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

10% of the total brain reductase activity in water-shocked myelin and about 60% of the activity in the microsomal fraction. Another possibility therefore is that 7-dehydrocholesterol released from myelin is transported to the endoplasmic reticulum, there reduced to cholesterol and reincorporated into the myelin sheath. ^{14}C -labelled myelin preparations from young rat brain were incubated with normal 29-day-old rat brain suspensions and post-mitochondrial preparations. The samples were incubated at 37°C for 30 min and recentrifuged to separate myelin and microsomes. About 10–12% exchange of radioactive cholesterol and total phospholipid and 6% exchange of cerebroside has been found in the unlabelled microsomes isolated after incubation. Similar results were obtained with 7-dehydrocholesterol-containing myelin. 2':3'-Cyclic nucleotide 3'-phosphohydrolase was used as a measure of myelin contamination in microsomes isolated after incubation at 37°C for 30 min. This exchange was not due to intact myelin contamination in the microsomal fraction, since no gain in 2':3'-cyclic nucleotide 3'-phosphohydrolase activity in the microsomal fraction and no loss from the myelin fraction was observed after 30 min incubation at 37°C . Exchange between serum lipoprotein cholesterol and adult myelin (Graham & Green, 1967) or erythrocyte stroma (Ashworth & Green, 1964) has been demonstrated. Phospholipid exchange in liver mitochondrial membrane is also now well established (Wirtz & Zilversmit, 1968; Beattie, 1969; McMurray & Dawson, 1969). Our observations may explain the apparent persistence of labelled cholesterol in myelin and brain mitochondrial membranes and suggest that the sterol is only loosely bound within the membrane structure.

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Further Studies on the Biosynthesis of Androsta-5,16-dien-3 β -ol and the Subcellular Location of the Site of Biosynthesis

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In continuing earlier studies of the biosynthesis of C_{19} Δ^{16} -steroids from C_{21} steroids by boar testis (Ahmad & Gower, 1968; Katkov & Gower, 1970) the possible involvement of a 20-hydroxy derivative as an intermediate has been investigated. 4- ^{14}C -labelled pregnenolone, pregn-5-ene-3 β ,20 α -diol and pregn-5-ene-3 β ,20 β -diol (I) were each incubated with boar testis homogenate in tris-HCl buffer, pH 7.4 (0.05M), containing NADPH (0.4mM) at 37°C for 20 min, and radioactive androsta-5,16-dien-3 β -ol (andien- β) was isolated and assayed (Katkov & Gower, 1970). The mean yields of andien- β formed from pregnenolone, the 20 α -hydroxy derivative and compound (I) were respectively 25.6, 2.7 and 16%. Subsequent short-term kinetic studies with pregnenolone and compound (I) as substrates seemed to indicate that the latter might be an intermediate in andien- β biosynthesis. However, attempts to isolate radiochemically pure compound (I) from chromatographic fractions by reverse isotope dilution were unsuccessful. Moreover, it was found from various micro-chemical studies that the chief component in these fractions was 17 α -hydroxypregnenolone, which does not give rise to andien- β (Katkov & Gower, 1970). In view of these findings experiments are in progress to evaluate the status of the other metabolites formed in the incubation as precursors for andien- β .

The enzyme system forming andien- β from pregnenolone ('andien- β synthetase') was found to be localized in the microsomal fraction of fresh boar testis homogenate. It was also found that the microsomal fraction gave the same pattern of metabolites as the original homogenate and that 'andien- β synthetase' activity was stable for at least 8 weeks when the microsomal suspension in 0.25M-sucrose was kept frozen at -20°C . An attempt to obtain an enzymically active powder preparation from the homogenate was unsuccessful, although such a preparation was found to possess 17 α -hydroxylase, 17 α -hydroxypregnenolone C-17–C-20 lyase and other enzyme activities. The latter finding, taken together with the recent observation that 17 α -hydroxypregnenolone is a very inefficient precursor for andien- β , suggests that 'andien- β synthetase' may be a separate enzyme system from that involved in androgen biosynthesis.

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