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A Source of Error in Gonadotropic Hormone Determinations.*

ALLAN PALMER. (Introduced by C. D. Leake.)

From the Department of Obstetrics and Gynecology, University of California Medical School, San Francisco.

This report is presented in an effort to show that determinations for gonadotropic hormone content of the urine are frequently erroneous due to the precipitation of contaminating amounts of fat-insoluble or combined estrogen.

Concerning urinary gonadotropins there is no doubt that pregnant women excrete a substance capable of producing corpora lutea in the ovaries of normal infantile animals. Some women in the menopause excrete a gonadotropic hormone capable of provoking follicle growth in the ovaries of hypophysectomized rats or mice. A gonadotropic hormone prepared from the pituitary and capable of interstitial cell stimulation has been described, but its detection if present in the urine, depends also upon the use of hypophysectomized animals. For studying the urine of non-pregnant women most workers have resorted to the use of intact animals and depend upon more cursory and unreliable criteria for the estimation of gonadotropic hormone. Speaking collectively, their criteria have been the appearance, in infantile rats or mice as old as 32 days and within periods up to as long as 130 hours after injection, of follicle growth, interstitial cell hypertrophy, increase in uterine and ovarian size and weight, establishment of vaginal introitus, and estrus. The reactions in the lower tubular tract have been interpreted as gonadotropic responses in some instances, in the absence of demonstrable changes in the gonads.

The uterus and its endometrium depend upon the ovaries for changes in histologic structure. If the uterus of an infantile rodent were stimulated directly without the medium of the ovaries but in their presence, by an estrogenic substance that does not in any way depress ovarian activity, then an independent activation of the ovaries by the animal's own pituitary might occur. Hypothetically this pituitary activity can be construed as being either independent and spontaneous, or as a result of stimulation by an estrogen. These are two hypotheses that should be considered in the interpretation of every gonadotropic hormone reaction in intact infantile rodents.

Emmenin is just such an estrogenic substance as described in the

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foregoing paragraph. Unlike estrone it fails to produce estrus in a castrated mouse in moderate dosage, while in the infantile intact mouse it does cause uterine development and estrus without an apparent depressive action on the ovaries. By hydrolysis I have converted a small amount of emmenin into an active fat-soluble estrogen, a substance that will produce estrus in a castrated mouse in the strength of one International Unit (calculated as estrone) per cc. of emmenin. I have also subjected emmenin to a gonadotropic hormone precipitation procedure, using sodium tungstate, and recovered its active principle.

In a series of tests done on the urine of a normal woman and published elsewhere, I found that the most marked reaction, characterized by estrus, marked uterine enlargement and some evidence of follicle growth, was seen in the animals injected with the gonadotropic hormone concentrate from the same specimen of urine containing the greatest amount of fat-insoluble estrogen during the menstrual cycle studied.

I have done the following experiment in further support of my opening statement. Fifty liters of urine, pooled from the voidings of obstetrical patients, were acidified to pH 5 with H_2SO_4 and treated in the usual manner of precipitation with sodium tungstate. The precipitate was washed many times with 100 volumes of ether and benzene, removing all fat-soluble estrogen. Following this the precipitate was suspended in distilled water, acidified to pH 1 with concentrated HCl, autoclaved for 1½ hours and finally extracted with benzene. The benzene extract was assayed in the usual manner and found to contain a large amount of estrogen.

Conclusion. At least one and possibly other methods of gonadotropic hormone precipitation are effective in recovering fat-insoluble estrogen as a contaminating substance with any gonadotropic substance that may also be present.