EXPERIMENTAL BIOLOGY

PROLIFERATION OF THE EPITHELIUM OF THE MAMMARY GLANDS IN VARIOUS STAGES OF THE SEXUAL CYCLE

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The study of the functional morphology of the mammary glands of laboratory animals, especially mice, is one of the current tasks of experimental oncology.

The mammary gland is an organ which, in female animals, is exposed to continual changes on the attainment of sexual maturity, with the interchanging phases of the sexual cycle, the occurrence of pregnancy and the period of lactation and, finally, on the cessation of the reproductive functions in old age.

The revelation of the laws governing the physiological proliferation of the epithelium of the mammary glands must help in the discovery of the causes of pathological proliferation, leading to the appearance of benign and malignant tumors of these organs.

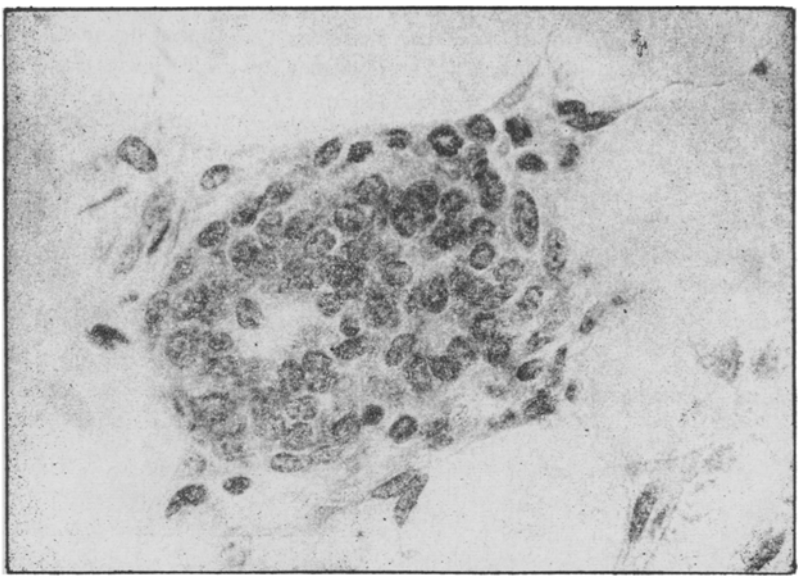

Fig. 1. A proliferative epithelial nodule in the mammary gland of a white mouse. Proestrus. Magnification 500 times.

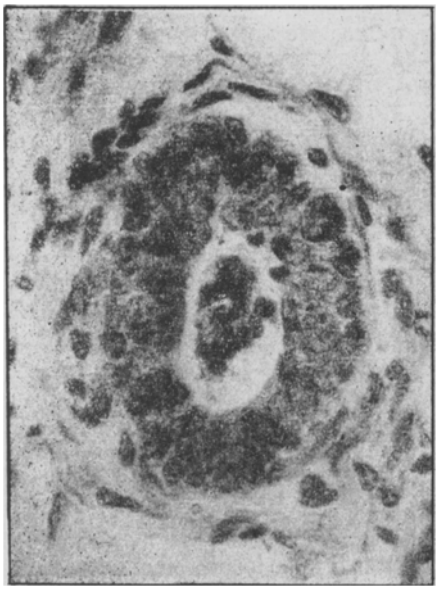

Fig. 2. Death of surplus epithelial cells in the mammary gland of the white mouse. Diestrus. Desquamation of epithelial cells into the lumen of an efferent duct. Magnification 500 times.

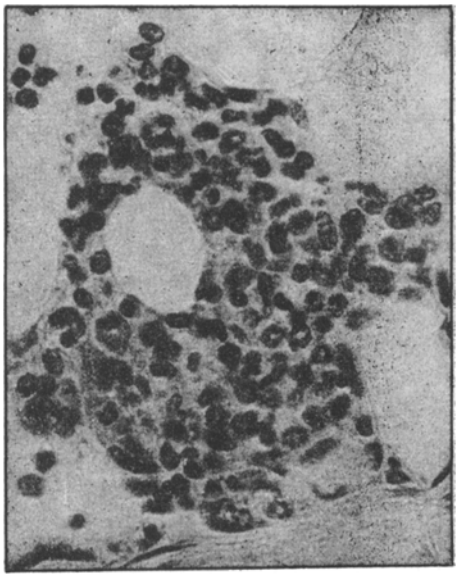

Fig. 3. Infiltration of an epithelial nodule with leucocytes. Metestrus. Magnification 500 times.

One of the most objective methods of study of the proliferation of cells is the method of determination of mitotic activity. However, despite the large number of investigations of the morphology of the mammary glands [2, 5, 6, 7] we were able to find only one work devoted to the study of the mitotic activity of the epithelium of the mammary glands of the mouse [3]. Bullough, the author of this paper, investigated the changes in the mitotic activity of the epithelium of the small ducts of the mammary glands of mice in the various phases of the sexual cycle. He found that in the course of the sexual cycle there are two periods of increase in the number of cell divisions: the first – at the end of diestrus, and the second – at the end of "full" estrus with a maximum after ovulation. The mitotic activity of the epithelium of the mammary glands during pregnancy and lactation has been studied in cows and rats by A. D. Al'tmas [1]. He came to the conclusion that mitotic activity is high only during pregnancy, whereas during lactation, when the number of mitoses is insignificant, proliferation of the cells is brought about by amitosis.

In the present investigation we set out to study the changes in mitotic activity of the epithelium of the terminal portions of the mammary glands in mice during the different stages of the sexual cycle, and also to trace the morphological changes in the glands which correspond to these stages.

EXPERIMENTAL METHOD

Experiments were carried out on female white mice, of mixed strains, aged roughly 4-5 months. In order to determine the stage of the sexual cycle the method of vaginal smears was used. At each stage of the cycle 10 animals were killed (by decapitation). The animals were killed at 12 noon. The second right thoracic mammary gland was fixed in Zenker's solution, and embedded in paraffin wax. Sections were cut to a thickness of 7 μ and stained with hematoxylin by Caracci's method. Every other section was examined under the oil-immersion system of the microscope, and 6000 cells of the terminal portions were counted, records being made of all mitoses, beginning with early prophase and ending with late telophase. The mitotic activity was expressed as the number of mitoses per thousand epithelial cells. The statistical significance of the differences in mitotic activity at the various phases was determined by the Fisher-Student method.

EXPERIMENTAL RESULTS

The mammary gland of sexually mature virgin female mice consists of a common outlet duct of the nipple, giving rise to a few long radial branches – the efferent ducts – which in turn give rise to a moderate number of branches which terminate in small single alveoli with a double row of cubical epithelium. However these alveoli are sharply distinguishable from the alveoli of the functioning gland. They are very small, they have hardly any lumen and no secretion is present in them. They are blind epithelial processes.

As we have observed, in the stage of proestrus dense proliferative nodules of epithelial cells, sharply demarcated from the connective tissue stroma, are formed at the ends and along the course of the smallest efferent ducts (Fig. 1). The epithelium of the small ducts and the alveoli proliferates and the alveoli increase in size. In the subsequent phases – estrus and metestrus – part of the proliferating nodules are converted into alveoli. In the course of diestrus the greater part of the alveoli undergo regression. The remaining alveoli and the cells of which they are composed, become greatly diminished in size. These processes take place without interruption in the mammary gland of sexually mature nonpregnant mice in accordance with the cyclic changes in the ovary. It must be pointed out that the degree of the changes described during the transition from one phase of the cycle to the next varies in different individuals and sometimes it can only be made out with difficulty.

The epithelial cells, and especially their nuclei, in the small ducts and terminal portions also undergo uninterrupted changes, showing in the majority of the phases of the sexual cycle an extreme polymorphism and very great variations in size. All intermediate stages can be found, from large, spherical, lightly stained nuclei to small, dense, darkly stained nuclei in the form of blacksmiths' nails or clumps of various shapes.

The terminal segments in the gland of the nonpregnant mouse are so few in number that in order to count the mitotic activity it is essential to examine a large number of sections. In this connection it cannot be excluded that in some cases small efferent ducts, cut across obliquely or tangentially, might have been taken by mistake for a terminal segment.

The process of proliferation of epithelial cells is balanced by the process of their physiological death. Death of surplus epithelial cells follows two paths in accordance with the differing situation of the epithelium: the first path is desquamation of epithelial cells with pyknotic nuclei into the lumen of the efferent ducts and alveoli (Fig. 2), the second path is swelling of the epithelial cells of the proliferative nodules, followed by their lysis. The first path is quicker, occurs during diestrus and proceeds without the intervention of leucocytes. The second path is of longer duration, lasting throughout metestrus, diestrus and even proestrus. It may be that this process does not come to an end in the course of one cycle and extends into the next. However such a suggestion requires closer definition. It is of interest to point out that the second path of destruction of epithelial cells takes place only with the obligatory participation of granulocytic leucocytes and lymphocytes, which infiltrate the resolving epithelial nodules (Fig. 3).

We shall give a short account of the state of the mammary gland, mainly so far as the epithelium of its terminal segments is concerned, in the various stages of the cycle.

Proestrus. At the ends of the small ducts of the gland and along their extent, proliferative nodules arise, in which mitotic figures are often found (see Fig. 1). The nodules are sharply demarcated from the connective tissue stroma. The nuclei of the epithelial cells are polymorphous. There are few dense, darkly staining nuclei and more large and lightly staining nuclei. Nuclei in the form of blacksmiths' nails are encountered rarely. Desquamation of cells into the lumen of the ducts is insignificant. There are few leucocytes in the connective

The Mitotic Activity of the Epithelium of the Mammary Glands of Mice in Various Stages of the Sexual Cycle

Stage of cycle	No. of mitoses	Mitotic coefficient (per 1000)	Early prophase		Prophase		Metaphase		Anaphase		Telophase		Late Telophase	
			absolute	in %	absolute	in %	absolute	in %	absolute	in %	absolute	in %	absolute	in %
Proestrus	369	6,8	40	10,84	92	24,93	91	24,66	9	2,43	85	23,03	52	14,02
Estrus	422	7,0	39	9,24	101	23,93	69	16,35	7	1,66	96	22,75	110	25,07
Metestrus	251	4,2	19	7,57	73	25,10	42	16,73	3	1,39	55	22,75	59	23,51
Diestrus	69	1,2	6	8,69	25	36,23	7	10,14	1	1,45	13	18,84	17	24,65

tissue stroma. Collections of large epithelial cells with lightly stained nuclei, not so sharply demarcated from the connective tissue stroma may be found. The chromatin in the cell nuclei is concentrated in granules and clumps, irregular in shape. Between the cells are many granulocytes and lymphocytes. The impression is created that some of these collections are not connected with the general epithelial framework of the gland. We believe that these are old, disintegrating nodules of epithelium.

Estrus. Part of the proliferative nodules have acquired a lumen and are forming alveoli. The nuclei of the epithelial cells of the terminal segments are polymorphous and there are many dense, darkly staining nuclei, part of which is shaped like a blacksmith's nail. Desquamation of cells into the lumen of the ducts is insignificant. Leucocytes are encountered rarely in the stroma.

Metestrus. The proliferative nodules consist of cells with round, not so clearly demarcated nuclei. Between the epithelial cells are many leucocytes. In the wall of the alveoli the cell nuclei are round and lightly stained. Dense, darkly stained nuclei are rarely found. Desquamation of cells into the lumen of the ducts is almost unnoticeable. The connective tissue stroma of the gland is infiltrated by leucocytes to a greater extent than in the other stages of the cycle.

Diestrus. The number and size of the alveoli are diminished. The nuclei of the epithelial cells of the terminal portions are polymorphous and variable in size. Many dense, darkly staining nuclei shaped like blacksmiths' nails and atrophic, pyknotic nuclei are present. Desquamation of epithelial cells into the lumen of the ducts and alveoli is observed (Fig. 2). There are fewer leucocytes in the stroma than in metestrus, but more than in the phases of estrus and proestrus.

Determination of the mitotic activity (see Table) shows that it does not stay constant, but varies throughout the whole cycle. The lowest degree of mitotic activity is observed in the period of diestrus (1.2 mitoses per thousand cells on the average); it rises sharply during proestrus (on the average 6.8 mitoses per thousand cells) and remains high throughout estrus (7.0 mitoses per thousand cells on the average).

The difference in the mean values of the mitotic activity at the various stages are statistically significant (P=0.001). The exception is the difference between the mitotic activity in the periods of proestrus and estrus. Here they are not statistically significant. The smallest individual variations were observed in estrus (from 5 to 9 mitoses per thousand cells). In the remaining phases these variations were significant. The highest value of the mitotic activity was observed in certain animals in proestrus (15.2 mitoses per thousand cells). There are therefore grounds for believing that at a certain period of proestrus the mitotic activity is maximum on account of the intensive division of the cells of the proliferative nodules. In diestrus the mitotic activity, although it varies considerably in individual animals (from 0.3 to 3.5 mitoses per thousand cells), nevertheless remains low in comparison with the phases of estrus and proestrus. Evidently at the beginning and end of diestrus the mitotic activity is not the same, and this may account for its individual variations in animals killed at the various stages of this period. The low figures for the mitotic activity in diestrus enable us to deny categorically the existence of a second significant rise in mitotic activity as described in this period by Bullough.

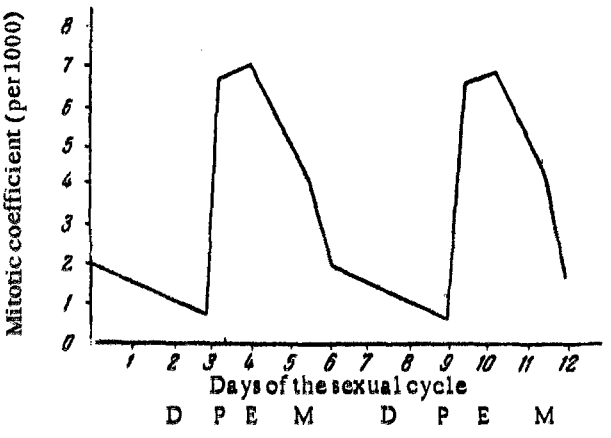

Fig. 4. The mitotic activity of the epithelium of the mammary gland of the mouse in different stages of the sexual cycle.

Comparison of the percentage relationships of the individual phases of mitosis of the epithelial cells of the mammary gland at different periods of the sexual cycle shows that in proestrus, when mitotic activity rises sharply, the percentage of metaphases increases. The mitotic activity is 8% higher than in the period of estrus; however this difference is not statistically significant.

The curve expressing the changes in the mitotic activity of the epithelium of the mammary gland (Fig. 4). shows that increased mitotic activity coincides with the secretion of estrogenic hormones from the ovaries into the blood. In diestrus, when estrogenic hormones are not secreted by the ovaries, the mitotic activity of the epithelium of the mammary glands is minimal.

In nonpregnant mice, in physiological conditions the appearance of estrogenic hormones in the blood leads to stimulation of cell division. However this still does not prove a direct action of estrogenic hormones on the epithelium of the mammary gland. Ferguson [4] showed in castrated and hypophysectomized female mice that the estrogenic hormones alone, without prolactin and pituitary growth hormone, do not have any stimulating effect on the mammary gland.

SUMMARY

The mitotic activity of epithelium of the end portions of the mammary glands was studied during various stages of sexual cycle in nonlinear white mice. The rise of mitotic activity corresponds to the maximal effect of estrogenic hormones in time. The mitotic activity is maximal in estrus and proestrus and is equal, on the average, correspondingly to 6.8 and 7.0 mitoses per thousand epithelial cells. In diestrus the mitotic activity is minimal and equals, on the average, 1.2 mitoses per thousand cells.

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