Summary. Trypsin accelerates the coagulation of hemophilic blood *in vitro*. Its action is similar to that of thrombin.

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Relation of Urinary Excretion of Estrone to Menstrual Cycle of Normal Woman.

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Although earlier work[1, 2] had failed to demonstrate a greater efficacy of the intravaginal method of assaying estrone, Berger[3] reported that 1/12th of the parenteral unit of estrone could be detected by intravaginal administration. Lyons and Templeton[4] reported that by their intravaginal method 1/200th of a rat unit of estrone could be detected. The latter workers reported estimates of the amount of urinary estrogenic substance excreted daily, at 4 periods in the normal menstrual cycles of 4 nulliparous women.

It seemed desirable to follow the daily excretion of estrogenic substance throughout the cycle, and for this purpose the cooperation of 2 normal nulliparous women was secured. For more than a full cycle these women collected 24-hour samples of urine preserving them with hexylresorcinol (1 part to 20 of urine) and storing them at 0°C. Crude extracts were made of a fraction of each sample according to the method formerly given,[4] and these extracts were administered vaginally to rats by means of a Breed and Brew 0.01 cc. micro-pipette to which was attached a short rubber tube such as is used on a blood-diluting pipette. Care was taken to remove any of the extract adhering to the outside of the pipette before inserting in the vagina.

Of a group of 40 ovariectomized adult rats, 24 were found sufficiently uniform and consistent in their reaction to 1/200th of a rat unit of Progynon B* administered intravaginally, to permit their

[1] Pratt, J. P., and Schmeltzer, M., *Endocrinology*, 1929, **13**, 320.

[2] Powers, H. H., Varley, J. R., and Morrell, J. A., *Endocrinology*, 1929, **13**, 395.

[3] Berger, M., *Klin. Wochnschr.*, 1935, **14**, 1601.

[4] Lyons, W. R., and Templeton, H. J., PROC. SOC. EXP. BIOL. AND MED., 1936, **33**, 587.

* The Progynon B (in oil) was supplied to Dr. H. M. Evans by the Schering Corporation of New Jersey. Dilutions were made with sesame oil. It is probable that differences encountered in standardizing estrone in oil and in water by the

use as assay animals. In standardizing the rats as well as in testing, the preparations were administered in 2 0.01 cc. daily doses and the reaction read 24 hours after the second administration.

The minimal effective dose was considered the smallest amount of extract that would produce a vaginal smear showing that definite epithelial growth and cornification had been stimulated in at least 2 standardized rats—lower doses giving negative reactions and higher doses producing more complete cornification.

Case A was studied throughout a 30-day cycle (an average length for this woman). Case B was studied throughout a 21-day cycle, the average length of cycle for this woman being 25 days. Given in the accompanying chart and Table I are the number of intravaginal units excreted daily by Case A, the 24-hour urinary volumes and the minimal effective urinary equivalent. It will be observed that the excretion of estrogenic substance reached two definite peaks during the menstrual cycle. One of these at mid-cycle (ovulation time), and the other, just before menstruation. In the case of the 21-day cycle, the mid-cycle peak occurred on day 9 and the premenstrual peak on day 17. Several other workers[5, 6, 7] have re-

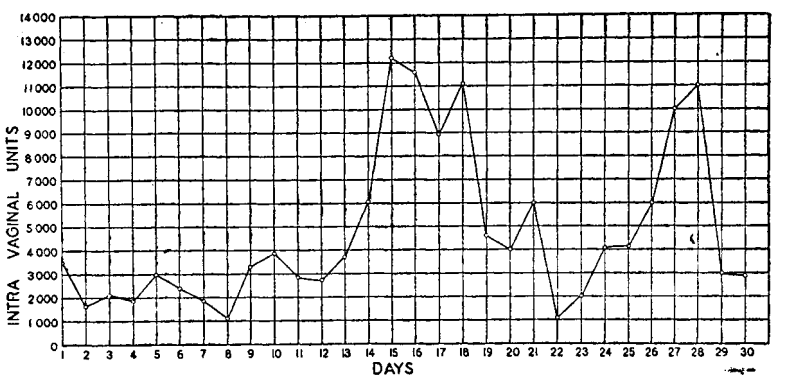

CHART 1.

Graph showing the number of intravaginal units of urinary estrogenic substance excreted daily throughout the menstrual cycle of a normal, nulliparous young woman (Case A).

subcutaneous method will be minimized by the intravaginal method, since rates of absorption and excretion are important variables to be considered in accounting for these differences. No attempt is made to identify the urinary estrogenic substance assayed in this research with any of the various estrones. Those interested in translating the unitages reported herein to one of the other systems should be able to do so through the medium of the Progynon B standardization (see also Schoeller, W., *et al.*, *Klin. Wchnschr.*, 1935, **14**, 826.).

[5] Gustavson, R. G., and Greer, D. F., *J. Biol. Chem.*, 1934, **105**, XXXIV.

[6] Smith, G. V. S., and Watkins, O., *Am. J. Physiol.*, 1935, **112**, 340.

[7] Pedersen-Bjergaard, K., *Zentralblat. f. Gynäk.*, 1936, **60**, 372.

ported finding 2 peaks of estrone excretion in woman. Fluhmann[8] reported that the blood estrone reached a peak at the 14th day, gradually declining until the 27th day.

TABLE I.

Day of Cycle	Volume of Urine	Min. Effective Urinary Equiv.	Units per 24 hr.	Day of Cycle	Volume of Urine	Min. Effective Urinary Equiv.	Units per 24 hr.
	cc.	cc.			cc.	cc.	
1*	1050	.3	3500	16	585	.05	11700
2*	670	.4	1675	17	880	.1	8800
3*	680	.3	2266	18	1120	.1	11200
4*	1290	.7	1843	19	1400	.3	4666
5*	1185	.4	2962	20	1220	.3	4066
6	1190	.5	2380	21	1800	.3	6000
7	930	.5	1860	22	1250	1.0	1250
8	640	.5	1280	23	600	.3	2000
9	690	.2	3450	24	1230	.3	4100
10	1140	.3	3800	25	1300	.3	4333
11	1400	.5	2800	26	1800	.3	6000
12	820	.3	2733	27	1600	.16	10000
13	750	.2	3750	28	1100	.1	11000
14	610	.1	6100	29	1200	.4	3000
15	1230	.1	12300	30	870	.3	2900
				1	Menstruated.		

* Menstruating.

The mid-cycle peak is probably due—as many investigators have suggested—to an increased production of estrone by the ripe Graafian follicle; but the subsequent drop and premenstrual rise will probably only be understood when a sensitive enough method will allow for the estimation of estrone and progesterone in the same sample. The figures submitted in this report represent the daily excretion by normal women of estrogenic substance detectable in urine by a given method; they do not represent the actual amount of estrone produced by the ovary.

[8] Fluhmann, C. R., *Endocrinology*, 1934, **18**, 705.