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Colorimetric Assay of Urinary Androgens.

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In earlier communications by the author[1, 2] on the colorimetric assay of urinary androgens no data were given on crystalline preparations of the pure hormones normally found in the urine. These data are of value in interpreting colorimetric work and are given here.

Crystalline androsterone (M.P. 180° u.c.) dehydroisoandrosterone (M.P. 135° u.c.) and testosterone (M.P. 152° u.c.) were obtained and weighed quantities made up into 60% alcohol. Color unit and capon assays were carried out on these preparations with the results recorded in Table I.

TABLE I.
Color Unit and Capon Comb Growth Equivalents of Crystalline Androgens.

Substance	Gamma equivalent to 1 color unit	Range	Gamma equivalent to 1 capon unit	No. of assays
Androsterone	90	78-100	8-12	10
Dehydroisoandrosterone	90	80-105	90-130	8
Testosterone	75	70-84	8-15	10

(One capon unit = the total dose distributed into 5 daily applications of equal size to the combs of at least 2 capons which will cause an average increase in comb area of 15%.)

The biological activity of a color unit from urine extracts was found to be approximately equal to 10 gamma of pure androsterone. This value varies as is evident from the data of Table I of our earlier report.[2] The range in comb growth for a color unit is of necessity wide. This is to be expected since:

1. The chemical reaction used is non-specific.
2. The various androgens found in urine are not always excreted in the same proportions.
3. The variations in the capon assay contribute to this factor and the number of capons used in this work was of necessity small.
4. There are probably other non-active androgen-like color-producing substances (such as undoubtedly result from the hydro-

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[1] Oesting, R. B., PROC. SOC. EXP. BIOL. AND MED., 1937, **36**, 524.

[2] Oesting, R. B., and Webster, B., *Endocrinology*, 1938, **22**, 307.

lysis treatments) which have evaded attention simply because of their lack of activity.

From the above data we see that one color unit of pure androsterone is represented by 90 gamma of the crystalline material, while one color unit of urine extract is equivalent in biological activity to only 10 gamma of androsterone. This is an expected finding, but one that must be remembered when using the colorimetric method as a guide to quantitative studies.

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Effect of Estrogen Injections on Lactogen Content of Female Rat Pituitary.

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Earlier work[1] has demonstrated that pituitary glands from sexually mature ovariectomized rats injected with estrogen contained more lactogen than glands from sexually mature ovariectomized non-treated rats. Ovariectomy itself, however, decreased the lactogen content of the rat pituitary gland.[1] We were interested, therefore, in ascertaining the influence of estrogen injections on the lactogen content of pituitary glands from animals with their ovaries intact.

Three series of rats, with 20 rats in each series, were employed in this study. The animals designated as experimental animals in the third series, however, are the same animals as those designated experimental animals in the second series. Rats were paired on the basis of body weight and they were all fed the same stock diet. Daily vaginal smears were made from injected animals, and from the control animals during the last 4 days of the experimental period. All animals were sacrificed the day following the last day of injection, their pituitaries and other endocrine organs removed and weighed. The lactogen content of the pituitary glands was determined by the assay method employed by Reece and Turner.[2]

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1 Reece, R. P., and Turner, C. W., PROC. SOC. EXP. BIOL. AND MED., 1937, **36**, 283.

2 Reece, R. P., and Turner, C. W., *Mo. Agr. Exp. Sta. Res. Bul.*, 1937, 266.