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## THE *IN VITRO* METABOLISM OF DESMOSTEROL WITH ADRENAL AND LIVER PREPARATIONS \*

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In a recent communication (2), it was demonstrated that in human subjects receiving triparanol therapy, 24-dehydrocholesterol (desmosterol) could serve as a direct precursor of several physiologically important compounds normally derived from cholesterol. The results indicated the direct conversion of desmosterol to both bile acids and to steroid hormones in these patients, and suggested that desmosterol was similar to cholesterol in its ability to serve as a precursor for these compounds.

The present study compares the metabolism of desmosterol and cholesterol in *in vitro* adrenal and liver preparations. The results support the conclusion of the previous *in vivo* studies.

### METHODS

*Preparation of labeled substrates.* Both C<sup>14</sup>-desmosterol and C<sup>14</sup>-cholesterol were biosynthetically prepared from 2-C<sup>14</sup>-mevalonic acid. The C<sup>14</sup>-mevalonic acid was purchased from the Volk Radiochemical Corp. as its dibenzylethylenediamine salt, and was converted to a solution of the potassium salt by alkalization with KOH, extraction of the diamine with light petroleum ether, and neutralization. Twenty microcuries of C<sup>14</sup>-mevalonate were injected intravenously into each of two normal rats and into two rats that had been given a diet containing 1 per cent triparanol for 2 months. The normal rats were used as the source of C<sup>14</sup>-cholesterol, and the triparanol-fed rats of C<sup>14</sup>-desmosterol. After 2.5 hours the livers were excised and extracted in a small blender with 25 vol (vol/wt) of acetone:ethanol (1:1, vol:vol). The total lipid extract so obtained was saponified with 2 per cent KOH in 50 per cent ethanol for one hour under nitrogen, and the nonsaponifiable fraction was then extracted with light petroleum ether. Further purification of the sterols was effected by chromatography of their *p*-phenylazobenzoyl esters on 40-cm columns of silicic

acid-Celite (2:1) as described by Avigan and co-workers (3). Chromatography of both samples was continued until the cholesterol zones had moved almost the entire length of the column. Only the cholesterol zone was visible in the sample from the normal rats; the sample from the triparanol-fed rats showed a desmosterol zone fairly well separated from the cholesterol zone. The contents of both columns were extruded, the central portion of the cholesterol zone collected from the sample from the normal rats and the center of the desmosterol zone from the sample from triparanol-fed rats. The latter was rechromatographed on a smaller column to obtain desmosterol of high purity. The labeled cholesterol and desmosterol esters were then hydrolyzed, the free sterols extracted, and analyzed for mass and for radioactivity. The yields of the two sterols were: a) cholesterol, 22 mg, with specific radioactivity 74,500 dpm per mg; b) desmosterol, 7 mg with specific radioactivity 57,000 dpm per mg.

A second specimen of C<sup>14</sup>-desmosterol was prepared later by injection of 40  $\mu$ c of 2-C<sup>14</sup>-mevalonate into one triparanol-fed rat. Desmosterol was purified from the nonsaponifiable fraction of the liver by thin-layer chromatography of the free sterols and of the sterol acetate esters (4). The yield of desmosterol was 6.5 mg, with specific radioactivity 192,000 dpm per mg. This preparation was demonstrated to contain pure desmosterol by gas-liquid chromatography. In the experiments reported below, this second desmosterol preparation was used only in the second experiment with adrenal homogenates.

Both cholesterol and desmosterol synthesized from 2-C<sup>14</sup>-mevalonate contain five labeled carbon atoms, three in the sterol nucleus—carbon atoms 1, 7, and 15—and two in the side-chain—carbon atoms 22 and 27 (5, 6). Since both labeled sterols were synthesized from the same precursor and purified by the same methods, it was felt that the two substrates were absolutely comparable.

*Experiments with liver mitochondria.* The oxidation of the terminal carbon atoms at the side-chain of cholesterol and desmosterol by mouse liver mitochondria was studied by the method of Horning, Fredrickson, and Anfinsen (7). In these experiments, the substrate sterol was added to the incubation mixtures as a serum-albumin stabilized suspension containing 0.9  $\mu$ mole of sterol per ml of 1 per cent bovine serum albumin. Mitochondria were obtained from livers of adult male mice by homogenization of the livers with a solution of 0.25 M sucrose and 0.01 M nicotinamide in a Potter-Elvehjem homogenizer, dilution of the homogenate to 75 ml per 10 g liver,

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and differential centrifugation to settle the mitochondria as sediment at  $10,000 \times G$ . The mitochondria were washed once by centrifugation and suspended in a volume equal to the original weight of liver.

Incubations were carried out in 25-ml Erlenmeyer flasks sealed with rubber serological caps. From each cap a small glass well was suspended. Each flask contained, in a volume of 6 ml: 300  $\mu$ moles Tris-HCl buffer at pH 8.4, 100  $\mu$ moles nicotinamide, 4  $\mu$ moles DPN, 2  $\mu$ moles TPNH, 20  $\mu$ moles glutathione, 10  $\mu$ moles 5-adenylic acid, 1 ml supernatant fraction of boiled liver homogenate—see (7)—, 3 ml washed mitochondrial suspension, and 0.9  $\mu$ mole substrate. After preparation of the incubation mixtures, 0.3 ml 10 per cent KOH was added to each hanging well, and the flasks were sealed and gently shaken in a metabolic incubator for 3.5 hours at  $37^\circ C$ . Subsequently, 0.3 ml 6N  $H_2SO_4$  was injected through the cap into the incubation medium and the flasks were shaken for 1 hour at room temperature. The caps and wells were then removed and the KOH quantitatively removed and diluted to 2.5 ml with water. In order to measure the  $C^{14}O_2$  trapped in the KOH, 2.0 ml of the diluted KOH was added to 10 ml of the scintillation mixture described by Bray (8) and the resultant solution assayed in a Packard liquid scintillation spectrometer with an efficiency of about 40 per cent. Corrections for quenching were made by again counting the samples after the addition of internal standard. All other radioassays reported employed 0.5 per cent diphenyl-oxazole in toluene as scintillation solvent, with a counting efficiency of 50 to 55 per cent. The two counting systems were compared by assay of the same amount of  $C^{14}$  absolute standard in each.

At the end of the experiments calculations were made, from the recovery of  $C^{14}O_2$ , of the percentage conversion of the terminal carbons of the sterol side-chain to  $CO_2$ . As mentioned above, the terminal carbons of the side-chain of each sterol contained one-fifth the total radioactivity in the sterol molecule.

*Experiments with adrenal homogenates.* The *in vitro* conversion of  $C^{14}$ -cholesterol and  $C^{14}$ -desmosterol to steroid hormones by homogenates of adrenal glands from triparanol-fed rats was studied in two experiments. Adrenal glands were obtained from 8 male Sprague-Dawley rats. The rats had been fed 1 per cent triparanol for 2 to 3 weeks, and weighed about 200 g each at the time of study. After decapitation of the animals, the adrenal glands were excised, rinsed in ice-cold saline, blotted dry, and weighed. The combined weight of the 16 glands was 348 mg in the first experiment, and 388 mg in the second. The glands were homogenized in a small Potter-Elvehjem homogenizer with 5 ml of a solution of 0.05 M potassium phosphate buffer at pH 7.4, 0.015 M nicotinamide, 0.05 M KCl, and 0.005 M  $MgCl_2$ . One-half ml of the homogenate was extracted with acetone-ethanol (1:1) and saponified; the nonsaponifiable fraction was then extracted with light petroleum ether and analyzed by gas-liquid chromatography. In each experiment this analysis showed the endogenous adrenal sterols to consist of

about 40 per cent desmosterol and 60 per cent cholesterol. The remainder of the homogenate, 4.5 ml, was divided into two equal parts in each of two 25-ml Erlenmeyer flasks, and to each flask were added: 5  $\mu$ moles TPNH, 5  $\mu$ moles TPN, and 20  $\mu$ moles glucose-6-phosphate. The final volume was 3 ml. The  $C^{14}$ -sterol substrate was then added to each flask in 100  $\mu$ L acetone solution. In the first experiment, 1.65  $\mu$ moles  $C^{14}$ -cholesterol was added to one flask, and the same amount of  $C^{14}$ -desmosterol to the second flask. In the second experiment 1  $\mu$ mole of each substrate was employed, and the second preparation of  $C^{14}$ -desmosterol was used. The flasks were flushed with oxygen, stoppered, and incubated in a shaking incubator at  $37^\circ C$  for 2 hours. The incubations were terminated by the addition of an equal volume of ethanol, and the lipids and steroids extracted twice with 10 ml  $CH_2Cl_2$  and once more with 20 ml acetone: ethanol (1:1).

Unlabeled carrier steroids were added to each total extract, and the solvent evaporated under nitrogen. The carrier steroids included approximately 100  $\mu$ g each of: progesterone, pregnenolone, corticosterone (compound B), 11-dehydrocorticosterone (compound A), and cortisone.<sup>1</sup>

The steroids were partially separated from the substrate sterols, and from other less polar lipids, by chromatography on a small column packed with 2 g silica gel (Davison Chemical Co., 100–200 mesh), with a slight modification of the method of Goldzieher, Baker, and Riha (9). Preliminary standardization showed that most of the cholesterol was eluted with 15 ml of 5 per cent ethyl ether in benzene. Progesterone, the least polar steroid expected, remained entirely on the column. All steroids, including cortisol, could then be eluted with 50 ml ethyl acetate. For chromatography of the samples from the incubations, most of the sterols were eluted with 5 ml hexane and 15 ml 5 per cent ethyl ether in benzene. The steroids were then eluted with 50 ml ethyl acetate.

Further purification of the steroids was effected by paper chromatography. The steroid samples were applied to separate strips of Whatman no. 1 paper, 46 cm in length. Polar steroids were separated first with the Bush B1 system—toluene: ligroin: methanol: water, 5:5:7:3—(10). These chromatograms contained compound B and compound A, fairly well separated from each other, and 11 $\beta$ -hydroxy-4-androstenedione near the end of the strip. The less polar steroids were all found in the effluents, or runoffs, from these chromatograms.

A second set of paper chromatographies was then carried out, in which the effluents from the first chromatograms, containing the less polar steroids, were reapplied to other paper strips and chromatographed with the Bush A1 system—ligroin: methanol: water, 10:9:1—(10). Progesterone was found in the distant third of these paper strips, and was separated from pregnenolone by about 7 cm. The effluents from these papers contained any contaminating cholesterol or desmosterol, and

<sup>1</sup>  $\Delta^4$ -pregnene-3,20-dione; 3 $\beta$ -hydroxy- $\Delta^5$ -pregnen-20-one;  $\Delta^4$ -pregnene-11 $\beta$ ,21-diol-3,20-dione;  $\Delta^4$ -pregnen-21-ol-3,-11,-20-trione; and 17 $\alpha$ ,21-dihydroxy- $\Delta^4$ -pregnene-3,11,20-trione.

were combined with the sterol-containing fractions from the silica gel columns.

Each paper chromatogram was scanned for radioactivity with an Actigraph II paper strip scanner (Nuclear-Chicago Corp.). Test portions 1 or 2 mm wide were cut off and tested for reducing areas and for  $3\beta$ -hydroxy- $\Delta^5$  steroids with the Allen reagent (11). All carrier steroids could be visualized under ultraviolet light.

Areas of radioactivity in the same positions as carrier steroids were cut out and eluted by descending capillary flow of ethanol. Adjacent regions of the papers, which were devoid of radioactivity, were also eluted as control areas, to determine the background level of radioactivity on the papers. All or part of each eluted sample was then evaporated and radioassayed in the scintillation spectrometer.

The pregnenolone formed from  $C^{14}$ -desmosterol in the first experiment was further characterized. One-tenth of the eluate from the pregnenolone area was used for radioassay, and the remainder was reduced, to form 5-pregnene- $3\beta,20\beta$ -diol, with 2 mg potassium borohydride in 4 ml 80 per cent ethanol. After standing for 6 hours at room temperature, the mixture was neutralized with acetic acid, diluted with 2 ml water, and the products extracted with  $CH_2Cl_2$ . Chromatography on paper resulted in a band giving a yellow color with the Allen reagent and located directly opposite a reference standard of 5-pregnene- $3\beta,20\beta$ -diol. The strip scanner showed radioactivity over the same area. This region was eluted and radioassayed, together with the eluate from a control area.

The corticosterone formed from both  $C^{14}$ -desmosterol and  $C^{14}$ -cholesterol in the second experiment was also characterized further. From each chromatogram of polar steroids, a relatively wide area, which included all the corticosterone carrier, was eluted. One-tenth of the eluate was radioassayed and the remainder acetylated with acetic anhydride and pyridine. Chromatography of the product in the Bush B1 system for 2.5 hours showed one band absorbing ultraviolet light directly opposite the compound B acetate reference standard. The strip scanner showed definite peaks of radioactivity in the same areas in both chromatograms. Areas of weak radioactivity also appeared opposite compound A acetate. The regions corresponding to the acetates of compounds B and A were separately eluted, together with adjacent control areas, and were radioassayed.

## RESULTS

*Sterol side-chain oxidation by liver mitochondria.* Preliminary experiments with liver mitochondria from normal mice showed a consistent yield of 4.5 to 6 per cent of the terminal carbons of  $C^{14}$ -cholesterol as  $C^{14}O_2$ , when the experiments were conducted as described.

For the definitive comparison of  $C^{14}$ -cholesterol

and  $C^{14}$ -desmosterol as substrates, mitochondria were prepared from mice fed 1 per cent triparanol for 12 days. Since such mitochondria are incapable of reducing desmosterol to cholesterol (12), this ensured that the added  $C^{14}$ -desmosterol would not be converted to  $C^{14}$ -cholesterol during the incubation. Any radioactivity appearing as  $C^{14}O_2$  in the incubations with  $C^{14}$ -desmosterol would therefore have had to come directly from desmosterol. The effectiveness of the drug therapy was checked by assay of the serum sterols of one mouse after 9 days of drug feeding. Desmosterol comprised 35 per cent of the total serum sterols, indicating the presence of an effective block of desmosterol reductase.

The results of this study are shown in Table I. Incubations with each labeled sterol were carried out in duplicate, together with a control incubation lacking mitochondria. The results indicated that both sterols were effective substrates for oxidation by the mitochondria. Similar yields of  $C^{14}O_2$  were obtained with both sterols, with the yield from cholesterol being slightly greater than that from desmosterol. Even this slight apparent difference may not have been real, however, since the liver mitochondria in each incubation contained almost 1  $\mu$ mole of endogenous sterol, 60 to 70 per cent of which was desmosterol. The specific radioactivity of the added  $C^{14}$ -desmosterol was therefore probably more reduced than that of  $C^{14}$ -cholesterol by dilution with endogenous sterol. It is clear that the two sterols were comparable in their ability to serve as substrates in this system.

At the end of the experiment, the incubation mixtures were saponified and the nonsaponifiable compounds extracted and assayed for cholesterol

TABLE I  
*Oxidation of the terminal side-chain of cholesterol and desmosterol by liver mitochondria from triparanol-fed mice*

$C^{14}$ -sterol added	$C^{14}O_2$ collected		Yield of terminal $C^{14}$ as $C^{14}O_2$
	dpm	$\mu$ mole	%
Cholesterol	228	38	4.2
Cholesterol	247	42	4.6
Desmosterol	147	31	3.4
Desmosterol	163	34.5	3.8
Control: no enzyme + cholesterol	4	<1	<0.1

and desmosterol by differential colorimetry (3). Evidence that C<sup>14</sup>-desmosterol had not been converted to C<sup>14</sup>-cholesterol was obtained by chromatography of the sterols, from the incubations with C<sup>14</sup>-desmosterol, as their *p*-phenylazobenzoyl esters. No radioactivity was found in the cholesterol zones from these chromatographies.

*Steroid hormone biosynthesis by adrenal homogenates.* The first experiment with adrenal homogenates showed a definite conversion of both C<sup>14</sup>-sterols into C<sup>14</sup>-steroid hormones. The total yield of steroid hormones was in the range of 4 to 6 per cent of each labeled sterol. In each incubation, radioactivity was detected in compound B, compound A, pregnenolone, and progesterone. The greatest amounts of radioactivity were found in the chromatographic areas corresponding to compound B, although considerable radioactivity was found in the areas of both pregnenolone and progesterone. More radioactivity was found in the latter two steroids with desmosterol as substrate than with cholesterol.

Because of the relatively low specific radioactivity of the first preparation of C<sup>14</sup>-desmosterol, and because of some technical difficulties, exact quantitative data could not be obtained from the first experiment. The second experiment was hence conducted to obtain quantitative data on the yield of each steroid hormone from each C<sup>14</sup>-sterol. The results, summarized in Table II, showed conversion of both C<sup>14</sup>-cholesterol and of C<sup>14</sup>-desmosterol into each of the four steroids listed. In contrast to the first experiment, only very small amounts of radioactivity were detected in pregnenolone and progesterone, with almost all

the steroid radioactivity found in compounds B and A. No radioactivity was detected with the strip scanner in locations corresponding to other steroids. Very small amounts of radioactivity in such locations would not have been detected because of the low sensitivity (efficiency about 6 per cent) of the strip scanner. As described above, control elutions were made with portions of each paper strip that were on each side of the locations of the compounds listed in Table II. The radioactivity eluted from the areas corresponding to the compounds was much greater than that from the control areas, and was corrected by subtraction of the radioactivity in control areas of identical length.

Further evidence that the observed radioactivity actually resided in the steroid hormones was obtained with pregnenolone and with compound B. After reduction of the eluate from the pregnenolone area (in experiment 1) and rechromatography, the peak of radioactivity coincided with the location of 5-pregnene-3 $\beta$ ,20 $\beta$ -diol. Almost 70 per cent of the total radioactivity used in the reduction was recovered in this peak. Similarly, rechromatography of 90 per cent of the eluates from the areas corresponding to compound B (in experiment 2) after acetylation resulted in the recovery of 60 to 70 per cent of the radioactivity in areas corresponding to compound B-acetate and to A-acetate (mostly in B-acetate). This is approximately the expected recovery for the series of manipulations. For tabulation of the radioactivity recovered in each compound (Table II), the C<sup>14</sup> found in the A-acetate zone was added to the radioactivity found in the area of compound A

TABLE II  
*Biosynthesis of steroid hormones from cholesterol and desmosterol by adrenal gland homogenates from triparanol-fed rats*

C <sup>14</sup> -sterol added	Steroid product	Radioactivity		Yield	
		Observed	Corrected *	%	$\mu$ mole
Cholesterol	Compound B	384	640	2.1	22
Cholesterol	Compound A	228	379	1.3	13
Cholesterol	Pregnenolone	19	32	0.12	1.2
Cholesterol	Progesterone	23	38	0.13	1.3
Desmosterol	Compound B	972	1620	2.2	22
Desmosterol	Compound A	527	876	1.2	12
Desmosterol	Pregnenolone	162	271	0.36	3.7
Desmosterol	Progesterone	62	102	0.14	1.4

\* Corrected for loss of 2 of 5 labeled carbons during side-chain removal (see text).

in the first chromatography. The individual yields listed for compounds B and A may therefore be somewhat inaccurate, although the sum of the yields of compounds B plus A is accurate in each case.

In Table II the first column of figures lists the actual dpm observed (minus appropriate controls) in the areas corresponding to each steroid. Since two of the five labeled carbon atoms of the precursor sterol are lost during the removal of the terminal five carbons of the side-chain en route to steroid hormones, the observed dpm have been corrected for this loss in the second column.

The data in Table II indicate that C<sup>14</sup>-cholesterol and C<sup>14</sup>-desmosterol were remarkably similar in their abilities to act as substrates for the biosynthesis of steroid hormones. The yields of the different steroids from the two sterols were almost identical.

Direct evidence was again obtained that the added C<sup>14</sup>-desmosterol was not converted to C<sup>14</sup>-cholesterol during the incubation. The neutral lipids and sterols from each incubation, obtained in the first elution from the small silica gel columns, were saponified, the nonsaponifiable compounds extracted, and one-third of each nonsaponifiable fraction added to a 1-mg mixture of non-radioactive cholesterol and desmosterol. These mixtures were then acetylated, and cholesterol acetate and desmosterol acetate separated by thin-layer chromatography (4). Ninety-eight per cent of the radioactivity in the nonsaponifiable fraction from the incubation with C<sup>14</sup>-cholesterol was found in the cholesterol acetate zone. All of the radioactivity in the nonsaponifiables from the incubation with C<sup>14</sup>-desmosterol was found in the desmosterol acetate zone. These results, therefore, indicated that the radioactive steroids found in each incubation must have been directly derived from the substrate sterol added.

#### DISCUSSION

In our previous study, indirect evidence was presented for the conversion of desmosterol to bile acids and to steroid hormones in triparanol-treated men (2). The present study provides confirmatory direct evidence for this conclusion by *in vitro* experiments with tissues from triparanol-treated rats and mice. Drug-treated animals were

employed in order to prevent the conversion of C<sup>14</sup>-desmosterol to C<sup>14</sup>-cholesterol during the incubations. In each experiment, analysis of the sterols present at the end of the incubations indicated that conversion of desmosterol to cholesterol had not occurred. A quantitative comparison of the effectiveness of cholesterol and desmosterol as substrates was obtained by conducting simultaneous incubations with labeled sterol substrates that were prepared in the same manner.

Despite studies by several groups of workers (7, 13-17), the details of the *in vitro* oxidation, by liver mitochondria, of the terminal carbons of the cholesterol side-chain to CO<sub>2</sub> are only partly understood. This fact is reflected in the large number of cofactors employed in the incubation mixtures. It is, however, generally accepted that this oxidation is related to the reactions involved in the biosynthesis of bile acids. Evidence exists that bile acid biosynthesis involves the oxidative removal of the terminal three carbons of the sterol side-chain as propionylcoenzyme A (15). There is also evidence that the *in vitro* oxidation of the terminal side-chain of cholesterol by liver mitochondria involves removal of carbons 25, 26, and 27 as a three-carbon unit (14). The three-carbon unit is then presumably completely oxidized to CO<sub>2</sub>.

In the present experiments, very similar yields of C<sup>14</sup>O<sub>2</sub> were obtained with C<sup>14</sup>-cholesterol and with C<sup>14</sup>-desmosterol. If the production of this C<sup>14</sup>O<sub>2</sub> followed the removal of carbons 25 to 27 as an intact three-carbon unit, these results would suggest that the presence of a double bond between carbons 24 and 25 does not significantly affect the reactions involved in the splitting of the sterol side-chain at this site. It is, of course, also possible that the effects of the presence of the 24,25 double bond are obscured because other reactions in the total process may be rate-limiting.

The experiments with adrenal gland homogenates demonstrated the formation of 35 to 40 nμmoles of labeled steroid hormone on incubation with approximately 1 μmole of each labeled sterol precursor. Compound B, the major steroid normally produced by rat adrenal glands, was the main labeled product in each case. Recent studies on the biosynthesis of adrenal steroid hormones (18-22) have revealed that cholesterol is probably first hydroxylated to form 20,22-dihydroxy-

cholesterol, followed by cleavage of the side-chain to yield pregnenolone and isocaproic aldehyde. The enzymes involved are located in the adrenal mitochondria and require molecular oxygen and reduced triphosphopyridine nucleotide. It is generally accepted that the hydroxylation of the sterol side-chain is the rate-limiting reaction in the production of steroid hormones. The finding that identical yields of steroids were obtained from C<sup>14</sup>-cholesterol and C<sup>14</sup>-desmosterol therefore suggests that the enzymes involved in the side-chain hydroxylation, and possibly also the side-chain cleavage enzyme, are unaffected by the presence of a double bond in the side-chain.

Several reports have indicated that adrenal function seems to be reduced during triparanol administration (23, 24). The present demonstration that desmosterol is as good a steroid precursor as cholesterol in the triparanol-treated rat adds support to our previous conclusion, that the decline in adrenal activity during triparanol therapy is not due to the specific blocking of the conversion of desmosterol to cholesterol. The reason for the reduced adrenal function is hence not clear. In preliminary analyses we have, however, observed that the sterol content of the adrenals of triparanol-treated rats is less than normal, and that the relative content of free sterol in these adrenals is much greater than normal. Thus, analyses of two sets of pooled adrenals from triparanol-fed rats showed a total sterol content of 5 to 10 mg sterol (cholesterol plus desmosterol) per gram of wet weight tissue; the normal sterol concentration is close to 30 mg per gram wet weight (25). A reduced sterol concentration in adrenal glands from triparanol-treated rats has also been reported by Paoletti (26). Most striking, however, was the finding that 50 per cent or more of the adrenal sterol in the triparanol-treated rats was present as free sterol, with the same ratio of desmosterol to cholesterol in both free and total sterol. Normal rat adrenals contain most (90 per cent) of their sterol as esterified cholesterol (25). It is possible that this great relative increase in free sterol in the drug-fed animals is in some way related to the reduced adrenal function in these animals.

Finally, the finding that desmosterol is an effective substrate for adrenal steroid and bile acid biosynthesis raises the interesting possibility that even under normal conditions some of these com-

pounds may be synthesized directly from sterol precursors of cholesterol.

#### SUMMARY

The metabolism of C<sup>14</sup>-labeled 24-dehydrocholesterol (desmosterol) and cholesterol was compared with *in vitro* liver and adrenal preparations from triparanol-fed mice and rats. Both sterol substrates were biosynthetically prepared from 2-C<sup>14</sup>-mevalonic acid. The reactions studied included the oxidation of the terminal carbons of the sterol side-chain to CO<sub>2</sub> by mouse liver mitochondria and the conversion of sterol to steroid hormones by rat adrenal gland homogenates. Similar yields of CO<sub>2</sub> and of steroid hormones, were obtained with each of the two sterol substrates, demonstrating that desmosterol and cholesterol were quantitatively comparable as substrates in these *in vitro* enzyme systems. These results confirm our previous *in vivo* study (2), which suggested that desmosterol was similar to cholesterol in its ability to serve as a precursor for bile acids and for steroid hormones in patients on triparanol therapy.

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