CIRCADIAN RHYTHM OF CELL PROLIFERATION IN THE MOUSE ESOPHAGEAL EPITHELIUM

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Many investigations have shown changes in the duration of periods of the mitotic cell cycle (MC) in the course of the 24 hours [1-4]. As a rule, however, the time of injection of [^{3}H]-thymidine in the investigations cited above was not linked with any particular phase of the circadian rhythm (CR) of cell reproduction. The period of observation also was usually 1-1.5 days [3-4].

The purpose of the present paper is the investigation of the kinetics of cell populations after the introduction of [^{3}H]thymidine during periods of maximum and minimum DNA synthetic activity of the cells over the course of 4 days.

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.). In the experiments of series I (control) changes in the index of labeled nuclei (ILN) during the 24-h period were studied. For this purpose, every 2 h the mice (five animals in each group) were given an injection of [^{3}H]thymidine. The mice were killed 45 min after the injection. Animals of series II (n = 235) and series III (n = 205) received a single injection of [^{3}H]thymidine at 1 a.m. and 1 p.m. respectively (times of maximal and minimal number of DNA-synthesizing cells during CR, established from the data of the experiments of series I). Five animals from each experimental series were killed after 1, 2, 3, 4, 5, 7, and 9 h, and thereafter every 2 h for 90 h (series II) and 78 h (series III) after injection of the isotope. In all series [^{3}H]-thymidine was injected in a dose of 1 μCi/g body weight (specific radioactivity 8.8 Ci/mole). Paraffin sections through the esophagus were coated with type M emulsion (Moscow Technical Photographic Plate Factory) and exposed for 45 days. The kinetics of cells of the basal layer of the epithelium and the parameters of their MC were determined from the labeled mitoses (LM) curve on the basis of analysis of 25-100 mitotic figures in each case [5]. The intensity of labeling of the dividing cells (ILDC) also was determined. The results were subjected to statistical analysis by Student's method.

EXPERIMENTAL RESULTS

It will be clear from Fig. 1a that the cell population which incorporated the isotope during the period of the peak ILN in CR (ILN 155‰) formed five waves of LM. The height of the 2nd, 3rd, 4th, and 5th waves was about equal (24-28%). Analysis of ILDC showed that on average there were 25 grains of reduced silver above the dividing cells of the 1st wave of LM, 13 grains above mitoses of the 5th wave. This indicated that the 2nd, 3rd, and 4th waves of LM are formed mainly by cells which have passed through MC only during the first day, whereas the 5th wave is formed by cells which have passed through two successive cycles: on the 1st and on either the 2nd or 3rd day. In other words, the fate of cells which passed through MC on the 1st day differed: Some of these cells divided again on the 2nd day, others on the 3rd day of the experiments. Some of these repeatedly dividing cells can enter the cycle a 3rd time, to form the 5th wave of LM. Our results do not rule out the existence of cells which have passed through four successive cycles of division, and also cells starting to proliferate only on the 1st day and again on the 4th day of the experiments.

The LM curve, reflecting the kinetics of the cells incorporating isotope during the period of the ILN minimum (72‰, III series of experiments, Fig. 1b), differs in an essential manner from the

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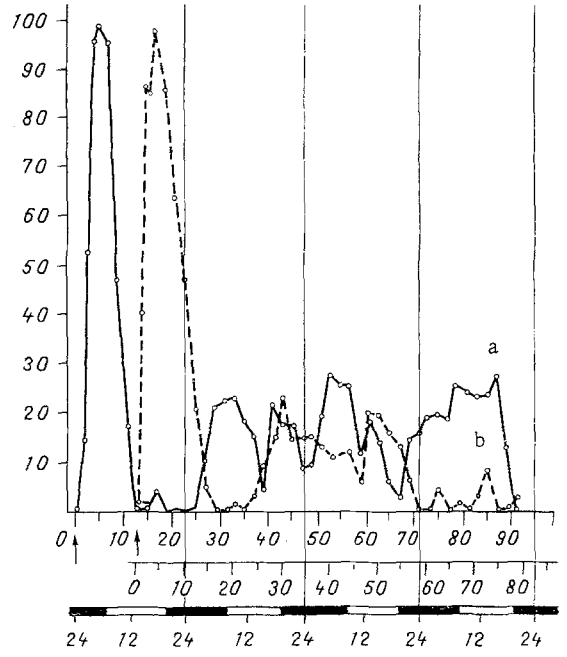

Fig. 1. Changes in percent LM in basal layer of esophageal epithelium after a single injection of [^{3}H]thymidine at 1 a.m. (experiments of series II, curve a) or at 1 p.m. (experiments of series III, curve b). Abscissa: top scale — time from moment of injection of [^{3}H]thymidine into animals of experiments of series II (in h), middle scale — the same, in experiments of series III, bottom scale — time of day (in h); ordinate, percent LM. Shades areas of bottom scale — period of darkness. Vertical lines indicate boundaries between 24-h periods. Arrows give time of injection of [^{3}H]-thymidine.

II series. After the second wave a sudden reduction of the percentage of LM does not occur. The magnitude of the third LM wave observed on the third day is less than the magnitude of the third wave in the II series (20 and 28‰, respectively). There are on the average 22 grains of reduced silver above the dividing cells of the first wave, while over mitoses of the second and third waves, 12 grains. This apparently indicates that the majority of the cells forming the third LM wave first enters into the MC only on the first day. Above the mitoses of the fourth wave, 5.5 grains of silver were observed. Consequently, cells forming the fourth wave before the menter into two MC — on the first and second day or on the third day. The small amplitude of the fourth LM wave, obtained in the III series of experiments (8%), attests to the fact that in the composition of the cell population incorporating isotope during 13 h, there are fewer cells capable of prolonged continuous proliferation.

Determination of the parameters of MC in the experiments of series II and III gave the following results: for cells passing through MC in the period of peak ILN: $T = 24.3$ h, $t_S = 6.0$ h, $t_{G_2 min} = 1.5$ h, $t_{G_2 + {}^1/_2 M} = 2.9$ h, $t_{G_1 + {}^1/_2 M} = 15.4$ h; for cells passing through MC during the period of minimum of ILN these values were: 25.6, 8.4, 1.0, 2.2, and 15.0 h respectively.

Investigation of the kinetics of the cell populations after injection of a single dose of [^{3}H]thymidine in different phases of CR of proliferation thus showed no significant differences in the duration of MC of cells taking up the isotope in periods of maximal and minimal values of ILN (although the duration of the synthetic period of these populations differed by 2.4 h). A more active part of cells labeled during the period of peak ILN in proliferation also was observed for a long time, possibly on account of their greater ability to pass through several successive MC compared with cells incorporating [^{3}H]thymidine during the period of a minimum of ILN, and also the more synchronized passage through successive MC of cells incorporating the isotope during the period of peak ILN. The results also show that after completion of the first MC most cells pass into the G_0, the duration of which differs for different cell populations.

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