THE CELL DIVISION PATTERN OF MAMMARY GLAND EPITHELIUM AT VARIOUS STAGES OF THE ESTRUS CYCLE IN MICE OF HIGH AND LOW CANCER LINES

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Many researchers have attempted to demonstrate a disturbance of the hormonal balance in mice of high cancer lines, and for this purpose they have compared the course of estrus cycles in different lines. However, attempts to discover an abnormality of the estrus cycle in mice of high cancer lines by means of the vaginal smear technique have yielded equivocal results [1, 6, 9, 11, 13, 14]. Mice of some lines have shown a prolongation of estrus and a shortening of diestrus, while in mice of other lines no such changes have been observed.

R. E. Kavetskii and N. M. Turkevich [3, 10], working with mice of the high cancer line C_3HA, found a disturbance of the regular rhythm of excretion of gonadotropic hormones. They consider that the rhythm of excretion of mammotropic hormones by the anterior lobe of the hypophysis is also disturbed in the mice of this line, but this is no more than conjecture. Meanwhile, it must be remembered that the mammotropic action of the ganodotropins themselves has not yet been proved, and is by no means accepted by everybody [15].

The principal factors regulating cell division in the organs of the female reproductive system are hormonal influences. It has been shown that after castration and hypophysectomy, cell division in the epithelium of the mammary glands and uterus ceases completely, and the number of divisions falls to a negligible value [2, 5]. Administration of ovarian hormones after castration, and also after castration and hypophysectomy, causes a rise in mitotic activity in the epithelium of the mammary glands [5, 7]. Evidence of the hormonal regulation of cell division in the organs of the reproductive system is also given by the change in the mitotic activity of the mammary gland epithelium in the course of the estrus cycle, pregnancy, and lactation [4, 8, 12]. It has been shown that the highest level of mitotic activity of the epithelium of the mammary glands coincides with the maximal excretion of estrogenic hormones from the ovary. It must therefore be recognized that a change in the mitotic activity of the mammary gland epithelium, like the cytological changes in vaginal smears, reflects the individual balance of the ovarian and hypophyseal hormones and the rhythm of their excretion.

If the modern ideas concerning the causes of mammary gland tumors are taken as a starting point, according to which they arise as a result of disturbance of the correlation of the hormonal activity of the hypophysis and ovaries, it is to be anticipated that the patterns of mitotic activity in mice of high and low cancer lines will differ significantly in different physiological states.

The object of the present work was to discover if any abnormal changes in the mitotic activity of the mammary gland epithelium in mice of high and low cancer lines took place in the various stages of the estrus cycle.

EXPERIMENTAL METHOD

Experiments were conducted on 40 sexually mature nulliparous mice aged from 3 to 6 months. Nineteen of these animals belonged to the low cancer line C57 and 21 to the high cancer line C_3H. The incidence of cancer of the mammary gland among female mice of line C_3H is known to be 90% even if they remain virgin. Consequently, if hormonal pathology is in fact a determining influence, it must show itself in this particular line in the course of the estrus cycle.

Mitotic Activity of the Mammary Gland Epithelium of Mice of High and Low Cancer Lines and Mongrel Albino Mice in Estrus and Diestrus

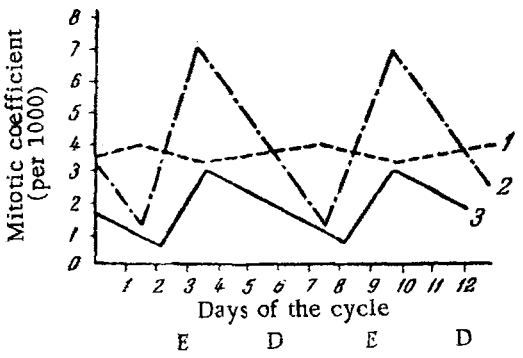
Mouse line	Stage of cycle	Animal No.	Mitotic coefficient (per 1000)	Mean mitotic coefficient for group (per 1000)	Statistical significance of difference between mean values of mitotic coefficient
C57 low cancer	Estrus	4	2	3.18	P = 0.001
		6	5.33		
		9	2.66		
		14	2.33		
		15	2.33		
		17	6.66		
		18	2		
		20	4		
		21	1.3		
	Diestrus	1	0	0.66	
		3	0.33		
		5	0		
		7	0.33		
		10	0		
		11	1.33		
		12	0.33		
		13	2		
		16	1.66		
		19	0.66		
Mongrel albino mice	Estrus			7	P = 0.001
	Diestrus			1.2	
	Estrus	1	2	3.41	P = 0.77
		6	2.33		
		7	1.33		
		12	12.33		
		13	4		
		17	4.66		
		20	2.66		
		22	0.66		
		23	0.66		
C_3H high cancer	Diestrus	4	0.33	3.94	
		8	7		
		9	0		
		11	0.66		
		14	1		
		16	17.33		
		18	6.66		
		19	3		
		27	4.66		
		28	1.33		
		30	1.33		

Vaginal smears were taken from the animals once every 24 hours for 2-3 weeks. Mice were sacrificed at 12 noon in stages of the estrus cycle when the mitotic activity in albino mice of mixed lines is maximal or minimal. These stages are estrus and diestrus respectively [4]. In the stage of estrus 9 mice of line C57 and 10 mice of line C_3H were sacrificed. We included no mice in the experiment in a state of prolonged diestrus. Ten mice of line C57 and 11 mice of line C_3H were sacrificed in dietrus. The mice were kept in a vivarium with no males, and they were fed on a diet made up into bricquettes. The technique of preparation of histological specimens, of determining mitotic activity, and of statistical interpretation of the results is described in previous articles [4, 7].

EXPERIMENTAL RESULTS

Determination of the character of the estrus cycles in mice of line C57 confirmed previous findings that the mean duration of diestrus in the females of this line is significantly longer than the mean duration of estrus [6]. We made no special study of the pattern of the estrus cycle in the females of line C_3H, but observations made over a period of 1-2 weeks showed that the natural sequence of the stages of the estrus cycle was by no means invariably maintained in the mice of this line. The stage of metestrus was that most frequently omitted, and diestrus followed immediately after estrus. Strictly speaking, metestrus was considerably shortened, and therefore was not detected. Sometimes the stage of proestrus was not found. In some cases a cycle, beginning with proestrus, was broken off and next day the vaginal smear again showed diestrus. These findings alone are sufficient to postulate hormonal disturbances in mice of the line C_3H.

Examination of histological preparations of the mammary glands of females of line C57 revealed the same patterns as we described earlier in the case of albino mice of impure lines [4, 8]. In the stages of proestrus and estrus nodules of proliferating epithelium appeared at the ends and along the course of the small ducts of the gland. These nodules were always absent in the stage of diestrus. Desquamation of epithelial cells into the lumen of the alveoli and ducts, and a decrease in the size of the epithelial cells composing the terminal segments were observed. It must be mentioned that the changes described in mongrel mice during the change from one stage of the cycle to another were not always clearly defined and sometimes it was difficult to classify them. In females of line C57, on the other hand, these changes were more clearly outlines, and in nearly every case the stage of the cycle could be determined by study of the histological section of the mammary gland.



Changes in the mean mitotic activity of the mammary gland epithelium of mice of different lines in estrus (E) and diestrus (D). 1) Line C_3H; 2) impure lines; 3) line C57.

The mammary glands of the line C_3H females were usually much more developed than those of mice of the low cancer line or of mixed lines. The terminal segments in the females of this line were considerably larger. In 4 mice sacrificed in the stage of diestrus, nodules of proliferating epithelium were present in the mammary glands. Consequently, in the females of this line, the epithelium frequently proliferates not only during the period of estrus, but also when estrus is absent. Thus, in the females of the line C_3H, the usual synchrony in the cyclic changes in the mammary glands and vaginal epithelium is disturbed. Moreover, in the mammary glands of the females of line C_3H atypical areas were found, uncharacteristic of the glands of nonpregnant mice. For instance, in 6 of the 21 mice, focal collections of alveoli could be seen with cells containing secretory vacuoles. Neither the presence of large numbers of alveoli nor of secretory activity in their cells are normally observed in nonpregnant or nonlactating mice. A carcinoma of the mammary gland was found in one mouse, consisting of a microscopic nodule formed partly of closely packed, small, terminal segments, and partly of solid collections of cells. The figures showing the mitotic activity in this animal are excluded from the table.

Determination of the mitotic activity in the females of line C57 showed that in these mice, as in mongrel albino mice, a considerable increase in the number of cell divisions takes place in the stages of proestrus and estrus, and in diestrus the mitotic activity falls to very low figures. A characteristic feature of the low cancer line is that the mean mitotic activity in estrus for the whole group is much lower than the mean mitotic activity in the same stage in mongrel mice. In mongrel mice, for instance, it amounted to 7 mitoses per 1000 epithelial cells, and in mice of line C57 it was only 3.18 mitoses per 1000 cells. This difference is statistically significant (see figure and table). In

diestrus the mitotic activity of the mongrel mice was 1.2 mitoses, and that of the C57 line 0.66 mitoses per 1000 cells. This difference is not statistically significant. Thus in female mice of line C57 the curve expressing the change in the mitotic activity of the mammary gland epithelium is of the same character as that for the mongrel mice (see figure). However, the rise in mitotic activity in estrus in the animals of the low cancer line is not as high. Two reasons for this may be suggested: 1) either the hormonal stimulus in the period of estrus is weaker in the females of line C57, or 2) in this line the epithelial cells of the mammary glands react more weakly to an equal hormonal stimulus, and fewer of them begin to take part in mitosis.

The determination of the mitotic activity of the mammary gland epithelium of female mice of the high cancer line C_3H showed significant abnormal trends. For instance, the mean mitotic activity in the mice sacrificed in estrus was 3.41 mitoses, while that in the mice sacrificed in diestrus was 3.94 mitoses per 1000 epithelial cells, i.e., it was not lowered. It is clear from the table that the mitotic activity of the mammary gland epithelium varied very considerably in the individual animals. In the stage of diestrus the individual indices of mitotic activity varied from 0 to 17 mitoses, and in the stage of estrus, from 0.7 to 12 mitoses per 1000 cells. It is difficult to decide from the results obtained whether the hormonal regulation of mitotic activity was disturbed in all the animals of the line C_3H. Since there is no difference between the mean values of the mitotic activity in estrus and diestrus, but normally the mitotic activity is always higher in estrus, it may be assumed that in many females of the line C_3H the mitotic activity either changes only very little in the different stages of the estrus cycle, or it is higher in diestrus than in estrus.

In determination of mitotic activity, like the analysis of the morphological findings characteristic of the mammary glands, provides evidence that in many female mice of line C_3H the cyclic changes in the epithelium of the vagina and mammary glands frequently do not coincide in time. Evidently in the mice of line C_3H there are abnormalities in the production of the mammotropic hormones of the anterior lobe of the hypophysis. It may be postulated that it is not only the hormones of the ovary that can increase the mitotic activity of the mammary gland epithelium, for in many females the line C_3H the mitotic activity is high in the stage of diestrus, when the concentration of estrogenic hormones is at its lowest level. The high mitotic activity cannot be explained by the action of progesterone, for in nonpregnant mice after ovulation the corpora lutea do not develop and do not secrete progesterone.

These results indicate a disturbance in the hormonal regulation of the changes in the organs of the reproductive system of virgin female mice of the high cancer line C_3H in the course of the estrus cycle.

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