METABOLISM OF THE STEROID HORMONES

II. THE CONVERSION OF α-ESTRADIOL TO ESTRONE AND β-ESTRADIOL
BY THE OVARIECTOMIZED-HYSTERECTOMIZED RABBIT*

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The biological relationship between α-estradiol and estrone has been established by the isolation of the latter compound from the urine of normal and ovariectomized female and normal male guinea pigs (2, 3) and man (4, 5) following the administration of α-estradiol. In view of the conclusion of Pincus (6), that α-estradiol is not converted to estrone by the ovariectomized rabbit, it was considered of interest to investigate the fate of α-estradiol in the rabbit by methods involving the isolation of crystalline urinary metabolites. The results of such an investigation are the subject of the present communication and demonstrate that, as in the guinea pig and man, α-estradiol is converted to estrone. Furthermore, this transformation does occur in the absence of both the ovaries and uterus.

We have also been able to isolate β-estradiol from the urine of ovariectomized-hysterectomized rabbits to which α-estradiol was administered. β-Estradiol has thus far been found in nature only in the urine of pregnant mares (7–9). Whether it is synthesized de novo in the ovaries, the placenta, or the adrenal cortex of the mare or arises as a metabolite of estrone has not been established. From the urine of normal rabbits to which estrone was administered, Stroud (10) obtained an impure phenolic compound which he believed to be β-estradiol. The work reported here demonstrates that α-estradiol is metabolized to β-estradiol in the rabbit, estrone presumably being an intermediate. The ovaries and uterus are not essential

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to the conversion but the possibility that these organs are involved in this transformation in the normal rabbit is not excluded.

Since the completion of this work, Heard, Bauld, and Hoffman (11) have reported, in a preliminary communication, the isolation of estrone and $\beta$-estradiol from the urine of intact estrous and ovariectomized-hysterectomized rabbits to which $\alpha$-estradiol or estrone was administered alone or simultaneously with progesterone.

EXPERIMENTAL

100 mg. of $\alpha$-estradiol dipropionate (equivalent to 70.8 mg. of $\alpha$-estradiol) were administered by subcutaneous injection daily for 5 days to each of two adult ovariectomized-hysterectomized rabbits. During the period of injection and for the following 3 days, the urine was quantitatively collected under toluene. After acidification by the addition of 10 cc. of concentrated hydrochloric acid per 100 cc. of urine, hydrolysis and extraction were carried out simultaneously by a modification of the method of Dingemanse, Borchardt, and Laqueur (12). The acidified urine, in 1 liter quantities, was refluxed for 6 to 8 hours with 250 cc. of benzene. This process was repeated once more, with a fresh portion of benzene. The benzene solutions were combined and the solvent distilled under reduced pressure.

The total benzene-soluble portion of the urine was dissolved in ether and treated according to the outline given in the accompanying scheme.

Isolation of Estrone—Fraction I consisted of an orange-colored crystalline residue and contained 225,000 I.U. of estrogenic activity. The material was dissolved in 25 cc. of acetone and adsorbed on a column of activated alumina (10 X 140 mm.). Elution was effected by passing through the column 50 cc. quantities of acetone containing progressively greater concentrations of absolute ethanol. Five fractions obtained by eluting the column with acetone containing 2.5 per cent by volume of absolute ethanol yielded an oily residue which crystallized upon standing. Elution with greater concentrations of ethanol in acetone yielded insignificant amounts of material. Upon crystallization from dilute methanol, a crop of 19.5 mg. of crystals, m.p. 245-254°, was obtained from the combined crystalline eluates. The melting point was raised to 251-256° by one recrystallization from dilute methanol. After drying 16 hours at 100° in vacuo over phosphorus pentoxide, the compound melted at 255-257°. When mixed with

1 We wish to express our thanks to Dr. H. R. Catechpole for performing the operations.
2 The method of bioassay is described in Paper I of this series (3).
3 The activated alumina used throughout this work was procured from the Aluminum Ore Company, East St. Louis, Illinois; it is designated grade A, mesh 40.
a sample of estrone (m.p. 256–258°), the melting point of the mixture was 256–258°. The benzoate melted at 215–217° and did not depress the melting point of a sample of authentic estrone benzoate. All melting points are uncorrected.

Isolation of β-Estradiol—Fraction II contained 56,000 r.u. of estrogenic activity. The orange-colored oil was dissolved in 8 cc. of 80 per cent benzene-soluble residue dissolved in 500 cc. ether

Extracted 5 times with 100 cc. quantities of
10% NaOH

Total phenolic and acidic compounds in 10% NaOH

Acidified with 16% sulfuric acid and extracted 5 times with 100 cc. quantities of ether

Ether-extracted 5 times with 100 cc. quantities of saturated aqueous NaHCO₃

Neutral fraction

Acidic fraction

Ether-extracted 5 times with 100 cc. quantities of 0.1 N NaOH

0.1 N NaOH-soluble compounds

Ether-washed with water and evaporated

Residue treated with Girard-Sandulesco ketone reagent, trimethylacethydrazide ammonium chloride

Ketonic phenols not extracted from ether solution by 0.1 N NaOH (Fraction I) Non-ketonic phenols not extracted from ether solution by 0.1 N NaOH (Fraction II)

ethanol. 100 mg. of digitonin in 5 cc. of 80 per cent ethanol were added and the mixture was allowed to stand at room temperature overnight. The digitonide, amounting to about 1 mg., was separated by centrifugation and discarded. The supernatant fluid was evaporated to dryness, dissolved in pyridine, and warmed on the steam bath for 1 hour. Ether was then added slowly and the precipitated digitonin separated by centrifuga-
tion. The supernatant ether solution was washed with dilute hydrochloric acid, saturated sodium bicarbonate solution, and water. The ether was evaporated and the residue dried. The residue was dissolved in 25 cc. of acetone and adsorbed on a column of activated alumina (10 X 140 mm.). Elution of a fraction containing solid material was effected by passing through the column a 5 per cent solution of absolute ethanol in acetone. After two recrystallizations from dilute methanol, a product melting at 209-216° was obtained. A precipitate of fine needles, m.p. 209-217°, appeared in the mother liquors and was separated. The two fractions were combined. After the material was precipitated from solution in ethyl ether by the addition of petroleum ether, the melting point was raised to 215-218°. The compound was dried in vacuo over phosphorus pentoxide at 100° for 16 hours. It weighed 4.8 mg. and melted sharply at 218-219°. When mixed with a sample of authentic β-estradiol (m.p. 215-216°) the melting point was 215-216°. The diacetate melted at 140-141° and did not depress the melting point of a sample of β-estradiol diacetate.

DISCUSSION

Estrone and estriol are generally assumed to be the principal active urinary metabolites of α-estradiol. The experiments of Heard, Bauld, and Hoffman (11) and those reported from this laboratory ((3), and the present work) support this assumption with respect to estrone. There is no direct evidence, on the other hand, that estriol is a metabolite of α-estradiol or estrone in the animal body. To the present time estriol has been isolated only from the human placenta (13) and the urine of pregnant women (14-16). There is yet no chemical evidence that this estrogen arises by any process other than the direct secretion of the placenta. We have been unable to isolate estriol from the urine of normal or ovariecmtomized female and normal male guinea pigs (3) or ovariecmtomized-hysterectomized rabbits (present work) following α-estradiol administration. Likewise, Heard, Bauld, and Hoffman (11) could not isolate estriol from the urine of estrous or ovariecmtomized-hysterectomized rabbits which received α-estradiol or estrone alone or simultaneously with progesterone.

Since in our experiment the β-estradiol isolated represents 19.8 per cent of the total amount of crystalline estrogens recovered, this diol must be considered an important metabolite of α-estradiol in the rabbit. According to Heard, Bauld, and Hoffman (11) β-estradiol represents, in fact, a major active urinary product of α-estradiol and estrone metabolism in the rabbit, for they were able to isolate 4 to 5 times as much β-estradiol as estrone following α-estradiol or estrone administration.
The low titers obtained by the biological assay of Fraction II (non-ketonic phenols not extracted from ether solution by 0.1 N NaOH) and the formation of a small amount of digitonide in this fraction indicate the completeness with which \( \alpha \)-estradiol is metabolized by the rabbit. That the human is not capable of metabolizing \( \alpha \)-estradiol so thoroughly is indicated by the work of Huffman, MacCorquodale, Thayer, Doisy, Smith, and Smith (17), who isolated appreciable quantities of \( \alpha \)-estradiol from the urine of pregnant women. Furthermore, Heard and Hoffman (5) recovered 3.9 per cent of injected \( \alpha \)-estradiol unchanged in the urine of a normal man.

The sample of estradiol dipropionate used in our work was prepared from \( \alpha \)-estradiol, synthesized by the catalytic reduction of estrone. It is known that small amounts of the \( \beta \)-isomer are produced during the latter process, and for this reason the question arises as to whether the \( \beta \)-estradiol we have isolated from rabbit urine was administered to the animals as a contaminant of the \( \alpha \)-estradiol. Dr. Scholz and Dr. Fischer, chemists of Ciba Pharmaceutical Products, Inc., have assured us, however, that with the three purification and recrystallization processes included in the manufacturing procedure of estradiol dipropionate, their estradiol dipropionate can contain only the smallest trace of \( \beta \)-estradiol dipropionate, if it is present at all.

**SUMMARY**

In the rabbit, as in the guinea pig and man, estrone is a urinary metabolite of \( \alpha \)-estradiol. \( \beta \)-Estradiol also arises from \( \alpha \)-estradiol when the latter compound is administered to the rabbit, estrone presumably being an intermediate. These conversions occur in the absence of both the ovaries and uterus, but the possibility that these organs participate in the transformations in the normal animal is not excluded.

We wish to acknowledge our indebtedness to Dr. Oskar Wintersteiner, for the samples of \( \beta \)-estradiol and \( \beta \)-estradiol diacetate; to Ciba Pharmaceutical Products, Inc., for the supply of estradiol dipropionate and estrone benzoate; and to the Schering Corporation for estrone.

**BIBLIOGRAPHY**


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\( ^4 \) According to our method of assay, \( \alpha \)-estradiol is about 4 times more potent than estrone in the ovariectomized mouse. On this basis, Fraction II contained less than 1.4 mg. of \( \alpha \)-estradiol if all the activity was due to this compound. However, since 4.8 mg. of \( \beta \)-estradiol were isolated, the maximal possible quantity of \( \alpha \)-estradiol present in Fraction II is further reduced.
10. Stroud, S. W., *J. Endocrinology*, 1, 201 (1939).
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