1970; Hornby & Boyd, 1970). Certain C₂₈ and C₃₀ substrates give C₂₈ and C₂₉ acids respectively under conditions that prevent oxidation at C-3. Thus the formation of a 3 β -hydroxy-4 β -methyl-5 α -cholestene-4 α -carboxylic acid from lanost-8-enol has been reported (Hornby & Boyd, 1970). The double bond in this acid can now be located at Δ^8 .

A microsomal acetone-dried powder and a 105000g supernatant were prepared from rat liver as previously described (Hornby & Boyd, 1970; Scallen, Dean & Schuster, 1968). Each incubation mixture (4.5ml) comprised a suspension of 50mg of acetone-dried powder in 4ml of 105000g supernatant supplemented with glucose 6-phosphate (2.2mm), NADP⁺ (0.22mm), glucose 6-phosphate dehydrogenase (0.2 unit) and glutathione (4.3mm). No NAD⁺ was added. After 1 h at 37°C incubations were terminated with methanol. Carboxylic acids were recovered from the lipid product by t.l.c.

[2-³H₂]4,4-Dimethyl-5 α -cholesta-8,14-dienol was synthesized (Klein & Knight, 1965) and incubated under these conditions to give the same acid as that obtained from lanost-8-enol, as judged by gas chromatography-mass spectrometry of their trimethylsilyl ether methyl esters. This acid cannot be $\Delta^{8(14)}$, since $\Delta^{8(14)}$ -monoenes, if involved in the biosynthetic pathway, would precede $\Delta^{8,14}$ -dienes. Unlabelled 4,4-dimethyl-5 α -cholest-7-enol gives 3 β -hydroxy-4 β -methyl-5 α -cholest-7-ene-4 α -carboxylic acid (J. L. Gaylor, personal communication), which has different gas chromatography-mass spectrometry characteristics. The compound from lanost-8-enol must therefore be 3 β -hydroxy-4 β -methyl-5 α -cholest-8-ene-4 α -carboxylic acid. The Δ^8 acids contained no Δ^7 acid by gas chromatography, which suggests that the $\Delta^8 \rightarrow \Delta^7$ isomerase is unable to accommodate a substrate with C-4 α carboxyl and C-4 β methyl groups. 4,4-Dimethyl compounds can be isomerized when anaerobic conditions prevent further demethylation (Gaylor, 1966). During cholesterol biosynthesis in rat liver, therefore, the $\Delta^8 \rightarrow \Delta^7$ isomerization seems to follow the loss of the C-14 and one of the C-4 methyl groups.

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The Effect of Dietary Cholesterol on Cholesterol Biosynthesis and other Processes in Mammalian Liver

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Cholesterol biosynthesis in mammalian liver is stimulated by injection of thyroid hormone (Guder, Nolte & Wieland, 1968) or phenobarbital (Jones & Armstrong, 1965); it is influenced by the concentration of dietary cholesterol and is depressed during starvation (Bucher, 1959). There is a diurnal variation in the rate of cholesterogenesis between a maximum at midnight and a minimum at midday (Hamprecht, Nüssler & Lynen, 1969). There is good evidence that the rate of cholesterol biosynthesis is dictated by the activities of those enzymes converting acetate into mevalonate; hydroxymethylglutaryl-CoA reductase (EC 1.1.1.34) in particular is stimulated or depressed by procedures that respectively accelerate or retard cholesterogenesis. There is little indication of how the activity of this enzyme is controlled.

Hydroxymethylglutaryl-CoA reductase is a microsomal enzyme and many of the factors affecting its activity are not specific in that they cause a variety of changes in the endoplasmic reticulum. For example, thyroid hormones are known to induce growth of the endoplasmic reticulum (Tata, 1968) and their effect of stimulating the reductase is one of the consequences. It is important that this should be so, because only by stimulating the reductase will increased production of cholesterol, necessary to sustain membrane proliferation, be accomplished. Indeed it is highly significant that the enzymes responsible for synthesizing other structural lipids, such as phospholipids, are themselves constituents of the microsomal membrane (Wilgram & Kennedy, 1963); in consequence the co-ordinated production of lipids and proteins, characteristic of growth, can be achieved. The action of other factors affecting hydroxymethylglutaryl-CoA reductase will be discussed in relation to their effects on other aspects of endoplasmic reticular metabolism.

During studies of the mode of action of cholesterol on hepatic cholesterogenesis, the influence of dietary cholesterol on certain microsomal functions has been investigated in the rat. The effect of dietary cholesterol on the rate of cholesterol biosynthesis is found not to be accompanied by changes in constitutive microsomal enzymes such as glucose 6-phosphatase or NADPH-cytochrome *c* reductase, or substances such as cytochrome *b*₅. The action of dietary cholesterol therefore appears to be rather specific in nature and the significance of these

observations to cholesterol metabolism will be discussed.

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The Identification of 17 α -Hydroxy-17 β -methyl-androsta-1,4-dien-3-one as a Metabolite of 17 β -Hydroxy-17 α -methylandrosta-1,4-dien-3-one in Man

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After the administration of the anabolic steroid 17 β -hydroxy-17 α -methylandrosta-1,4-dien-3-one (methandrostenolone) to man, two major metabolites have been found in the chloroform extract of fresh urine. The least polar metabolite has now been identified as 17 α -hydroxy-17 β -methylandrosta-1,4-dien-3-one, the 17-epimer of methandrostenolone. The polar metabolite has already been identified as 6 β -hydroxymethandrostenolone by Rongone & Segaloff (1963).

The 17-epimer was synthesized from 3 β -hydroxy-androst-5-en-17-one by reaction with triphenylmethylphosphonium bromide to form the 17-methylene derivative, which was oxidized and then isomerized to form the 4-en-3-one; this was oxidized with *m*-chloroperoxybenzoic acid to yield 17 β -methyl-17 α ,20-oxidoandrost-4-en-3-one. The oxido-ketone was reduced with lithium aluminium hydride to a 3 β ,17 α -diol, which was oxidized and dehydrogenated in one step with dichlorodicyanobenzoquinone to give 17 α -hydroxy-17 β -methylandrosta-1,4-dien-3-one. This was clearly distinguished from methandrostenolone by its n.m.r. spectrum and melting point, 221°C, which was similar to that of the metabolite isolated by Rongone & Segaloff (1963).

The freely extractable neutral fraction was obtained from a fresh 24h collection of urine from a

normal man after oral administration of 5mg of methandrostenolone. The component with the same 'carbon skeleton' as methandrostenolone (Adhikary & Harkness, 1969), which behaved chromatographically like methandrostenolone, was separated by t.l.c. followed by g.l.c. and then subjected to mass spectrometry (A.E.I. MS902). The epimer and metabolite showed a characteristic and similar pattern; for example, the molecular-ion peak at *m/e* 300 was always smaller than the peak at *m/e* 282. This was reversed for methandrostenolone.

No 17-epimer was found in the administered drug, nor was there any detectable conversion during the isolation procedure. It therefore seems justifiable to conclude that the 17-epimer is a metabolite apparently produced by a new pathway, which is relatively important since about 3.5% of an oral dose can be recovered as the epimer.

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Exchange of Sterols between Rat Brain Membranes

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The long-term persistence and negligible turnover of [4-¹⁴C]cholesterol and other radioactive precursors in the white matter of the brain has been taken to indicate the relative metabolic stability of myelin (Davison, Dobbing, Morgan & Payling-Wright, 1959; Torvik & Sidman, 1965; Simon, 1966). However, Rawlins, Hedley-Whyte, Villegas & Uzman (1969) have evidence of reutilization of [1,2-³H]cholesterol in the peripheral nervous system. Recent studies on the metabolism of brain membrane sterols suggest that during development they undergo relatively rapid replacement. Thus substantial amounts of cholesterol precursors have been shown to be accumulated in brain and myelin after developing rats have been injected with hypocholesterolaemic drugs (Banik & Davison, 1967; Fumagalli, Smith, Urna & Paoletti, 1969). After injection of AY-9944 [*trans*-1,4-bis-(2-chlorobenzylaminomethyl)cyclohexane dihydrochloride] into 5-day-old rats myelin and all subcellular fractions of the brain were found to contain 50–60% of total sterol as 7-dehydrocholesterol. Within a week much of the 7-dehydrocholesterol was replaced by cholesterol. After administration of [1-¹⁴C]-acetate most of the radioactivity of the myelin 7-dehydrocholesterol could be subsequently recovered in cholesterol. These results suggested that 7-dehydrocholesterol is reduced *in situ* by 7-dehydro-reductase to cholesterol, but we found only about