# ONCOLOGY

# Relationship between Changes in the Photoperiod and Proliferation of Ehrlich Ascitic Tumor Cells

**O. G. Mashanova, Yu. A. Romanov, V. V. Evstaf'ev, and M. V. Semenova**

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The effect of changed photoperiod (constant darkness) on the proliferation of Ehrlich ascitic tumor cells was demonstrated. Some mechanisms maintaining the proliferative homeostasis in Ehrlich ascitic tumor under conditions of constant darkness were studied, explaining more leveled pattern of circadian rhythms of cell proliferation in animals kept under conditions of constant darkness and indicating the existence of circadian rhythm in sensitivity of Ehrlich ascitic tumor cell to permanent darkness.

**Key Words:** *chalones; photoregimen; constant darkness; Ehrlich ascitic tumor*

Study of the time regularities of cell proliferation is one of modern and important trends of chronobiology [2-4]. The hierarchic structure of regulation levels of the time organization of the proliferative system includes external and internal factors. Of external factors, the time indicators for these rhythms are particularly important for the regulation of cell multiplication rhythm [1,6], specifically photoperiodicity [5,7,8].

The aim of this study was chronobiological investigation of the effect of changed photoregimen (constant darkness) on the proliferation of Ehrlich ascitic tumor (EAT) cells.

## MATERIALS AND METHODS

The study was carried out on 90 random-bred male mice. Five to seven animals per point were examined. Diploid EAT strain was the object of the study.

Methods for evaluating the mitotic index (MI), graphic parametrical method for biological rhythm studies, analysis of correlations, and standard statistical methods for data processing [5] were used.

Russian State Medical University, Moscow. ***Address for correspondence:*** vladolg@email.ru. O. G. Mashanova

The parameters of circadian changes in the EAT cell mitotic activity were studied under conditions of standard photoregimen and constant darkness. Group 1 animals were kept under conditions of standard photoregimen (light:darkness (L:D)=12:12 h, light from 6.00 to 18.00) for 21 days before the experiment, after which the animals were transplanted EAT and the rhythm of mitotic activity was studied on day 5 of tumor development (L:D animals). Group 2 animals were kept under conditions of constant darkness during the same period (D:D animals). MI was evaluated at 11.00, 15.00, 16.00, 17.00, 18.00, 20.00, 23.00, 3.00, 4.00, 5.00, 6.00, 7.00, 9.00 and 11.0.

## RESULTS

The rhythmic structure of circadian changes in the integral mitotic index (ICI) was biphasic in L:D and D:D animals, but differed by many parameters (Fig. 1). For instance, the duration of active phase (AP) was 1.3 times longer in D:D animals in comparison with L:D ones, the absolute and relative amplitudes were 2.4 times lower (ICI fluctuation rhythm was more smooth in D:D animals). On the other hand, the mesor, number of dividing EAT cells over 24 h, and the pool

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of cells dividing during the rhythm AP were similar in the two groups. The number of EAT cells dividing over 24 h was 525.3 and 514.8% in L:D and D:D animals, respectively. This attests to intensely proliferating cell system in EAT under conditions of constant darkness. Opposite changes in the amplitudes and AP duration in D:D animals compensate for each other, while the ratio between cell numbers dividing during the AP and over 24 h remained unchanged and served as the mechanisms maintaining the proliferative homeostasis in EAT under conditions of constant darkness.

Circadian rhythms of indexes of individual phases of mitosis in EAT cells are coordinated in L:D animals (Fig. 1, *a*) and desynchronized with the ICI rhythm and between each other in D:D animals (Fig. 1, *b*). EAT cells of D:D animals unevenly move through the mitosis phases, while EAT cells in L:D animals demonstrate regular kinetics in the mitosis phases. The prophase index (PrI) rhythm of D:D animals is shifted to the right by the phase in comparison with PrI rhythm in L:D animals. The metaphase index rhythm of D:D animals was also changed: the first maximum was 4 h shifted to the right and the second was shifted by 2 h in comparison with L:D animals. The second maximum of telophase index rhythm was 3 h shifted to the left and the second maximum was shifted by 5 h to the left.

Analysis of correlations showed that exposure to constant darkness appreciably changed the ratio of individual mitosis phases in comparison with photoperiodicity conditions. The correlation between changes in the indexes of the neighboring mitosis phases in EAT of L:D animals was not high, but positive ($r$=0.29-0.33), while in D:D animals it was minor, or even absent. Hence, the passage of EAT cells through all mitosis phases sharply changed in D:D animals compared to L:D animals and became irregular. This resulted in notable leveling of circadian rhythms of EAT cell proliferation in D:D animals.

The rhythm of EAT ICI sensitivity to the constant darkness regimen was detected. There were no appreciable changes in ICI of D:D animals in comparison with ICI of L:D animals for 3 time points (15.00, 20.00, and 5.00). In the rest hours ICI of D:D animals differed from that of L:D animals, these changes opposite in different hours of day and night. In the morning and day hours cell reaction to darkness manifested in intense proliferation, while during the evening and night hours it was weaker in comparison with L:D animals.

Hence, EAT is a rapidly renewing cell system. High level of its proliferation is largely maintained by the AP of circadian rhythm of cell multiplication. The kinetics of EAT cells is characterized by even movement through mitosis phases.

The main changes in the 24-h kinetics of EAT cells under conditions of constant darkness are decreased amplitude of circadian rhythm of their multiplication and prolongation of its AP. These opposite shifts in the rhythm parameters of EAT proliferation maintain unchanged (*vs.* control) number of dividing cells in EAT, which provides the proliferative homeostasis.

The reaction of EAT cells to factors leading to inhibition and stimulation of their proliferation is observed during the same hours of the day and night in animals exposed to photoperiodicity and constant darkness, but this reaction is weaker in animals exposed to constant darkness. EAT cells are characterized by

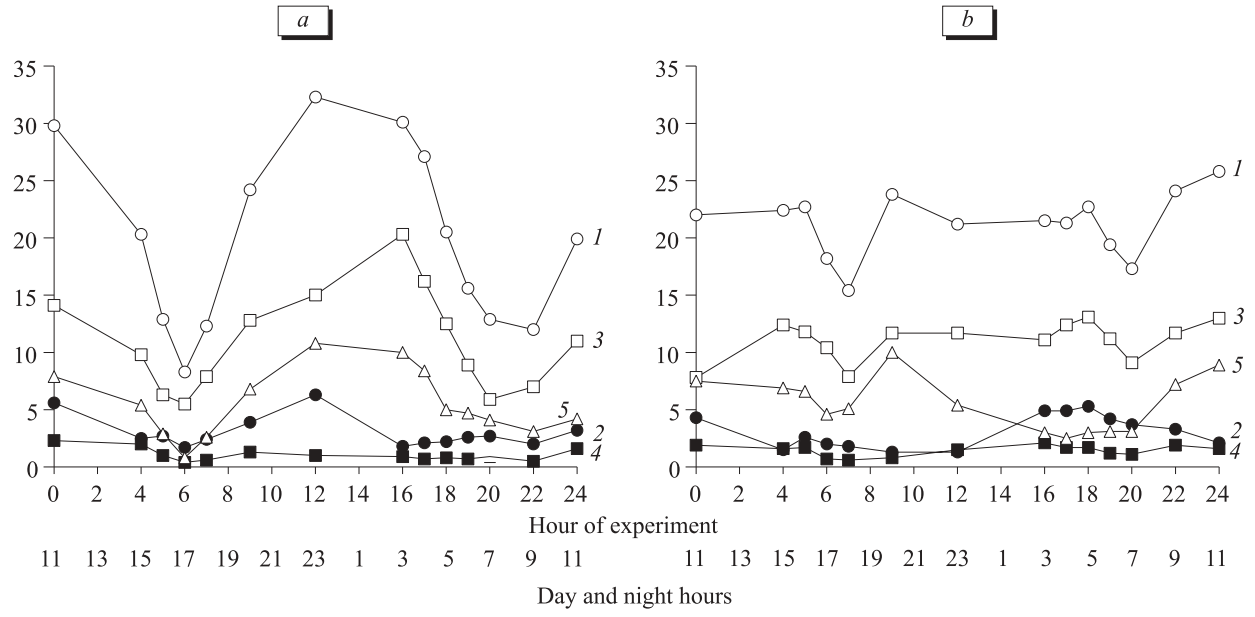


**Fig. 1.** Changes in integral mitotic index (IMI) and indexes of individual mitosis phases (%) in Ehrlich ascitic tumor cells during 24 h in L:D (*a*) and D:D animals (*b*). *1*) ICI; *2*) prophase; *3*) metaphase; *4*) anaphase; *5*) telophase index.

circadian rhythm of sensitivity to constant darkness: increased of proliferation during the morning and day hours and decreased proliferation during the evening and night hours in comparison with animals kept under conditions of photoperiodicity. Opposite reactions of EAT proliferative system in different hours is also a mechanism maintaining the proliferative homeostasis in the tumor under conditions of constant darkness.

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