CHANGES IN THE NUMBER OF CELLS SYNTHESIZING DNA AND IN MITOSES IN THE MOUSE CORNEAL EPITHELIUM DURING THE 24-HOUR PERIOD

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A circadian rhythm was demonstrated in the number of cells of the mouse corneal epithelium synthesizing DNA, with a maximum between 10 a.m. and 7 p.m. and a minimum between 10 p.m. and 7 a.m. The mitotic index reached a maximum at 10 a.m. and a minimum at 10 p.m.

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Few investigations have been carried out to study changes in the circadian rhythm of DNA synthesis in mammalian tissues. The number of cells synthesizing DNA has been shown to reach a maximum during the evening, 12-14 h before the mitotic activity in the tissues reaches its maximum [12]. More recent evidence has not confirmed the view that the interval between the maximum of DNA synthesis and the maximum of mitotic activity is constant. The largest number of cells synthesizing DNA can be found at different times of day, and can even coincide in time with the maximum of mitotic activity [4, 5, 8, 14].

Because of the importance of establishing the principles governing the diurnal changes in cell proliferation [1] and the need for its precise determination in order to establish the rates of renewal of the tissues, the present investigation was carried out in order to compare the character of the circadian rhythms of mitosis and of the number of cells synthesizing DNA in the basal and more highly differentiated cells of the stratum spinosum of the corneal epithelium in mice.

EXPERIMENTAL METHOD

Experiments were carried out on 38 male C57BL mice preliminarily separated into eight groups, with four-six mice in each group. One hour before sacrifice the mice received an intraperitoneal injection of thymidine-H^3 with specific activity 8.6 Ci/mmole in a dose of 20 μCi per animal. The animals were sacrificed at 10 a.m., 1, 4, 7, and 10 p.m., and 1, 4, and 7 a.m. Transverse sections through the cornea were coated with type "M" (NIIKhimfoto) liquid emulsion. The sections were exposed in a refrigerator for 3 months. In each layer, 3000-5000 nuclei were examined and the number of labeled nuclei, the number of mitoses in the stratum basale and stratum spinosum were counted. Degenerating cells in the third (surface) layer were not counted. The nucleus was regarded as labeled if it had at least 5 silver grains above it. The index of labeling of the nuclei, or radioactive index (RI), was calculated in percent and in promille, and the mitotic index (MI) in promille relative to the total number of cell nuclei in the particular layer.

EXPERIMENTAL RESULTS

The results of determination of the number of labeled nuclei and dividing cells in the corneal epithelium of mice during the 24 h period are given in Figs. 1 and 2.

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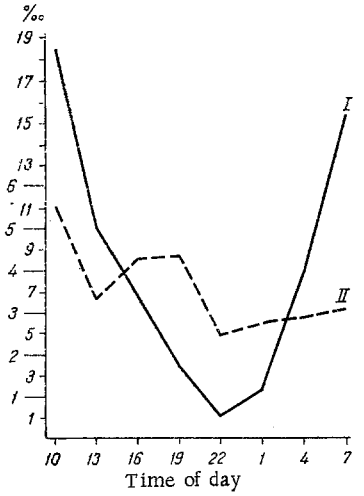

Fig. 1.

Fig. 2.

Fig. 1. Changes in number of cells synthesizing DNA and number of mitoses in stratum basale of mouse corneal epithelium during the 24 h period. I) Mitotic index; II) radioactive index.

Fig. 2. Changes in number of cells synthesizing DNA and number of mitoses in stratum spinosum of mouse corneal epithelium. I) Mitotic index; II) radioactive index.

Regular changes occured in the indices of labeled cells and mitoses in the corneal epithelium during the 24 h period. The number of labeled nuclei in the stratum basale and stratum spinosum reached a maximum at 10 a.m. and a minimum at 10 p.m. ($P = 0.015$ and 0.11, respectively). In the stratum basale, RI remained at a high level with only very slight fluctuations until 7 p.m. after which the index of labeled cells fell considerably. Analysis of the changes in the number of labeled cells during the 24 h period showed that between 10 a.m. and 7 p.m. a larger number of cells (RI = 44.3%) in the corneal epithelium passes through the stage of DNA synthesis than in the period from 10 p.m. to 7 a.m. (RI = 26.0%). The difference between RI values in these time intervals is significant ($P = 0.003$). A similar picture was observed in the cells of the stratum spinosum of the corneal epithelium. The mean value of RI in the period from 10 a.m. until 7 p.m. was 10.6%, and in the period from 10 p.m. to 7 a.m. 6.4% ($P = 0.026$). The mean value of RI for the 24 h period in the stratum basale was 35.4% and in the stratum spinosum 8.5%. Similar data were obtained by Scheving and Parely [14], who found a mean RI value of 4.2% for the 24 h period in the stratum basale of the rat corneal epithelium. According to figures given by most workers investigating incorporation of thymidine-H^3 into the basal cells of the corneal epithelium, the RI value for this tissue is 3-4% [3]. The mean value of MI for the stratum basale was 8.1%, and for the stratum spinosum 2.6%.

The circadian rhythm and absolute values of MI at different times of day were identical with those in a group of mice not receiving thymidine-H^3 [6].

MI in the stratum basale reached its maximum at 10 a.m. and minimum at 10 p.m. ($P = 0.006$). By 4 a.m., MI had started to rise and by 7 a.m. it was near its maximum. The number of mitoses also reached a maximum at 10 a.m. in the cells of the stratum spinosum of the corneal epithelium. MI reached its minimum at 10 p.m. (for the interval 10 a.m.-10 p.m., $P = 0.003$). The ratio between the mean values for the 24 h period RI/MI for the stratum basale was 4.4 almost identical with the value obtained in the corneal epithelium of rats [14]. However, this ratio fluctuated at different times of day from 2.0 to 24.4 in the stratum basale and from 1.4 to 17.4 in the stratum spinosum. Considerable variability in the RI/MI ratio has also been demonstrated for other tissues [2].

The intensity of labeling, reflected in the number of silver grains per nucleus, remained essentially unchanged during the 24 h period.

In view of these results, an attempt was made to determine the time of renewal of cells in the stratum basale of the corneal epithelium.

Chumak [8] has shown that the duration of the S-phase in this tissue is 8.5 h. This figure has been confirmed by the method of double labeling [11]. Using the equation in [13]:

$$\frac{n}{N} = \frac{t_s}{T},$$

where n is the mean index of labeling for the 24 h period, N the total number of cells, t_S the duration of DNA synthesis, the value of T was found to be 240 h, or 10 days. Introducing a correction for the proliferative pool, namely 70% [7], the value of T = 168 h or 7 days, was obtained. This result is close to that obtained by determining the renewal time in the mouse corneal epithelium by observing the rate of migration and desquamation of labeled cells [10] and by the use of a colchicine method [9].

It is interesting to note that the occurrence of the largest number of labeled cells in the corneal epithelium at a different time from that in the epithelium of the lower surface of the tongue in the same animals [5] coincides with the maximum of mitotic activity. In the lingual epithelium the number of nuclei incorporating label was highest between 7 p.m. and 7 a.m., with a maximum at 4 a.m. The number of labeled nuclei reached a minimum during the afternoon.

In the corneal epithelium of rats [14], as in the present experiment on the corneal epithelium of mice labeled cells were most numerous in the morning and afternoon and least numerous between 10 p.m. and 1 a.m. Unlike other investigations [12], marked variability of individual values of RI was found at all times of the investigation.

On the basis of known evidence that cells differ in their passage through the mitotic cycle and that individual variability of RI is high, it can be accepted as a working hypothesis that the "burst" of DNA synthesis can occur in different animals at different times. The highest labeling index at a particular time of day in this case will be due to the occurrence of high indices for individual animals. Consequently, the time when DNA synthesis reaches its maximum may differ in different groups of animals.

These findings are not in agreement with the view [7] that differentiated cells in stratified squamous epithelia are incapable of synthesizing DNA. On the contrary, the presence of labeled nuclei in the stratum spinosum, the more highly differentiated layer of cells of the corneal epithelium, is evidence that these cells can synthesize DNA.

Cells of the stratum spinosum can thus multiply in two different ways: by migration of cells from the stratum basale which have doubled their DNA content, and by synthesis of DNA in cells of the stratum spinosum itself.

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