 10 min at 85–90\textdegree. Precipitated material was removed by centrifugation for 10 min at 10 000 \texttimes g.

The composition of the incubation mixture was as follows: 10 ml of mitochondrial suspension (corresponding to mitochondria from 15 g of liver); 5 ml of "boiled juice"; 3 ml of 0.05 M tris (hydroxymethyl)aminomethane-hydrochloric acid buffer, pH 7.8; 3.3 ml of serum albumin stabilized emulsion of \textsuperscript{14}C-desmosterol (2.6 mg of \textsuperscript{14}C-desmosterol, about 30 \textmu C, dissolved in 0.3 ml of ethanol and added to 3 ml of a 1 \%, solution of bovine serum albumin in saline); and 50 \(\mu\) moles of NADPH (Sigma Chemical Co., St. Louis, Mo.). Incubations were terminated by addition of 35 volumes of chloroform/methanol 2:1.
Analysis of incubation mixtures. The chloroform/methanol extract was filtered and extracted with 0.2 volumes of saline solution. The chloroform phase was evaporated to dryness under reduced pressure and the residue subjected to reversed phase partition chromatography using phase system I. In the further analysis of the metabolites formed chromatography was performed with phase systems III, F 1, and C 1 and also on columns of aluminum oxide, grade III (Woelm, Eschwege, W.-Germany).

Analysis of bile from bile fistula rats. The strain of animal used in experiments with bile fistula rats and the procedures used for analysis of bile were the same as those recently described.

RESULTS

Incubation of $^{14}$C-desmosterol with mouse liver mitochondria fortified by addition of boiled liver juice and NADPH resulted in the conversion of 10—18% of the added isotope into more polar products. These products were separated from unchanged desmosterol and from cholesterol by subjecting the extracts of the incubation mixtures to column chromatography using phase system I. The more polar products were subsequently chromatographed with phase system III. Fig. 1 shows a typical chromatogram. The radioactivity is eluted as three peaks at 8, 25, and 34 ml of effluent and as a broad band between 50 and 85 ml of effluent. The last-mentioned labeled material appears at a place characteristic of 7α- and 7β-hydrocholesterol and was not further analyzed. The labeled material appearing between 6 and 15 ml of effluent and that appearing between 20 and 40 ml of effluent were combined in two fractions, Fraction I and II, respectively.

Analysis of Fraction I. Fraction I, together with 5 mg of unlabeled chenodeoxycholic acid, was chromatographed with phase system F 1 (cf. Fig. 2). The radioactivity was eluted as several peaks none of which coincided with the titration peak of the added chenodeoxycholic acid (peak at 48 ml of effluent). The labeled material appearing between 12 and 32 ml of effluent was combined and chromatographed on phase system C 1 together with unlabeled cholic acid. None of the radioactivity eluted from this column coincided with the titration peak of cholic acid.
Analysis of Fraction II. Fraction II and 28 mg of unlabeled cholestane-3β,5α,6β-triol were dissolved in 1 ml of pyridine and 0.5 ml of acetic anhydride. After standing for 24 h at room temperature the solution was diluted with water and extracted with ether. The ether extract was washed with dilute hydrochloric acid, sodium carbonate solution and finally with water until neutral. The ether extract was evaporated to dryness and the residue chromatographed on a column of aluminum oxide, grade III. The column was eluted with increasing concentrations of benzene in hexane (cf. Fig. 3). Two radioactive peaks were obtained in this chromatogram; one (acetylated compound A) was eluted with 40% benzene in hexane, the other with 80% benzene in hexane appearing shortly before the diacetate of cholestane-3β,5α,6β-triol. In view of previous experience that cholesterol is partly autoxidized under similar conditions of incubation, one experiment was performed in which 14C-desmosterol was incubated with boiled liver juice and NADPH. The products formed were analyzed as described above. In this case, chromatography of acetylated Fraction II yielded one main radioactive peak, apparently identical with that eluted with 80% benzene in hexane in the chromatogram shown in Fig. 3. The identity of this labeled compound has not been established. It is possible that it is the diacetate of cholest-24-ene-3β,5α,6β-triol. However, this compound was not available for further identification.

Compound A. The polarity of acetylated compound A, being eluted with 40% benzene in hexane from a column of aluminum oxide, grade III, is similar to that of the diacetate of 26-hydroxycholesterol. Upon chromatography of part of the acetate of compound A with this latter compound the isotope did not separate from the carrier. After hydrolysis and subsequent chromatography on aluminum oxide, grade III, using increasing concentrations of ethyl acetate in benzene as eluant, the radioactivity and the carrier were again eluted as one peak. However, when compound A was crystallized with unlabeled 26-hydroxycholesterol, the specific activity of the crystals did not remain constant. After ten crystallizations from different solvent mixtures (methanol/water and acetone/water) the specific activity of the crystals was such that the amount of 26-hydroxycholesterol in compound A was less than

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Fig. 3. Chromatography of acetylated Fraction II (20–40 ml of effluent of column shown in Fig. 1) together with the diacetate of cholestane-3β,5α,6β-triol. Column: 10 g of aluminum oxide, grade III. Eluting solvents: H = hexane; B = benzene. Fractions (50 ml each): 1 = H/B, 8:2; 2–6 = H/B, 6:4; 7 = H/B, 4:6; 8–14 = H/B, 2:8. Broken line: radioactivity. Bars: weight.

Fig. 4. Chromatography of first 24 h portion of hydrolyzed bile from bile fistula rat injected with compound A. Column: 4.5 g of hydrophobic Hyflo SuperCel. Phase system: F 1. Broken line: radioactivity. Solid line: titration values.

10%. The crystals and the mother liquors were then combined and hydrogenated as described by Scheer et al. A series of crystallizations (from methanol/water and acetone/water) was again performed. After two crystallizations the specific activity of the crystals remained constant through four additional recrystallizations. The melting point of the crystals was 180–181°C, the melting point reported for 5α-cholestane-3β,26-diol is 179–181°C. The yield on hydrogenation of compound A of the product identical with 5α-cholestane-3β,26-diol was calculated to be 70%. It should be mentioned that in this hydrogenation the carrier 26-hydroxycholesterol had been converted to 5α-cholestane-3β,26-diol in a yield of about 80%, as shown by gas chromatography of an aliquot of the hydrogenation mixture.

The metabolism of compound A in the bile fistula rat was examined using procedures recently described. Compound A was found to be metabolized to chenodeoxycholic acid and to two acids with chromatographic properties in phase system C 1 similar to those of the 6β-hydroxylated metabolites of chenodeoxycholic acid (3α,6β,7α-trihydroxy- and 3α,6β,7β-trihydroxy-5β-cholanic acids). Fig. 4 shows a chromatogram with phase system F 1 of the first 24 h portion of bile from a bile fistula rat injected with compound A. About 45% of the radioactivity eluted from the column coincides with the titration peak of chenodeoxycholic acid (40 ml of effluent). Rechromatography of the early radioactive peak with phase system C 1 showed that it consisted mainly of the 6β-hydroxylated metabolites of chenodeoxycholic acid.

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The products formed in incubations of \(^{14}\text{C}\)-desmosterol with fortified preparations of mouse liver mitochondria were found to be similar in chromatographic properties to those isolated from incubations of \(^{14}\text{C}\)-cholesterol conducted under the same conditions.\(^{12,13}\) Several of the compounds isolated from such incubations of \(^{14}\text{C}\)-cholesterol were shown to be autoxidation products of cholesterol, viz. 7α-hydroxycholesterol, 7β-hydroxycholesterol, 7-ketcholesterol, cholestane-3β,5α,6β-triol, and small amounts of unidentified compounds more polar than the above-mentioned compounds.\(^{12}\) Among the metabolites of \(^{14}\text{C}\)-desmosterol isolated in the present investigation compounds were present that had chromatographic properties similar to these autoxidation products of \(^{14}\text{C}\)-cholesterol. It appears probable therefore that \(^{14}\text{C}\)-desmosterol had been autoxidized to some extent with formation of compounds structurally similar to the autoxidation products of cholesterol. This contention is further strengthened by the results of experiments in which \(^{14}\text{C}\)-desmosterol was incubated with boiled liver juice, where the conversion to more polar products was about 4%. These products consisted of a number of compounds chromatographically very similar to the autoxidation products of cholesterol.

From incubations of \(^{14}\text{C}\)-desmosterol with mouse liver mitochondria one main enzymically formed product was isolated. The structure of this compound has not been established conclusively but is proposed to be cholesta-5,24-diene-3β,26-diol. Thus, the isolated metabolite had chromatographic properties very similar to those of 26-hydroxycholesterol but was not identical with this compound as shown by recrystallization with carrier 26-hydroxycholesterol. However, after hydrogenation the metabolite was identical with 5α-cholestane-3β,26-diol. In this connection it should be mentioned that the hydrogenation experiment indicated that the metabolite was not homogeneous but contained small amounts (\(\sim 10\%\)) of another compound(s). When administered to a bile fistula rat the metabolite was excreted rapidly in bile and had been transformed mainly into chenodeoxycholic acid. It had thus been metabolized in a manner very similar to that of 26-hydroxycholesterol,\(^{13}\) as could be expected from the structure proposed for the metabolite.

Acknowledgements. This work is part of investigations supported by Statens Medicinska Forskningsråd and the National Institutes of Health, Bethesda, Md. (Grant H-2842).

REFERENCES


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Received January 21, 1964.