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Nature of the Urinary Androgens of Castrate Men.

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A number of investigators have reported small amounts of urinary androgens in human male castrates[1-5] but the nature of the active material has not been established. A comparison of this androgenic material with that from the urine of normal men is of interest in view of the increasing use of urinary androgen values as measures of testis activity.

The present report describes the measurement of the androgens in the urine of male castrates and the fractionation of this material with digitonin in an effort to characterize the androgens present.

A complete urine collection was made from two male surgical castrates for 9 and 7 days respectively and the urines pooled. One subject had been castrated 10 years previously at the age of 37, the other 35 years before at the age of 26. The urine was kept in the refrigerator without added preservative and used within 3 days after its collection. Acid hydrolysis, benzene extraction and separation into androgenic and estrogenic fractions were carried out according to the methods of Gallagher, *et al.*[6] The androgenic fraction was taken up in 60% alcohol and assayed by inunction on the capon comb in parallel with pure androsterone as a standard.† Assays on groups of 4 to 6 capons showed an androgen excretion of 6 international units per day.

One-half of the androgenic fraction, the equivalent of 8 days' excretion, was treated with digitonin to separate any dehydroisoandrosterone (or other sterols having an hydroxyl group at position 3 in the beta configuration) as the insoluble digitonide.[7] The

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1 Koch, F. C., *J. Urology*, 1936, **35**, 382.

2 Chou, C. Y., and Wu, H., *Chinese J. Physiol.*, 1937, **11**, 429.

3 Callow, R. K., *Proc. Roy. Soc. Med.*, 1938, **31**, 841.

4 Hansen, E. H., *Endokrinologie*, 1938, **21**, 9.

5 McCullagh, E. P., *J. A. M. A.*, 1939, **112**, 1037.

6 Gallagher, T. F., Peterson, D. H., Dorfman, R. I., Kenyon, A. T., and Koch, F. C., *J. Clin. Invest.*, 1937, **16**, 695.

† We are grateful to the Ciba Corporation, Lafayette Park, New Jersey, for the androgens used and to Robert Kinloch for his assistance with the assays.

7 Butenandt, A., Dannenbaum, H., Hanisch, G., and Kudszus, H., *Z. Physiol. Chem.*, 1935, **237**, 57.

TABLE I.
Androgen Content of Urine-fractions of Normal and Castrate Men.

	Untreated urine (I.U./day)	Digitonin-treated urine extracts: Androsterone γ/day	Androsterone I.U./day	Dehydroisoandrosterone γ/day *Obs.*	Dehydroisoandrosterone γ/day *Corr.*	Dehydroisoandrosterone I.U./day	% A.‡ by wt	% D.§ by wt	Ratio A. to D. by wt
Normal	42	2925	29.25	1400	2000*	6.25†	59	41	3:2
Castrate	6	450	4.5	70	100*	0.3†	82	18	4:1

*Observed dehydro. values divided by 0.7 to correct for incomplete separation as measured on known solutions.

†Gamma of dehydro. converted to I.U. (100 gamma of androsterone) on basis of activity ratios by the inunction assay method. 100 gamma of androsterone equal to 320 gamma of dehydro.

‡A. equals androsterone.

§D. equals dehydroisoandrosterone.

digitonide was decomposed with pyridine and the dehydroisoandrosterone and androsterone fractions assayed in parallel with the pure androgens. An extract of normal male urine was treated similarly. Control experiments with known solutions of dehydroisoandrosterone gave only a 70% recovery with digitonin treatment so the values observed for the urine fractions have been corrected for this loss. The results are shown in Table I.

The value found for the total androgen excretion of male castrates, 6 I.U. per day, agrees with the values previously reported, most of which are between 1 and 10 I.U. per day although Callow reports one case with 30-39 I.U. per day. The ratio of androsterone to dehydroisoandrosterone in male castrate urine, 4:1, contrasts with the approximately 1:1 ratio previously reported for normal male urine[7, 8] and the 3:2 ratio found here. Attempts to isolate androgens from these fractions were unsuccessful. In the absence of actual identification of androsterone or dehydroisoandrosterone considerable caution must be used in interpreting these figures. On the other hand, in view of the presence of these two androgens in the urine of normal men and women and female castrates[7, 9, 10] it seems reasonable to assume that these two compounds make up the greater part of the biologically active material. (An inactive epimer of androsterone, alpha-3-hydroxyetiocholanone-17, has been isolated in amounts equal to androsterone from the urine of normal men and of female castrates.[7, 10]) Hansen[4] suggests that the androgenic material in male castrate urine resembles Δ-4-androstenedione in its biological action. He did not consider the possible presence of dehydroisoandrosterone and the properties he mentions can probably be explained as due to the combined actions of dehydroisoandrosterone and androsterone rather than to androstenedione.

It is apparent from Table I that the sum of the androgenic activities in the androsterone and dehydroisoandrosterone fractions is about 20% less than the total androgens as determined on the unfractionated urine. This difference is due to errors arising from the manipulations involved, the biological assay, the rather arbitrary correction factor applied to the dehydroisoandrosterone values and the activity ratio used in converting gamma of dehydroisoandrosterone to gamma of androsterone (I.U.). The existence of this difference and the nature of the methods used make it inadvisable to theorize from the present data but at the same time these ratios have interesting implications.

8 Dingemanse, E., and Laqueur, E., *Biochem. J.*, 1938, **32**, 651.

9 Callow, N. H., and Callow, R. K., *Biochem. J.*, 1939, **33**, 931.

10 Hirschmann, H., *J. Biol. Chem.*, 1939, **130**, 421.

The isolation of steroids from the adrenal cortex closely resembling the common androgens, some having androgenic activity, and the high levels of urinary androgens in some cases of adrenal tumor seem to point to the adrenal as the source of the extragonadal urinary androgens. Callow[3] has discussed this possibility and in view of the isolation of large quantities of dehydroisoandrosterone from the urine of patients with adrenal tumors suggests that all the dehydroisoandrosterone and most of the other androgenic activity in normal male urine is derived from the adrenal and not the testis. With this suggestion in mind it is surprising to find that the male castrate urine contained a smaller rather than a larger proportion of dehydroisoandrosterone than normal urine. It is conceivable that the testis normally transforms a portion of the steroids elaborated by the adrenal cortex into the testis hormone which is subsequently changed by other tissues into suitable excretion forms, androsterone and its inactive epimer and dehydroisoandrosterone. A small part of the steroids appears to go through this or a similar series of reactions independently of the testis and can be found in the urine when the testis is absent as the same androgens but in slightly different proportions.

The measurement of the relative amounts of these two types of urinary androgens by digitonin fractionation is being carried out on the urine of female castrates, boys and girls, in an effort to extend the data.

Summary. A pooled urine collection from 2 male castrates contained 6 I.U. per day of androgenic activity. Capon assay of the results of digitonin fractionation indicated an androsterone to dehydroisoandrosterone ratio of 4:1 compared with the approximately 1:1 ratio found in normal male urine. In the absence of the testis smaller amounts of the same or similar androgens are excreted in slightly different proportions.