CHARACTERISTICS OF MITOTIC ACTIVITY IN CELL POPULATIONS OF STRATIFIED SQUAMOUS EPITHELIUM OF DIFFERENT DEGREES OF DIFFERENTIATION

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A diurnal rhythm of mitosis was found in the stratum basale and stratum spinosum of the corneal and esophageal epithelium in mice. A diurnal rhythm of DNA synthesis was found in the esophageal epithelium. Proliferative ability is sharply reduced in the stratum spinosum.

The ability of cells to divide by mitosis is determined not only by the effect of factors such as hormones, metabolites, etc., on cell reproduction but also to a large extent by the specific features of intracellular metabolism. It is therefore important to study the division of cells in the course of their development and differentiation, which are accompanied by modifications of cell metabolism.

The object of the present investigation was to study mitotic activity in the cells of the stratum basale and stratum spinosum of the corneal epithelium of rats and the esophageal epithelium of mice. The ability of cells of the stratum basale and stratum spinosum of the esophageal epithelium of mice to synthesize DNA also was investigated.

EXPERIMENTAL METHOD

In the experiments of series I the intensity of cell reproduction in the stratum basale and stratum spinosum of the corneal epithelium was determined by calculating the mitotic index (MI: MI_1 and MI_2 respectively, in pro mille) in 68 male albino rats (mean weight 115 g). For this purpose, between 20,000 and 25,000 cells in sections of the cornea were examined in each case. The rats were sacrificed at different times of day: 10 A.M., 1, 4, 7, and 10 P.M., and 1, 4, and 7 A.M.; 8 or 9 animals were sacrificed at each time of the experiment.

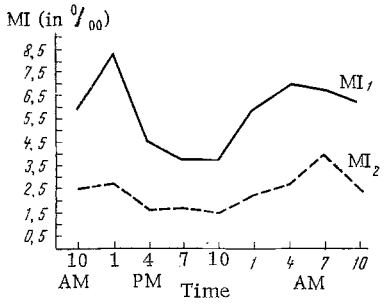

Fig. 1. Changes in mitotic activity during the 24-h period in the corneal epithelium of albino rats. Here and in Figs. 2 and 3: MI_1) mitotic activity of cells of stratum basale; MI_2) mitotic index of cells of stratum spinosum.

In the experiments of series II MI was determined (in pro mille) in the stratum basale (MI_1) and stratum spinosum (MI_2) of the esophageal epithelium of 40 male C57Bl mice (mean weight 22 g; group 1) and 40 noninbred male albino mice (mean weight 25 g; group 2). From 20,000 to 25,000 cells in sections of the esophagus from each animal were examined. The animals of group 1 were sacrificed at 10 A.M., 1, 4, 7, and 10 P.M., and 1, 4, and 7 A.M., the animals of group 2 at 11 A.M., 2, 5, 8, and 11 P.M., and 2, 5, and 8 A.M.; 5 mice were studied at each time.

In the experiments of series III the values of MI_1 and MI_2 and the labeling index were calculated for cells of the stratum basale and stratum spinosum (LI_1 and LI_2 respectively, in pro mille) of the esophageal epithelium in 32 male C57Bl mice (mean weight 20 g) after inspection

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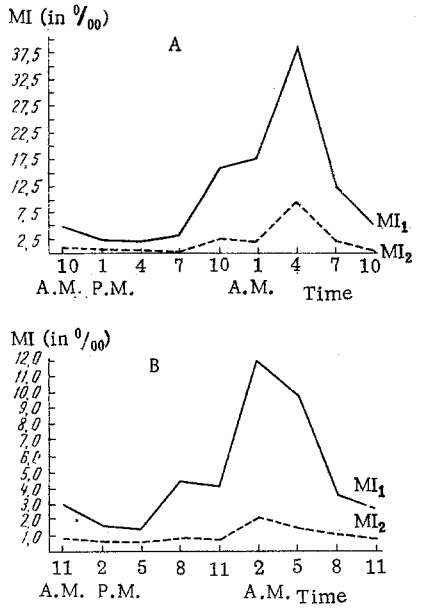

Fig. 2

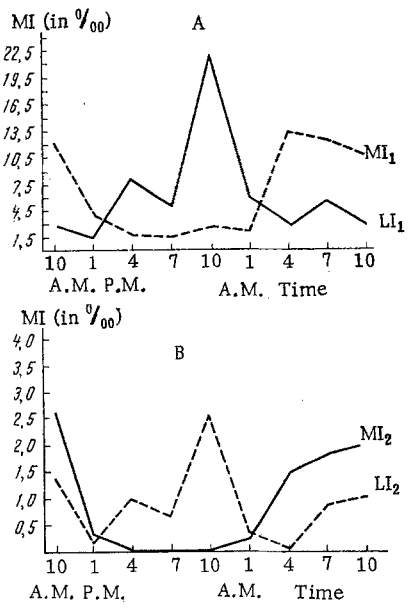

Fig. 3

Fig. 2. Changes in mitotic activity during 24-h period in esophageal epithelium of C57Bl mice (A) and noninbred albino mice (B).

Fig. 3. Changes in mitotic activity and labeling index during 24-h period in cells of stratum basale (A) and stratum spinosum (B) of esophageal epithelium of C57Bl mouse. LI_1) labeling index of cells of stratum basale, LI_2) labeling index of cells of stratum spinosum.

of between 20,000 and 25,000 cells in each case. These animals were sacrificed at different times of day (10 A.M., 1, 4, 7, and 10 P.M., 1, 4, and 7 A.M.) and 1 h before sacrifice they received a single intraperitoneal injection of thymidine-H^3 in a dose of 1 μCi/g body weight (specific activity 8.6 Ci/mmole). Four animals were used at each time of the experiment. Cell nuclei were taken as labeled if not less than 5 grains of silver were found above them on the autoradiograph (NIKFI type R emulsion, exposure 180 days).

EXPERIMENTAL RESULTS

In the cells of the stratum basale and stratum spinosum of the rat cornea mitotic activity varied with the time of day (Fig. 1). In the stratum basale the largest number of mitoses was observed between 1 A.M. and 1 P.M. and the smallest number between 4 and 10 P.M. ($P = 0.012$–0.05). The number of mitoses in the stratum spinosum was greatest between 4 A.M. and 1 P.M. and smallest between 4 and 10 P.M. ($P = 0.048$–0.05). The mean diurnal MI_1 was twice as high as the mean diurnal MI_2 (5.9 and 2.4 ‰ respectively, $P < 0.0001$). These results indicate the existence of diurnal rhythms of mitosis in the stratum basale and stratum spinosum of the rat corneal epithelium which correspond graphically to unimodal curves. Investigations by other workers (1-3, 5-10] have shown that mitotic activity in the rat corneal epithelium is intensified in the second half of the night and morning and is weaker in the evening and first half of the night. The present results show that the period of increased mitotic activity is prolonged (12 h), suggesting that the factors acting on the cells to synchronize the beginning of their mitosis do not act simultaneously. It is interesting to note that at the times when MI_1 in the stratum basale was increased, there was a corresponding increase in the number of mitoses whose axes were directed perpendicularly to the basement membrane (54-58% in the period from 4 P.M. to 4 A.M. and 64-72% in the period from 7 A.M. to 1 P.M.). The cells of the stratum spinosum of the corneal epithelium show much weaker mitotic activity, presumably because of their transfer from the basement membrane to a different cell system, with the accompanying development of differentiation and its resulting effects on the mitotic cycle of the cell.

The highest number of mitoses in the basal cells of the esophageal epithelium of C57Bl mice (Fig. 2A) was observed between 10 P.M. and 10 A.M. (maximum at 4 A.M.), and substantially fewer mitoses were found between 1 and 7 P.M. ($P = 0.0001$–0.036). Similar changes in mitotic activity during the 24-h period also occurred in the cells of the stratum spinosum. The mean diurnal MI_1 was 5.5 times greater than the

mean diurnal MI_2 (11.7 and 2.1 ‰ respectively, $P=0.001$). In noninbred mice (Fig. 2B) the maximum mitotic activity in the basal cells of the esophageal epithelium occurred between 8 P.M. and 5 A.M. (maximum at 2 A.M.), and fewer mitoses in this case were observed between 8 A.M. and 5 P.M. ($P=0.0001$–0.02). An increase in the number of mitoses in the cells of the stratum spinosum was observed at 2 and 5 A.M., while at other times the mitotic activity of these cells was low ($P=0.004$–0.02). The mean diurnal MI_1 was 6.2 times greater than the mean diurnal MI_2 (5.1 and 0.8 ‰ respectively, $P<0.0001$).

Both in the stratum basale and stratum spinosum of the esophageal epithelium of the mice diurnal rhythms of mitosis corresponding graphically to unimodal curves were therefore observed. The dynamics of changes in the number of mitoses was similar in the two layers of cells, although in the mice of group 2 the increase in MI_2 occupied a shorter time than that in MI_1, and the increase in MI_2 occurred 6 h later than the increase in MI_1. It is stated in the literature [2, 4, 15] that an increase in the number of mitoses in the esophageal epithelium of mice takes place during the morning. The present investigation showed that the increase in mitotic activity in the esophageal epithelium may occur for a long time and it may still be found in the evening. Just as in the corneal epithelium, the differentiating cells of the stratum spinosum in the esophagus are characterized by a considerably diminished mitotic activity.

The results of the experiments of series III show an increase in the number of mitoses in the stratum basale (Fig. 3A) of the esophageal epithelium between 4 A.M. and 1 P.M. compared with the values of MI_1 from 4 P.M. to 1 A.M. ($P=0.0001$–0.018). The number of cells labeled with thymidine-H^3 in the stratum basale is higher between 4 P.M. and 1 A.M. than at other times of day ($P=0.022$–0.044). The number of mitoses in the stratum spinosum (Fig. 3B) reached a maximum between 4 and 10 A.M., while the number of labeled cells was increased between 4 and 10 P.M., and again between 7 and 10 A.M. The mean diurnal MI_1 was 7.9 times greater than the mean diurnal MI_2 (5.8 and 0.7 ‰ respectively, $P<0.0001$), while the mean diurnal LI_1 was 8.4 times greater than the mean diurnal LI_2 (8.4 and 1 ‰ respectively, $P<0.0001$). The ratio between the values of LI and MI in the stratum basale and stratum spinosum were 1.5 and 1.3, respectively.

The results indicate an increase in the mitotic activity in the esophageal epithelium of the mice of this series in the morning and the first half of the afternoon. They also indicate the existence of a diurnal rhythm in the number of cells synthesizing DNA in the stratum basale and stratum spinosum of the esophageal epithelium. The increase in number of DNA-synthesizing basal cells takes place on the average 12 h before the increase in mitotic activity in this layer. The duration of the period of maximum mitotic activity and of the period when the largest number of basal cells with labeled nuclei is found is the same, namely 9 h. If the diurnal rhythm of mitosis in the cells of the stratum spinosum is shown graphically by a unimodal curve, the diurnal rhythm of DNA-synthesizing cells of the stratum spinosum is represented by a bimodal curve.

It has been claimed [13, 14, 16] that only cells of the stratum basale in the stratified squamous epithelium are capable of synthesizing DNA. However, Mamontov [11, 12] found DNA-synthesizing cells in the stratum spinosum of the corneal and lingual epithelium of mice. The present results showed that cells of the stratum spinosum of the esophageal epithelium can also synthesize DNA and, consequently, can start on a cycle of mitosis. However, the number of such cells is unusually low by comparison with the number of DNA-synthesizing basal cells. It is possible that DNA synthesis in the stratum spinosum is actually carried on by even fewer cells than would appear from the results of these experiments, if a correction is introduced for the probability of migration of cells which have started DNA synthesis in the stratum basale into the stratum spinosum. The small number of cells in the stratum spinosum exhibiting the ability to synthesize DNA is evidently one reason for the extraordinarily low mitotic activity in this layer. Differentiation processes thus exert a marked influence on the entry of cells into the mitotic cycle, and they thus control the intensity of cell reproduction.

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