THE SEXUAL CYCLE OF RATS AFTER DEGENERATION OF THE OVARY

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When studying the structure of the ovaries in cats after their denervation by extirpation of the corresponding sensory roots and removal of the celiac ganglion of the solar plexus we have been struck by the many pathological changes which as a rule developed subsequently; these changes included dilatation of the blood vessels, extensive degeneration of the sex cells in the follicles at various stages in development, the absence of any mature Graafian follicles ready to ovulate, the absence of corpora lutea, and the spread of the connective tissue stroma, etc. [1].

Such ovarian changes would be expected to lead to functional changes, and influence the course of the estrus cycle.

A cytological study of the vaginal contents gives an indication of ovarian function [3-5, and others]. We have undertaken a systematic study of the phases of the sexual cycle by making parallel observations on vaginal smears and of ovarian structure in normal and in operated animals, in order to reveal the degree and the nature of the ovarian functional disturbances.

METHOD

We used 52 female rats weighing 150-200 g. Initially, for a month we made daily vaginal smears by washing out the vaginal contents with distilled water from a pipette.

In 34 animals we denervated the ovaries by removal of the celiac ganglia, and we recorded the phase of the estrus cycle at which the operation was performed. The animals were killed at various times from three days to three months after the operation. The controls were two groups of animals, one consisting of ten unoperated rats, and the other of eight animals in which the abdominal cavity was opened, but the celiac ganglion was not removed; the rats of the second group were used to determine any possible effect due to trauma at operation.

The experimental and control animals were kept under absolutely identical conditions. Vaginal smears were made right up to the time the animals were killed.

The ovaries were fixed in a 12% solution of neutral formol, were stained in hematoxilin-eosin, and embedded in paraffin. The smears were prepared by the usual method, and were also stained in hematoxylin-eosin.

RESULTS

Apart from a very few exceptions, as regards the duration of the sexual cycle and the length of each phase, and the cytological appearance of the smears in each of the phases, our results corresponded to those which have frequently been reported [2, 5].

A daily analysis of the cellular constitution of the smears after removal of the celiac ganglion showed that the normal course of the estrus cycle was disturbed, and that there were changes both in the cellular constitution, and in the duration of the phases of the cycle.

If the denervation was carried out in the proestrus, metestrus, or estrus phases, then the next phase of diestrus occurred, but its duration was 4-6 days. If the denervation was carried out in the phase of diestrus, this phases was not altered, and occurred normally 50-57 hours after proestrus, and continued for 4-6 days.

Subsequently, in both groups of animals the cycle continued with the same deviations from normal. These deviations were shown as an increase in the time of all phases of the cycle, particularly in the proestrus phase, which

occupied 2-3 days instead of the normal 12 hours, and in the diestrus phase, which lasted for 5-6 days as compared with the normal 57 hours. The total duration of the cycle was extended to 8-12 days as compared with the normal 5 days.

The cellular composition of the smear in these phases differed from the normal. In the proestrus phase, in addition to the epithelial cells typical of this stage, in the smear there were also a large number of small oval epithelial cells with a dark, flattened, frequently deformed nucleus representing abnormal cells from the deeper layers of the vaginal epithelium (Fig. 1). Then, when the estrus phase was due to occur, in some of the animals a small number of squamous cells appeared, so that estrus was barely detectable. In other animals, the squamous cells never appeared

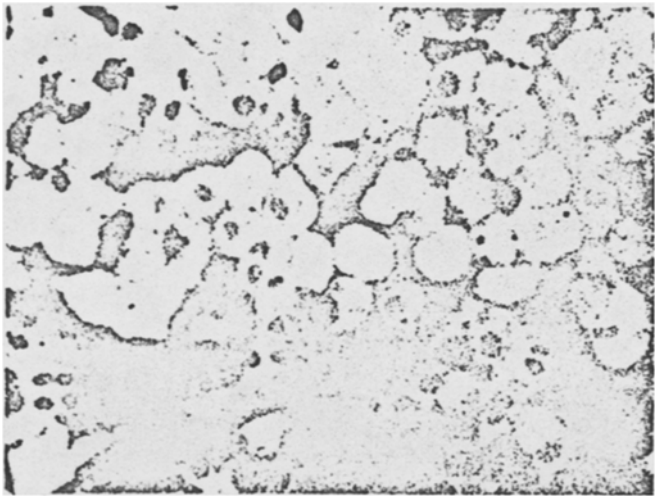

Fig. 1. Vaginal smear of a rat in the phase of proestrus made on the 10th day after extirpation of the solar plexus. In addition to the usual cells, there are a large number of atypical small dark cells from the deep epithelial layers, some of which have a deformed nucleus. Micrograph. Stain hematoxylin-eosin. Objective 40 ×, ocular 15 ×.

at all, i.e., the cycle terminated in them at the proestrus phase with the appearance of the atypical cellular constitution just described.

In the next phase of metestrus, among the cells typical of this stage there were also altered cells from the deeper layers. These cells appeared also in the smears made at diestrus.

The picture as described was observed on average for one month. At the end of the first or occasionally at the beginning of the second month, the cytological appearance of the smear was already close to the normal (Fig. 2), but the total length of the cycle was still extended on account of the prolongation of di- and proestrus. Complete normality was not restored until the end of the second month after the operation.

In the ovaries of these animals, in the first 4-6 weeks after the operation, there were false corpora lutea, some falling away of the membranes, and a marked dilatation of the blood vessels containing static blood; as a rule, there were no developing follicles. However, as early as the middle, or more often at the end of the second month after the operation the structure of the ovary corresponded to a definite phase of the estrus cycle; in other words, at this time, the normal cycle had been restored.

We observed such a cycle in 19 of the 34 operated animals. In 11 of the 34, operated at various phases of the cycle, after the operation the estrus phase appeared and was maintained for 6-7 days, and the cellular composition of the initial and terminal stages was typical. In two animals, killed on the 5th and 6th days after the operation, occasional Graafian follicles and immature follicles were found.

From the second week after the operation onwards, the course of the cycle and the cytology of the smears of these animals corresponded to those in the 19 animals described above, i.e., the estrus cycle was extended, and the

constitution of the smears altered. In the ovaries of animals killed at these times, there were many false corpora lutea, the vessels were still widely dilated and contained static erythrocytes. At approximately the same times, i.e., at the second month after the operation, the estrus cycle returned to normal.

In four animals, denervation of the ovaries led to no marked changes of the sexual cycle, although the duration of the phases was somewhat increased, the appearance of the vaginal smears remained typical, and the ovarian structure, as a rule, corresponded to the cytological appearance of the smear.

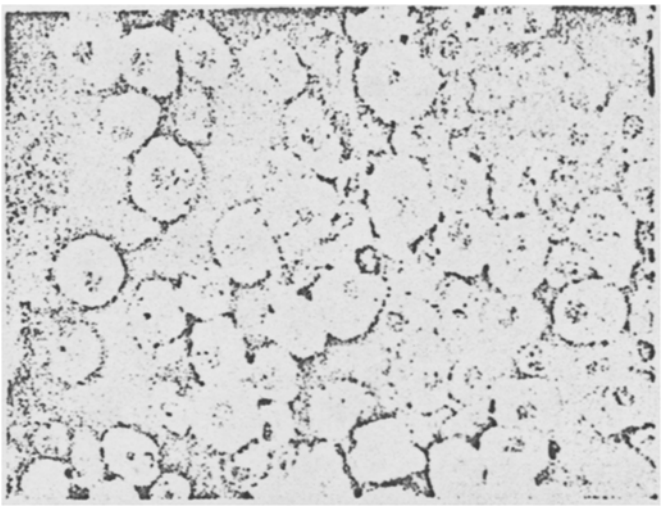

Fig. 2. Vaginal smear made during proestrus on the 29th day after extirpation of the solar plexus. A few cells from the deep epithelial layers can be seen. Micrograph. Stain hematoxylin-eosin. Objective 40 ×, ocular 15 ×.

In the control group, in which the semilunar ganglia were not extirpated, there was no abnormality of the cycle.

It can be seen therefore that denervation of the ovaries leads to various degrees of disturbance of the estrus cycle. The number of developing follicles is reduced, Graafian follicles ready to ovulate are almost completely absent, and there is an impairment of the blood supply to the ovary.

It would appear that the false luteinizing effect is also a result of denervation, and the explanation that this change is simply a reaction to the mechanical stimulation of the vagina by the pipette [6, 7] is most unconvincing, because in the control animals under the same conditions no such luteinizing effect was observed.

The general course of the estrus cycles was prolonged, particularly the stages of diestrus, and there were changes in the cellular composition of the smear.

The appearance in the vaginal smears of cells from the deeper epithelial layers, their degenerate form, and the absence or paucity of cornified squames at the phase when actual estrus should occur, and the fact that a particular cytological appearance of the smear was maintained longer than normal (we recorded this effect as a prolongation of the phases of the cycle) – are all changes due to impaired growth of the vaginal epithelium.

Such disturbances of the cellular composition of the vaginal epithelium are not of course directly due to denervation of the vagina itself, and are merely the manifestations of the effect of denervation on ovarian function.

Thus, the trend of the changes, and particularly those of the vaginal smears indicate an altered ovarian function, and a reduction in its production of estrogen. The fact that even incomplete denervation of the ovary alters estrogenic function is important in connection with the problem of the effect of nervous influences on the structure and hormonal activity of the ovary.

We are inclined to attribute the small variations from one animal to another in the course of the cycles to some difference in the degree of denervation effected at the operation, differences which were due to variation of the innervation of the ovary from the celiac ganglion and from other sources.

SUMMARY

Denervation of the rat ovary by removal of the semilunar ganglia of the solar plexus disturbs the estrus cycle; it increases to 10-12 days, and there are abnormalities of the smears caused by disturbed growth of the vaginal epithelium. These changes are connected with reduced ovarian estrogenic function; the development of the follicles is retarded, and circulatory disturbances occur. After 1½-2 months, regular estrus cycles are restored, and they become completely normal.

LITERATURE CITED

1. O. V. Volkova, Dokl. AN SSSR, Vol. 113, p. 2, p. 430 (1957).
2. I. D. Rikhter, Histophysiological Features of the Mucous Membrane of the Female Sex Ducts of Rats and Mice. Dissertation for Doctorate. Kursk (1949).
3. A. Dănilă-Muster and I. Maxim, Stud. Cercet. Endocr., Vol. 3, p. 431 (1952).
4. S. Geist, U. Salmon, and M. Mintz, Am. J. Obstet. Gynec. Vol. 36, p. 67 (1938).
5. G. N. Papanicolaou et al., Am. J. Obstet. Gynec. Vol. 31, p. 806 (1936).
6. J. R. Slonaker, Am. J. Physiol., Vol. 89, p. 406 (1929).
7. I. Szabo, Endocrinology, Vol. 27, p. 125 (1940).
