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Determination of Luteinizing and Follicle-Stimulating Principles in Castrate and Menopause Urine.

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Method. By the following method it has been possible to demonstrate the presence of luteinizing (in 62.5% of 16 women) as well as follicle-stimulating principle in the urine of both castrates and spontaneous menopause.

Two hundred cc. of urine is taken from a fresh 24 hours' specimen and acidified with concentrated acetic acid to pH 3.5 (Congo Red). Four volumes of cold acetone are added to the urine, shaken vigorously and allowed to stand overnight in the refrigerator. The supernatant fluid is poured off and the precipitate extracted with weak NaOH. The pH of the mixture is adjusted to between 8 and 8.5, the residual precipitate stirred thoroughly, the mixture centrifuged, and the final precipitate discarded as the supernatant fluid contains the gonadotropic principle. The fluid is then adjusted to pH 7 with dilute acetic acid. The equivalent of 100, 50, and 25 cc. is injected into immature rats weighing 22 to 24 gm. The injections in each rat are divided into 5 doses, given over 3 days. The animals are autopsied at the end of 96 hours. The ovaries are examined in serial section.

The same procedure can be performed using alcohol instead of acetone. Acetone is preferable to alcohol as no deterioration or destruction of the hormone occurs on standing, whereas deterioration of the gonadotropic principle occurs when alcohol is used.

The opinion is gaining ground that "the principle in castrate or menopause urine seems to be identical with the follicle-stimulating fraction prepared from the pituitary glands."[1] However, it should be emphasized that Lassen and Brandstrup[2] reported a luteinizing reaction in the urine (diagnosis by gross inspection of the mouse ovaries), and Fluhman[3] in the blood of such patients.

Katzman and Doisy's benzoic acid precipitation method[4] does

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1 Smith, P. E., *J. A. M. A.*, 1935, **104**, 553.

2 Lassen, H. C. A., and Brandstrup, E., *Acta Scand. Obst. et Gynec.*, 1934, **14**, 89.

3 Fluhman, C. F., *J. A. M. A.*, 1929, **93**, 672.

4 Katzman, P. A., and Doisy, E. A., *J. Biol. Chem.*, 1934, **106**, 125.

not extract the factor found in the menopause or castrate urine. By using our new method, 16 cases, partly surgical castrates, X-ray castrations, and spontaneous menopause were studied.

The luteinizing factor was found in the urine of 6 out of 9 surgical castrates, in 3 of 5 spontaneous menopause cases and in 1 of 2 X-ray castrates, 62.5% of 16 cases. Luteinization was produced with from 25 to 100 cc. of urine. With diminishing quantities of extract, the luteinizing reaction disappeared, the follicle-stimulating effect becoming more marked. Fluctuation in excretion was noted in individual women, equal quantities, varying between strong luteinizing, follicle-stimulating and negative effect.

In 3 cases examined, the luteinizing factor was demonstrated in the blood.

No demonstrable correlation between the symptoms complained of and the quantity or quality of the gonadotropic hormone excreted in the menopause, whether spontaneous or induced (surgical or X-ray) could be noted. Two patients who had no symptoms, excreted 10 to 40 R.U. per liter of both factors. Four cases with mild symptoms excreted 10 to 55 R.U. per liter of only follicle-stimulating factor. In the 10 cases with severe symptoms, no correlation was apparent.

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Micromethod for Determining Insect Hemolymph Specific Gravity (Periplaneta americana Linn).*

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This is a preliminary report of a falling drop method (based on Stokes's law) that permits the determination of the specific gravity (S.G.) of a single drop of insect hemolymph (4.85 $mm.^3$ or less) within the temperature range of 15°C.-40°C., with an average accuracy of ±0.0021 (1 determination) or ±0.0008 (mean of 10 determinations), and with an average determination time of about 8 minutes. The diameter of the hemolymph drop at a beeswax-mineral oil surface is measured with a calibrated ocular micrometer scale. The hemolymph drop, surrounded by oil, is sucked into a

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