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.

We are grateful to the Multiple Sclerosis Society of Great Britain for their support.

- Ashworth, L. A. E. & Green, C. (1964). *Biochim. biophys. Acta*, **84**, 182.
Banik, N. L. & Davison, A. N. (1967). *J. Neurochem.* **14**, 594.
Beattie, D. S. (1969). *J. Membrane Biol.* **1**, 383.
Davison, A. N., Dobbing, J., Morgan, R. S. & Payling-Wright, G. (1959). *Lancet*, **i**, 658.
Fumagalli, R., Smith, M. E., Urna, G. & Paoletti, R. (1969). *J. Neurochem.* **16**, 1329.
Graham, J. M. & Green, C. (1967). *Biochem. J.* **103**, 16c.
McMurray, W. C. & Dawson, R. M. C. (1969). *Biochem. J.* **112**, 91.
Rawlins, F. A., Hedley-Whyte, E. T., Villegas, G. & Uzman, B. G. (1969). *Proc. 2nd int. Meet. International Society for Neurochemistry, Milan*, p. 334.
Simon, G. (1966). *Lipids*, **1**, 369.
Torvik, A. & Sidman, R. L. (1965). *J. Neurochem.* **12**, 555.
Wirtz, K. W. A. & Zilversmit, D. B. (1968). *J. biol. Chem.* **243**, 3596.

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

By K. H. LOKE* and D. B. GOWER. (*Department of Biochemistry, Guy's Hospital Medical School, London S.E.1, U.K.*)

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.

- Ahmad, N. & Gower, D. B. (1968). *Biochem. J.* **108**, 233.
Katkov, T. & Gower, D. B. (1970). *Biochem. J.* **117**, 533.

* On leave from the University of Malaya.