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The Fate of Testosterone in the Human.*

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When testosterone is administered to men with deficient testicular secretion, the principal androgen excreted in the urine is androsterone.[1,2] However, another substance, etiocholan-3(α)-ol-17-one, an isomer of androsterone, has also been isolated from the urine of a patient receiving injections of testosterone.[1] In a previous communication presence of this substance was indicated but not definitely established.[2] This communication is concerned with the isolation and identification of etiocholan-3(α)-ol-17-one from the urine of a patient receiving testosterone, thus confirming the original observation. The conversion of testosterone into androsterone in the complete absence of the testis forms the second part of this report.

Isolation of Etiocholan-3(α)-ol-17-one. Twenty-four liters of urine was collected from a patient who showed symptoms of deficient testicular secretion during a period when this individual received 30 mg of testosterone propionate† per day intramuscularly. The urine was treated by the method previously described.[2] That fraction of the urinary extract which contained the neutral ketonic substances was subjected to chromatographic adsorption using carbon tetrachloride as the solvent. Selective elution was carried out using mixtures of carbon tetrachloride and ethanol. The column was eluted with carbon tetrachloride containing 0.1%, 0.2%, and 0.3% ethanol. From the 0.2% ethanol fraction was obtained 150 mg of pure androsterone, melting point 179-181°C, after three recrystallizations from 95% ethanol.

From the 0.3% ethanol in carbon tetrachloride fraction, a reddish viscous oil was prepared which on sublimation at 3 micra pressure

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1 Callow, N. H., *Biochem. J.*, 1939, **33**, 559.

2 Dorfman, R. I., Cook, J. W., and Hamilton, J. B., *J. Biol. Chem.*, 1939, **130**, 285.

† Testosterone propionate was furnished by Ciba Pharmaceutical Products, Inc., under the trade name Perandren.

and 120°C yielded a crystalline sublimate. This material was recrystallized from aqueous ethanol and yielded 40 mg of a compound which melted at 144-146°C. The benzoate melted at 161-163° while the acetate melted at 95-96°C. This compares favorably with previously published constants on etiocholan-3(α)-ol-17-one, M.P. 147-151°C, the benzoate M.P. 161.5-163.5°C, and the acetate M.P. 91-94.5°C.[1]

Conversion of Testosterone into Androsterone in the Male Castrate. The patient employed in this study was a male castrate aged 40 years, who had been castrated for the past 20 years. This individual received orally 60 mg of testosterone propionate daily for 5 days during which time all the urine was collected. The urine was treated in the usual manner to prepare a concentrate of the neutral ketonic compounds. This fraction containing the neutral ketonic compounds was sublimed at 1 micron pressure and 125°C. The crystalline sublimate weighed 15 mg and was slightly colored. After 3 recrystallizations from ethanol, the compound melted at 175-177°C. On 2 further recrystallizations, the melting point was increased to 180-181°C. The melting point of the benzoate was 172-175°C. Neither the melting point of the free compound nor its benzoate was depressed when mixed with authentic samples of androsterone and androsterone benzoate, respectively.

Discussion. The conversion of testosterone into androsterone and etiocholan-3(α)-ol-17-one is quite unique since it points to a rather unusual biological reduction; that is, in the reduction of the double bond in ring A at carbons No. 4 and 5, two isomers are formed. It seems that the enzyme systems participating in this reaction do not exhibit the usual specificity. Since we have been successful in isolating etioallocholanol-3(β)-17-one from the urine of guinea pigs after the administration of testosterone,[3] it appears likely that the lack of specificity extends also to the reduction of the ketone group at carbon 3.

The conversion of testosterone to androsterone in the castrate definitely rules out the testis as the only site of this reaction. It does not rule out, however, the possibility that the reaction may also proceed in this tissue.

Summary. The conversion of testosterone to etiocholan-3(α)-ol-17-one in addition to androsterone in the human has been confirmed. It has been demonstrated that the conversion of testosterone to androsterone may take place in the complete absence of the testis.

[3] Dorfman, R. I., and Fish, W. R., *J. Biol. Chem.*, 1940, **135**, 349.