CELL-POPULATION INTERRELATIONS IN MECHANISMS OF THE CIRCADIAN RHYTHM OF PROLIFERATION IN LINGUAL EPITHELIUM OF MICE EXPOSED TO CONTINUOUS LIGHT

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Separate cell populations, participating in the formation of a circadian rhythm of proliferative activity, and differing in the degree of their participation in this process, are known to exist [2-4] in mammalian tissues.

The aim of this investigation was to study whether the principles of cell-population interrelations found in normal animals are also observed in animals kept for a long time during constant exposure to light, in which [1] the circadian rhythm of cell proliferation is disturbed.

EXPERIMENTAL METHOD

Experiments were carried out on 380 male mice weighing 22-25 g. The animals were kept in the animal house with an intensity of illumination of 250 lx and with food ad lib. Mice of group 1 were kept in alternating light and darkness (light from 8 a.m. to 8 p.m.). Animals of group 2 were kept for 1.5 months in constant light. At 1 a.m. (the time of maximal DNA synthesis in the circadian rhythm [3]) all animals were given a single injection of [^{3}H]-thymidine in a dose of 0.7 μCi/g body weight (specific activity 4.1 Ci/mole). Animals of each group were killed 1, 2, 3, 4, 5, 6, 7, 9, 10, 11, and 13 h, and thereafter every 2 h until 37 h after injection of the isotope. Histological sections of the tongue 5 μ thick were covered with type M liquid emulsion. Exposure lasted 25 days. In sections stained with Mayer's hematoxylin the basal layer of the dorsal surface of the tongue was studied and the mitotic index (MI), percentage of labeled mitoses (PLM), number of labeled mitoses as a ratio of the total number of basal cells — the index of labeled mitoses (ILM) and, in some cases, the intensity of labeling of dividing cells (ILDC) were determined. MI and ILM were determined after examination of up to 15,000 cells in each animal and were expressed in promille; to determine PLM 25-100 mitoses were analyzed in each case. Parameters of the mitotic cycle (MC) were found by the method of Quastler and Sherman.

EXPERIMENTAL RESULTS

A circadian rhythm of MI of cells of the basal layer of epithelium of the dorsal surface of the tongue (Fig. 1) with maximal values at 6 a.m. and minimal at 6 p.m. ($P < 0.001$) was found in the animals of group 1. ILM in these animals changed in the course of the experiment, forming two peaks coinciding in time with an increase in MI in the circadian rhythm (Fig. 1). The first rise of ILM virtually repeated the MI curve. This is evidence that injection of the isotope occurred during the period of intensification of DNA-synthesizing activity of the epithelial cells in the circadian rhythm. During the next rise of MI, ILM was 18-25‰. The results of determination of ILDC showed that during this period this fraction of the cells divided a second time (at the 5th-7th hour of the experiment ILDC was 26.4-30.1, falling by the 29th-33rd hour to 11.6-14.0, i.e., by about half). These results indicate that on the 2nd day of the investigation the curve of circadian rhythm of MI, during the period of its stimulation, is formed by two cell populations which differ from one another in their preceding fate. One cell population, accounting for about 18-25%, during the previous day

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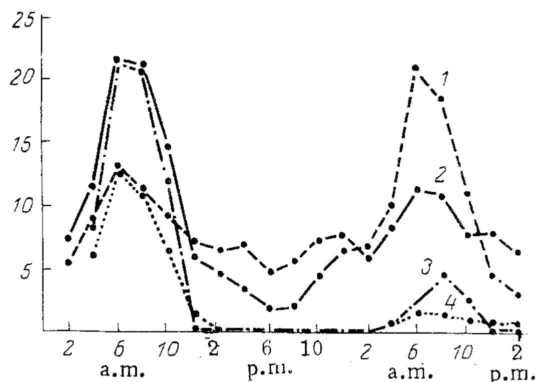

Fig. 1

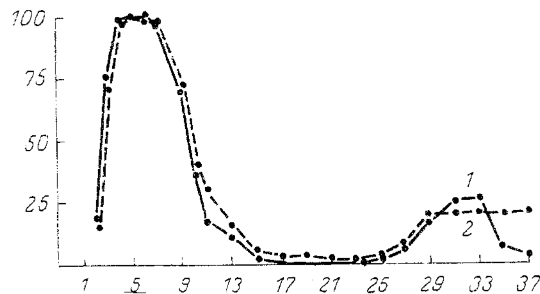

Fig. 2

Fig. 1. Circadian changes in MI and ILM of basal layer of dorsal surface of tongue of mice receiving a single dose of [^{3}H]thymidine. Abscissa, clock time; ordinate, MI and ILM (in %). 1, 2) MI; 3, 4) ILM. 1 and 3) Mice of group 1; 2 and 4) mice of group 2.

Fig. 2. Changes in PLM of cells of basal layer of dorsal surface of mouse tongue with time after a single injection of [^{3}H]thymidine. Abscissa, time after injection of isotope (in h); ordinate, PLM. 1) Mice of group 1; 2) mice of group 2.

passed synchronously through MC, whereas the other (about 75%) was outside MC at that time. Analysis of the PLM curve (Fig. 2) enabled the parameters of MC to be determined. The minimal value of tG_2 was 2.5 h and tS was 7.1 h. The total duration of MC for cells passing synchronously through two successive cycles was about 26 h, i.e., about equal to the period of the circadian rhythm.

Rhythmic changes in MI also were observed in the animals of group 2, with maxima at the same times of day as in the mice of group 1 (Fig. 1). However, these fluctuations were much smaller in amplitude, possibly evidence of the desynchronization of cell proliferation in the course of the 24-h period. The amplitude of MI decreased on account of both a decrease in its maximal values and an increase in minimal. As a result the mean values of MI for the 24-h period were about equal in mice of the two groups (8.5 ± 1.0 and 7.6 ± 1.1, respectively). This evidently means that the number of cells capable of dividing in the course of 24 h was about the same in animals of the two groups. The alternation of light and darkness was the factor synchronizing cells already competent to divide.

ILM in the animals of group 2, just as in the mice of group 1, formed two peaks in the course of the experiments, which coincided in time with periods of an increase in MI (Fig. 1). The first peak of MI was 70-93% attributable to labeled mitoses, the second only 16-17%. Consequently ILM, like MI, points to the presence of desynchronization.

Determination of the duration of phases of MC from the PLM curve in mice of group 2 (Fig. 2) gave the following results: tG_2 = 2.5 h, tS = 7.3 h. The total duration of MC for 16-17% of cells was close to the duration of the circadian rhythm, namely about 27 h (ILDC at the 5th-7th hour of the experiment was 27.9-30.4, at the 29th-33rd hour 11.4-13.8, i.e., reduced by about half). Analysis of PLM curves for the animals of the two groups revealed some small degree of desynchronization of the cell kinetics in mice of group 2.

The study of cell kinetics thus revealed that it was desynchronized in mice of group 2 compared with those of group 1. The desynchronization process was much more characteristic of the total cell population (reflected in MI) than for the population of labeled cells (reflected in PLM). This indicates that desynchronization of cell division in the circadian rhythm of animals kept exposed to constant light was evidently not connected with desynchronization of cell kinetics in one population chosen separately. The process of desynchronization of the general cell kinetics in the course of the 24-h period is probably due to differences in the state of the separate cell populations entering MC at different times of day. It can be concluded from the results that interpopulation relations in mice of this group were modified, but they continued to function, as a result of which cell division under the conditions mentioned above follows a rhythmic pattern.

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