The Biosynthesis of 3 β -Hydroxyandrost-5,16-diene and its Further Metabolism in Boar Testis

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The biosynthesis of C₁₉ Δ^{16} -steroids from the C₂₁ precursors pregnenolone and progesterone in boar testis tissue is now well documented (Ahmad & Gower, 1968; Katkov & Gower, 1968). These experiments have also shown that more androst-4,16-dien-3-one (I) is formed from pregnenolone than from progesterone *in vitro* and led Ahmad & Gower (1968) to suggest 3 β -hydroxyandrost-5,16-diene (II) as a possible intermediate in the conversion of pregnenolone into compound (I).

In view of the present interest in C₁₉ Δ^{16} -steroids in the pig (Patterson, 1969) and in order to elucidate the mechanism of the formation of these compounds, the biosynthesis of compound (II) from pregnenolone has been studied in more detail.

Boar testis tissue was homogenized in 10 vol. (v/w) of ice-cold sucrose (0.25M). Then 1ml. of this homogenate was pipetted into the reaction medium, which contained [4-¹⁴C]pregnenolone, NAD⁺, NADPH and ATP (each 0.4mM) in a final volume of 5ml. of tris-HCl buffer, pH 7.4 (0.05M). After shaking for 10 min. at 38°, carrier steroids were added and the aqueous mixture was extracted with ethyl acetate. After column chromatography on alumina (Gower & Ahmad, 1967) the combined fractions containing C₁₉ Δ^{16} -steroids were applied successively to thin-layer plates coated with Kieselgel G, developed in benzene-ether (4:1, v/v), and to AgNO₃-impregnated plates (Lisboa & Palmer, 1967), developed in benzene-ethyl acetate (1:2, v/v). This procedure separated compound (II) from other radioactive metabolites. Further proof of its identity was obtained by column chromatography on AgNO₃-impregnated Kieselgel H. Allowing for analytical losses, pregnenolone was found to be metabolized to compound (II) and to 3 β -hydroxy-5 α -androst-16-ene (III) in yields of 8% and 2% respectively. NADPH was an essential cofactor for the formation of compound (II); NADH had less activity and ATP was unnecessary. The amount of compound (II) formed from pregnenolone by boar testis homogenate increased linearly during 15 min. and with increasing protein concentration. No conversion occurred in the absence of boar testis homogenate or in the presence of boiled homogenate.

Radioactive compound (II) was prepared biosynthetically from [4-¹⁴C]pregnenolone and its conversion into compounds (I) and (III) by boar testis tissue was demonstrated. This indicates that

compound (II) is a key intermediate in the biosynthesis of other C₁₉ Δ^{16} -steroids.

Patterson (1968) has shown that C₁₉ Δ^{16} -steroids are present in boar submaxillary glands and saliva. Studies *in vitro* have now shown that boar submaxillary gland can metabolize [4-¹⁴C]pregnenolone to compound (II).

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Phospholipases in Cerebrospinal Fluid and Cell Fractions of Human Brain

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Phospholipases A₁, A₂ and a trace of C were observed in fresh cerebrospinal fluid from human subjects. In an investigation of the possible origin of the above, samples of human brain tissue were examined. The grey matter from post-mortem cases was dissected out, comminuted in a Potter-Elvehjem homogenizer and fractionated into nuclei, mitochondria, lysosomes, microsomes and supernatant components (de Duve, Pressman, Gianetto, Wattiaux & Appelmans, 1955). Appropriate samples containing 2-8mg. of protein from each of these fractions were incubated aerobically with [β -1-¹⁴C-oleyl]lecithin (I), [*Me*-¹⁴C-choline]lecithin (II), [*Me*-¹⁴C-choline]lysolecithin (III) and [*Me*-¹⁴C-choline]sphingomyelin (IV) in turn for 3hr. periods at 37° in tris buffer, pH 7.4. After incubation, the residual phospholipids and their degradation products were extracted from the medium (Bligh & Dyer, 1959), and isolated by t.l.c. on Kieselgel H for the fat-soluble components and by ion-exchange chromatography on Zeo-Karb 225 (Webster & Cooper, 1968) for the water-soluble components.

With compound (I) as substrate, radioactive lysolecithin (a measure of phospholipase A₁ activity) and radioactive fatty acids (a measure of phospholipase A₂ activity and the combined activities of phospholipases A₁ and B) were formed by all fractions. The further addition of deoxycholate (1mg./ml.), an inhibitor of phospholipase B, to parallel incubations only reduced by 20% the radioactivity in the fatty acid fractions.