THE INFLUENCE OF OVARIAN HORMONES ON GROWTH OF THE EPITHELIUM OF THE MAMMARY GLANDS

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According to the present-day views of the majority of oncologists and endocrinologists tumors of the mammary gland arise as the result of a disturbance of the balance of the hormones of the ovary and the anterior lobe of the hypophysis. Hormonal disturbances distort the proliferative and destructive processes in the epithelium of the mammary glands, as a result of which a tumor develops. In order to clarify the role of the individual hormones and of disturbance of their interrelationships in the processes of carcinogenesis, investigation of the hormal regulation of the growth of the mammary glands in normal conditions assumes great importance. This problem has been intensively studied in many countries [1, 2, 4, 6, 10, 11, 12, 16, 17, 18]. In the more recent work it has also been shown that all carcinogens producing carcinoma of the mammary glands possess to some degree the characteristic activity of a sex hormone, and some of them are very close in their chemical structure to the sex hormones [13]. It has been shown experimentally that the ovarian hormones – estrogens – stimulate proliferation of the epithelium of the mammary glands, and the hormone progesterone promotes formation of the secretory divisions of the glands [8, 9]. The lactigenic action of an extract of the anterior lobe of the hypophysis on the mammary gland was first discovered by Stricker and Grueter [19] in 1928. It was shown later, when the pituitary hormones had been separated and purified, that prolactin – a hormone from the anterior lobe of the hypophysis – – possesses a mammotropic action. Lyons [14] first obtained secretion of milk and observed active growth of the alveolar epithelium of the mammary gland after injecting prolactin directly into the gland.

Lyons and his coworkers later showed that the injection of ovarian hormones alone, without prolactin, into rats from which the ovaries, adrenals, and hypophysis had been removed, does not cause growth of the mammary glands [15]. When the growth hormone of the hypophysis had been purified, Lyons found that it acts directly on the mammary glands, causing some degree of stimulation of their growth, and that the activity of prolactin, estrogens, and progesterone is increased in its presence [16]. Later experiments led this worker to the conclusion that the normal development of the mammary glands requires the combined action of all these hormones, the prolactin of the hypophysis being the most important [17]. Lyons's findings were confirmed by Ferguson in experiments on mice [11].

In contrast to the conclusions of Lyons and his supporters, Ahren and Jacobsohn [7] showed that complete development of the mammary glands in hypophysectomized females may be indiced by ovarian hormones alone, i.e., in the absence of prolactin, if insulin is injected at the same time as the ovarian hormones. N. I. Lazarev also does not share Lyon's point of view and considers that gonadotropic hormone and estrogens have a dominant influence on growth of the mammary glands [4, 5]. Our work on the change in the mitotic activity of the epithelium of the mammary glands in pregnant and lactating mice [3] also contradicts Lyons's findings. We showed that the high mitotic activity of the epithelium of the mammary glands observed in the later stages of pregnancy

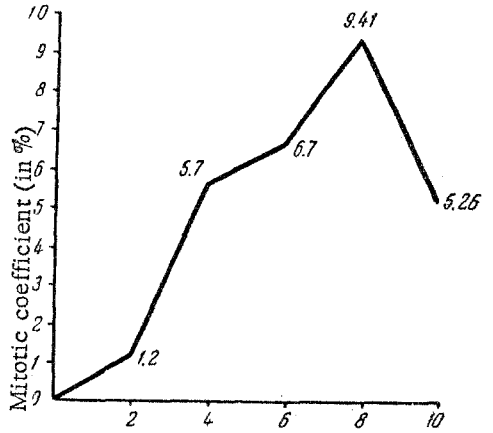

Figure. Change in the mitotic activity of the epithelium of the mammary glands after administration of folliculin to ovariectomized female mice.

falls sharply after birth and the onset of lactation. In this case prolactin, although it stimulates the secretion of milk, does not increase the mitotic activity of the epithelium. Much evidently depends on the conditions under which a given hormone acts and on the state of the cells which it affects. There is every reason to believe that the hormonal regulation of the growth of the mammary glands is more complex than might be gathered from the scheme suggested by Lyons. In recent years many papers have been published on the study of the action of the hormones of the adrenal cortex and the adrenocorticotropic hormone of the hyophysis (ACTH) on the mammary gland. The findings obtained are, however, contradictory. Flux [12] found that glucocorticosteroids inhibit the growth of the mammary glands in intact and ovariectomized animals. Selye [18], on the other hand, reported that ACTH and the glucocorticosteroids stimulate growth of the mammary glands and induce the secretion of milk.

We studied the effect of folliculin and progesterone on the processes of destruction and proliferation of the epithelium of the mammary glands in mice, using the method of determination of the mitotic activity. The results of these experiments are described in the present paper.

EXPERIMENTAL METHODS

Experiments were carried out on 122 sexually mature female mice. Bilateral ovariectomy was performed on all the animals in a period of 2 days. Twenty days after operation, when a persistent diestrus had become established in all the mice, a certain number of them were given daily subcutaneous injections of folliculin dissolved in apricot oil. The remaining control animals received daily injections of apricot oil. After 2, 4, 6, 8, and 10 injections of folliculin respectively, 9-10 experimental mice and 7-9 controls were sacrificed. The second and third right mammary glands were taken for histological examination. The mitotic activity of the epithelium of the terminal divisions of the glands was determined by the same method as in our previous research [2], and the mitotic coefficient was calculated (number of dividing cells per 1000). In one group (11 mice) administration of folliculin was discontinued after 6 daily injections. The 9 control mice to this group received 6 daily injections of oil and were sacrificed 5 days after the 6th injection. The action of the corpus luteum hormone — progesterone — on the intensity of cell division in the epithelium of the mammary glands was determined in two groups of animals. The first group of ovariectomized females received 6 daily injections of folliculin and then, on each of the next 5 days they were given a subcutaneous injection of 0.2 mg progesterone in 0.1 ml of oil. The animals were killed 24 hours after the last injection. The second group of ovariectomized females received 6 daily injections of oil and then, for the next 5 days, they were given subcutaneous injections of 0.2 mg progesterone in 0.1 ml oil.

EXPERIMENTAL RESULTS

After only the second injection of folliculin into the ovariectomized mice, diestrus was replaced by estrus. The vaginal smears of a small number of animals at this time still showed signs of the proestrus stage. After the third injection, all the mice save three were firmly in the stage of estrus. This provided convincing evidence of the activity of the preparation which we used. Determination of the mitotic activity in the control mice showed that ovariectomy completely arrested the mitotic division of the epithelial cells in the mammary glands, and that the act of injecting apricot oil subcutaneously did not itself cause an increase in the mitotic activity of these cells.

The mitotic activity of the epithelium of the mammary glands of the experimental mice increased considerably from one injection of folliculin to the next (see figure). After the second injection it rose on the average from zero to 1.2 mitoses per 1000 cells, and after the 4th injection it reached 5.7 mitoses per 1000 cells.

The differences between the mean values of the mitotic activity here were statistically significant ($P < 0.0001$). Although the mean mitotic activity after the 6th injection had increased to 6.7 mitoses per 1000 cells, comparison of this figure with the mean mitotic activity of animals sacrificed after 4 injections showed that this increase was not statistically significant. The further increase in the mitotic activity after 8 injections of folliculin was statistically significant ($P = 0.006$). In animals sacrificed after 10 injections of folliculin a considerable fall was observed in the mitotic activity of the epithelium of the mammary glands. Whereas the mitotic activity after the 8th injection averaged 9.41 mitoses per 1000 cells, after 10 injections it was only 5.26 mitoses per 1000 cells. The difference between these mean values was statistically significant ($P = 0.006$).

Histological investigation of the mammary glands in the control mice showed that bilateral ovariectomy causes marked atrophy of the glands. In our experiments the earliest time at which mice were sacrificed after ovariectomy was the 22nd day. At this and later times, no significant destructive changes could be seen in the gland for they had mainly been completed beforehand. Atrophy of the mammary glands after ovariectomy was shown as a considerable fall in the number of terminal divisions and a sharp decrease in the diameter of the major efferent ducts. The number of rows of nuclei in the epithelial lining of the terminal divisions and ducts was less than in the glands of normal nonpregnant mice. Among the nuclei of the cells of the terminal divisions there were many which were dark and pyknotic. The nuclei of the epithelial cells were smaller in size than in normal glands. Proliferative epithelial nodules, so characteristic of the progressively developing gland, were completely absent. Accumulations of desquamated epithelial cells could often be seen in the lumen of the ducts.

In the experimental animals sacrificed after 2 injections of folliculin the glands had the same structure as those of the control animals. However, the mitotic activity of the epithelium of the mammary glands in the control animals was equal to zero, and that of the experimental animals was increased by the action of folliculin to 1.2 mitoses per 1000 cells. The important conclusion thus followed that the general morphological examination of the structure of the mammary glands gives evidence of the action of the hormone much later than does the method of determination of the mitotic activity. After 4 injections of folliculin the morphological changes in the gland were now marked. Proliferative epithelial nodules were apparent. The epithelial lining of the alveoli and ducts was thicker and the lumen of the ducts wider. After 6 injections, and especially after 8 injections, the proliferative epithelial nodules continued to increase in number and size. This was evidently connected with failure of the cells, accumulating as the result of mitosis, to differentiate into typical organs.

The usual pattern of formation of bunches of alveoli, with a lumen and a single layer of epithelium, from the solid epithelial nodules, that is characteristic of the development of the glands in pregnancy was not observed. After 10 injections, although many proliferative nodules could be seen in the glands, the number of mitoses was appreciably reduced. After 8 and 10 injections secretory vacuoles appeared in the cells of the terminal divisions and the small ducts in a few animals. In the series of experiments in which ovariectomized females received only 6 injections of folliculin, after which the administration of folliculin ceased, the mitotic activity on the 6th day after the last injection fell to a negligible figure — 0.18 mitoses per 1000 cells. Evidently the mitotic activity did not fall to zero because the estrogens were gradually excreted from the animals. This was confirmed by examination of vaginal smears. In the first few days after discontinuation of the folliculin injections, estrus was observed in all the mice. After the 4th day diestrus supervened in some mice. Only on the 6th day was a state of diestrus found in all the mice.

Histological examination of the mammary glands revealed well marked signs of destruction. Proliferative epithelial nodules were absent. In many of the terminal divisions the epithelium had become separated from the basal membrane. Only the connective tissue framework remained of some alveoli. Many desquamated epithelial cells had accumulated in the fluid filling the ducts. The nuclei of the epithelial lining of the ducts showed considerable polymorphism. The nuclei of many epithelial cells were pyknotic. The number of leucocytes in the stroma of the gland and in its epithelial formations was perceptibly increased. In the control animals of this series of experiments the mammary glands were atrophied and no mitoses were present in the epithelial cells.

In the last series of experiments the animals received 6 daily injections of folliculin, followed by 5 injections of progesterone. The mean mitotic activity of the epithelium of the mammary glands in these animals was 0.97 mitoses per 1000 cells. This mitotic activity was much below the mitotic activity observed in normal mice in the stage of diestrus, but yet it was higher than in the previous series of experiments, the difference being statistically significant ($P = 0.006$). The results of the control experiments in which injections of progesterone followed

administration of oil were completely unexpected. In this case the mitotic activity was greater than in the experimental series, amounting on the average to 3.3 mitoses per 1000 cells. It was found that progesterone caused only a slight increase in the mitotic activity of the mammary glands, and its action was weaker when it was given immediately after folliculin.

In our previous research we showed that growth of the epithelium in nonpregnant mice with a normal sexual cycle and in the first days of pregnancy is similar in its character. This growth consists of the accumulation of epithelial cells by mititic division but with no differentiation of the typical epithelium. In the second half of the first week of pregnancy, differentiation of the typical organ is observed alongside profiferation. The epithelium enters into relationships with the connective tissue characteristic of the mammary gland, as a result of which the terminal divisions of the organ are formed. Alveoli with two rows of epithelial cells are formed from the epithelial anlage – the proliferative nodule [2, 3].

In our opinion, when speaking of growth of the epithelium of the mammary glands and of the regulation of this growth it is above all essential to know which of the two types of growth mentioned above is concerned. None of the authors cited in the survey of the literature have made such a distinction.

From the results of our experiments it may be concluded that the first type of growth is stimulated by estrogenic hormones. As our experiments showed, the presence of estrogens in the body is absolutely essential for mitotic division of the epithelial cells of the mammary gland. The repeated injection of folliculin into ovariectomized mice causes growth of the epithelium but does not stimulate the development of the gland as an organ, since no formation of alveoli takes place. The prolonged administration of folliculin ultimately leads to a lowering of the mitotic activity of the epithelium of the glands. We have no grounds at present for suggesting the courses of this lowering.

Lyons [15] and Ferguson [11] have shown that the injection of estrogens alone into animals after hypophysectomy does not cause growth of the mammary glands. Injection of prolactin alone under the same circumstances brings about growth of the epithelium with the formation of alveoli. From the illustrations in Ferguson's paper it can be clearly seen that the administration to ovariectomized and hypophysectomized females of estrogenic hormones in conjunction with growth hormone gives a well marked picture of the first type of epithelial growth. Evidently the second type of epithelial growth requires, in addition to hormones causing cell division, stimulation by differentiating hormones. In view of the findings of Cowie [10] and Lyons [15], prolactin and progesterone must be considered to be hormones of this type.

The apprehensions of Lyons and Ferguson that the adrenal cortex of ovariectomized females would begin to produce estrogens very soon after operation are greatly exaggerated. Our findings show that the adrenal cortex of mice 20-30 days after ovariectomy does not yet produce estrogens in amounts sufficient to cause keratinization of the epithelium of the vagina or mitoses in the epithelium of the mammary glands.

We were unable to confirm the observations of Cowie and Benson that progesterone causes obvious stimulation of the formation of alveoli in the mammary gland. In our experiments the administration of progesterone to ovariectomized mice led to the appearance of mitoses in the epithelium of the mammary glands, but this increase in the number of mitoses was insignificant by comparison with that due to the action of folliculin. We were not convinced that the progesterone preparation which we used was sufficiently active, for we did not carry out control tests of mucification of the uterine endometrium. In consequence of this, the results of the action of progesterone obtained in our experiments must be regarded as purely preliminary.

SUMMARY

The author studied the effect of ovarian hormones (folliculin and progesterone) on the mitotic activity of the terminal portions of the mammary glands in ovariectomized mice. He showed that the mitotic activity of the epithelium in the mammary glands increases from zero to 9.41 mitoses per 1000 cells after daily administration of folliculin in a dose of 25 I. U. to ovariectomized females. The maximal mitotic activity is noted after 8 folliculin injections. Following 10 folliculin injections the mitotic activity becomes reduced. In comparison with folliculin, progesterone provokes a lesser rise of mitotic activity.

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