The Enzymic Conversion of 13-Hydroperoxy-*cis*-9-*trans*-11-Octadecadienoic Acid into 13-Hydroxy-12-Oxo-*cis*-9-Octadecenoic Acid

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The formation of hydroperoxides of unsaturated fatty acids containing a *cis,cis*-1,4-pentadiene system is catalysed by lipoxygenase (EC 1.13.1.13). The metabolic role in the plant of the hydroperoxides is still unknown. Zimmerman (1966) reported that flax seed should contain an enzyme that is able to isomerize linoleic acid hydroperoxides to oxo-hydroxy compounds. The structure of these substances has not been completely proved (e.g. the u.v. spectrum is not in accordance with the proposed structures). Following the work of Zimmerman (1966) we tried to prepare these substances as follows. A mixture of the linoleic acid hydroperoxides was obtained from linoleic acid by incubation with soya-bean lipoxygenase (EC 1.13.1.13). After acidification and extraction the hydroperoxides were isolated by thin-layer chromatography on silica gel. The molar ratio of 13-hydroperoxy-*cis*-9-*trans*-11-octadecadienoic acid to 9-hydroperoxy-*trans*-10-*cis*-12-octadecadienoic acid was 10:1. This mixture of hydroperoxides was incubated with a 0.1M-phosphate buffer, pH 7.4, extract of an acetone-dried powder of flax seed. After acidification and extraction the reaction products were separated by thin layer chromatography on silica gel. The main spot was analysed. With the aid of i.r. and u.v. spectroscopy, nuclear-magnetic-resonance analysis of this substance, and by mass-spectrometric analysis of the methyl ester and its toluene-*p*-sulphonyl derivative, the compound was identified as 13-hydroxy-12-oxo-*cis*-9-octadecenoic acid. This structure was further confirmed by ozone and periodate degradation. The optical activity of this component was determined in chloroform as solvent [$(\alpha)_D^{20}$ -10.4]. In contradiction to Zimmerman (1966), we found that the crude enzyme fraction reacts only with the 13-hydroperoxy-*cis*-9-*trans*-11-octadecadienoic acid component of the mixed linoleic acid hydroperoxides.

Further investigations of the substrate specificity with the peroxides of other unsaturated fatty acids are in progress. We attempted to prepare 13-hydroxy-12-oxo-*cis*-9-octadecenoic acid by the oxidation of vernolic acid (*cis*-12,13-epoxy-*cis*-9-octadecenoic acid) with dimethylsulphoxide (Tsuiji, 1966). However, the reaction product turned out to be 12-hydroxy-13-oxo-*cis*-9-octadecenoic acid.

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Biosynthesis of Steroids in Rat Testis Seminiferous tubules

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In the mammalian testis, the interstitial tissue (Leydig cells) is commonly considered to be the principal source of androgens. This concept stems both from the relationship of Leydig-cell appearance with peripheral androgenic manifestations (Albert, 1961), and also from experiments in which interstitial preparations, freed from tubule structures, were incubated *in vitro* to give various steroid products (Christenson & Mason, 1965; LeBourhis & Hofman, 1968; Bell, Vinson, Hopkin & Lacy, 1968).

Recent work from this Department (Bell *et al.* 1968) showed that when rat testis interstitium was incubated with equimolar concentrations of [^3H]-pregnenolone and [^{14}C]-progesterone, a clear indication of the pathways by which the androgens were formed was gained from the sequential incorporation of label into intermediates and end products. The results were considerably less clear when whole-testis preparations were incubated. This may have been due either to the very pronounced selective binding of steroid by the tubule, or to its separate steroidogenic role.

Further experiments were designed in which rat testis seminiferous tubules (without Leydig tissue) were incubated with equimolar concentrations of [7α - ^3H]-pregnenolone and [4 - ^{14}C]-progesterone, and the products examined after different time intervals. [4 - ^{14}C]-Progesterone was readily converted into ^{14}C -labelled 17-hydroxyprogesterone, androstenedione and testosterone. These compounds, together with [^3H]-progesterone, were also formed from [^3H]-pregnenolone, although with considerably less efficiency. This may indicate the relative deficiency of the 3β -hydroxy steroid Δ^5 -dehydrogenase-isomerase in the tubule. Nevertheless, yields of [^3H]-progesterone were substantially greater than yields of 17-hydroxy[^3H]-pregnenolone, indicating that, as in the isolated interstitium incubations, progesterone could well be an important intermediate in the elaboration of androgens from pregnenolone. This, together with the lack of alternative intermediates, suggests that the data are fundamentally compatible with the classical pathway for the formation of androgens from pregnenolone: pregnenolone $\rightarrow$ progesterone $\rightarrow$ 17-hydroxyprogesterone $\rightarrow$ androstenedione $\rightarrow$ testosterone.

No clear sequence of incorporation of either label

into successive intermediates could be discerned, however, since only about 20% of the added radioactivity could be recovered from the incubation medium; this was possibly due to the pronounced binding of steroid by the tissue.

It was noteworthy that, in contrast with the interstitium findings, yields of androstenedione were at all times near the limits of detectability, apparently indicating the rapid turnover of this compound.

In further contrast with the interstitium results, two additional products were isolated from the tubule incubations in substantial yields from both precursors. These were 20 α -hydroxypregn-4-en-3-one and 5 α -pregnane-3 β ,20 α -diol; they indicate the existence in the tubule of a steroid-reductive pathway. Two other products, also not found in interstitial incubations, await identification.

These observations suggest a steroidogenic role for the rat testis seminiferous tubule that is distinct and independent from interstitial hormone production. This concept is contrary to the commonly held view that the interstitial tissue (Leydig cells) is the principal site of testis hormone production in the mammal (Albert, 1961).

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Subribosomal Particles in Newborn-Rat Liver

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Shortly after birth in mammals there is a rise in the activity of a number of liver enzymes and a rapid increase in the rate of nuclear RNA synthesis (Sereni & Barnabei, 1967). In this study, labelling

of cytoplasmic ribosomal subparticles and monomers, polysomes and RNA was investigated at intervals after birth after intraperitoneal injection of [6-¹⁴C]orotic acid.

Optical scanning of postmitochondrial supernatants (PMS) revealed peaks of material sedimenting at approximately 80s, 65s and 45s corresponding to ribosomal monomers and subunits. After a 30min. pulse there was a progressive rise in the total acid-insoluble radioactivity of PMS during the first 24hr. after birth. At 0, 2, 12 and 24hr. after birth the proportion of radioactive material sedimenting more slowly than 80s, in the 45s region, was 13%, 23%, 39% and 40% respectively. The equivalent values for material sedimenting more rapidly than 45s were 9%, 21%, 21% and 22% respectively. In the different age groups there was no significant difference in the proportion of material sedimenting more rapidly than 80s (approximately 67% of total). Selectively isolated polysomes were poorly labelled but their radioactivity rose during the first 24hr. after birth.

RNA was extracted and analysed by 5–20% sucrose-density-gradient centrifugation; 45s subribosomal particles contained 18s RNA only and 65s particles contained both 18s and 28s RNA.

The specific radioactivity of RNA extracted from whole PMS was greater than that from polysomes. But the specific radioactivity of both rose progressively at increasing intervals after birth. After 30min. pulses, radioactivity in the PMS was found mainly in 18s RNA and to a lesser extent in poly-disperse RNA. After 24hr. pulses, radioactivity was predominantly in 28s RNA and the differences between age groups disappeared. Thus, as in steroid-induced protein biosynthesis in adult rat livers (Finkel, Henshaw & Hiatt 1966), the 45s particles containing 18s RNA were labelled selectively after short pulses. The rate of labelling rose rapidly during the 24hr. after birth.

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