OVARIAN HORMONES AS A FACTOR ESSENTIAL TO MITOSIS OF EPITHELIAL CELLS OF REPRODUCTIVE ORGANS

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Bilateral castration of sexually mature female mice has been found to cause cessation of mitotic division of the epithelial cells of the mammary glands [5]. This effect, verified many times, arises as a result of a sharp fall in the concentration of estrogens and progesterone in the internal environment of the organism. In the same conditions, however, solitary cell divisions continue to occur in the epithelium of the vagina. The mitotic activity of the uterine epithelium also falls sharply after castration [2], although to a lesser degree than in the vagina.

We have suggested that the estrogen concentration in castrated female mice does not fall to zero, but is maintained at a low level, too low to stimulate cell division in the mammary gland but sufficient to maintain mitotic activity in the uterine epithelium at a low level.

Investigation of the epithelium of the mammary glands of noninbred albino mice has shown that no mitoses can be found even 6 months after castration. This demonstrates that the adrenal cortex is unable to compensate estrogen synthesis after castration. Uncertainty remains about the way in which castrated animals produce the necessary concentration of estrogens to stimulate the isolated cell divisions taking place in the uterine epithelium. We have suggested that a small amount of estrogens may enter the body with the food and may influence an organ so highly sensitive to the action of these hormones as the uterus.

In this investigation an attempt was made to prevent castrated mice from obtaining estrogens with their diet, and to ascertain how this affects the mitotic activity of the vaginal and uterine epithelium.

EXPERIMENTAL METHOD

Experiments were carried out on sexually mature female mice weighing 30 g, in three series. In the principal series of experiments, 15 days after castration, the mice received nothing but water for 3 days (8 animals). In the first control series normal mice were kept in the same conditions of fasting for 3 days (6 animals). In the second control series castrated mice received their normal diet (7 animals). All the animals were sacrificed 3 days after the beginning of the experiment at 11 A.M. One cornu of the uterus and the vagina were fixed in Zenker's fluid. Longitudinal sections were cut through the uterus and vagina, selecting the middle third. In the sections 3000 cells were counted for each animal and the number of mitoses was counted and expressed in promille. The mitotic activity in the uterus was determined separately in the epithelium lining the uterine cavity and in the epithelium of the uterine glands. The degree of significance of the differences between the mean values in the experimental and control series was determined by the Fisher-Student method.

EXPERIMENTAL RESULTS

All the animals used in the experiment tolerated fasting for 3 days. In the first control series (Table 1), the mitotic activity in the vaginal and uterine epithelium of the normal rats fasted for 3 days remained at a fairly high level. Although considerably lower than that counted in proestrus, the mitotic activity was at a much higher level than that found in late diestrus. In most animals the mitotic activity was roughly equivalent to that observed in the stage of metestrus. Keratinization of the epithelium was not present in the vaginal sections, but numerous epithelial

TABLE 1. Mitotic Activity (in ‰) in Normal Animals Fasted for 3 Days (1st control series of experiments)

Animal No.	Epithelium of vagina	Epithelium of uterine cavity	Epithelium of mammary glands
1	6,33	7	17,33
2	0,66	0,33	12
3	4	6	8,33
4	4	4,33	6,33
5	8,66	5,66	5,33
6	2,33	7,66	15,33
Mean . . .	4,33	5,16	10,78

TABLE 2. Mitotic Activity (in ‰) in Castrated Animals Receiving a Normal Diet (2nd control series of experiments)

Animal No.	Epithelium of vagina	Epithelium of uterine cavity	Epithelium of mammary glands
1	0,66	2	3
2	0,66	1	1,66
3	1	2	3
4	0,66	1,66	3
5	0,33	0	2,33
6	1	2	3
7	0,66	3	1,66
Mean. . .	0,71	1,67	2,52

TABLE 3. Mitotic Activity (in ‰) in Castrated Animals Fasted for 3 Days (Principal series of experiments)

Animal No.	Epithelium of vagina	Epithelium of uterine cavity	Epithelium of mammary glands
1	0	0	1
2	0	0,33	0,66
3	0	0	0,33
4	0	1	2
5	0	0	0,33
6	0	0,33	1
7	0	0	1
Mean . .	0	0,23	0,90

cells filled with mucus were observed, together with polymorphonuclear leukocytes. The epithelium was of considerable thickness. Desquamation of cells into the lumen was evidently inhibited for some reason.

It may be concluded from the results of this series of experiments that total fasting for 3 days, although arresting the normal sexual cycle in female mice, does not lower the intensity of mitotic activity in the epithelium of the vagina and uterus below the level corresponding to metestrus. This demonstrates that in this particular experiment the ovaries of the fasting mice continued to produce estrogens.

In the second control series the mitotic activity in the castrated animals kept on a normal diet was very low (Table 2). Such a low level was not observed at any stage of the sexual cycle. In this series of experiments the anticipated result was obtained. The sharp fall in the concentration of ovarian steroids after bilateral castration led to a corresponding fall in the mitotic activity in the epithelium of the reproductive organs of the female mice.

In the principal series of experiments fasting caused a considerable decrease in the mitotic activity of the epithelium of the vagina and uterus (Table 3). In the vaginal epithelium no mitoses were found in any of the animals. In the uterine epithelium solitary mitoses were found in only three animals. The mean mitotic activity was negligible and differed by a statistically significant amount from the results of the second control series of experiments ($P = 0.002$). In the epithelium of the uterine glands the mitotic activity also fell significantly ($P = 0.0001$) although its level was higher than in the epithelium of the uterine cavity.

Conflicting reports of the effect of fasting on the level of mitotic activity are to be found in the literature. Some authors found a lowering of mitotic activity in starvation [1, 8, 9, 12], while others reported that it continued for a long time almost at its normal level [3, 4, 10]. Total disappearance of mitoses as a result of fasting has never been described. O. T. Movchan [6, 7] showed that the change in mitotic activity during fasting is dependent primarily on the state of the animals' nutrition and their weight loss during the experiment, and also on the fall in the blood sugar and the carbohydrate content of the investigated tissue. At the beginning of fasting the mitotic activity may actually rise for a short time.

It is probable that our experimental conditions were not severe enough to cause a sharp fall in mitotic activity in the epithelium of the vagina and uterus. It is also possible that during fasting the mitotic activity of the epithelium of the reproductive organs does not fall so sharply, or falls at later periods.

It may be concluded from the relatively high mitotic activity in the normal fasting animals that the mitotic activity in the principal series of experiments was so low, not because of the starvation, but because of the absence of estrogens entering the body with the lipid fraction of the diet. Two circumstances convince us that this explanation is correct. There are no reports in the literature of the total cessation of mitosis in any organ during starvation, whereas in our experiments no mitoses whatever were found in the vaginal epithelium of the castrated mice during fasting (Table 3). In the same vaginal sections in which no mitoses could be found in the epithelium, mitoses were present in the stratum basale of the epidermis and in the cells of the hair bulbs. The fact that a few mitoses continued to occur in the epithelium of the uterus and of the uterine glands indicates that small amounts of estrogens are present in the internal environment of the organism. It appears that the attempt to rid the organism completely of estrogens is doomed to failure.

It may be concluded that differences in the level of estrogenic stimulation are necessary to maintain mitotic division in the epithelium of the reproductive organs. The highest concentration of estrogens is required for the mammary gland, a lower level for the vaginal epithelium, lower still for the uterine epithelium, and the lowest concentrations are necessary for the uterine glands.

The generally accepted view that mitotic cell division takes place without the participation of hormones, and that these substances can do no more than stimulate or depress mitosis, and are therefore regulators of mitosis of the second order is not true in respect of cell division in the epithelium of reproductive organs such as the mammary gland, the vagina, and the uterus. In these organs the onset of mitosis is impossible without the presence of a definite concentration of steroid ovarian hormones in the body. We know that spermatogenesis and orgenesis are impossible without the action of the gonadotropic hormones of the hypophysis. In the course of evolution, however, it is not only the spermatogenic epithelium and the oogonia of the ovary that have come under hormonal control. The female sex hormones also control processes of proliferation in the epithelium of the reproductive organs of the female mammal. As a result of the differential sensitivity of the epithelium of the mammary glands, the vagina, and the uterus to a fall in the concentration of female sex hormones, as measured by the intensity of the residual mitotic activity, the organism is able to stimulate or depress the proliferation of the epithelium in these organs in a manner best suited to the reproductive function.

SUMMARY

An attempt was made to arrest mitosis of the epithelial cells of the mammary glands, vagina and uterus of sexually mature female mice by castration and the subsequent deprivation of food estrogens. In control castrated animals the mitotic activity in the epithelium of the mammary glands disappears and it decreases considerably in the vaginal and uterine epithelium. After 3 days of starvation the mitotic activity remains high in the vaginal and uterine epithelium of control intact animals. In the castrated animals left without food for 3 days mitosis disappeared entirely in the vaginal epithelium and decreased considerably in the uterine epithelium (in comparison with control castrated animals). Statistical treatment confirmed the authenticity of these results.

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