MITOTIC ACTIVITY OF MOUSE MAMMARY GLAND EPITHELIUM AFTER REMOVAL OF A CONSIDERABLE PORTION OF THE GLAND

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Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 54, No. 12, pp. 84-89, December, 1962
Original article submitted March 10, 1962

It is known that when parts of an organ (liver, kidney, spleen, pancreas, etc.) are removed, the number of dividing cells in the remaining part is increased. This process begins after a certain latent period, reaches a maximum rate a few days after the operation, and subsequently the mitotic activity in the damaged organ gradually abates [3]. However, it is not known whether the mitotic conditions become normal, or how the damaged organ behaves in this respect when demands are made upon it which increase its rate of function. In most reports dealing with the mitotic activity of regenerating organs no account is taken of the cyclical changes normally occurring in them.

We have set out to study the mitotic conditions in epithelium of mouse mammary gland after removal of approximately half of it and a considerable proportion of the tissue of the others. Both portions were removed at the same period in the sexual cycle; we have made the same studies when the gland showed increased development during pregnancy and lactation.

The mitotic activity of the mammary gland has already been studied at various periods of the sexual cycle, pregnancy, and lactation [2]. The change of activity induced by removal of part of the gland has not been studied in mice. In rabbits and guinea pigs the operation causes an increased proliferation in the part of the organ remaining. Coen [7] followed this process up to the 17th day. Vlasov [1] made a study 5-7 days after the operation.

We thought that in our experiments the removal of a large proportion of the tissue of the mammary glands would reveal the regenerative and compensatory processes in the remaining parts.

EXPERIMENTAL METHODS

For the experiments we used sexually mature females of an impure strain of white mice weighing 18-27 g. The operation for removal of the mammary glands was made at diestrus. On the right side, the outer halves of the second and third thoracic and both inguinal mammary glands were removed; on the left side we attempted to remove the second and third thoracic and inguinal glands entirely, but some tissue usually remained. The first pair of thoracic glands was left intact. In the first and second experiments, incisions in the skin were made at the level of the glands to be removed; on the right the incision was made outside the nipple, on the left, medial to it (Fig. 1A). In the third set of experiments the glands were removed through two incisions made along the middle of the back (Fig. 1B). The wounds were studied after arrest of the hemorrhage. In this way, about 30-50% of the tissue of all the mammary glands remained. In the control animals no operation was performed.

In the first experiment, operations were made on 45 mice. The animals were killed on the 6-15th day after the operation at various stages of the sexual cycle (the spread in the dates of fixation was due to the problem of selecting animals at the appropriate part of the cycle).

In the second experiment, 86 mice underwent the operation. The females in estrus were fertilized by males 4-6 days after the operation. The animals were killed on the 8th and 18th days of pregnancy (40% were pregnant).

In the third experiment, operations were performed on eight females between the 10th and 13th days of pregnancy. They were killed 1-5 days after littering (12-13 days after the operation). The corresponding control group

was killed at the same time. Except for six mice of the third series, in all the experiments the mice were killed from 2 p.m. onwards. For histological treatment we used the second and third right mammary glands, which were fixed in Zenker, and then embedded in paraffin. Sections 8 μ thick were then cut and stained in hematoxylin-eosin.

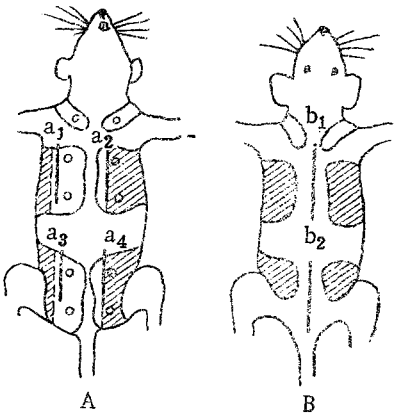

Fig. 1. Diagram of the operation to remove part of the mammary gland tissue. A) View from the ventral side; B) view from the dorsal side. a_1, a_2, a_3, a_4 – Line of incision of skin in the first and second experiments; b_1, b_2 – line of incision of skin in third experiment. Shaded area indicates glands removed at operation.

In the first experiment, mitoses were counted in characteristic epithelial thickenings formed by the terminal portions of the growing ducts (Fig. 2a); in most of the mice, the formation of alveoli was not observed. In the second experiment, mitoses were counted and related to the degree of development of the gland in the terminal portions of ducts, or in the epithelium of the alveoli which were forming or had formed; in the third experiment the mitoses were counted in the epithelium of the alveoli. In each experiment we examined 6,000 cells, and calculated the mitotic index (MI) representing the number of mitoses per thousand. The results obtained were treated by the Fisher-Student method.

EXPERIMENTAL RESULTS

Experiment I. From the large number of cases of each stage of the cycle we were not able to find a sufficient number of terminal sections in every fixed portion. Still, we were definitely convinced that the removal of 50-70% of the glandular tissue did not appreciably influence the mitotic activity of the remaining half of the organ at the period between 5 and 15 days after the operation (Table 1).

Experiment II. As in the first experiment, no difference between the mitotic activities of the mammary glands of the experimental and control groups could be found. On the eighth day of pregnancy the mean MI in the experimental group was 11.2% and in the control group 10.1%; on the 18th day the corresponding figures were 4.0 and 4.5% (Table 2). These results are at variance with those of S. S. Laguchev [2]. In our experiments the mitotic activity fell considerably between the 8th and 18th day of pregnancy ($P < 0.01$), while in Laguchev's the MI increased during pregnancy. In order to determine more precisely the nature of the changes in the mitotic activity during pregnancy, we compared the level of this activity with the state of development of the gland in pregnant mice. In this part of the work, in addition to the results of the principal experiment, we also used material from the preliminary experiment, where the date of pregnancy was not always precisely known. All the treated mammary glands were divided, according to their degree of development, into five states. Stage I included glands in which alveoli had not yet been formed (see Fig. 2a, b). In the glands of stage II, the alveoli were arranged in small clusters separated from each other by broad layers of fat. The protoplasm of the epithelial cells was homogeneous (Fig. 2c). In the glands of stage III, the alveoli occupied a large area of a section of the gland. The protoplasm of most of the cells of the alveoli contained vacuoles (Fig. 2d). In glands of stage IV, the fatty tissue between the groups of alveoli had disappeared completely. The lumen of the alveoli was filled with a secretion (Fig. 2e). In the glands of stage V the alveoli were greatly distended by a secretion, and the epithelial glands were flattened (Fig. 2f). The results of a count of mitotic activity are shown in Table 3.

The mitotic activity of the epithelium of the mammary gland was higher during the period of the increased growth of the duct (stage I) and of the formation of alveoli (stage II) than during the subsequent development of the alveoli, when secretory vacuoles appeared in the cells, and the secretion was liberated into the lumen of the alveolus (stages III, IV, and V). In the control mice, the differences between the mean MI in glands at stages I and II was highly significant ($P < 0.00$); the differences between the mitotic activity of glands in stage II and the next stage were also significant ($P = 0.015$). The mean values for the glands of the experimental and control mice at a given stage of development were closely similar.

Experiment III. In the operated mice which were feeding their young, the mitotic activity of the epithelium of the mammary gland did not differ appreciably from that of control mice also feeding their young (Table 2). No differences were found between four experimental and two control mice killed at 5 a. m. – the time at which other organs show greatest mitotic activity – and other mice killed at 2 p. m. (see Table 2).

TABLE 1. Mitotic Index (MI) of the Epithelium of the Terminal Portions of the Mammary Gland in the Experimental and Control Mice at Various Stages of the Sexual Cycle (number per thousand)

Group of animals	Proestrus		Estrus		Metaestrus		Diestrus					
							first hours		2-4th day		17-24th day	
	MI	day after operation	MI	day after operation	MI	day after operation	MI	day after operation	MI	day after operation	MI	day after operation
Control	12 5.8 11.1 7.5	No operation	13.2 13.1 16.1 11.1 14.3 8.7		10.7 3.6 16.1 2.7 5.3 10		9.3 11.5 6.2 15.6 5.2		0.4 0.3 0.3			
Mean	9.1		12.7		8.1		9.6		0.3			
Experimental	8.9	8th	7.7 13.6 13.7 5.6 5.9 11 15.7	7th 5th 6th 6th 9th 9th 6th	2.9 8.9 1.0 2.8	15th 12th 6th 11th	– – – –	– – – –	0.2 0.4	7th 8th	6.5 9.9 2.5	7th 6th 6th
Mean	8.9		10.5	3.9	3.9				0.3		6.8	

TABLE 2. Mitotic Index of the Epithelium of the Mammary Gland in Experimental Control Mice during Pregnancy and Lactation (number per thousand)

Pregnant mice						Lactating mice				
8th day of pregnancy			18th day of pregnancy							
control	experimental		control	experimental		control		experimental		
MI	MI	day after operation	MI	MI	day after operation	MI	day of lactation	MI	day of lactation	day after operation
11.7	13.8	14th	7.3	3	22th	0	5	0.2	4th	12th
11.3	7.2	13th	2	1.2	22th	0.7	5	6.5	3th	12th
6.2	9	14th	5.3	3.7	23rd	0.2	5	1.3	3th	12th
7.5	13.3	14th	2.3	3.8	23rd	2.3	2	0.8	1st	12th
8.7	18.5	14th	7	8	23rd	2	2	0.1*	5th	13th
15.7	10.5	13th	2.2	3.3	28th	0.1*	6	0.2*	4th	13th
9.3	5	13th	5.7	5.3	28th	0.3*	6	0.2*	2nd	13th
								0.3*	2nd	13th
Mean 10.1	11.2		4.5	4		0.79		1.19		
Significance of the difference between the control and the experimental groups	P > 0.05		P > 0.05			P = 0.69				

*Mice killed at 5 a. m.

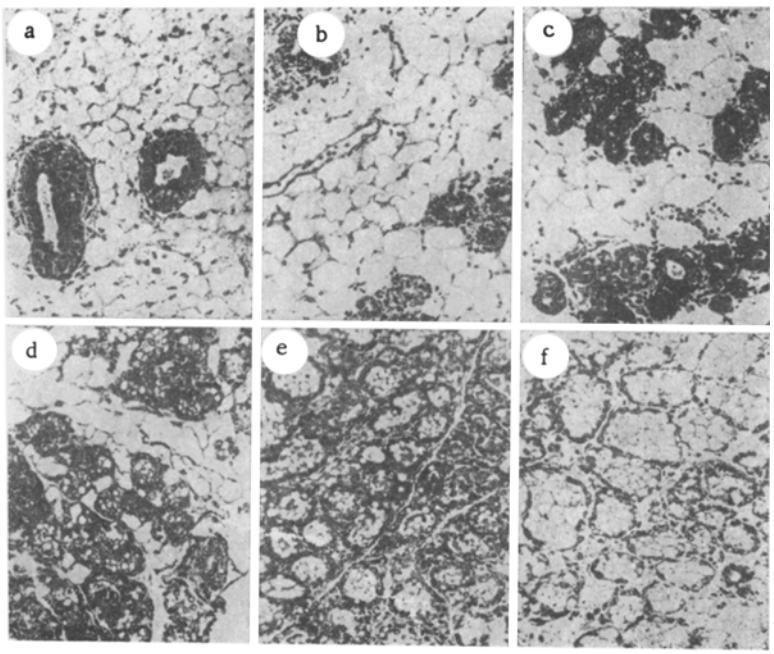

Fig. 2. Sequence of changes in the structure of a mammary gland during pregnancy (a – f).

TABLE 3. Relationship of the Mitotic Index of the Epithelium of the Mammary Gland to the Degree of Development during Pregnancy

Animals	Stage I			Stage II			Stage III			Stage IV			Stage V		
	Extent of pregnancy (in days)														
	6-15th			15-20th			15-20th			18-20th			18-20th		
	no. of mice	MI	day after operation	no. of mice	MI	day after operation	no. of mice	MI	day after operation	no. of mice	MI	day after operation	no. of mice	MI	day after operation
Control animals	11	9.8	—	9	6.8	—	5	3.6	—	10	3.4	—	3	2.6	—
Experimental animals	8	9.7	13-25th	16	6.4	13-34th	4	4.3	24-34th	8	4.2	23-42nd	1	3.3	23rd

Thus, when 50-70% of the mass of the mammary glands was removed, we found no increase of mitotic activity in the remaining portions. In the experimental animals there was a change corresponding to the alteration of the physiological condition, and the index was close to that found in the control mice in an identical physiological state. It is true that the earliest observations in our experiments were made 5-6 days after the operation, and possibly mitotic activity was raised before then and subsequently returned to normal. We must however note that according to published reports, after removal or damage of a portion of some other organs, its mitotic activity remains raised for a long time. For example, in rats, many mitoses are found in the lungs on the seventh day [6], and in the pancreas up to the 15th day after the operation [5]; in mice, in the liver and spleen, up to the 14th day the mitotic activity was higher than in the control group [4, 8]. In our experiments, most of the animals were killed between five and 14 days after the operation. It is therefore possible that in the case of the mammary gland, the removal of a portion has a different effect on the mitotic activity than is the case elsewhere.

In our experiments we made no special study of regeneration of the mammary gland. We can say only that in females feeding their offspring (3-5th day of lactation) the weight of the inguinal glands was 60% of the corresponding weight in the control lactating mice. Similar relative weights were found for the total weight of the mammary glands of mice which had ceased to feed their young and which were killed 40 days after littering (2 months after the operation); the values held too for mice which had not litered. However, the weight of the mammary gland can scarcely be considered as any kind of satisfactory criterion of the increased mass of tissue of this organ, because a large amount of fatty tissue is contained in an extirpated gland.

SUMMARY

From 50 to 70% of the total mass of mammary glands was excised from female mice; a study was made of the mitotic activity in the remaining portion of the second and third right mammary glands. In glands with undeveloped alveoli, counts were made of mitoses of the terminal portions of the ducts or in the alveolar epithelium. The average mitotic index for the experimental mice was 10.5% during estrus (5 to 9 days after the operation), 11.2% on the 8th day of pregnancy (13-14th postoperative day), 4% on the 18th day of pregnancy (22nd-23rd postoperative day), and 1.19% during the first five lactation days (the 12-13th day after the operation on pregnant females). In nonoperated mice the average mitotic indices for the corresponding groups were 12.7, 10.1, 4.5, and 0.79%. There were therefore no significant differences between the control and experimental animals.

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All abbreviations of periodicals in the above bibliography are letter-by-letter transliterations of the abbreviations as given in the original Russian journal. *Some or all of this periodical literature may well be available in English translation.* A complete list of the cover-to-cover English translations appears at the back of this issue.
