

STIMULATION OF THE PROLIFERATION OF THE EPITHELIUM
OF THE MAMMARY GLANDS WITH FOLLICULIN IN HYPO-
PHYSECTOMIZED, OOPHORECTOMIZED MICE

S. S. Laguchev

Laboratory of Growth and Development (Head — Prof. L. D. Liozner),
Institute of Experimental Biology (Dir. — Prof. I. N. Maiskii) of the
AMN SSSR, Moscow

(Presented by Active Member AMN SSSR N. N. Zhukov-Verezhnikov)

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The study of the hormonal regulation of the growth of the epithelium of the mammary glands in normal conditions is of great interest to oncology. In the view of the majority of contemporary oncologists and endocrinologists, tumors of the mammary glands arise as a result of pathological changes in the balance of the mammotropic hormones of the pituitary and ovaries [4, 6, 7, 8]. So far, however, there is no sufficiently clear idea of the normal hormonal regulation of growth and involution of the mammary glands. Naturally, it is impossible to understand the essence of the hormonal disturbances leading to the formation of tumors of the mammary gland before the normal hormonal relationships have been discovered.

The view most widely accepted amongst non-Soviet scientists is that of Lyons, who considers that the normal development of the mammary glands requires the combined action of prolactin, growth hormone, folliculin and progesterone. Lyons, however, considers prolactin to be the most important hormone, for without it, in his opinion, the ovarian hormones have no influence on the mammary glands. After administration of prolactin alone to oophorectomized, hypophysectomized rats, Lyons observed growth of the mammary glands [15]. Lyons's findings were confirmed by Ferguson [13] in experiments on C_3H line mice.

Lyons's conclusion that estrogens are of secondary importance in the stimulation of growth of the mammary glands are to some extent contrary to the facts accumulated by oncologists. It has been shown that carcinoma of the mammary glands may be produced by the prolonged administration of estrogens in mice and rats, whereas carcinoma develops spontaneously in these animals extremely rarely [8]. Removal of the ovaries from high-cancer line mice at a certain age prevents the development of carcinoma of the mammary gland in 100% of cases, regardless of the obvious presence of milk factor [1].

Clinical oncologists are well aware that mastopathies and carcinoma of the mammary gland are almost always accompanied by hyperestrinization or disturbance of the rhythm of secretion of estrogenic hormones [6, 7, 9, 10]. Taking into consideration these findings, it is difficult to imagine that estrogens, which may cause pathological hyperplasia of the epithelium of the mammary gland and even malignant growth of the epithelium, have no significant effect on the growth of the mammary glands in normal conditions.

Many workers do not share Lyons's views and cite facts contrary to his scheme [2, 3, 4, 5, 11].

In a previous paper we showed that castration of female mice causes the cessation of the mitotic division of the epithelial cells of the mammary glands, and that the administration of folliculin to such animals causes a considerable increase in mitotic activity, the formation of proliferative epithelial nodules, but not the differentiation of their terminal divisions [3]. The problem of whether folliculin in this case acts directly on the

epithelium of the mammary glands or whether its action is mediated through the pituitary and its hormones has, however, remained unsolved.

The object of the present work was to find out whether folliculin may or may not stimulate the processes of proliferation of the epithelium of the mammary glands in hypophysectomized mice.

METHOD

Experiments were carried out on 102 sexually mature female mice. In the first series of control experiments the animals were hypophysectomized and sacrificed ten days later. Two or three thoracic mammary glands on the right side were taken for histological examination. Besides studying the structure and state of the glands, we determined the mitotic activity of the epithelium of the terminal divisions and ducts. Vaginal smears were taken from the mice during the period of ten days. The absence of an estrus cycle indicated total suppression of the pituitary gland.

In the second series of control experiments, 30 days after castration the mice received 8 daily injections each of 25 i. u. of folliculin in 0.1 ml of peach oil. As our previous experiments showed [3], the greatest increase in mitotic activity in the epithelium of the mammary glands in castrated mice takes place after 8 injections of folliculin. The animals were sacrificed on the ninth day. The same glands as in the preceding series of experiments were taken for histological examination and for determination of the mitotic activity.

In the main series of experiments, the ovaries were removed from the animals and, 30 days after this operation, the pituitary was removed. Ten days after hypophysectomy, the mice received subcutaneous injections of 25 i. u. of folliculin in 0.1 ml of peach oil daily for 8 days. Histological examination, determination of the mitotic activity and the statistical treatment of the results were carried out by the methods described previously [2].

Hypophysectomy was performed under sodium amytal anesthesia by Bahner's method [12]. After the operation a considerable proportion of the mice died. Only 25% of the animals survived operation.

After the operation the animals received intraperitoneal injections of 1 ml of 5% glucose and 100 units of penicillin. These injections were repeated on the next two days.

For four days after operation the animals were kept in a cage heated by means of a 25 w electric lamp with a reflector.

Many previous experiments have shown that the subcutaneous injection of peach and apricot oil alone into mice in the doses which we used have no effect on the mitotic activity of the epithelium of the mammary glands [3]. In the present investigation we therefore dispensed with controls in which oil alone was injected.

The criterion of total extirpation of the pituitary in our experiments was the stability of the body weight of the hypophysectomized animals, macroscopic and histological control of the site of the pituitary gland and also the absence of an estrus cycle in the animals of the first series.

RESULTS

Of the 42 animals of the first series, only ten tolerated hypophysectomy relatively well. In mouse No. 8 estrus developed on the fifth day after operation, and at postmortem the presence of one quarter of the anterior lobe of the pituitary was demonstrated. This animal was excluded from the experiment. In the remaining nine mice no remnant of the anterior lobe of the pituitary was found macroscopically or histologically. Vaginal smears showed a state of persistent diestrus. The body weight of the hypophysectomized mice remained stable, whereas the majority of the healthy mice used as controls gained appreciably in weight over the period of ten days.

The results of the first series of experiments showed that the technique used for hypophysectomy gave satisfactory results.

Ten days after hypophysectomy, histological examination of the mammary glands of the animals showed severe atrophy, resembling in its character the atrophy arising immediately after castration. The diameter of the ducts and terminal divisions was greatly diminished by comparison with the normal. The number of terminal

divisions was very small. The epithelial lining of the ducts and alveoli was flattened. The cells were reduced in size. The nuclei of many cells were pyknotic. Ducts were often seen with desquamation of the epithelium. The mitotic activity of the epithelium of the mammary glands fell to zero ten days after hypophysectomy in all the mice except No. 6, but in this animal too the mitotic coefficient was low, amounting to 0.66. The effect of hypophysectomy thus resembles the action of bilateral castration [3] on the mammary glands also by the inhibition of mitotic activity.

The second series of experiments conducted on ten control mice gave results as anticipated. As in the previous investigation [3], the injection of folliculin into castrated mice caused a significant increase in mitotic activity (on the average by 9.8 per thousand). It is interesting to observe that, despite some difference in the conditions of performance of this series of experiments and those of the previous investigation (the age of the animals and the time elapsing after castration), the figures obtained for the mean mitotic activity were very close, amounting to 9.8 and 9.41 mitoses per 1000 cells respectively.

Histological sections of the mammary glands showed the picture of the typical action of folliculin. In the terminal segments and along the course of the ducts proliferative epithelial nodules appeared. The epithelial lining of the ducts became thicker and the lumen of the ducts wider than in the glands of the castrated mice. The formation of terminal segments from the compact proliferative nodules did not take place, as it does in pregnancy.

In the last and main series of experiments, the mice were subjected to bilateral castration followed by hypophysectomy, after which folliculin injections were given. Of the 50 mice undergoing operation, only 13 survived. At postmortem on mouse No. 1 an unexcised portion of the pituitary was found, so that mammary glands were taken from only 12 mice for investigation. Histological examination of the glands showed that administration of folliculin produced a marked reaction of the epithelium in the mammary gland. The character of this reaction was similar to that of the reaction of the mammary glands of castrated females to folliculin. It must be pointed out, however, that the reaction was much weaker in this series of experiments. Although proliferative epithelial nodules developed, they were fewer in number. The thickening of the epithelial lining and the dilatation of the lumen of the ducts were less pronounced than in the castrated animals with intact pituitary gland. The mitotic activity of the epithelium of the mammary glands rose on the average to 4.38 mitoses per 1000 cells, whereas in the control series of experiments it was practically equal to zero. Comparison of the mean values of the mitotic activity in the animals of the second and third series shows that the difference between the results obtained was statistically significant ($P < 0.0001$).

Analysis of the results shows that folliculin, independently of prolactin and growth hormone, in certain conditions may stimulate proliferation of the epithelium of the mammary glands.

At first sight this conclusion is contrary to the results of the work of Lyons [15] and Ferguson [13]. These contradictions may, however, be readily explained. In his experiments, Ferguson gave injections of estradiol and stilbestrol to hypophysectomized mice for periods of 6 months or more. Such experimental conditions are far from physiological. We have shown that the continuous administration of folliculin to castrated mice first stimulates proliferation of the epithelium of the mammary glands, but that later, starting on the tenth day, this stimulation is greatly weakened [3]. It may naturally be considered that the mammary glands of the mice in Ferguson's experiments were in a state of atrophy at the time of sacrifice, since the stimulating action of the estrogens on growth had long ago ceased.

When he speaks of growth of the mammary glands of mice, Lyons means by this only that lobular and alveolar growth observed in the second half of pregnancy. It is this growth which he attempts to reproduce, choosing for this purpose a combination of pituitary and ovarian hormones [14]. Meanwhile the study of the hormonal regulation of proliferation of the epithelium of the mammary glands in nonpregnant animals and in the first half of pregnancy is of the greatest interest to oncology. At this time proliferation of the undifferentiated epithelium takes place, leading to the formation of compact proliferative epithelial nodules. In the second half of pregnancy growth of the glands and differentiation of the epithelium, typical of the organ in question, takes place. It must not be forgotten that carcinoma of the mammary gland is found more frequently in women who have had few or no children and in women who have not practiced breast-feeding. The second half of pregnancy and especially lactation thus have a normalizing influence on the epithelium of the mammary glands.

SUMMARY

Sexually mature female mice were subjected to hypophysectomy and bilateral ovariectomy. Following this the animals received 25 i.u. of folliculin daily for 8 days and were killed on the 9th day. Mitotic activity of the epithelium in the acinin and ducts of the mammary glands was assessed histologically. In the control series of experiments hypophysectomy was followed by atrophy of the mammary glands and arrest of mitotic activity in the epithelial cells. In hypophysectomized and ovariectomized animals mitotic activity of epithelium of the mammary glands increased on the average, to 4.38 mitoses per 1000 cells after injection of folliculin. However, this rise of the mitotic activity was considerably less than in the control group of castrated animals with intact hypophysis (9.8 mitoses). The above experiments show that in female rats which lack hypophyseal hormone folliculin will definitely stimulate proliferation, of epithelium in the mammary glands, though it fails to produce growth typical of the latter organ.

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