CONVERSION OF 16-^{14}C-ESTRADIOL-17β TO ^{14}C-LABELLED ESTRIOL BY AVIAN LIVER SLICES

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The generally accepted pathway for estradiol - 17β metabolism as it involves the three classical estrogens is :

estradiol ⇌ estrone ⟶ estriol.

The conversion of estradiol to estriol is well established in vivo, this being an apparently irreversible reaction (Stimmel, 1947; Stealy and Stimmel, 1948). It is only recently that this transformation has been reported to occur in vitro, the tissues employed being human fetal liver (Engel et al, 1958) and rat liver (Hagopian and Levy, 1958). This conversion of estradiol is apparently of more than academic importance since it has been shown to proceed to a greater extent than normal in human males with prostatic cancer (May and Stimmel, 1948) and myocardial infarction (Bauld et al, 1957).

Certain evidence has accumulated in favour of the existence of an additional biosynthetic pathway for estriol involving neither estrone nor estradiol (Brown, 1957; Ryan, 1958). Besides these observations, interest in estriol has arisen from its apparent non-occurrence in a number of mammalian species. This is of particular interest in the rabbit which does not exhibit spontaneous atherosclerosis.

In view of the finding of estriol in avian excreta (Hurst, 1957) and also in ovarian extracts from the laying hen (Layne and Common, 1958) it seemed that in vitro experiments on hepatic tissue from such a source might yield significant results.

Experiments and Results

Slices of liver (500 mg.) obtained from a laying hen were incubated with 40 μg. of 16-^{14}C-estradiol-17β (2.7 μc./mg.) in 5 ml. of Krebs-Ringer-Phosphate solution, pH 7.4, for 2 hours at 37° with shaking. After incubation 1 mg. of pure unlabelled estriol was added as carrier. Following the preparation of a lipid extract a 4-transfer distribution in C_6H_6/H_2O was performed with single withdrawal, and subsequent saponification of the estriol-containing aqueous phase. Part of this material was subjected to paper chromatography in the system C_6H_6:C_6H_{14}(1:1)/CH_3OH:H_2O(7:3) followed by radioautography. This showed all of the radioactivity to be associated with a spot of the same chromatographic mobility as pure estriol. A second portion of the aqueous extract was subjected to a 24-transfer countercurrent distribution in $CHCl_3$:CCl_4(4:1)/CH_3OH:H_2O(7:3) yielding a curve for radioactivity which corresponded in position to that of the carrier estriol as measured by the Kober reaction (Fig. 1). The estriol

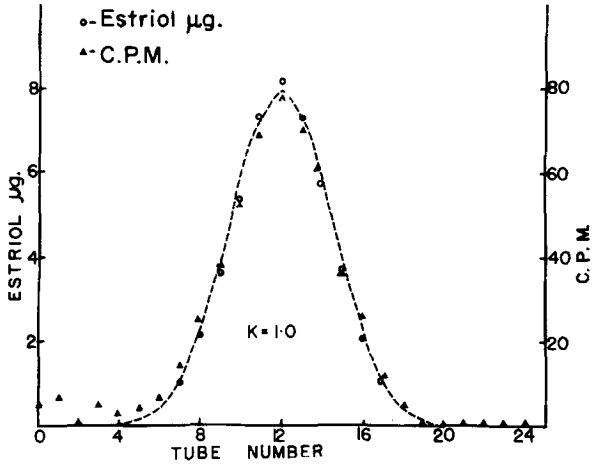

Fig. 1. 24-Transfer countercurrent distribution in 70% $CH_3OH/CHCl_3$:CCl_4 (4:1) of carrier estriol (open circles) and the radioactive product obtained after incubating 16-^{14}C-estradiol - 17β with avian liver (triangles). The broken line represents the theoretical distribution curve.

had a specific activity of 9,800 counts/min./mg. at this stage. The remainder of the material was chromatographed on paper in $CHCl_3/HCONH_2$ against pure estriol as standard. Elution of the estriol-containing zone

was followed by chromatography on a celite partition column (Bauld, 1956) in $ClCH_2CH_2Cl/CH_3OH:H_2O$. The resulting estriol had a specific activity of 10,000 counts/min./mg. Part of this was methylated to yield the methyl ether derivative which was subsequently partitioned in C_6H_6/H_2O. The organic extract, when chromatographed on alumina (Brown, 1955) yielded estriol methyl ether of specific activity 9,800 counts/min./mg.

Comment

The data outlined above constitutes good evidence for the conversion of estradiol - 17B to estriol by avian liver, thus indicating that some, at least, of the estriol associated with avian metabolism probably arises by this route. The extent of the conversion found here was approximately 6%.

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