glycolytic intermediate, to be formed by the action of a specific fructose 1-phosphate kinase (Slein, Cori & Cori, 1950). It is, however, difficult to speculate on the activity of the enzyme under conditions obtaining in intact muscle owing to the lack of knowledge of steady-state concentrations of glyceraldehyde and of glycerol in muscle cytoplasm. In phosphate buffer, pH 7.4 (0.05M), with saturating concentrations of D-glyceraldehyde and NADPH the glycerol dehydrogenase in homogenates of skeletal muscle metabolized 2800 μ moles of D-glyceraldehyde/hr./g. of muscle. Under optimum conditions, without provision for the removal of the glyceraldehyde, the rate of the reaction in the reverse direction (glycerol to D-glyceraldehyde) is one-tenth of this. The rate of [14 C]glycerol conversion into $^{14}\text{CO}_2$ (113 μ moles/hr./g. of muscle) and incorporation into lipids (29 μ moles/hr./g. of muscle) in diaphragm slices (Table 1) is therefore apparently of a similar order of magnitude to the activity of glycerol dehydrogenase in skeletal-muscle homogenates.

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The Conversion of 7-Dehydrocholesterol into Cholesterol

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Previous investigations from several Laboratories have established the sequence of reactions leading to synthesis of cholesterol (II) from acetate (Clayton, 1965). The mechanisms of a number of the reactions, especially those between acetate and lanosterol, have also received attention (Clayton, 1965). However, details of the reactions that result in the formation of cholesterol from lanosterol are still obscure. In the biosynthesis of cholesterol from lanosterol three methyl groups at positions 14, 4 α and 4 β are replaced by three hydrogen atoms, the $\Delta^{24,25}$ -double bond is reduced and the $\Delta^{8,9}$ -double bond is replaced by one between C₍₅₎ and C₍₆₎.

Some recent reports suggest that the last intermediate in the biosynthesis of cholesterol (II)

is 7-dehydrocholesterol (I) (Dempsey, Seaton, Schroepfer & Trockman, 1964; Dvornik, Kraml & Bagli, 1964); however, it has not yet been unambiguously established that 7-dehydrocholesterol is an obligatory intermediate in the biosynthesis of cholesterol.

The present communication describes the origin and stereochemistry of the two hydrogen atoms at positions 7 and 8 during the conversion of 7-dehydrocholesterol (I) into cholesterol (II) with rat-liver homogenates by the enzyme 7-dehydrocholesterol reductase. Optimum conditions for the conversion of 7-dehydrocholesterol (I) into cholesterol (II) were studied by using 7-dehydro[19- ^3H]cholesterol (Akhtar & Gibbons, 1965). In all experiments

cholesterol was isolated by preparative thin-layer chromatography and purified to constant radioactivity by the dibromide method (Fieser, 1953).

Incubation of non-radioactive 7-dehydrocholesterol (I) in the presence of $[4\text{-}^3\text{H}]\text{NADPH}$ (Popják, Goodman, Cornforth, Cornforth & Ryhage, 1961) gave radioactive cholesterol. Although there were individual variations, the direct transfer of hydrogen from NADPH to cholesterol was demonstrated by six independent experiments. The position of the hydrogen atom thus biosynthesized was established by the following sequence of reactions.

Cholesterol (II), on treatment with acetic anhydride, gave the acetate (III), which on treatment with NaNO_2 and HNO_3 yielded the nitro compound (IV). On reduction with zinc and acetic acid at 80° the nitro compound gave the ketone (V), which retained 88% of the radioactivity of the parent acetate (III). The ketone (V), when treated with methanolic KOH, lost at least 93% of the activity of the original acetate (III). These experiments conclusively establish that in the conversion of 7-dehydrocholesterol (I) into cholesterol (II) one hydrogen atom is transferred directly from NADPH to position 7 of cholesterol.

In the second series of experiments non-radioactive 7-dehydrocholesterol (I) was incubated in the presence of tritiated water and non-radioactive NADPH. The cholesterol was processed to the ketone (V) as described above. Radioactivity measurement of the base-equilibrated ketone (V) revealed that the cholesterol biosynthesized in the presence of tritiated water contained 74% of the radioactivity at $\text{C}_{(7)}$ and hence the remaining 26% at $\text{C}_{(8)}$. Since we have already shown that the hydrogen at $\text{C}_{(7)}$ is derived from NADPH, the presence of radioactivity at $\text{C}_{(7)}$ in the tritiated-water experiment remains to be explained. It has been suggested by Popják *et al.* (1961) that there is a soluble enzyme system in rat-liver homogenates that directs an equilibration of hydrogen at $\text{C}_{(4)}$ of NADPH with those of water by the following sequence of reactions:

The radioactivity at $\text{C}_{(7)}$ in cholesterol (Expt. 2 in Table 1) biosynthesized in the presence of

Table 1. *Incorporation of ^3H into cholesterol from $[4\text{-}^3\text{H}]\text{NADPH}$ and tritiated water on incubating 7-dehydrocholesterol with rat-liver homogenate*

Samples (10 ml.) of a 500g cell-free supernatant of rat-liver homogenates (1.25 ml. of 0.1 M-sodium phosphate buffer, pH 7.3/g. of liver) were incubated with 300 μg . of 7-dehydrocholesterol at 37° for 3 hr. under anaerobic conditions. In Expt. 1, 2 μmoles of $[4\text{-}^3\text{H}]\text{NADPH}$ (6.5 μC) were used, giving cholesterol of activity 2500 counts/min. In Expt. 2, 2 μmoles of NADPH and 15 μmoles of glucose 6-phosphate together with 100 mc of tritiated water were used, giving cholesterol of activity 23700 counts/min. The radioactive samples were dissolved in Nuclear Enterprise 213 or 220 liquid-scintillation fluid and counted in an I.D.L. 2022 liquid-scintillation counter at 15% efficiency. Experimental error was less than 3%.

	Activity of compounds (counts/min./m-mole)						
	Cholesterol (II)	6-Nitro compound (IV)	6-Oxo compound (V)	6-Oxo compound after base treatment (V)	5 α -Hydro- peroxy compound (VI)	Percentage of total radioactivity	
						At C ₍₇₎	At C ₍₈₎
Expt. 1: Cholesterol from [4- ³ H]NADPH	5800	—	5120*	440	—	93	7†
Expt. 2: Cholesterol from tritiated water	9050	8800	7540*	2320	2520	74	26

* Loss due to partial equilibration under acid conditions.

† The corrected value for the radioactivity at C₍₈₎ in this experiment is considerably less than 7%. However, this correction involves a few assumptions and therefore we prefer to quote the uncorrected value.

tritiated water can only have originated from NADPH formed by the equilibration reaction (3). The equilibration reaction (3) does not affect the main conclusion based on the $[4\text{-}^3\text{H}]\text{NADPH}$ experiment because any equilibration of the label in this experiment would hardly affect the net radioactivity of the hydrogen atoms of water. The equilibration reaction, however, decreases the incorporation of label from $[4\text{-}^3\text{H}]\text{NADPH}$ into cholesterol (II).

We now attempted to determine the stereochemistry of the hydrogen at C₍₇₎. Cholesterol obtained from the tritiated-water experiment was converted into the 5 α -hydroperoxide by the method of photo-oxidation (Nickon & Bagli, 1961). This conversion resulted in a 97% loss of label originally present at C₍₇₎ (deduced from Table 1). It has been shown (Nickon & Bagli, 1959) that in the conversion of cholesterol into the hydroperoxide (VI) the 7 α -hydrogen is stereospecifically lost. We therefore conclude that in the conversion of 7-dehydrocholesterol (I) into cholesterol (II) the 7 α - and 8 β -hydrogen atoms are derived from NADPH and a suitable proton source respectively. The overall addition is *trans*-diaxial, as shown in formula (VII).

The relative isotope effect, 3:1, in the formation of C₍₈₎-H and C₍₇₎-H bonds respectively, suggests that the crucial step in the 7-dehydrocholesterol reductase-catalysed conversion of 7-dehydrocholesterol (I) into cholesterol (II) is the formation of the C₍₈₎-H bond, thus generating a highly stable allylic

carbonium ion at C₍₇₎ that in accordance with chemical expectations is neutralized by the delivery of a hydrogen by NADPH from the α -side of the steroid molecule.

Avigan & Steinberg (1961) have shown that an NADP-dependent dehydrogenase is concerned in the reduction of the side-chain double bond between C₍₂₄₎ and C₍₂₅₎ of steroids, and our results suggest that in this reduction the hydrogen atoms at C₍₂₄₎ and C₍₂₅₎ would be derived from a proton source and NADPH respectively.

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