

Conclusions. 1. The injection of benzoic acid into rabbits which have been starved for 24 hours causes an increase of glutathione in the blood. 2. Using bromobenzene, the results, though less striking, are suggestive that this detoxication process is accompanied by a definite increase of glutathione in the blood.

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Hormones in Urine of a Normal Non-Pregnant Woman.*

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The gonadotropic and estrogenic hormone content of 24-hour urine specimens from normal women has been studied at weekly intervals.^{1, 2} More recently the daily urinary excretion of hormones has been determined³⁻⁸ with results which have demonstrated a point not shown in weekly assays, namely, that the gradual rises and falls in estrin excretion curves are sudden in their occurrence and are confined to certain days. If hourly examinations could be made perhaps still sharper peaks might be found. Cohen, *et al.*,⁹ reported their observations pertaining to the combined and free state of estrin in the urine of pregnant women at the onset of labor. Is there sudden freeing and combining of estrogenic substances associated with events of the menstrual cycle? My report, developed from the study of the urine excreted by one woman during one menstrual cycle, suggests that ovulation and menstruation are possibly associated with changes in the chemical state of estrin. Gonad-

* Supported by the Christine Breon Fund for Medical Research.

¹ Frank, R. T., *The Female Sex Hormone*, 1929, Charles C. Thomas, Springfield, Ill.

² Frank, R. T., and Salmon, U. J., *PROC. SOC. EXP. BIOL. AND MED.*, 1935, **32**, 1237.

³ Gustavson, R. G., and Green, D. F., *J. Biol. Chem.*, 1934, **105**, xxxiv.

⁴ Smith, G. V. S., and Smith, O. W., *Am. J. Physiol.*, 1935, **112**, 340.

⁵ Gustavson, R. G., Wood, T., and Hays, E., *J. Biol. Chem.*, 1936, **114**, xlvi.

⁶ Smith, G. V. S., and Smith, O. W., *New England J. Med.*, 1936, **215**, 908.

⁷ Gustavson, R. G., Hays, E. E., and Wood, T. R., *Proc. Am. Soc. Biol. Chemists*, 1937, **31**, xlii.

⁸ Yerby, L. D., *PROC. SOC. EXP. BIOL. AND MED.*, 1937, **36**, 496.

⁹ Cohen, S. L., McMaster, B. A., Marrian, G. F., and Watson, M., *Lancet*, 1935, **1**, 674.

otropic hormone studies in non-pregnant women have been less satisfactory due to the variation in methods and interpretations of results.

My method of estrogenic hormone extraction combines important features of several other methods.^{4, 10-13} Each 24-hour urine specimen is made up to 1200 cc. with distilled water if it does not already exceed that volume. Of this, 700 cc. are used for estrin assay and 500 cc. for gonadotropic hormone assay. The 700 cc. portion is put directly into a continuous extraction apparatus and extracted with benzene for 24 hours. The collecting flask contains 100 cc. of a saturated sodium carbonate solution as a wash for the benzene extract containing the hormone. The latter is then transferred to a separatory funnel and washed with 100 cc. of 2½% HCl, saturated with NaCl, to remove any trace of alkali. Olive oil is added to the benzene solution, the benzene removed by steam heat and the final extract made up to 7 cc. with olive oil, thus making a 100-fold concentration of urine estrin in its free state. One hundred cc. of concentrated HCl are next added to the 700 cc. portion of urine and the mixture autoclaved for 1½ hours under 15 lb. pressure at 120°C. After autoclaving, the urine is reextracted with benzene for 24 hours and the extract washed and evaporated as before and put into olive oil as a 100-fold concentration of urine estrin in its combined state. Injections and assays are carried out on castrated mice and corrections for the 24-hour volume calculated exactly as described previously.¹⁴ Values for less than 4.0 gamma of estrone per liter of urine are not determined in this series.

Gonadotropic hormone extraction is carried out on each 500 cc. portion of urine by sodium tungstate precipitation, following a procedure described by Freed and Hechter.¹⁵ Here, also, an extract representing a 100-fold concentration of urine is prepared. This allows the use of 2 immature mice for each specimen, the equivalent of at least 200 cc. of urine being injected into each test animal.

There is a difference of opinion in the interpretation of findings in gonadotropic hormone assay of the urine. All agree that production of corpora lutea in the ovaries of immature normal mice or rats after 100 hours indicates the presence of luteinizing hormone, the

¹⁰ Kurzrok, R., and Ratner, S., *Am. J. Obst. and Gynec.*, 1932, **23**, 689.

¹¹ Schmulovitz, M. J., and Wylie, H. B., *J. Lab. and Clin. Med.*, 1935, **21**, 210.

¹² Cohen, S. L., and Marrian, G. F., *Biochem. J.*, 1935, **29**, 1577.

¹³ Schmulovitz, M. J., and Wylie, H. B., Mimeographed Supplement to Reference No. 11.

¹⁴ Palmer, A., *PROC. SOC. EXP. BIOL. AND MED.*, 1937, **36**, 123.

¹⁵ Freed, S. C., and Hechter, O., *Endocrinology*, 1936, **20**, 396.

amount being calculated according to the quantity of urine injected. This reaction denotes the presence of the gonadotropic hormone characteristic of pregnancy. There is also agreement that in a similar manner the production of many uniformly large follicles, without hypertrophy of the interstitial or theca cells in the ovaries of immature hypophysectomized mice or rats after 100 hours, indicates the presence of follicle stimulating hormone in the preparation injected and that this reaction denotes the presence of the urinary gonadotropic hormone characteristic of the menopause.

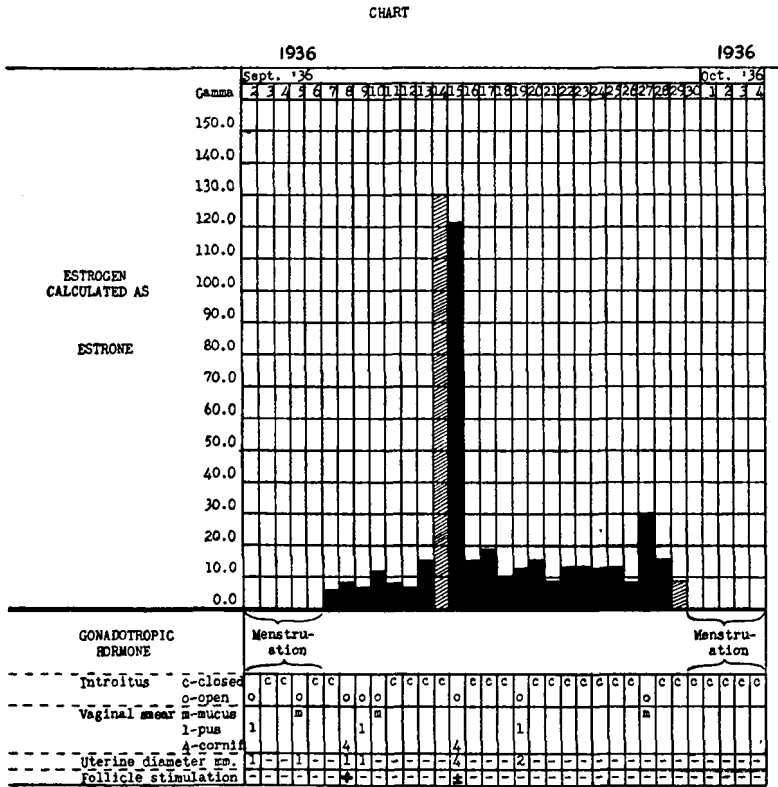


FIG. 1.

The chart shows graphically the excretion of estrogenic hormone. The solid columns represent combined estrin and the shaded columns free estrin both of which values are calculated as estrone. The lower section of the chart lists the findings in gonadotropic hormone detection.

Others believe that such criteria as opening of the vagina, increase in uterine and ovarian size and weight, histologic evidence of some follicle development and theca and interstitial cell hypertrophy in immature normal female mice or rats are reactions on which may be

based the assay of gonadotropic hormone in the urine when neither pregnancy nor the menopause is concerned. The finding of uterine enlargement in immature test animals by some workers has been their basis for reporting the presence of gonadotropic hormone even though follicle growth has been absent.¹⁶ My gonadotropic hormone extracts, tested on normal immature female mice, were effective in producing the above named criteria in a few instances during the one menstrual cycle studied. An interstitial cell stimulating hormone of the pituitary in the urine apparently is not demonstrable when immature normal rodents are used.¹⁷ Follicle growth, as characterized by many thin-walled, uniformly enlarged follicles without an associated hypertrophy of interstitial and theca cells was not seen. Corpora lutea were never found. In brief, neither the gonadotropic hormone characteristic of pregnancy nor that characteristic of the menopause was found at any time during the menstrual cycle studied.

Estrin equivalent to amounts greater than 4.0 gamma of estrone per liter of urine was not excreted in the urine in either chemical form during menstrual bleeding. The sudden excretion of free estrin for one day (9-14-36) might be explained on the basis of sudden peritoneal absorption of follicular fluid and excretion of free estrin before adequate detoxication or combination with glucuronic acid could occur, which process may have occurred by the following day (9-15-36). The second occurrence of free estrin in the urine is hardly great enough to be significant. However, a comparison is made of this finding with those of Cohen, *et al.*,⁹ pertaining to the chemical state of estrogenic hormones at the onset of labor. The latter and the onset of menstruation can be considered as similar physiologic processes. In both instances uterine activity is preceded by the occurrence of free estrin in the urine. At the onset of labor the best outlet for the estrogenic hormone is by kidney excretion which probably accounts for the remarkable rise in free urinary estrin. At the time of menstruation free urinary estrin has occurred, but the onset of bleeding, which possibly effects a ready outlet for the hormone, might account for the disappearance of the hormone from the urine during the menstrual period. The high estrin content of menstrual blood has been demonstrated.^{1, 18}

Summary. This report concerns the daily excretion of estrogenic hormone in both free and combined chemical forms during one men-

¹⁶ Hinman, F., and Powell, T. O., *Tr. Am. A. Genito-Urin. Surgeons*, 1934, **27**, 359.

¹⁷ Evans, H. M., Korpi, K., Simpson, M. E., Pencharz, R. I., and Wonder, D. H., *Univ. California Publ. Anat.*, 1936, **1**, 255.

¹⁸ Frank, R. T., and Goldberger, M. A., *J. A. M. A.*, 1926, **86**, 1686.

strual cycle of a normal multiparous woman. The possible excretion of gonadotropic hormone is reported merely that comparisons may be made with the reports of other workers. I feel that the excretion of this has not been demonstrated. Evidence of a significant association between the events of the menstrual cycle and changes in the amount and chemical form of estrin is discussed.