THE ACTION OF FOLLICULIN AND PROGESTERONE ON THE MITOTIC ACTIVITY OF THE UTERINE EPITHELIUM IN MICE

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In our previous researches [2, 4] we showed that the mitotic activity of the epithelium of the uterine mucous membrane in mice undergoes regular changes during the sexual cycle and can be represented graphically in the form of a curve with the largest number of cell divisions in proestrus and in the transition to diestrus.

The first increase in mitotic activity in proestrus is evidently due to an increase in the concentration of estrogens in the blood. There are reports in the literature [1, 7, 8] of the stimulating action of estrogens on cell division in the epithelium of the uterine mucosa. It has not yet been explained, however, what causes the rapid fall in the mitotic activity and its maintenance at a low level during estrus, when the blood folliculin level continues to remain high.

Even less clearly understood are the principles involved in the mechanism of the second increase in mitotic activity in metestrus, when the blood estrogen concentration falls. The problem of the role of progesterone in this increase has necessitated special investigation, for the corpora lutea in rats and mice, according to the existing opinion [6], becomes functionally active only after mating or after the action of certain other factors. Attempts to investigate this problem during natural activation of the corpora lutea in mice [3] did not reach any definite conclusions. It was found that at early periods of pregnancy the mitotic activity of the uterine epithelium is no higher than at the same periods after ovulation in nonpregnant mice. Meanwhile, there are reports in the literature [9, 10] that progesterone may exert a stimulating action on division of the cells of this tissue.

In the present work we investigated the action of the sex hormones – folliculin and progesterone – on the mitotic activity of the epithelium of the uterine mucosa in mice.

From analysis of our previous results [2, 3, 4] and also from data in the literature [5] it is considered that the influence of the sex hormones on cell division even within the confines of the reproductive system is much more complex than is apparent from the existing information, and that the final effect of the action of the hormone depends on the state of the tissue under investigation.

EXPERIMENTAL METHODS

For the experiments we used 130 sexually mature female mice, weighing 22-23 g. Ovariectomy was performed on all the animals, which were then divided into 8 groups with 8-10 experimental and 7 control animals in each group. Starting on the 20th day after removal of the ovaries, the experimental mice of groups I-V received daily 25 units (2.5 γ) of folliculin in 0.1 ml of apricot oil. The animals of these groups were given different doses of this preparation (2, 4, 6, 8, and 10 injections). The control mice received the same number of

TABLE 1

Mitotic Coefficient in the Uterine Epithelium of Mice Receiving Injections of Progesterone

Hormone used	No. of animals	No. of injections	MC
Progesterone	9	5	0.36
Progesterone after 6 injections of folliculin	10	5	0.08
Folliculin	8	6	0.05

TABLE 2

Number of Mitoses by Phases in a Conventional Unit of Area of Section

Nature of experiment	Area of epithelium in conventional units	No. of mitoses per 1000 conventional units	MC	Phases of mitosis						PC
				early prophase	prophase	metaphase	anaphase	telophase	late telophase	
2 injections of folliculin	3 000	267.2	0.30	86.3	28.3	67.3	23.7	28.3	33.3	2.1
4 injections of folliculin	3 670	182.3	0.16	81.7	21.2	34.9	11.9	16.3	16.3	3.1
6 injections of folliculin	2 800	54.4	0.05	24.6	7.8	12.1	2.1	5.0	2.8	4,4
8 injections of folliculin	2 840	70.4	0.06	22.5	8.8	25.0	3.5	8,1	2.5	4.0
10injections of folliculin	3 700	94.8	0,08	40.5	14.3	19.5	5.7	8.9	5.9	3,6
Cessation of folliculin injections	690	371.8	0.38	131.9	34.8	131.9	28.9	36.2	8.1	4.0
Injection of progesterone after 6 injections of folliculin	2 600	93.8	0.08	29.6	15.4	30.0	6.1	8.1	4.6	3.9
Injection of progesterone	1 600	336.2	0.36	88.7	33.8	123.7	21.9	45.6	22.5	2.7

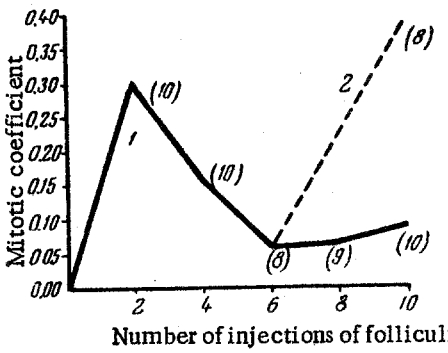

Fig. Changes in the mitotic coefficient in the uterine epithelium of mice. 1) During injection of folliculin; 2) after cessation of folliculin injections. The figures in brackets represent the numbers of experimental animals.

injections respectively of oil. The animals were sacrificed 24 hours after the last injection. The animals of group VI received 6 injections of folliculin and they were then sacrificed 5 days after the last injection. After receiving 6 injections of folliculin, the animals of group VII were given injections of 0.2 mg progesterone in 0.1 ml oil daily for 5 days. The animals of group VIII also received progesterone injections for 5 days, but after 6 injections of oil. The animals of groups VI-VIII were sacrificed simultaneously.

The effectiveness of action of the preparations was checked throughout the whole experiment by taking daily vaginal smears. The animals were killed by decapitation, all at the same time of day (11.00-12.00 hours).

The uterus was fixed in Zenker's fluid. Serial transverse sections were cut through the upper half of the right cornu of the uterus, to a thickness of 7 μ,

and stained with Carazzi's hematoxylin; the number of mitoses in the epithelium of the mucous membrane was counted in 10 sections (at intervals of 3 sections), as described in previous papers [2, 4].

The mitotic coefficient (MC) was determined as the number of mitoses per unit area of epithelium. The degree of significance of the differences observed was calculated by the Fisher-Student method.

EXPERIMENTAL RESULTS

Only isolated mitoses were found in the uterine epithelium of the ovariectomized mice whatever the time of sacrifice. After only 2 injections of folliculin (see figure) the number of cell divisions reached considerable proportions (MC = 0.30). With further injections, however, the mitotic coefficient fell; after 4 injections MC = = 0.16 and after 6 injections MC = 0.05; the fall in the number of mitoses in the interval between the second and 6th injections was significant (P = 0.01). This low value of the mitotic coefficient was maintained, with slight fluctuations, until the end of the course of injections of folliculin. With cessation of the administration of folliculin at the 6th injection (group VI) there was a sharp rise in the number of cell divisions (MC = 0.38, $P < 0.0001$).

The administration of progesterone to ovariectomized mice (Table 1) also had a considerable stimulating action on mitosis in the uterine epithelium (MC = 0.36). During the action of progesterone after preliminary administration of folliculin to animals, the mitotic coefficient remained almost the same (MC = 0.08) as after the 6th injection of folliculin (MC = 0.05).

Besides determining the mitotic coefficient, we also calculated the phase coefficient (PC), i.e., the ratio between the sum of the early phases of division (early prophase, prophase, and metaphase) and the sum of the late phases (anaphase, telophase, and late telophase).

As shown by Table 2, the value of PC more than doubled as the mitotic coefficient fell between the second and 6th injections of folliculin. Analysis of the causes of the changes in PC suggested that the fall in the value of the mitotic coefficient after two injections of folliculin was to a large extent connected with acceleration of the course of the late phases of mitosis, since the number of cells engaging in division was only very slightly less after 4 injections than after 2 injections of folliculin.

After 6-10 injections of folliculin, when the mitotic coefficient was very low, the number of early phases of mitosis became smaller, although PC rose to 4.4. Obviously the course of later phases of mitosis was accelerated in addition to the onset of depression of impending cell division. Cessation of administration of folliculin after 6 injections, when PC was 4.4, led to a sharp and proportional increase in all phases of mitosis (PC = 4). In this case, therefore, there was a universal stimulation of mitotic activity.

During administration of progesterone after 6 injections of folliculin approximately the same trend of changes in the phases was observed as after 8 and 10 injections of folliculin. The phase coefficient during the action of progesterone alone was close to that which was found after 2 injections of folliculin, apart from the number of metaphases, which was doubled.

The changes observed in the mitotic activity were accompanied by definite morphological changes affecting the uterine mucous membrane throughout the period of administration of the estrogen.

On the 20th day after ovariectomy the uterus was very small and its lumen narrow. The epithelial cells were cubical and sometimes flattened, their nuclei round or polymorphic and often shrivelled. The stroma was very compact and the glands feebly developed.

After 2 injections of folliculin the uterus increased sharply in size; its cavity was large and its contours had become indented. The epithelial cells were high-prismatic and their nuclei elongated, staining intensively and uniformly. The stroma was friable and a small number of glands with a well marked lumen was observed.

After 4, and occasionally after 6 injections of folliculin, the uterus in the majority of cases was elongated and its cavity had attained a very large size; secretion was often present in the cavity. The epithelial cells were cylindrical and cubical, with round or polymorphic nuclei. The glands were well developed.

Starting after 6, and especially after 8 and 10 injections of folliculin, the borders of the cavity became broken up and the epithelial layer composed of several rows of cells, arranged in folds, or sometimes it became syncitial in character. Pyknosis and rhexis of the nuclei were frequent. Vacuolation of the epithelium was seen

with desquamation of its cells and sometimes of whole areas into the lumen; occasionally a marked leucocytic infiltration could be observed. The glands developed to an extraordinary degree, and in isolated cases they showed cystic hyperplasia.

On the 5th day after cessation of the folliculin injections the uterus had almost regained its original size. The epithelial layer became less complex, the stroma was compact and the glands reduced in size.

In the normal sex cycle the process of destruction of the epithelium begins at the end of estrus and reaches its full development in metestrus; at the same time regeneration of the epithelium is proceeding, its reconstruction being completed in diestrus. At this time the blood estrogen concentration is known to be decreased. Our experiment after cessation of folliculin administration was to some extent an attempt to reproduce this part of the sex cycle. The high mitotic activity observed under these conditions was evidently connected, as in the normal animal, with regeneration of the epithelium and restoration of its original state. It is natural that, during the normal cycle, when the folliculin concentration falls gradually, the changes in mitotic activity are themselves more gradual in character.

This comparison thus confirms our previous hypothesis [2, 3, 4] that the mitotic activity is closely connected with the functional state of the organ.

The uterus of those mice which received injections of progesterone after folliculin, showed characteristic changes, known as progestin changes [6]. The uterine cavity was relatively large in size. The surface of the epithelial layer became nodular in appearance. The epithelial cells were mainly high-prismatic and their nuclei elongated and uniformly stained. The stroma was compact close to the lumen and the glands well developed.

When progesterone was given to the ovariectomized animals without previous treatment with folliculin, the size of the uterine cavity remained small. The epithelial cells were cylindrical and their nuclei oval. The stroma was very compact and the glands negligible in number and reduced in size. The experiment in which progesterone was injected without previous folliculin administration gave results suggesting that the mitotic activity is a more sensitive index of the tissue reaction than its proliferative changes. This hypothesis is in agreement with the experimental findings of Haskins and Leong [7], who observed the highest mitotic activity in the uterine epithelium of rabbits 48 hours after injection of progesterone, whereas the full completion of proliferation required 72-96 hours.

The results obtained suggest that folliculin has a stimulating effect on mitotic activity only after the first injections of the drug. Continuing saturation of the tissue with the hormone not only causes no further increase in the number of dividing cells, but, on the contrary, it leads to a fall in their number. Cessation of the folliculin injections at a time when its possibilities of stimulating this particular tissue are evidently exhausted, leads to an increase in mitotic activity.

Progesterone also had a stimulating action on cell division in the uterine epithelium. Its stimulating action in the conditions of our experiments, however, was observed only when no preliminary injections of folliculin had been given to the animals.

There are, therefore, at least two hormonal stimulators of the mitotic activity of the uterine epithelium, the effectiveness of which differs in accordance with the initial state of the tissue.

SUMMARY

Only individual mitoses are observed in the epithelium of the uterine mucous membrane of mice on the 20th day after ovariectomy. Following 2 folliculin injections the number of cell divisions is considerably increased; after 6 injections the number of mitoses becomes diminished and this reduction holds with further administration of the hormone (up to 10 injections).

Injections of progesterone to ovariectomized mice also have a considerable stimulating effect on cell divison in the uterine epithelium. If progesterone is administered after preliminary folliculin injections no such effect is observed.

Consequently, there are at least two hormone stimulants of mitotic activity of the uterine epithelium, possessing different degrees of efficacy depending upon the initial condition of the tissue.

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*See C. B. Translation.