CIRCADIAN RHYTHMS OF KINETICS OF MOUSE ESOPHAGEAL EPITHELIAL CELLS

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The circadian rhythm of cell division and DNA synthesis and parameters of the mitotic cell cycle during the maximum and minimum of proliferation in the course of the 24-h period were studied in the epithelium of the mouse esophagus. The number of mitoses and DNA-synthesizing cells increased regularly at 1-7 a.m. and 10 p.m.-4 a.m. respectively. When [^{3}H]-thymidine was injected into the mice at 2 a.m. the duration of the periods of the mitotic cycle was as follows: $G_{2\,min}$ = 1 h, $G_2 + {}^1/_2M$ = 2 h, S = 7.1 h, and $G_1 + {}^1/_2M$ = 15.9 h. When [^{3}H]-thymidine was injected at 2 p.m. the duration of the S-period was increased to 8.2 h, and that of $G_1 + {}^1/_2M$ to 14.8 h. The total duration of the mitotic cycle in both series of experiments was 25 h. The duration of the individual phases of the mitotic cycle thus depends on the time of day and correlates with rhythmic changes in the number of dividing and DNA-synthesizing cells. The duration of the mitotic cycle is the same for cells passing through it at different times of day and is approximately the same as the period of the circadian rhythm of mitosis and DNA synthesis in the esophageal epithelium.

KEY WORDS: circadian rhythm; mitosis; mitotic cycle.

One aspect of the kinetics of cell populations which is attracting attention at the present time is the problem of correlation between parameters of the mitotic cycle (MC) and the circadian rhythm of cell reproduction [2, 5]. Some workers have found [8-10] that the duration of the periods of MC is constant at different times of the 24-h period. According to other workers [1, 6, 7, 11, 14], on the other hand, the duration of the G_2 and S periods of MC varies in the course of the 24 hours.

The object of this investigation was to study the circadian rhythm of cell division and DNA synthesis in the esophageal epithelium of mice and parameters of the mitotic cell cycle during the wave of proliferation and its decline in the course of the 24-h period.

EXPERIMENTAL METHOD

Experiments were carried out on 190 noninbred male albino mice with a mean body weight of 23 g, after adaptation for 3 weeks to constant conditions (light:darkness 12:12 h, light from 8 a.m. to 8 p.m.; temperature 23 ± 1°C; access to food ad lib.). The mice were divided into three groups. In the mice of group 1 the circadian rhythms of dividing (mitotic index – MI) and DNA-synthesizing (radioactive index – RI) cells were investigated. The animals were killed at 3-hourly intervals over a period of 48 h. [^{3}H]Thymidine was injected 1 h before sacrifice. The duration of the periods of MC was determined in the mice of groups 2 and 3 from the curve of change in the percentage of labeled mitoses [13]. [^{3}H]Thymidine was injected into these animals simultaneously at 2 p.m. (group 2) or at 2 a.m. (group 3), and the animals were sacrificed 1, 2, 3, 4, 5, 7, 9, and 11 h after the injection, and thereafter at 3-hourly intervals until the 32nd hour of the experiment. [^{3}H]Thymidine was injected intraperitoneally into the mice of all groups in a dose of 0.7 μCi/g body weight (specific activity 4.1 Ci/mmole). The esophagus was removed for investigation and sections through it, 5 μ in thickness, were coated with type M (State Research Institute of Photographic Chemistry) photographic emulsion and exposed for 3 weeks at 4°C. The sections were stained with Ehrlich's hematoxylin. MI was counted in 10,000 cells in the basal layer of the esophageal epithelium, RI in 3000-5000 cells, and the index of labeled mitoses in 50-100 mitoses in each animal. MI and RI were expressed in pro mille, and the index of labeled mitoses in per cent. - Nuclei and mitoses with four or more grains of reduced silver above them were regarded as labeled. Statistical analysis of the data was carried out by the Fisher–Student method.

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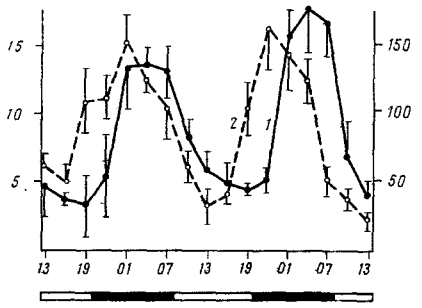

Fig. 1. Changes in MI (1) and RI (2) in the course of 48 h. Abscissa, time of day (hours); ordinate: left, MI (in ‰), right, RI (in ‰).

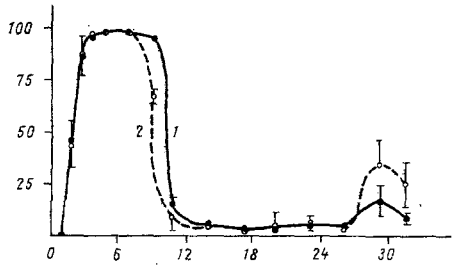

Fig. 2. Curve of labeled mitoses after a single injection of [^{3}H]thymidine. 1) Thymidine injected at 2 p.m.; 2) thymidine injected at 2 a.m. Abscissa, time after injection of [^{3}H]thymidine (in h); ordinate, index of labeled mitoses (in %).

EXPERIMENTAL RESULTS

Changes in MI and RI in the course of 48 h are illustrated in Fig. 1. It is clear that the curve of the circadian rhythm of cell division is monomodal, with a maximum at night and in the early morning (1-7 a.m.) and a minimum in the evening (4-7 p.m.; $P = 0.002$). A rhythm of DNA synthesis was also found, with a maximum at 10 p.m.-4 a.m. and a minimum at 1-4 p.m. ($P = 0.001$), so that its phases precede those of the rhythm of cell division by 3-6 h. According to data in the literature, the rhythm of cell division and the rhythm of DNA synthesis in the mouse esophageal epithelium are similar in character [3, 4, 12].

Changes in the percentage of labeled mitoses in the course of 32 h after injection of [^{3}H]thymidine into the mice of groups 2 and 3 are shown in Fig. 2. In animals receiving [^{3}H]thymidine during the period of maximal DNA-synthetic activity (2 a.m.) the duration of the periods of MC was as follows: G_2 min = 1 h, $G_2 + 1/2M = 2$ h, $S = 7.1$ h, $G_1 + {}^1/_2M = 15.9$ h; $T = 25$ h. In animals receiving [^{3}H]thymidine during the period of minimal DNA-synthetic activity (2 p.m.) the corresponding values were: $G_2 = 1$ h, $G_2 + {}^1/_2M = 2$ h, $S = 8.2$ h, $G_1 + {}^1/_2M = 14.8$ h; $T = 25$ h.

At different times of day and night cells thus pass through different phases of MC at different speeds. Shortening of the S-period in esophageal epithelial cells during the period of maximal DNA-synthetic activity, as found in the present investigation, was also observed in the epithelium of the hamster retrobuccal pouch [11], mouse cornea [7], and rat thyroid gland [6]. In the present experiments no changes were found in the duration of the G_2 period, but in other experiments not described in this paper the duration of the G_2 period also showed circadian variations.

Differences in the size of the second wave of labeled mitoses in the animals of groups 2 and 3 were noted. In the cell population tagged with [^{3}H]thymidine at night, twice as many cells took part in the second cycle as in the cell populations tagged with the isotope during the day (Fig. 2). Meanwhile the second maximum of labeled mitoses in both cases occurred 29-32 h after injection of [^{3}H]thymidine and the total duration of MC

was the same. Consequently, changes in one phase of the cycle correspond to opposite changes in the other phase, which suggests that mutual compensation of the phases of MC evidently takes place.

It can be concluded from the results described above that the duration of the individual phases of MC depends on the time of day or night and correlates with changes in the number of DNA-synthesizing and dividing cells in the esophageal epithelium during the 24-h period. Meanwhile, the duration of MC is virtually identical for cells passing through it at different times of the 24-h period and is close to the period of the circadian rhythm of DNA synthesis and mitotic activity in the epithelium of the mouse esophagus.

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