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The Chemistry of the Estrus Producing Hormone.

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The publications of Allen and Doisy in which estrin (folliculin) has been shown to be present in the follicular fluid of castrated mice,¹ have been followed by publications from other authors who detected this hormone in the ovary and, more particularly, in the placenta. Loewi and his coworkers have since shown the hormone to be present in the urine of pregnant women,² and Doisy and Veler have devised a convenient method for extracting it.³

Little seems to be known of the chemistry of this hormone. Earlier authors considered estrin to be a lipoidal substance present in the non-saponifiable fraction (sterol fraction). Somewhat later several investigators (particularly Zondek⁴) prepared the substance in a water-soluble form. No detailed description of the method used in preparing the substance in water-soluble form seems to have been given; and it seems somewhat difficult to understand how a substance first described as lipoidal in character can be rendered entirely water-soluble by an extraction process.

Some observations made by the writer during the past year may throw light on the matter. It has been found that estrin is very sparingly soluble even in hot water, and that most of the hormone occurs in the free state in the ovary, placenta or urine. Saponification is not necessary. The reason why the hormone has been supposed to reside in the unsaponifiable (cholesterol) fraction is because it forms salts with strong alkalies which are fairly well soluble in ether. Estrin forms soluble sodium, potassium, ammonium, and barium salts. Making use of the property just described, estrin fractions weighing 0.01 mg. per rat unit are easily obtainable. Such preparations show an acidity equivalent to 43.2 cc. of N/10 sodium hydroxide per 100 mg. of substance. The similarity in chemical behavior of estrin and phenols renders further purification not easy.

Using urine as starting material, one often encounters further difficulty, due to the frequent use of phenolphthalein as a laxative,

¹ Allen and Doisy, *Am. J. Anat.*, 1924, xxxiv, 133.

² Loewi and Lange, *Klin. Wochenschr.*, 1926, v, 1038; Loewi and Voss, *Ibid.*, 1928, vii, 1376.

³ Doisy and Veler, *Proc. Soc. Exp. Biol. and Med.*, 1928, xxv, 806.

⁴ Zondek, *Klin. Wochenschr.*, 1928, vii, 485.

and, therefore, to its presence in mixed urines. It is very difficult to separate the hormone from the phenolphthalein.

Estrin appears to be a phenol or an alcohol forming easily soluble salts with strong alkalis.

Aqueous solutions of estrin appear to be slightly less active (as measured on castrated rats) than solutions in oil of equal strength. This might be due to a more rapid elimination of the aqueous extract.

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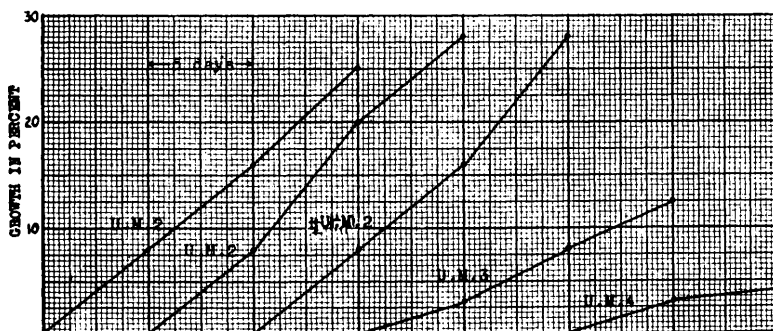
The Male Hormone. II.

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In a previous communication¹ we reported that the male urine contains appreciable quantities of the male hormone, as evidenced by the increased size of comb in castrated cocks.

In the course of the work we noticed that the male hormone (testiculin) shows chemical properties similar to those of estrin. For example, testiculin, like estrin, can be extracted from urine by means of chloroform. The urine to be used is adjusted to a pH of 4.0-4.5 and continuously extracted with chloroform for 6 hours. The separated chloroform layer is evaporated and the residue dissolved in an appropriate amount of olive oil.



Action of the daily injection of chloroform extracts (representing 100 cc. urine) on groups of six castrated cocks.

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¹ Funk and Harrow, *PROC. SOC. EXP. BIOL. AND MED.*, 1929, xxvi, 325.