COMPARATIVE STUDY OF DIURNAL RHYTHMS OF MITOTIC ACTIVITY IN HEALTHY AND TUMOR TISSUES

M. V. Berezkin

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A distinct diurnal rhythm of mitosis, reaching a maximum at 10 A. M. and 1 P. M. and a minimum at 7 P. M. and 1 A. M. was found in a carcinoma of the forestomach transplanted into C3HA mice. Changes in the number of mitoses in the stratified squamous epithelium of the forestomach, both in mice affected by a tumor and in intact mice, show a well-defined diurnal periodicity; the highest mitotic index was observed at 10 A. M. and the lowest at 10 P. M.

Comparative investigation of the level of mitotic activity and the principles governing the diurnal rhythm of mitosis in normal tissues and tumor tissues is of great importance in connection with the discovery that inhibitors of cell proliferation are not equally effective in their action when administered to animals at times when the number of mitoses in the tissues is at a maximum or minimum [3, 4].

A diurnal rhythm of mitoses is found in most normal tissues. Meanwhile, neither the existence of a mitotic rhythm nor its character in malignant neoplasms has been adequately explained. Some workers [10, 11, 12] found no significant differences between the number of mitoses in tumors at different times of day and night, while others [1, 6-8, 13] observed a definite rhythm of mitosis in them.

The effect of tumor growth on mitotic activity and the character of the diurnal rhythm of mitosis in tissues unaffected by malignant growth are problems of considerable interest. Some workers [5, 9] have observed a decrease in the number of mitoses in the corneal epithelium of mice inoculated subcutaneously with tumors. However, this decrease was not apparent in every case [9]. In all these investigations, the observations were made without regard to possible changes in the diurnal rhythm of cell division.

As yet very little attention has been paid to the study of the similarity or difference in character of the diurnal rhythm of mitosis in tumors and in healthy tissues derived from a common origin.

The object of the present investigation was to study the level of mitotic activity and character of the diurnal rhythm of mitosis in a transplanted carcinoma of the mouse forestomach, strain OZh-5, and in the stratified squamous epithelium of the forestomach of normal mice and mice inoculated intramuscularly with a tumor of this strain.

EXPERIMENTAL METHOD

Sexually mature male and female C3HA mice were used in the experiments. A suspension of tumor cells, minced in physiological saline, was injected into the femoral muscles in a dose of $2 \cdot 10^5$ cells in a volume of 0.2 ml. The animals were sacrificed on the ninth day after inoculation, over a period of 24 h at intervals of 3 h, 6-8 animals at each time. Material was fixed in Carnoy's fluid and embedded in paraffin wax, and sections cut to a thickness of 7-8μ were stained with hematoxylin. The cells and mitoses were counted (binocular microscope, 900×) in areas of tumor free from necrosis. In each case about 10,000 cells were examined. Early prophases were not counted.

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TABLE 1. Diurnal Rhythm of Mitosis in Carcinoma of Forestomach and in Epithelium of Forestomach in Intact Mice and Mice Inoculated with Tumors

Time of day	Tissue studied					
	carcinoma of forestomach		epithelium of forestomach of mice with tumors		epithelium of forestomach of intact mice	
	MI	P	AM	P	MI	P
10 AM	20,6	0,08	7,4	0,05	8,2	0,70
1 PM	16,7	0,09	4,8	0,03	7,8	0,004
4 PM	13,7	0,06	2,7	0,04	3,1	—
7 PM	11,4	0,02	1,7	0,10	2,6	0,14
10 PM	8,7	—	1,2	0,04	1,3	—
1 AM	8,5	0,004	2,3	0,22	1,6	—
4 AM	12,0	0,13	3,0	0,08	1,8	0,03
7 AM	14,1	0,004	3,9	0,006	3,6	0,001
Mean	13,2		3,4		3,7	
10 AM-4 PM $P=0,0001$ 10 AM-1 AM $P=0,0001$ 1 AM-7AM $P=0,0001$			10 AM-4 PM $P=0,001$ 10 AM-10 PM $P=0,0001$ 10 PM-7 AM $P=0,0001$		10 AM-4 PM $P=0,0001$ 10 AM-10 PM $P=0,0001$ 10 PM-7 AM $P=0,001$	

After the abdomen and thorax had been opened, a ligature was applied to the esophagus, and fixing fluid was injected from a syringe with a blunt needle into the stomach through the duodenum, as a result of which the stomach was slightly distended. After additional fixation, a strip was cut from the wall of the forestomach, wound into a coil, and embedded in paraffin wax. Sections were stained with hematoxylin. Cells (about 10,000 in each case) and mitoses were counted without differentiation of the epithelium into layers.

To assess mitotic activity, the mitotic index (MI) was calculated in promille. Statistical analysis of the results was carried out by the Fisher-Student method.

RESULTS

The number of cell divisions in the carcinoma of the forestomach and in the epithelium of the forestomach varied during the 24-h period (Table 1).

The number of mitoses in the carcinoma reached a maximum at 10 A. M. It then gradually decreased, the difference becoming significant by 4 P.M. The number of mitoses reached a minimum between 10 P.M. and 1 A.M. The subsequent increase in MI became significant by 4 A.M. Consequently, highest values of MI were found between 10 A.M. and 1 P.M., and lowest between 7 P.M. and 1 A.M. The diurnal changes of MI in the epithelium of the forestomach in mice with tumors and in intact mice were similar both to each other and to the changes observed in carcinoma of the forestomach.

The number of mitoses found in the epithelium of the forestomach of intact mice and of mice with a tumor reached a maximum at 10 A.M. and a minimum in the forestomach of mice with tumors between 7 P.M. and 1 A.M. and in the forestomach of intact mice between 7 P.M. and 4 A. M.

In all the investigated tissues the diurnal rhythm of mitosis can thus be described by a unimodal curve.

Differences between the mean values of MI for the 24-h period in the epithelium of the forestomach of mice with tumors (3.4‰) and intact mice (3.7‰) are not significant.

The amplitude of the diurnal fluctuations in the number of mitoses in the carcinoma was smaller than in the epithelium of the forestomach. In the tumor, for instance, the value of MI at its maximum was 2-4 times greater than at its minimum, while in the epithelium of the forestomach the difference was 6.3 times.

It follows from these results that transplanted carcinoma of the forestomach has no significant effect on the character of the diurnal rhythm of mitosis or the level of mitotic activity in the epithelium of the forestomach. These results are in agreement with observations [2] showing the absence of significant differences in the character of the diurnal rhythm of mitosis in the cornea of normal mice and of mice with leukemia. These results concerning the character of the diurnal rhythm of mitosis in the epithelium of the forestomach agree with results [10] obtained with the same tissues using colchicine.

However, they do not agree with the results of a careful investigation conducted by Kolomina [6-8]. This worker studied diurnal changes in mitotic activity of a tumor of the forestomach of the same strain. She found a diurnal rhythm of mitosis, but the number of mitoses reached a maximum at 8 P.M.-midnight and a minimum at 4 A.M.-noon. These differences are difficult to explain at present. They may perhaps be due to different conditions under which the experimental animals were kept and differences in the number of generations of the strain. However, these hypotheses require special verification.

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