PN 1262

The reduction *in vitro* of [16-^{14}C]-16-ketoestradiol-17β mammalian blood

Reduction of the 17-keto group of estrone by blood from various species has been recognized for some years[1,2]. PORTIUS AND REPKE[3] reported the reduction product to be estradiol-17β and/or estradiol-17α depending on the source of the blood. BREUER[4] has furnished evidence for the reduction of 16-ketoestradiol-17β to 16-*epi*-estriol by human erythrocytes.

In this laboratory [16-^{14}C]16-ketoestradiol-17β, when incubated with whole blood from the cat or the rat, showed, on thin layer chromatography[5], 2 radioactive zones corresponding in mobility to estriol and 16-*epi*-estriol. The former predominated in cat blood, the latter in the rat. Most of the radioactivity was recoverable in an ether extract of the plasma following incubation. In order to substantiate the finding 35 ml of cat blood and 20 ml of rat blood were each incubated with 3.6 μg of [16-^{14}C]16-ketoestradiol-17β (58400 counts/min) for 2 h at 37° in an atmosphere of $O_2 - CO_2$ (93:7). A parallel blank experiment was performed in which the steroid was incubated with saline. The whole blood was precipitated by 10 vol. of ethanol - acetone (1:1) and the supernatant evaporated. The dried residues to which 25 μg of unlabelled estriol and 22 μg of unlabelled 16-*epi*-estriol had been added as carriers, were fractionated by a modified Girard reaction[6], the non-ketonic fraction saponified and the steroids extracted with ether. Estriol and 16-*epi*-estriol were separated by column partition[6] and each was further partitioned in separatory funnels between benzene and water in order to exclude trace contamination of one with the other. In the cat-blood experiment 8500 counts/min were in the estriol fraction and 1700 counts/min in the 16-*epi*-estriol fraction. In the experiment with rat blood the corresponding counts were 1000 and 26000. The blank experiment yielded a total of 100 counts/min in the estriol fraction and 300 counts/min in the 16-*epi*-estriol fraction, *i.e.* 0.17 and 0.51 % of the incubated activity, respectively.

For the purpose of determining radiochemical homogeneity of the major transformation products 15.7 mg of pure unlabelled estriol were added to 7500 counts/min of the cat-estriol fraction and 840 μg of unlabelled 16-*epi*-estriol were added to 13000 counts/min of the rat 16-*epi*-estriol fraction. Successive crystallizations of the estriol from methanol gave specific activities of 494 and 495 counts/min/mg for the crystals and 482 and 465 counts/min/mg for the corresponding mother liquors, respectively. Methylation[7] of the pooled crystals and mother liquors followed by successive crystallizations yielded crystals of specific activities 432 and 446 counts per min/mg corrected to the free steroid. One crystallization of the 16-*epi*-estriol resulted in specific activities of 14.4 and 14.8 counts/min/μg for crystals and mother liquor, respectively, quantitation being made by the Ittrich spectrophotometric procedure[8]. Methylation of the pooled crystals and mother liquor gave a product of specific activity 15.1 counts/min/μg again corrected to the free steroid. Taking the specific activity of the pure estriol to be 440 counts/min/mg and that of the pure 16-*epi*-estriol to be 14.5 counts/min/μg it can be calculated that at the stage of benzene–water partition there were 7800 counts/min in the estriol from the cat blood (13 % conversion) and 24800 counts/min in the 16-*epi*-estriol from the rat blood (42 % conversion). These figures neglect losses incurred prior to the partition.

It is evident that blood from these 2 species possesses considerable ability to reduce the 16-keto group of 16-ketoestradiol-17β, the main products being the 16-α

isomer in the cat and the 16β isomer in the rat. It has not yet been ascertained whether the radioactivity associated with 16-*epi*-estriol from cat blood and the estriol from rat blood really represents these compounds. Preliminary experiments with separated white and red cells from rat blood have shown the reduction to occur in the latter. Studies are now in progress on detailed aspects of the mechanism involved.

This study is supported by the Banting Research Foundation.

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Received March 18th, 1963

Biochim. Biophys. Acta, 71 (1963) 748–749

PN 1264

The conversion of androst-4-en-3,17-dione into testololactone and other substances by incubation in bovine blood

As part of a study of steroidogenesis in perfused bovine adrenals and ovaries, we have incubated androst-4-en-3,17-dione in bovine blood alone and have examined the nature of the transformation products.

[4-^{14}C]Androst-4-en-3,17-dione, (I), was purified first by partition chromatography on a column of diatomaceous earth (Celite 545), using methanol–water (4:1) as the stationary phase (0.67 ml/g) and hexane as the mobile phase, and then by multiple crystallizations in ethyl acetate.

In 1 incubation, 50.0 mg of I, 3000 counts/min/mg, dissolved in 10 ml of propylene glycol, were mixed with 800 ml of oxygenated, citrated whole blood, taken from a pregnant cow at slaughter, to which 20 mg of oxytetracyclinehydrochloride (Terramycin) had been added to inhibit bacterial growth. The mixture was then circulated through the aeration chamber of a perfusion apparatus[1] at 39–40° for 3 h under an atmosphere of 95 % O_2–5 % CO_2. A second incubation was performed in the same manner but for only 160 min. The steroids were extracted from both incubates by isopropyl acetate and the extract residues fractionated by, successively, partition between hexane and methanol–water (7:3); column-adsorption chromatography on silica gel (Grade 62, Davison Chemical Corp.); and column-partition chromatography (chrom. 2) in the same system used to purify I, except that hexane–benzene mixtures, as well as hexane alone, were used for the mobile phase.

Certain of the hexane–benzene (9:1) eluate residues of chrom. 2 were crystallized from ethyl acetate to yield a substance, 69.8 mg, m.p. 173–175°, whose infrared-absorption spectrum was identical with that of androst-4-en-3,17-dione.