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Hypothalamic Control of Pituitary Function and Corpus Luteum Formation in the Rat.* (23101)

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Following bilateral hypothalamic lesions in the region of the ventro-medial nucleus in the rat, persistent cornification of the vaginal epithelium is observed in a large number of the rats which eventually become obese, as well as many of those failing to develop obesity. Interference with production of corpora lutea following destructive lesions in the anterior hypothalamus of the guinea pig has been described by Dey, Fisher, Berry and Ranson (1). Brookhart, Dey and Ranson (2) demonstrated that such ovaries, characterized by follicles only, became luteinized following ad-

ministration of a single dose of pituitary luteinizing factor, substantiating the conclusion that this type of lesion interfered with pituitary secretion of luteinizing hormone. Hillarp (3) localized a center controlling luteinization in the rat in the anterior hypothalamic area immediately anterior and ventral to the paraventricular nucleus. Bilateral lesions in this area resulted in ovarian changes characterized by normal follicular development and abundant interstitial tissue but no corpora lutea.

It was the object of the present study to re-investigate hypothalamic control of corpus luteum formation in the rat. As complete

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an endocrine study as practical was made to determine the functional state of the pituitary in the presence of such lesions, and replacement therapy with luteinizing (or interstitial cell stimulating) hormones was instituted.

Materials and methods. Female rats of the Sprague-Dawley strain, 80-90 days of age, were used. All animals were maintained on Diet I[†] *ad libitum*, with the exception of one group in which diet was restricted to reduce weight. Bilateral hypothalamic lesions were produced in 200 g rats using the Horsley-Clarke instrument adapted by Clark(4) for use on the rat. Under ether anesthesia, electrolytic lesions were made with a direct current of 1.8 milliamperes for 15 seconds, using a unipolar electrode introduced through a drill hole in the parietal bone. The lesions were placed ventral to the ventro-medial nucleus of the hypothalamus. Daily vaginal smears and weekly check of body weight were recorded for 5 months on operated rats as well as on a group of unoperated controls. Twenty-four hours prior to autopsy (5 months post-operative), metabolic rates were determined with a closed-circuit multiple respiration apparatus, patterned after that described by Kleiber(5).[‡] All rats were fasted 4 hours and allowed to adjust to the apparatus for one hour prior to oxygen consumption measurements, which extended over a 2.5 hour period. The liters of dry oxygen, at standard temperature and pressure, consumed by each rat per hour were converted to calories per hour using 4.80 as the caloric equivalent. The calories per hour thus obtained were expressed per square meter of body surface using Benedict's formula(6) for the rat:

$$\text{area in m}^2 = \frac{9 \times \sqrt[3]{(\text{body wt in g})^2}}{10,000}. \text{ To}$$

[†] Diet K (modified from McCollum's formula) consists of 67.5% whole wheat, 15% casein, 10% whole milk powder, 0.75% NaCl, 1.5% CaCO₃, 5.25% hydrogenated vegetable oil, and a concentrate of fish oil in amount to give 19 U.S.P. units of vit A and 2.5 A.O.A.C. chick units of vit D per g of diet. This diet was supplemented by fresh lettuce twice weekly.

[‡] We wish to thank Dr. E. S. Evans for determinations of metabolic rate.

measure I¹³¹ uptake of the thyroid, 1 μ c of I¹³¹ was given (intraperitoneally, 16 hours prior to autopsy) and activity of thyroid determined by the scintillation counter. Just prior to autopsy blood volume and total circulating red cell volume were determined by the Fe⁵⁹ labeled cell dilution method(7). Following blood volume determination, the animals were killed with chloroform. Their specific gravity was measured by the method of volume displacement using a graduated cylinder containing water. The pituitary, thyroid, adrenals, thymus, ovaries, uterus and oviducts were carefully dissected and weighed. After weighing, the adrenals were fixed in neutral formalin, imbedded in gelatin, cut in frozen section and stained with Sudan black for demonstration of lipid distribution. Thyroids and ovaries were fixed in Bouin's fluid, imbedded in paraffin and stained with hematoxylin-eosin. The pituitaries were fixed in Zenker-formalin, weighed, imbedded in nitrocellulose, cut at 4 μ and stained with modified Mallory-Azan stain(8). The calvarium was cut away and the brain carefully lifted from the cranium, fixed in 95% alcohol, imbedded in paraffin and the hypothalamic area sectioned serially at 10 μ . Every 10th section was mounted and stained with thionin.

Results. Excessive increase in body weight began immediately in the majority of the 53 rats, in which bilateral lesions had been placed ventral to the ventro-medial nucleus. The animals which eventually became obese gained 2 to 8 g per day in the first week after operation. Of rats which became obese, most (80%) developed persistent cornification of the vaginal epithelium within 2 weeks following the operation. The remainder showed either persistent diestrus smear or normal cycles. Of the operated animals which did not become obese, 1/2 developed persistent cornification, and the remainder continued to show either a normal rhythm, or a persistent diestrus smear. Those animals which became obese were irritable and difficult to handle.

After 5 months, groups of 5 to 7 rats, representing each type of response, were autopsied, 1) obese showing constant vaginal cornification; 2) obese, showing normal cycles;

TABLE I. Comparison of Body Size, Reproductive and Endocrine Systems of Rats with Hypothalamic Lesions. (Avg 5 rats/group.)

Category	Obese, constant vaginal cornification	Obese, normal cycles	Not obese, constant vaginal cornification	Unoperated controls, normal cycles
Body wt (g)	*414 ±12 †	467 ±30	248 ±10	287 ± 7
Specific gravity	*.98± .02	.98± .01	1.05± .01	1.04± .01
Red cell vol (cc/100 g body wt)	1.30± .11	1.37± .11	1.71± .10	1.68± .08
Thyroid wt (mg)	29 ± 3	24 ± 2	17 ± 2	22 ± 2
I ¹³¹ uptake (%)	22.1 ± 4.8	14.6 ± 5.8	12.1 ± 1.9	7.2 ± 2.3
Stand. metabolic rate (Cal./m ² /hr)	32.9 ± 3.1	30.1 ± 2.1	32.7 ± 1.7	30.8 ± .7
Adrenal wt (mg)	57 ± 2	62 ± 5	56 ± 3	60 ± 4
Thymus " "	150 ±34	125 ±21	123 ± 8	132 ± 12
Pituitary " "	14.1 ± 1.4	12.1 ± .4	12.5 ± .8	13.0 ± .8
Ovarian wt (mg)	43 ± 5	62 ±17	36 ± 2	81 ± 5
Follicles†	s, m, l cysts	s, m, l	s, m, l cysts	s, m, l
Corpora lutea	0	Several	0	Several
Uterus + oviducts (mg)	800 ±53	598 ±38	818 ±52	654 ±119

* Two rats, whose body wt was reduced by restricting food, were not included in the average.

† Mean and stand. error.

‡ Small (s), medium (m), large (l); cysts, *i.e.* in excess of size of mature follicle.

3) not obese, showing constant vaginal cornification; 4) unoperated controls. The results of the various determinations made are summarized in the Table. Animals designated as obese weighed 60% more than the non-obese groups, and specific gravity of the obese rats was significantly less than that of the non-obese rat.

The ovaries of the animals showing persistent vaginal cornification contained no corpora lutea, although follicles were abundantly present. The interstitial cells of the ovary were epithelioid, appearing healthy except for an excess lipid content. The follicles were of all sizes, including several in excess of normal size; many were in different stages of atresia. Infolding of the granulosa was present in some of the largest or cystic follicles.

The ovaries of rats which had shown normal vaginal cycles contained corpora of different ages, also growing follicles and normal appearing epithelioid interstitial tissue. The weight of ovaries, which contained follicles, was considerably less than that of ovaries containing corpora lutea.

In obese rats with constant cornification, I¹³¹ uptake of the thyroid was 3 times that of controls. The thyroids of this group weighed only slightly more than the controls, but gave histological evidence of increased activity. There was no significant difference in stand-

ard metabolic rate of the 4 groups. This is in contrast to the findings of Greer(9), Bogdanove and Halmi(10), Ganong *et al.*(11) and D'Angelo and Traum(12) who found evidence of decreased TSH secretion following lesions in the hypothalamus.

Adrenal, thymus, pituitary weights and tail lengths were not different in the 4 groups. The total circulating red cell volume was low in the obese animals.§ As red cell volume is expressed in relation to body weight this low figure is undoubtedly attributable to adiposity. It is known that increase in blood volume accompanying fat deposition is less than that accompanying increase in lean body weight.¶

The pituitaries from animals showing constant vaginal cornification were distinguishable histologically from those of normal controls, even though normal rats were in different stages of the estrous cycle at time of autopsy. They were characterized by a decrease in number of normally granulated, deeply staining acidophils and by an increase in number of almost completely degranulated acidophils, which were indistinguishable from chromophobes except after careful examina-

§ Total circulating red cell volume of the controls was considerably less than that for adult rats of the Long-Evans strain(7).

¶ Garcia, J. F., USAEC, UCRL 3516.

tion. These cells differed from chromophobes by their larger size, by distinctness of cellular outlines, by enlarged negative image of the Golgi apparatus, as well as persistence of a few acidophilic granules. The pituitaries of animals with lesions also showed decreased numbers of basophils; those which remained were irregular in contour and were situated within cords and not in intimate contact with the blood vessels. Their nuclei were vesicular, they had prominent nucleoli and the Golgi apparatus was in close proximity to the nucleus. This distribution and morphology suggest that they are thyrotropic basophils, since gonadotropic basophils are oval or rounded in shape, without prominent nucleoli, and with the negative image of the Golgi apparatus situated definitely away from the nucleus. The location of gonadotropic basophils is also described as bordering the sinusoids not within the cellular cords. The decrease in the number of basophils was therefore possibly due to decrease of gonadotropic basophils. In rats which were obese, but with normal vaginal cycles, the pituitaries showed normal basophils of both thyrotropic and gonadotropic types. Some degranulation of acidophils was present but not to the same degree as in the animals showing constantly cornified vaginal smear.

Serial sections of the hypothalamic area were examined and the lesions were diagrammed using the schematic graphs of Krieg. It was found that of 10 animals examined, which showed constant vaginal cornification (5 obese and 5 not obese), the lesion lay ventral to the ventro-medial nucleus in 9 and ventral to the anterior nucleus in 1 (obese). Of 4 animals examined which were obese but maintained normal vaginal cycles (several crops of corpora present in the ovary), 3 had lesions placed more anteriorly than those in the constant estrous group (ventral to the anterior nucleus) and in 1 the lesion was more posterior (ventroposterior to the ventro-medial nucleus).

Progesterone. According to Greer(13) rats showing obesity and constant vaginal cornification, following bilateral lesions in the rostral hypothalamus, could be made to cycle

by administration of progesterone. For this reason, progesterone was given to 3 rats which had developed obesity and persistent vaginal cornification; one was given 0.5 mg of progesterone (the dose used by Greer), and 2 were given 5 mg, each dose given subcutaneously in cottonseed oil daily for 4 days. By 24 hours leucocytes had appeared in the vaginal smear and by 48-72 hours the smear was typical of diestrus. On the fifth day, the animals were autopsied and ovaries examined grossly and histologically. No corpora lutea were present and there was no evidence of luteinization of follicle walls. The incidence of follicular atresia was, however, definitely increased in all progesterone treated animals.

Chorionic Gonadotrophin. Five rats which became obese following operation and showed a persistent cornification of the vaginal smear, were given graded doses of chorionic gonadotrophin in the hope of inducing corpus luteum formation and ovulation. A total dose of 5, 10, 25, 50 or 150 rat units was given in divided doses subcutaneously for 4 days. On the third day of injection, leucocytes were present in the vaginal smear and by the fourth day the smear was typical of diestrus in those rats receiving 25 to 150 rat units, but remained cornified at the lower doses. The ovaries of rats given 25 rat units or more when observed on the fifth day, contained a single group of large, newly formed corpora lutea of approximately the same age. No luteinization was observed at the 2 lower doses. The oviducts were not distended. The interstitial tissue had become more normal in appearance; the excess lipid characteristic in the interstitial tissue of untreated animals had disappeared. The uterine mucosa of animals given 150 rat units of chorionic gonadotrophin showed very active glands, with wide openings. Such functional change in uterine mucosa would not be expected unless lactogenic hormone had been secreted from the pituitary with resultant progesterone secretion from the luteinized structures. At 25 rat units, the uterine mucosa showed less transformation, and at the lower doses no such change occurred.

Beef pituitary interstitial cell stimulating hormone (ICSH). Since administration of an ICSH-like urinary hormone (chorionic gonadotrophin) repaired what appeared to be a defect in gonadotrophic secretion resulting from the hypothalamic lesion, it was decided to attempt restoration with pituitary ICSH. Purified beef ICSH prepared by salt fractionation and having a minimal effective dose of 0.025 mg was given to 2 rats which were obese and showed constant vaginal cornification. A total dose of 1 mg of ICSH was given intraperitoneally in 4 daily doses. Twenty-four hours after first injection the vaginal smear contained leucocytes and by 48 hours the smear was typical of diestrus and remained so. On the fifth day the ovaries were examined grossly and histologically and showed a single set of newly formed corpora lutea and the interstitial tissue was indistinguishable from that of normal controls. Distended oviducts were not observed and ova were not found in sections of oviducts. Breeding behavior was not investigated.

Discussion. The demonstrations by Dey *et al.*(1) and by Hillarp(3) that destructive lesions in the hypothalamus may result in failure of corpus luteum formation have been confirmed in this study. Replacement therapy with luteinizing hormone (chorionic gonadotrophin or pituitary ICSH) resulted in the formation of functional corpora lutea. A similar result was obtained in the guinea pig by Brookhart, Dey and Ranson(2). Whether the location of lesions represents the actual center of control of luteinization or a point along the pathways from the center, as suggested by Hillarp, has not been investigated.

The changes seen in the pituitaries of the rats with prolonged cornification were suggestive of the changes found after prolonged estrinization from exogenous source(14) *i.e.*, disappearance of gonadotrophic basophils and profound degranulation of the acidophils. However, the animals with hypothalamic lesions, although showing effects of chronic estrinization, appeared to be continuously forming new follicles, indicating that secretion of

follicle stimulating hormone must then have been occurring to some extent in spite of the decrease in gonadotrophic basophils. The location of the hypothalamic lesions could not be correlated with the variability in adiposity and ovarian function.

Summary and conclusions. 1. Bilateral hypothalamic lesions ventral to the ventromedial nucleus in female rats resulted in persistent vaginal cornification in the majority of animals. Thyroid morphology and I^{131} uptake in obese rats with constant cornification indicated increased thyroid activity. The ovaries of those showing constant cornification contained follicles of all sizes but no corpora lutea. The interstitial tissue was not atrophic. 2. The follicles were readily converted into corpora lutea by administration of luteinizing substances, both human chorionic gonadotrophin and pituitary interstitial cell stimulating hormone. The interpretation is made that secretion of luteinizing factor by the pituitary may be impaired by small, bilateral hypothalamic lesions ventral to the ventro-medial nucleus.

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