INVESTIGATION OF PARAMETERS OF THE MITOTIC CYCLE IN EPITHELIUM OF THE MOUSE ESOPHAGUS ON REVERSAL OF THE PATTERN OF LIGHT AND DARKNESS

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One of the main problems in the chronobiology of cell division is the study of regulation of biological rhythms of cell proliferation by time detectors. In some studies of reorganizations of the rhythm of cell proliferation during changes in the principal external time detector — the cycle of daylight and darkness — it was shown that rhythms of mitotic activity and DNA synthesis in different tissues are resynchronized with the new photoperiodic conditions in the course of 10-40 days [1, 3, 10-12]. However, the mechanisms of the reorganization have not yet been explained. To do so requires, in particular, a study of the state of the parameters of the mitotic cycle (MC) in the period of reorganization of the rhythm of cell division. Yet there are no data on this problem in the literature.

In the investigation described below parameters of MC of esophageal epithelial cells of mice were investigated at different times after reversal of the cycle of daylight and darkness.

EXPERIMENTAL METHOD

Experiments were carried out on 500 noninbred male albino mice (average weight 24 g), adapted for 3 weeks to standard conditions (12 h daylight — 12 h, darkness, daylight from 8 a.m. to 8 p.m.; temperature 23 ± 1°C; food *ad libitum*), and divided into four groups. The mice of group 1 were controls, whereas the animals of groups 2, 3, and 4 were kept under conditions of reversal of daylight and darkness (daylight 12 h — daylight 12 h, light from 8 p.m. to 8 a.m.) for 5, 10, and 17 days, respectively. The animals of all groups were divided into two subgroups. In each group the mice of the first subgroup received an injection of ^{3}H-thymidine all at the same time, at 2 p.m., whereas those of the second subgroup received the same injection at 2 a.m.; the animals were sacrificed 1, 2, 3, 4, 5, 7, 9, and 11 h later, and thereafter at 3-hourly intervals until 32 h after the beginning of the experiment. All mice received ^{3}H-thymidine by intraperitoneal injection in a dose of 0.7 μCi/g body weight (specific activity 4.1 Ci/mmole). The esophagus was taken for investigation, and sections 5 μ thick were coated with type M photographic emulsion and exposed for 3 weeks at 4°C. The preparations were stained with Ehrlich's hematoxylin. The percentage of labeled mitoses (LM) was counted in 50-100 mitoses from each animal in the basal layer of the esophageal epithelium. Mitoses were counted as labeled if there were four or more granules of reduced silver above them. The parameters of MC were determined by the method of Quastler and Sherman [9]; data obtained in each group on the animals of the first and second subgroups were averaged. Statistical analysis was carried out by the Fisher–Student method.

EXPERIMENTAL RESULTS

Data on changes in the percentage of LM in the esophageal epithelium of the mice of groups 1-4 during 32 h after injection of ^{3}H-thymidine are in Fig. 1. They show that the character of the curves is similar for all groups of the experiment. Both in the control and after reversal of daylight and darkness the number of LM at the time of the first maximum, 5-7 h after the beginning of the experiment, was 100%. The labeling intensity was between 40

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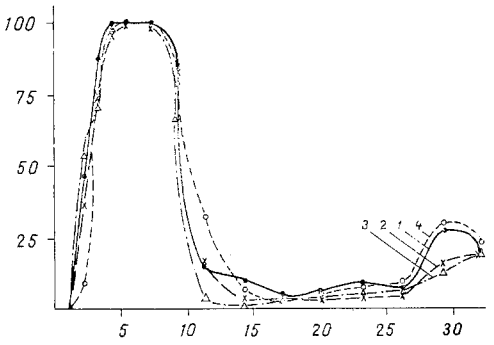

Fig. 1. LM in cells of basal layer of esophageal epithelium of mice in control and at various times after reversal of daylight and darkness. 1) Control; 2) 5 days, 3) 10 days, 4) 17 days after reversal of daylight and darkness. Abscissa, time after injection of ^{3}H-thymidine (in h); ordinate, index of LM (in %).

TABLE 1. Duration of MC and Its Periods in Basal Layer of Esophageal Epithelium of Mice in Control and at Various Times after Reversal of Daylight and Darkness

Periods of MC, h	Control	Time after reversal, days		
		5	10	17
G_2 min	1,0	1,0	1,0	2,0
$G_2+{}^1/_2$ M	2,0	2,4	2,4	2,7
S	7,7	7,4	7,1	7,3
$G_1+{}^1/_2$ M	15,3	15,2	15,5	14,0
T	25,0	25,0	25,0	24,0

and 50 grains of silver. A second maximum of LM, reaching between 19 and 31% in different experimental groups, was observed 29-32 h after the injection of thymidine. The number of grains of silver above mitoses was 20-25. The number of LM between the two maxima was small, on average 5%; as a rule, moreover, the intensity of labeling above mitoses at this period was low (4-6 grains of silver).

The results are evidence that the principles governing the kinetics of cells in MC are unchanged after reversal of daylight and darkness although at that time significant changes are known to take place in the rhythm of cell division [1, 3, 10-12]. On the basis of the observed 100% level of LM during the first maximum and the very small number of weakly labeled mitoses between the first and second maxima of LM, it can be concluded that in both the control and the experimental animals, only those cells which have previously passed through the S-phase of MC enter into mitosis. A small proportion of cells, on completing division, immediately enter the next MC, as shown by the presence of a second maximum of LM and by dilution of the label above LM 29-32 h after injection of ^{3}H-thymidine.

Results of determination of the duration of the different phases of MC in the control and after reversal of daylight and darkness are given in Table 1. They show that the duration of the phases of MC in epithelial cells of the mouse esophagus after reversal do not differ significantly from the control values, in agreement with data in the literature [2, 4-8].

The results of these investigations thus indicate that reorganization of the rhythm of cell proliferation observed after reversal of daylight and darkness takes place without any appreciable changes in the kinetics of the cells in MC. Under these conditions intracyclic mechanisms regulating proliferative processes in cell populations are evidently not significantly disturbed.

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