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ROLE OF DIFFERENT MOUSE ESOPHAGEAL EPITHELIAL CELL POPULATIONS IN FORMATION OF THE CIRCADIAN RHYTHM OF PROLIFERATION

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KEY WORDS: circadiam rhythm; cell populations; heterogeneity.

The degree of participation of the cell population passing synchronously through the mitotic cycle (MC) in the formation of the circadin rhythm of mitotic activity was studied previously [1, 2] over a period of several days. However, the role of the asynchronous population in the formation of the circadian rhythm of cell proliferation has not yet been elucidated [3], and the investigation described below was undertaken to study this problem.

EXPERIMENTAL METHOD

Noninbred male albino mice weighing 25 g were kept in the animal house under conditions of 12 h daylight and 12 h darkness (daylight from 8 a.m. to 8 p.m.). There were two series of experiments. The animals in both series were given a single injection of [^{3}H]thymidine in a dose of 1 μCi/g body weight (specific radioactivity 8.8 Ci/mole). The mice of series I (n = 235) were given the isotope at 1 a.m., the mice of series II (n = 205) at 1 p.m., at times of maximal and minimal DNA-synthetic activity of cells of the stratum basale of the esophageal epithelium; the index of labeled nuclei (ILN) was 155 and 72°/₀₀ respectively. The animals were killed 1, 2, 3, 4, 5, 7, and 9 h later, and thereafter every 2 h for 90 h (series I) and 78 h (series II) after injection of the isotope. Paraffin sections of the esophageal epithelium were coated with type M emulsion (Moscow Technical Photographic Plate Factor) and exposed for 45 days. On the basis of analysis of 5000-10,000 cells of the stratum basale in each case the mitotic index (MI) and the index of labeled mitoses (ILM — the number of labeled mitoses divided by the total number of cells of the stratum basale) were determined and expressed in pro mille. The intensity of labeling of dividing cells (ILDC) also was determined. The numerical results were subjected to statistical analysis by Student's test.

EXPERIMENTAL RESULTS

A circadian rhythm of MI was found for the animals in both series, with maxima at the end of the dark and beginning of the light periods of the day (Figs. 1a and 2a).

Investigation of ILM in the animals of series I showed (Fig. 1b) that it changed in the course of the experiment, with four peaks corresponding in time with periods of maxima of MI in the circadian rhythm. The first peak of ILM practically repeated the MI curve (about 76%

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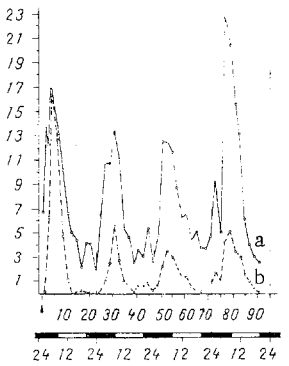

Fig. 1

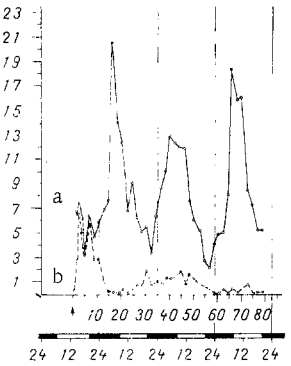

Fig. 2.

Fig. 1. Changes in ILM and MI in stratum basale of esophageal epithelium during an experiment lasting 78 h (experiments of series I). a) MI, b) ILM. Abscissa: top scale — time after injection of [^{3}H]-thymidine (in h), bottom scale — time of 24-h period; ordinate, ILM and MI (in $^0/_{00}$). Shaded regions of bottom scale indicate dark time of 24-h period. Vertical lines mark boundaries between days. Arrow — time of injection of [^{3}H]thymidine.

Fig. 2. Changes in ILM and MI in stratum basale of esophageal epithelium during an experiment lasting 78 h (experiments of series II). Legend as to Fig. 1.

of all mitoses were labeled in the interval from 4 a.m. to noon, and at the peak of MI the fraction of labeled mitoses reached 99%). This is evidence that injection of the isotope in fact occurred at a peak period for the number of DNA-synthesizing cells in the circadian rhythm. On the 2nd-4th days the fraction of labeled mitoses participating in the formation of acrophases of the rhythm, MI was 24.3, 23.4, and 22.3% respectively. Analysis of ILDC showed that above mitoses of the first wave of ILM there were on average 25 grains of reduced silver, compared with 13 grains above mitoses of the second and third waves, and 7.5 grains above mitoses of the fourth wave. These data confirm that the second, third, and fourth peaks of ILM on the 2nd and subsequent days were formed by different groups of cells. Peaks of ILM on the 2nd and 3rd days of the experiment were evidently formed by cells which passed through MC only on the 1st day, whereas the peak of ILM on the 4th day was formed mainly by cells which had passed previously through two MC (the first on the 1st day, the second on the 2nd or 3rd day of the experiment).

Changes in ILM found in animals of series II (Fig. 2b) differed significantly from changes in ILM in the mice of series I. The cell population incorporating the isotope during the minimum of DNA-synthesizing activity in the circadian rhythm did not take part in formation of the acrophase of MI on the 2nd day of the experiment (14-22 h after injection of [^{3}H]thymidine). The reason may possibly be that at this time the labeled cell population had only just completed the first MC and could be only in the G_1-S periods of the next MC, but not in mitosis. On the 3rd day of the investigation (38-46 h after injection) elevation of the ILM level was observed at the same times as the acrophase of MI, but the fraction of labeled mitoses taking part in the formation of the MI peak was small, only 8.1%. The data for ILDC indicate that at this time there was a second division of some of the cells of the population which had passed through MC on the 1st day (ILDC 3-7 h after injection was 22 grains per nucleus, and 38-46 h after injection it was 12 grains). On the 4th day of the investigation ILM was very low (0.1-0.6$^0/_{00}$) and, consequently, the contribution of the labeled population to formation of the acrophase of the MI rhythm was extremely small (0.03%). From five to nine grains of silver were observed above the dividing cells in the acrophase period of the rhythm on the 4th day. This may indicate that some of the cells which divided on the 4th day had passed previously through two MC, but some had passed through only one.

The results thus indicate that the role of the labeled populations in formation of the acrophases of the circadian rhythm of MI differs. For instance, the cell population which

passed synchronously through MC during the 1st day played a considerable part in the formation of acrophases of MI on subsequent days (experiments of series I). This suggests that some cells of the synchronous population preserved relatively high proliferative ability for several days. This, in turn, can be explained on the grounds that the duration of MC of these cells was comparable with the period of the rhythm [2], and for that reason these cells could be restimulated to pass through the next MC after completing the previous cycle. It must be noted that some cells which passed synchronously through the first MC probably then passed into the G_0 phase, in which they remained for 2-3 days, and were capable of responding to the synchronizing stimulus on the 3rd-4th day of the experiment.

Meanwhile the contribution of the population proliferating asynchronously for 24 h to the formation of acrophases of the circadian rhythm of MI was very small (experiments of series II). However, this population also remained capable, to a certain degree, of responding later (on the 3rd day) to the action of the synchronizer.

Investigation of ILM also showed that an approximately equal fraction of the cells of the labeled populations of the two series takes part in proliferation during periods of low mitotic activity in the circadian rhythm For instance, in the interval from 2 p.m. to 2 a.m. every day 10-17% of mitoses were labeled. Consequently, the population of cells proliferating synchronously during the 1st day of the experiment took part on subsequent days in the formation both of acrophases and of troughs of MI. Meanwhile the asynchronously proliferating population, which played virtually no part in acrophase formation, proliferated sufficiently actively during troughs of MI.

It can be concluded from the facts described above that the kinetics of populations passing through MC at different times of the 24-h period differs significantly.

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CHANGES IN RESPONSE OF CIRCULATING COLONY-FORMING UNITS TO ACTH IN THYMECTOMIZED MICE

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With increased concentrations of endogenous glucocorticoids the number of hematopoietic stem cells circulating in the peripheral blood decreases and migration of colony-forming units (CFUs) from a region of bone marrow screened during irradiation is inhibited [1, 2]. There is experimental evidence also of the role of T lymphocytes and thymus hormones in regulation of the function of hematopoietic stem cells, including in their recirculation *in vivo* [5-9, 11].

The object of this investigation was to study responses of circulating CFUs to elevation of the endogenous glucocorticoid level in thymectomized mice.

EXPERIMENTAL METHOD

Experiments were carried out on (CBA × C57Bl)F_1 mice of both sexes weighing 22-28 g. To determine the number of circulating CFUs blood was collected in a heparinized vessel from five decapitated donor mice and the number of leukocytes in it was counted. The blood thus obtained was diluted with medium 199 in the ratio of 1:1 and injected in a volume of 0.4 ml into the caudal vein of syngeneic lethally irradiated recipients. The number of macro-

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