

## 40. EXCRETION OF ANDROGENS BY EUNUCHS: THE ISOLATION OF 17-KETOSTEROIDS FROM THE URINE

BY NANCY HELEN CALLOW AND  
ROBERT KENNETH CALLOW

*From the National Institute for Medical Research, London, N.W. 3*

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THE result of investigations in the last few years has been to show that the excretion in human urine of material with androgenic action is an imperfect index of the androgenic activity of the gonads. In spite of some degree of correlation of androgen excretion with sex and age, it would be impossible in practice to determine from the androgen content of the urine either the sex or the age of a human individual. Even after castration, although reports have been made to the contrary, it seems that the excretion of androgens still occurs, and may be within normal limits [Callow *et al.* 1940]. Very low levels of androgen excretion may be associated with castration, Addison's disease or severe pituitary insufficiency. A high level, on the other hand, is the only observation with a definite and independent diagnostic value; it occurs in cases of adrenal cortical tumour.

In a series of papers from this laboratory, efforts have been made to apply chemical methods to the analysis of the androgenic material in extracts of human urine, with the object of replacing the unspecific biological test. It was found that the androgenic material in extracts of hydrolysed urine was concentrated in the ketonic fraction, confirming Butenandt & Tscherning [1934], and that the biological activity was actually parallel to the content of the 17-ketosteroids determined colorimetrically [Callow *et al.* 1938]. The attempt to separate individual compounds from the ketonic fraction led to the isolation of androsterone, aetiocholan-3( $\alpha$ )-ol-17-one and *transdehydroandrosterone* from men's and women's urine in similar amounts [Callow & Callow, 1939]. It had been found that, of these substances, androsterone and aetiocholan-3( $\alpha$ )-ol-17-one could probably be derived from testosterone given by injection [Callow, 1939], although it seemed improbable that in women they were derived from secreted testosterone, whilst *transdehydroandrosterone* was found in large amount in the urine of cases of adrenal cortical tumour [Croke & Callow, 1939]. In a recent paper [Callow & Callow, 1939], we expressed the hope that chemical investigation of the nature of the compounds excreted by eunuchs and ovariectomized women would give a clue to the contribution of the adrenal cortex to the urinary 17-ketosteroids. Hirschmann [1939] has now isolated androsterone, aetiocholan-3( $\alpha$ )-ol-17-one and *transdehydroandrosterone* from the urine of ovariectomized women by methods similar to ours, and in yields only slightly below those obtained from normal women's urine. Hansen [1938] first attempted to identify the androgens in eunuchs' urine, but could only conclude, from the rather uncertain evidence of type of biological activity, that they resembled those of normal men's urine but differed from androsterone.

We made a preliminary examination of a small quantity of mixed extracts from the urine of eunuchs and found that it yielded androsterone, aetiocholan-3( $\alpha$ )-ol-17-one and *transdehydroandrosterone*, though in quantities insufficient for certain identification. In the investigation described in the present paper, we examined a large amount of urine from an individual eunuch and confirmed the presence in it of these three compounds which occur in bulked collections of normal urine. The only difference from the normal shown by the eunuch's urine was an increase in the total amount of *transdehydroandrosterone* and in its proportion to the other two 17-ketosteroids. It is, therefore, clear that androsterone and aetiocholan-3( $\alpha$ )-ol-17-one are not of purely gonadal origin in either sex, whether derived from testosterone or from some other compound, and they may be transformation products of substances from the adrenal cortex.

The individual eunuch whose urine we examined may not be a typical case, since the level of his excretion of 17-ketosteroids was the highest among the eunuchs we examined, but the results give support to the supposition that *transdehydroandrosterone* is of adrenal cortical origin, since the relative amount of this compound excreted appears to be increased when the alternative source of urinary 17-ketosteroids is absent. Further, this observation is consistent with the hypothesis that increased activity of the adrenal cortex may, in some cases, compensate for the absence of gonads. This is analogous to the hypothesis put forward by Hamblen *et al.* [1939], that the adrenal cortex compensates by increased activity for the regression of gonadal activity at the onset of the climacteric in women.

#### EXPERIMENTAL

##### *Source of urine*

The eunuch, E. C., aged 20, was a patient of Dr A. W. Spence at St Bartholomew's Hospital. A brief case history has been published [Case 5, Callow *et al.* 1939, 1]. He had been treated with testosterone propionate, but when collection of urine for the present investigation was begun he had received no recent treatment, and a 24 hr. collection showed a daily excretion of 9.4 mg. of 17-ketosteroids, as compared with 10.9 mg./day a year previously, before any hormone therapy; the oestrin excreted was also lower, according to biological assays, for which we are indebted to Dr C. W. Emmens. Subsequent collections were made discontinuously and sent to the laboratory at fortnightly intervals; the specimens were fairly concentrated (average sp. gr. 1.021). Toluene was used as a preservative, and the specimens on receipt had pH 5-6. We are very grateful to Dr A. W. Spence for the opportunity of obtaining this material, and to the subject himself, who very patiently continued with the collections over a period of 6 months.

##### *Extraction of ketonic material*

The separate collections of urine were extracted as soon as received by the combined hydrolysis and extraction method [Callow *et al.* 1939, 2], and the neutral fractions from about 24 l. of urine, with colorimetric values for 17-ketosteroid content ranging from 10.0 to 15.4 mg./l., were combined and treated with the Girard-Sandulesco reagent P. From 1.35 g. there were obtained 0.296 g. of ketonic and 0.706 of non-ketonic material.

##### *Separation by adsorption and elution*

The ketonic fraction was dissolved in carbon tetrachloride ("Analar") and the solution put through a chromatograph column, 20 x 1 cm., prepared with carbon tetrachloride and alumina (Merck's "Aluminium oxide standardized

according to Brockmann<sup>21</sup>). The column was developed first with carbon tetrachloride and then with carbon tetrachloride containing 0.1% ethanol. The weights of fractions obtained after the collection and evaporation of successive portions of eluate are shown in Table I. Further elution with carbon tetrachloride containing 0.2% ethanol yielded only traces of gummy material in a concentration of 0.01 mg./ml.

Table I

Fraction	Vol. of eluate ml.	Solvent	Wt. of fraction mg.	Conc. mg./ml.	Remarks
I	300	CCl <sub>4</sub>	12.5	0.042	Crystalline. Chloro-compound?
II	275	CCl <sub>4</sub>	13.7	0.05	Gum
III	500	CCl <sub>4</sub>	2.8	0.0056	Gum
IV	220	0.1% EtOH in CCl <sub>4</sub>	19.3	0.086	Gum, containing <i>trans</i> -dehydroandrosterone
V	230	0.1% EtOH in CCl <sub>4</sub>	52.8	0.23	Crystalline <i>trans</i> -dehydroandrosterone
VI	220	0.1% EtOH in CCl <sub>4</sub>	15.7	0.071	Crystalline. Androsterone
VII	190	0.1% EtOH in CCl <sub>4</sub>	7.0	0.037	Gum
VIII	570	0.1% EtOH in CCl <sub>4</sub>	22.7	0.0398	Red gum, yielded aetiocholan-3(α)-ol-17-one
IX	280	0.1% EtOH in CCl <sub>4</sub>	5.3	0.019	Crystalline

#### Isolation of steroids from crude fractions

(a) *trans*-Dehydroandrosterone. Fraction IV was treated in 90% alcohol with digitonin. The precipitate, after drying and decomposition of the pyridine solution with ether, yielded 5.7 mg. of material, which was benzoylated. The benzoate, after recrystallization from ethyl acetate, melted at 233–245°. This was presumably *trans*-dehydroandrosterone benzoate, but the amount was too small for further purification and confirmation of its identity. The main bulk of the *trans*-dehydroandrosterone was contained in the following fraction. Fraction V had  $[\alpha]_D + 36^\circ$ ,  $[\alpha]_{5461} + 47^\circ$  in EtOH. It was converted into the benzoate by treatment with benzoyl chloride in pyridine. The crude, crystalline benzoate (60 mg.) obtained by diluting with water, yielded, with two successive crystallizations from ethyl acetate, fine needles having m.p. 250–254° (24 mg.) and finally with m.p. 253–254° (16 mg.), unchanged when mixed with an authentic specimen of *trans*-dehydroandrosterone benzoate. Transition of the needle form to a platy form at 236–239° was observed. The total amount of *trans*-dehydroandrosterone present in Fractions IV and V is estimated to be about 40–50 mg. or about 2 mg./l.

(b) *Androsterone*. The crystalline material from Fraction VI melted at 140–170° but a portion which sublimed on the cover-slip melted at 178–182°. The whole fraction had  $[\alpha]_D + 74^\circ$  and  $[\alpha]_{5461} + 81^\circ$  in EtOH. After successive recrystallizations from methanol and from ethyl acetate material was obtained with m.p. 184–185°. Mixed with an authentic specimen of androsterone (m.p. 184–185°) it had m.p. 183.5–184.5°. By evaporation of the mother liquors from the above recrystallization crystals were obtained which were rapidly washed with a little ether to remove gummy, yellow material, and then treated with benzoyl chloride in pyridine. The reaction mixture was diluted with water, extracted with ether, and the extract washed, dried and evaporated. The residue, after two recrystallizations from methanol, yielded needles, m.p. 172–

175°, unchanged when mixed with an authentic specimen of androsterone benzoate. A rough estimate of the amount of androsterone present, assuming that some also came in Fraction V, gives the figure of 0.5 mg./l. of original urine.

(c) *Aetiocholan-3(α)-ol-17-one*. Fraction VIII was treated with charcoal in methanol solution to remove the red colour, filtered and the filtrate evaporated to dryness. The residue, scratched after addition of a trace of methanol, yielded crystals, m.p. 136–145°,  $[\alpha]_D + 92^\circ$ ,  $[\alpha]_{5461} + 115^\circ$  in EtOH. After two recrystallizations from methanol the m.p. was 146–147.5° (soft at 145°). A mixture with an authentic specimen of aetiocholan-3(α)-ol-17-one (m.p. 151–152°), for which we are indebted to Prof. Ruzicka, melted at 147–151°. A benzoate was prepared from the mother liquors of the aetiocholan-3(α)-ol-17-one, which, after three recrystallizations from methanol, had m.p. 161–163.5° alone, and 161–164° when mixed with an authentic specimen of aetiocholan-3(α)-ol-17-one benzoate (m.p. 161.5–163.5°). The amount of aetiocholan-3(α)-ol-17-one present in the original urine is roughly estimated at 0.8–0.9 mg./l.

(d) *Unidentified material*. Fraction I was partly crystalline. Treated with methanol, a small fraction of it was sparingly soluble. The filtrate from this deposited crystals, m.p. 138–155°, which contained about 90% of 17-ketosteroid, determined colorimetrically. It showed only faint selective absorption in the ultraviolet in alcoholic solution. It contained halogen (Cu wire test). In view of the small quantity it was not examined further. Possibly this fraction consists of chlorinated compounds such as the 3-chloroandrosten-17-one obtained by Butenandt & Dannenbaum [1934]. Similar fractions have been obtained from extracts of normal urine.

*Note*. All melting points recorded were observed in Kofler's micro-melting point apparatus. The optical rotations were measured in a 4 dm. tube.

#### SUMMARY

Examination of the urine of a eunuch has been made by the same methods as those which have been applied to normal urines. From the ketonic fraction there were isolated the same compounds as are obtained from normal men's or women's urine, namely, androsterone, aetiocholan-3(α)-ol-17-one and *transdehydroandrosterone*.

The relative amounts of the compounds in a eunuch's urine, about 0.5, 0.9 and 2 mg./l., respectively, compared with those in mixed normal men's urine (1.6, 1.4 and 0.2 mg./l., respectively) show a probably significant increase in the amount of *transdehydroandrosterone*.

None of these compounds is of purely gonadal origin. The results are consistent with the assumption that *transdehydroandrosterone* is derived from the adrenal cortex, which may be more active in a eunuch than in a normal subject.

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