The Conversion of Steroid 7,9-Dienes into Cholesterol

By A. D. RAHIMTULA, D. C. WILTON and M. AKHTAR

Department of Physiology and Biochemistry, University of Southampton, SO9 5NH

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Our interest in the mechanisms of biosynthesis in the steroid field led us to investigate the possible role of a 7,9-diene system of the type (I) in cholesterol (V) biosynthesis. The diene system of the type (I) is present in naturally occurring mammalian steroids agnosterol (IV) and dihydroagnosterol (III) (Fieser & Fieser, 1959), and both compounds (IV) and (III) have been biosynthesized from [^{14}C]acetate (Clayton & Bloch, 1956). In this communication it is shown that cholesta-7,9-dien-3 β -ol (I) and dihydroagnosterol (III) are efficiently converted into cholesterol (V) by an enzyme system prepared from rat liver homogenate. The crucial event in these conversions (I $\rightarrow$ V; III $\rightarrow$ V) is the involvement of an enzyme system that catalyses the reduction of the 9,11-double bond in 7,9-dienes of the type (I) and (III).

Cholesta-7,9-dien-3 β -ol (I) (Heusser, Heusler, Eichenberger, Honegger & Jeger, 1952) was oxidized with Jones reagent (Bowden, Heilbron, Jones & Weedon, 1946) to the corresponding 3-ketone; this ketone was then reduced with tritiated NaBH_4 to

give [$3\alpha\text{-}^3\text{H}$]cholesta-7,9-dien-3 β -ol. The experimental details for the synthesis of tritiated compounds by the above sequence are described by Dewhurst & Akhtar (1967). The latter compound (I) (200 μg .; 931 000 c.p.m.) was incubated with 9 ml. of a 10 000 g supernatant of rat liver homogenate under aerobic conditions at 37° for 90 min. (Wilton, Munday, Skinner & Akhtar, 1968). Addition of carrier cholesterol (100 mg.) and purification as described by Wilton *et al.* (1968) showed that 56% of the original radioactivity had been incorporated into cholesterol. For comparison it was shown that under the conditions of the above incubation a related compound, cholest-7-en-3 β -ol (II), was converted into cholesterol in 67% yield. When [$3\alpha\text{-}^3\text{H}$]cholesta-7,9-dien-3 β -ol (I) (200 μg .; 931 000 c.p.m.) was incubated as above but under anaerobic conditions, no conversion into cholesterol occurred; instead cholest-7-en-3 β -ol (II) was formed in at least 20% yield. The formation of the latter compound was demonstrated in the following way. The steroidal fraction obtained from the above incubation was diluted with carrier cholest-7-en-3 β -ol (2 mg.) and acetylated with pyridine-acetic anhydride. The resulting mixture of acetates was applied to a silica gel GF254 thin-layer plate impregnated with 20% (w/v) AgNO_3 , which was run twice in light petroleum (b.p. 60–80°)–benzene (1:1, v/v). The band corresponding to cholest-7-en-3 β -ol acetate (R_F 0.7–0.8) was eluted and shown to contain 185 000 c.p.m. (in a control experiment with boiled enzyme no radioactivity was detected in the cholest-7-en-3 β -ol acetate region). The radioactive material could be crystallized with cholest-7-en-3 β -ol acetate. Conversion of the latter compound (200 mg.; 162 000 c.p.m./m-mole) into cholest-8,9-epoxy-3 β -ol-7-one acetate (12 mg.; 147 400 c.p.m./m-mole) (Akhtar & Rahimtula, 1968) resulted in a 91% retention of radioactivity, thus showing that the radioactivity in the anaerobic incubation was indeed incorporated into the compound (II). The cumulative evidence presented above shows the presence of an enzyme system that catalyses the reduction of the 9,11-double bond in the diene (I) to give the compound (II); the conversion of the latter compound into cholesterol then occurs by the conventional pathway (Akhtar, Watkinson, Rahimtula, Wilton & Munday, 1968). It is possible

(I) $\text{R} = \text{H}$

(II) 9,11-Dihydro-compound (I)

(III) $\text{R} = \text{CH}_3$

(IV) 24,25-Dehydro-compound (III)

(V) Cholesterol

that the reduction of the 9,11-double bond in the diene (I) occurs by the mechanism established for the reduction of the 7,8-double bond in cholesta-5,7-dien-3 β -ol (Wilton *et al.* 1968) and for the reduction of the 14,15-double bond in cholesta-8,14-dien-3 β -ol (Akhtar, Rahimtula, Watkinson, Wilton & Munday, 1969).

The conversion of dihydroagnosterol (III) (Bellamy & Doree, 1941; Doree, McGhie & Kurzer, 1949) was also studied. Dihydroagnosterol (III) was oxidized with Jones reagent (Bowden *et al.* 1946) and the derived 3-ketone was treated with ethanolic 2% (w/v) KOH containing tritiated water (200mc) overnight at room temperature. The product obtained was reduced with LiAlH₄ to give [2-³H₂]dihydroagnosterol (1512500 c.p.m./mg.). The latter compound (III) (200 μ g.; 302500 c.p.m.) when incubated under aerobic conditions as described above gave cholesterol in 4.23% yield. For comparison it was shown that under identical conditions a related compound, dihydrolanosterol, was converted into cholesterol in 12.96% yield.

The natural occurrence of a 7,9-diene system in mammals and the demonstration in the above work of the presence of an enzyme that participates in the reduction of the 9,11-double bond in compounds (I) and (III) highlights the biological importance of agnosterol and dihydroagnosterol and therefore the biosynthetic pathway through which the latter compounds may be formed.

Gaylor (1963) has shown that lanost-7-en-3 β -ol is formed when squalene is converted aerobically into sterols by rat skin. Further, the work of

Gaylor, Delwiche & Swindell (1966) indicates that the isomerization of the 8,9-double bond of lanosterol to the 7,8-position of lanost-7-en-3 β -ol could not take place *in vitro* owing to the presence of the 14 α -methyl group. It is therefore possible that lanost-7-en-3 β -ol may arise from agnosterol (IV) by saturation of the 9,11-double bond.

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