Preliminary Notes

Biogenesis of 6-hydroxylated oestrogens in human tissues

Phenolic steroids hydroxylated at carbon atom 6 have recently gained biological interest. Thus MUELLER AND RUMNEY[1] incubated oestradiol-17β with mouse liver microsomes in the presence of reduced TPN and obtained evidence for the formation of 6-hydroxyoestradiol-17β and 6-hydroxyoestrone. BREUER, NOCKE AND KNUPPEN[2] observed 6-hydroxylation of oestradiol-17β and oestrone on incubation with rat liver slices, and BREUER AND KNUPPEN[3], using the same system, isolated 6-hydroxyoestradiol-17β as a metabolite of oestradiol-17β. Recently MARRIAN[4] has reported the isolation of a new ketonic Kober chromogen (KC-6B) from pregnancy urine which appears to be identical with a 6-hydroxyoestrone.

There seem to exist two metabolic pathways for the biogenesis of 6-hydroxylated oestrogens in human tissues, one involving the conversion of 6-hydroxylated C_{19}-steroids to the corresponding C_{18}-steroids, and the second which involves the direct hydroxylation of oestradiol-17β. We have examined both possibilities (1) by using RYAN's enzyme preparation of human placenta[5] which converts readily Δ^4-androstene-3,17-dione to oestrone and (2) by carrying out incubations with human fetal liver slices.

Human placentae were prepared as described by RYAN[5]. The 10,000 $\times$ g supernatant fractions each equivalent to 15 g of tissue were incubated with 10 μmoles ATP, 2.5 μmoles DPN and 200 μg of 6α- or 6β-hydroxy-Δ^4-androstene-3,17-dione at 37° for 60 min in a total volume of 5 ml. The incubation mixtures were extracted with ether–chloroform (3:1, v/v) and the phenolic fractions chromatographed on paper in the formamide–chloroform system. The paper chromatograms were sprayed with FOLIN AND CIOCALTEU reagent[6,7]. After incubation of 6α-hydroxy-Δ^4-androstene-3,17-dione a phenolic metabolite (I) was detected which showed the same mobility (14.2 cm/24 h) as authentic 6'α'-hydroxyoestrone prepared by oxidation with chromic acid from 6'α'-hydroxyoestradiol-17β 3-monoacetate 6-monobenzoate[8]. (I) was a Kober chromogen and had the same absorption curve as 6'α'-hydroxyoestrone with the H_2SO_4–H_2O reaction ($\lambda_{max.}$ 270 mμ, 289 mμ, 462 mμ; $\lambda_{min.}$ 260 mμ, 275 mμ, 360 mμ). For further identification (I) was sublimed under normal pressure. The crystals obtained had a melting point (261–263°) which was identical with that of 6-dehydrooestrone; the same melting point was observed after sublimation of authentic 6'α'-hydroxyoestrone. The phenolic substance (II) detected after incubation of 6β-hydroxy-Δ^4-androstene-3,17-dione showed a similar mobility (14.6 cm/24 h) as 6'α'-hydroxyoestrone. On rechromatography in the system benzene–light petroleum–methanol–water (33:66:80:20) the mobility of (II) was slightly greater (7.6 cm/24 h) than that of 6'α'-hydroxyoestrone (6.8 cm/24 h). (II) gave a red colour with the Kober reaction. From the data reported here it is concluded that human

Abbreviations: TPN, triphosphopyridine nucleotide; DPN, diphosphopyridine nucleotide; ATP, adenosine triphosphate.

placenta can convert 6α-hydroxy-Δ^4-androstene-3,17-dione to 6α-hydroxyoestrone and 6β-hydroxy-Δ^4-androstene-3,17-dione to 6β-hydroxyoestrone. It is interesting to note that approx. 4 times as much 6α-hydroxyoestrone was formed as 6β-hydroxyoestrone.

In order to investigate the second metabolic pathway leading to the formation of 6-hydroxylated oestrogens, oestradiol-17β was incubated with human fetal liver. 200 mg of liver slices were shaken with 200 μg of steroid in 5 ml Krebs-phosphate saline at 37° for 60 min. The incubation mixtures were extracted with chloroform–ether (3:1, v/v) and the extracts evaporated. The residues were subjected to paper chromatography in the system formamide–chloroform. The "polar" fractions containing oestriol and possibly 6′α′-hydroxyoestradiol-17β were eluted from the paper and methylated with dimethyl sulfate; the methylated material was then applied to an alumina column (*cf.* ref. 2). Upon elution with 1.4% ethanol in benzene a Kober chromogen was obtained which showed in the H_2SO_4–H_2O reaction the same absorption curve as the 3-methyl ether of 6′α′-hydroxyoestradiol-17β ($\lambda_{max.}$ 288 mμ, 438 mμ, 462 mμ; $\lambda_{min.}$ 260 mμ, 350 mμ, 450 mμ). Further elution with 3.0% ethanol in benzene yielded the 3-methyl ether of oestriol. On the basis of these results it is concluded that oestradiol-17β is hydroxylated by human fetal liver not only to oestriol—as has already been shown by ENGEL, BAGGETT AND HALLA[9]—but also to 6′α′-hydroxyoestradiol-17β. It seems therefore that, with respect to the metabolism of oestradiol-17β, human fetal liver resembles very closely that of rat liver.

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