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.

Thus both phospholipases A₁ and A₂ were present in the cell fractions. In the supernatant, phospholipase A₁ activity (0.4 nmole/hr./mg. of protein) was greater than that of phospholipase A₂ (0.15 nmole/hr./mg. of protein), but in all others phospholipase A₂ activity exceeded that of phospholipase A₁. The greater phospholipase A₂ activity could arise partly from the fact that the pH used, 7.4, was closer to the optimum pH, 6.5, for phospholipase A₂ compared with the optimum pH, 4.4, for phospholipase A₁ (Smith, 1969). Experiments with compound (II) were consistent with the above findings and showed that phospholipases B and C were also present in all cell fractions, but mainly in the mitochondria and lysosomes. Compound (III) was degraded rapidly by phospholipase B by all fractions (3–10 nmole/hr./mg. of protein), so clearly this 'intermediate' in phospholipid metabolism would not tend to accumulate to an extent where it could damage organelle structure. Hydrolysis of compound (III) was strongest in mitochondria and lysosome fractions. From the experiments with compound (IV) sphingomyelinase activity was observed strongest in lysosomes, supernatant and mitochondria, the values being 0.95, 0.39 and 0.23 nmole/hr./mg. of protein respectively.

Since phospholipases A₁, A₂ and sphingomyelinase have been detected in the supernatant fraction of brain tissue cells, it is possible that this could be the source of the phospholipases found in cerebrospinal fluid, because it has been shown by Smith (1969) that the above can be released into the perfusate of the perfused adrenal gland.

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The Proteolytic Action of Arvin on Human Fibrinogen

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Arvin is a purified proteolytic enzyme fraction isolated from the venom of *Agkistrodon rhodostoma*, the Malayan pit viper (Esnouf & Tunnah, 1967). It possesses considerable clinical potential as a means of producing controlled defibrination in patients

requiring anti-coagulant therapy, but, unlike the usual anticoagulants, acts only at the fibrinogen-fibrin level. It is capable of decreasing the plasma fibrinogen concentration virtually to zero without apparently affecting other plasma clotting factors (Bell, Pitney & Goodwin, 1968; Sharp, Warren, Paxton & Allington, 1968; Bell, Bolton & Pitney, 1968).

The action *in vitro* of arvin on purified human fibrinogen results in the production of a clot that is much 'softer' and more readily dispersed than that produced by thrombin, indicating a different mechanism of action. Thrombin is known to release four soluble fibrinopeptides, designated A, AP, AY and B (see Blomback, Blomback, Edman & Hessel, 1966). It has now been shown that under identical experimental conditions a more complex pattern of fibrinopeptides results from the action of arvin preparations. This pattern can be resolved by chromatography of desalted clot supernatants on Dowex 50 resin or by high-voltage electrophoresis. Six acidic peptides can be separated in addition to a number of more basic ones. Three of the acidic peptides are identical with the fibrinopeptides A, AP and AY released by thrombin. Two of the remaining acidic peptides have also been characterized and are heptapeptides derived from the N-terminal ends of fibrinopeptides A and AP. Preliminary investigations indicate the remaining acidic peptide to have been derived from the N-terminal section of the fibrinogen β -chains. The structures of the basic peptides are currently under investigation.

The complexity of the peptide pattern obtained with arvin suggested the existence of more than one proteolytic enzyme in the preparation and it has been possible to demonstrate the presence of at least two such enzymes. The one present in largest amounts is responsible for the clotting phenomenon and liberates only fibrinopeptides A, AP and AY. Fibrinopeptide B is not released by this enzyme. Time-activity studies suggest that this enzyme is responsible for the initial attack on the fibrinogen molecule, leading to clotting.

The significance of these various findings will be discussed.

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