

# DURATION OF MITOSIS AND DIURNAL RHYTHM OF CELL DIVISION OF EHRLICH'S TUMOR AND LYMPHOMA NK/Ly

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Ehrlich's ascites tumor and lymphoma NK/Ly are characterized by a clearly defined diurnal rhythm of cell division. Curves showing the changes in mitotic activity of the tumor cells during the 24-h period are unimodal in character with a minimum of the number of mitoses at 9 a.m. and a maximum for Ehrlich's tumor at midnight and for lymphoma NK/Ly at 3 a.m. The increase in mitotic activity in the tumors takes place through the entry of a larger number of cells into mitosis and not through an increase in the duration of mitosis.

Normal and malignant tissues have a clearly defined diurnal rhythm of cell division [2, 4-6, 8, 13]. It is not yet clear, however, whether this increase in mitotic activity is caused by the prolongation of mitosis or by an increase in the number of cells entering into mitosis. The investigation described below was carried out to study this problem.

## EXPERIMENTAL METHOD

The test objects were Ehrlich's ascites tumor and lymphoma NK/Ly, which were inoculated intraperitoneally into noninbred male albino mice weighing 18-20 g. The experiments were carried out in the autumn and winter. The animals were kept under normal conditions of illumination and on a normal diet. They were taken for the experiment on the 10th day after inoculation of the tumor. The mice of the experimental group received an intraperitoneal injection of colchicine in a dose of 1  $\mu$ g/g 6 h before sacrifice. No late phases of mitosis were found in the preparations. Mice of the control and experimental groups were sacrificed at intervals of 3 h during the 24-h period (9 a.m., noon, 3, 6, and 9 p.m., midnight, 3 and 6 a.m.). From 5 to 7 animals were used at each period of the experiment. The ascites fluid was withdrawn from the mice and films prepared from it. These were fixed with a mixture of 3 parts methyl alcohol and 1 part glacial acetic acid, and then stained with 2% acetorcein solution. Mitoses were counted by phases in each film in 5,000 tumor cells under a magnification of 900 $\times$ . Mitotic activity was judged from the mitotic index (MI) - the ratio between the number of dividing cells and the total number of cells counted, expressed per thousand cells. The number and form of the pathological mitoses also were determined [3].

The duration of mitosis was calculated by the equation [7]

$$t_m = \frac{MI \cdot A}{MI_{colch.}},$$

where MI is the mitotic index in the control group,  $MI_{colch.}$  the mitotic index in the experimental group, and A the time of action of colchicine. The duration of the cell cycle was calculated by the equation

$$t_c = \frac{t_m \cdot N}{n},$$

where N is the total number of cells, n the number of dividing cells, and  $t_m$  the duration of mitosis.

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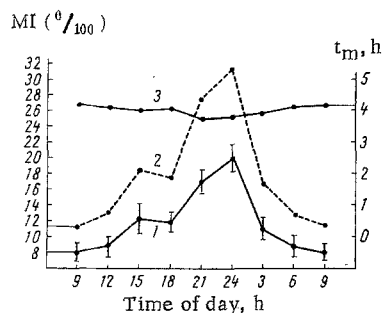


Fig. 1

Fig. 1. Change in MI (1),  $MI_{colch.}$  (2), and duration of mitosis (3) during 24-h period in cells of Ehrlich's ascites tumor.

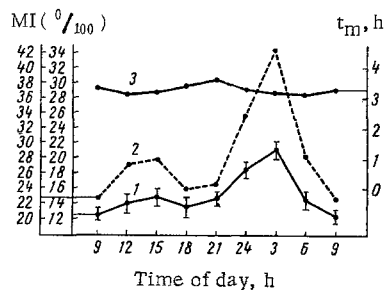


Fig. 2

Fig. 2. Change in MI (1),  $MI_{colch.}$  (2), and duration of mitosis (3) during 24-h period in cells of lymphoma NK/Ly.

Statistical analysis of the numerical results was carried out by the Student-Fisher method.

### EXPERIMENTAL RESULTS

It is clear from Fig. 1 that the minimal number of dividing cells of the Ehrlich's tumor was observed at 9 a.m. ( $MI = 8.0 \pm 1.0\%$ ). The number of mitoses then increased to reach a maximum at midnight ( $MI = 20.1 \pm 1.6\%$ ). Starting from midnight the number of dividing cells fell sharply. The mean diurnal MI was  $12.4 \pm 1.3\%$ .

Lymphoma NK/Ly also showed a well marked diurnal rhythm of cell division (Fig. 2). The minimal number of mitoses was observed, just as in Ehrlich's tumor, at 9 a.m. ( $MI = 12.6 \pm 0.6\%$ ). Later the number of dividing cells increased somewhat. A marked increase in mitotic activity occurred at 9 p.m. and reached its maximum at 3 a.m. ( $MI = 21.2 \pm 0.8\%$ ). Starting from this time and until 9 a.m. the number of dividing cells decreased.

The mean diurnal MI for the lymphoma NK/Ly was somewhat higher than that for Ehrlich's tumor, namely  $15.5 \pm 1.0\%$ .

The course of mitosis was disturbed in 11.5% of dividing Ehrlich's tumor cells and 13.6% of lymphoma NK/Ly cells. The forms of the pathological mitoses and their frequency are given in Table 1. No clear change in the relative number of pathological mitoses depending on variation of the mitotic activity in the tumors in the course of the 24-h period was detected.

These results show that for the ascites type of Ehrlich's tumor and lymphoma NK/Ly there is a clearly defined diurnal rhythm of cell division. Curves showing changes in mitotic activity of the tumor cells during the 24-h period are unimodal in character with a minimum of the number of mitoses of 9 a.m. and a maximum for Ehrlich's tumor at midnight and for lymphoma NK/Ly at 3 a.m. The number of C-mitoses also varied similarly during the 24-h period. The lowest values of  $MI_{colch.}$  in the Ehrlich's tumor and lymphoma NK/Ly were observed at 9 a.m., when they were  $11.4 \pm 1.5$  and  $22.8 \pm 4.7\%$ , respectively. The number of C-mitoses reached a maximum at the same times as the normal mitoses, i.e., at midnight in the Ehrlich's tumor and at 3 a.m. in the lymphoma NK/Ly; the corresponding values were  $31.7 \pm 3.0$  and  $42.0 \pm 5.1\%$ , respectively (Figs. 1 and 2).

Besides this rhythm of division of the tumor cells, some change in the duration of mitosis also took place in the course of the 24-h period. In the Ehrlich's tumor cells the duration of mitosis varied from 3.7 to 4.1, and in lymphoma NK/Ly from 3.1 to 3.6 h. The mean diurnal value of  $t_m$  for Ehrlich's tumor was 4 h, and for lymphoma NK/Ly 3.25 h (Figs. 1 and 2). So far as the duration of mitosis in the period of increased mitotic activity of the tumor cells is concerned, as Figs. 1 and 2 show, its duration was not increased. During this period mitosis in the cells of Ehrlich's tumor lasted 3.8 h and in the cells of the lymphoma NK/Ly it lasted 3.2 h.

The results of these experiments indicate that the change in the duration of mitosis in the cells of Ehrlich's tumor and lymphoma NK/Ly does not correlate with variations in their mitotic activity during

TABLE 1. Form and Number of Pathological Mitoses in Ehrlich's Tumor and Lymphoma NK/Ly

Test object	Total number of pathological mitoses (in %)	Form and number of pathological mitoses (mean diurnal value, in percent)								
		deletion of chromosomes in metaphases	deletion of chromosomes and their fragmentation on separation	bridge	scattering of chromosomes	monocentric mitosis	multipolar mitosis	asymmetrical mitosis	tubular metaphase	"group of three" metaphase
Ehrlich's tumor	11,5	6,9	12,1	12,3	6,2	18,5	3,1	4,3	30,2	6,4
Lymphoma NK/Ly	13,6	2,2	14,6	11,1	7,3	17,8	6,0	5,3	29,5	6,2

the 24-h period. Mitotic activity was increased not through the lengthening of mitosis, but through an increase in the number of dividing cells.

When the mean diurnal value of  $t_m$  had been determined, the duration of the cell cycle in Ehrlich's tumor and lymphoma NK/Ly also was calculated. It was assumed that all cells in the tumor population participated in division, i.e., the proliferative pool  $P_c = 100\%$ . The duration of the mitotic cycle for the cells of Ehrlich's tumor was 13.4 days and for lymphoma NK/Ly 8.7 days. The values of  $t_m$  and  $T_c$  for Ehrlich's tumor and lymphoma NK/Ly obtained in the present experiments were a little higher than those given in the literature [1, 10]. The reason evidently is that the present experiments were carried out at later periods of development of the tumors. During growth of ascites tumors the duration of all phases of the cell cycle, including mitosis [12], is increased. The traction of proliferating cells also is reduced. In addition, the duration of mitosis determined with the aid of colchicine must always be higher than its true value [9]. Nevertheless, the writer considers that the colchicine method can be used successfully to study problems similar to those which are the subject of this paper.

Ehrlich's tumor and lymphoma NK/Ly are characterized by a monophasic diurnal rhythm of mitosis with a maximum of cell division during the evening and night and a minimum in the morning. Meanwhile, in normal as opposed to malignant tissues, the maximum of mitotic activity occurs in the morning, and its minimum in the evening [2, 4-6, 8, 11]. The duration of mitosis in Ehrlich's tumor and in lymphoma NK/Ly varies somewhat during the 24-h period. However, the duration of mitosis was not increased at that time of day when the mitotic activity of the tumors was increased. The diurnal increase in mitotic activity in Ehrlich's tumor and lymphoma NK/Ly thus takes place, just as in some normal tissues [7], not by the prolongation of mitosis but because of the entry of more cells into mitosis.

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