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### Cyclic Inhibitory Influence of the Rat's Ovary on the Uterine Response to Estrin.

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We have recently demonstrated the participation of ovarian factors other than estrin and progesterin in the phenomenon of estrus in the rat.<sup>1</sup> One of these ovarian factors or hormones inhibits the uterine response to administered estrogenic substance, and accounts for the marked difference in the effect of such treatment on the uteri of normal and castrated rats respectively.

The present report offers: (a) Further evidence for the existence of an ovarian factor inhibiting uterine-estrus, (b) evidence that the inhibiting factor normally operates in a cyclic manner.

(a) **THE INHIBITORY FACTOR.** *Methods and Results.* Each of 16 adult female rats was injected with 250 international units of dihydroxyestrin benzoate per day, for 3 days. On the fourth day the animals were anesthetized and their abdomens opened. In 11 of the animals the uterus was in diestrus, in 2 there was evidence of early estrus, and in only 3 was there definite estrus. This distribution of results is similar to that found upon opening a corresponding group of normal, untreated adult female rats. We have previously shown that the administration of estrin for as long as 20 days shows a similar lack of effect.<sup>1</sup> After recording the state of the uteri, 10 of the animals were ovariectomized and the abdomens of all were sutured. Three days later, without any further treatment, all the animals were sacrificed for examination. In every one of the 10 castrated animals, the uterus had developed a typical estrus reaction. The uteri in the 6 control animals were essentially unchanged. It should be noted that in all the above animals, both before and after castration, the administration of estrin resulted in a heavy cornification of the vaginal epithelium.

*Comment.* It is evident that ovariectomy removed an inhibitory influence which, in the normal animals, prevented most of the uteri from responding to the administered estrin, although the

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<sup>1</sup> Freed, S. C., Garvin, T., and Soskin, Samuel, *PROC. SOC. EXP. BIOL. AND MED.*, 1936, **35**, 409.

vaginal response was not inhibited. However, since in a few of the normal animals the uteri did respond to the estrin, it occurred to us that the stage of the estrus cycle at which the animals were injected might account for this variation in results.

(b) THE CYCLIC ACTIVITY OF THE INHIBITORY FACTOR. *Methods and Results.* Thirty-two adult female rats of the Long-Evans strain, which were showing regular estrus cycles, were selected and divided into 3 groups. Each animal of the first group was injected with 25 international units of dihydroxyestrin benzoate on the first day of its estrus cycle. This dose was repeated on the second and third day, and each animal was opened for examination on the fourth day. The 2 remaining groups of rats were similarly treated, except that the estrin injections were begun on the third and fifth days of the estrus cycle respectively.

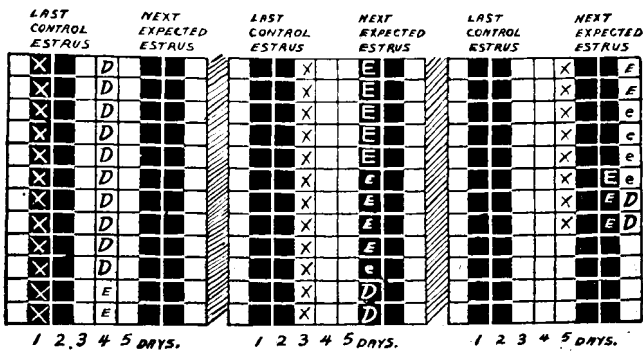


FIG. 1.

The influence of the time in the estrus cycle at which estrin is administered, upon the uterine response. The 3 blocks of results separated by the cross-hatched areas represent 3 different sets of experiments. Each horizontal row of squares in these blocks represents an experiment on an individual rat, each square being equivalent to one day of the estrus cycle. The last control estrus phase, and the next expected estrus, based on previous observation of a number of cycles, are shown in black.

X = day upon which administration of estrin was begun.

D = finding of a diestrous uterus.

e = " " " slight estrous uterus.

E = " " " moderate estrous uterus.

E = " " " full estrous uterus.

The results are graphically shown in Fig. 1. It may be seen that, with few exceptions, the state of the uteri depended upon the day of the estrus cycle upon which they were examined. The animals of the first group (whose day of examination fell on the fourth day of the cycle, *i. e.*, during the period of diestrus) exhibited the characteristic diestrus uterus. Microscopic examination showed the state of the uterine endometrium to be consistent with the gross picture, although the vaginal epithelium in the same animals was

heavily cornified. Most of the animals in the second group (whose day of examination fell on the first day of the next anticipated estrus) showed full-blown to moderate uterine estrus, with hyperemia and fluid distension. In the third group (whose day of examination fell on the third day of the cycle) the uteri were only slightly affected, and showed some hyperemia but no distension. The animals in this last group were in the terminal stages of estrus, as evidenced by the fact that several of them which were subjected to a preliminary laparotomy on the day before their routine examination, had shown much more pronounced changes.

*Comment.* These results clearly show that the administration of estrin to the normal rat causes little disturbance in its uterine estrus cycle, although the vagina is markedly affected. The state in which the uterus is found depends largely upon the day of the estrus cycle upon which it is examined. This might be interpreted to mean that administered estrin never has any significant effect on the uterus of the normal rat. However, there is no reason to believe that the estrin which we injected lacks the essential physiological properties of the estrin secreted by the rats' own ovaries. And, since the results during diestrus were not entirely negative, and in view of the definite though mild effects observed in early diestrus, it seems more logical to conclude that the uterus is resistant to any estrin during its diestrus phase, but responds during estrus.

*Discussion and Summary.* We have demonstrated the existence of an ovarian factor in the rat, which inhibits the uterine response to estrin, and which operates in a cyclic manner corresponding to the estrus cycle of the animal. Although the development of uterine inhibition during the cycle is paralleled by the development of corpora lutea in the ovary, we do not believe that the inhibition is due to progesterin. This conclusion is based on evidence that progesterin, in amounts sufficient to inhibit the effects of estrin on the uterus, always results in progestational phenomena in this organ.<sup>2, 3, 4</sup> But, at no time in the present or previous<sup>1</sup> work did we observe progestational changes in the uterus. Furthermore, the antagonism between progesterin and estrin also includes the inhibition of vaginal cornification,<sup>2, 3</sup> while in our work the vaginal mucosa never failed to become cornified after estrin administration.

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<sup>2</sup> Selye, H., Browne, J. S. L., and Collip, J. B., *PROC. SOC. EXP. BIOL. AND MED.*, 1936, **34**, 198.

<sup>3</sup> Hisaw, F. L., Meyer, R. K., and Weichert, C. A., *PROC. SOC. EXP. BIOL. AND MED.*, 1928, **25**, 754.

<sup>4</sup> Leonard, S. L., Hisaw, F. L., and Fevold, H. L., *Am. J. Physiol.*, 1932, **100**, 111.

To speculate briefly on the purpose served by this inhibitory factor, it may be pointed out that the estrus cycle of the rat is so short that the closely repeated stimulations of the uterus by estrin might lead to a chronic state of estrus, were it not for the presence of a mechanism which sharply limits the response of the uterus to this hormone, at the appropriate time in the cycle.

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### Activity of Insulin in Diabetic Hyperglycemic Animals.

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Hyperglycemia is a constant accompaniment of severe untreated diabetes mellitus. Does it of itself interfere with the ability of the organism to utilize carbohydrate? Previous to the use of insulin, a fall in the blood sugar and a decrease in the severity of the diabetes was noted with fasting.<sup>1, 2</sup> With the use of insulin the control of hyperglycemia is accompanied by a decrease in the daily insulin requirement of patients.<sup>3</sup> In view of the fact that in both of these conditions the prevailing blood sugar level is lowered, the following question naturally presents itself: Does the hyperglycemia *per se* interfere with the ordinary action of insulin?

The activity of insulin at normal blood sugar levels was studied by Greeley.<sup>4</sup> He found that with a certain hourly intravenous dose of insulin, the blood sugar of a diabetic animal could be maintained at a normal level without change. For the individual fasting, depancreatized dog, this dose was remarkably constant over a long period of time and was consequently termed the "Basal Insulin Requirement" and expressed in units of insulin per kilogram of body weight per hour. In the present work this "Basal Insulin Requirement" was first determined on the experimental dog (at a normal blood sugar level). Then on other experimental days the animal was given insulin at this rate (or less) when the blood sugar was at a hyperglycemic level. The essential difference in procedures is

<sup>1</sup> Allen, F. M., *J. Am. Med. Assn.*, 1914, **63**, 939.

<sup>2</sup> Newburgh, L. H., and Marsh, P. L., *Arch. Int. Med.*, 1920, **26**, 647; 1921, **27**, 699.

<sup>3</sup> Joslin, E. P., *Treatment of Diabetes*, Ed. 6, 305, Lea and Febiger, Phil., 1937.

<sup>4</sup> Greeley, P. O., *Am. J. Physiol.*, 1937, **120**, 345.