CHANGES IN MITOTIC ACTIVITY OF THE CORNEAL EPITHELIUM OF ALBINO RATS COOLED AT DIFFERENT TIMES OF DAY

N. B. Simon, S. S. Timoshin, and S. A. Alekseenko

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The effect of moderate hypothermia on mitotic activity of the corneal epithelium of albino rats was studied. The animals were cooled to 28°C by a contact method for 1 h. Cooling was carried out in the early morning (6 a.m.), at noon, and in the evening (6 p.m.). The response of the epithelium to cooling was found to depend on the time of day. The most marked inhibition of mitotic activity (by 14 times) occurred in the afternoon, 3 h after cooling at noon. A tendency toward restoration of normal cell division was observed 6 and 12 h later. The number of mitoses was reduced 3 h after cooling in the evening, but no changes in mitotic activity were discovered 6 and 12 h later. No changes were found 3 and 6 h after cooling in the morning, but 12 h later cell division was inhibited.

KEY WORDS: *Mitotic activity; stress; moderate hypothermia; cell division; circadian rhythms.*

Different changes in mitotic activity in response to the action of a stressor (pyrogenal) depending on the time of day were found in a previous investigation [3].

In this investigation the response of the corneal epithelium was studied to a different stressor, moderate hypothermia, which has a marked antimitotic action [2].

EXPERIMENTAL METHOD

Experiments were carried out on male albino rats weighing 150-200 g. The animals, having first been accustomed to the experimental conditions, were kept in wire cages and cooled by a contact method (by application of polyethylene ice bags) down to a body temperature of 28°C. The temperature was measured by an electrothermometer introduced into the rectum to a depth of 2.5 cm. After exposure to cold for 1 h, when the temperature was 27-30°C, the animals were allowed to warm up spontaneously. There were three series of experiments: I) The animals were cooled from 6 to 7 a.m., II) from noon to 1 p.m., III) from 6 to 7 p.m. The animals were then decapitated 3, 6, and 12 h after the end of cooling. At each time 15 to 19 animals (experimental and control) were used. To determine the reproducibility of the results, the main series of experiments were repeated once. The total number of animals used was 211. Total preparations were made from corneas fixed in a mixture of ethanol and acetic acid (3:1) and stained with Böhmer's hematoxylin. Mitotic activity was judged from the number of mitoses in 100 fields of vision. The total number of mitoses was expressed in absolute numbers and the ratio between phases in per cent. The results were subjected to statistical analysis by the Fisher—Student method and by determination of confidence limits, and they are illustrated in Figs. 1-3.

EXPERIMENTAL RESULTS

The experimental results show that the effect of cooling on mitotic activity of the corneal epithelium differed in character depending on the time of day. The strongest in-

Central Scientific-Research Laboratory, Khabarovsk Medical Institute. Laboratory of Cytology, Institute of Human Morphology, Academy of Medical Sciences of the USSR, Moscow.
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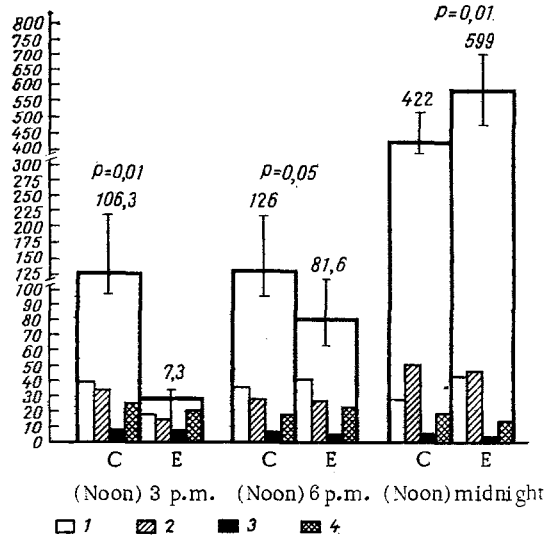

Fig. 1

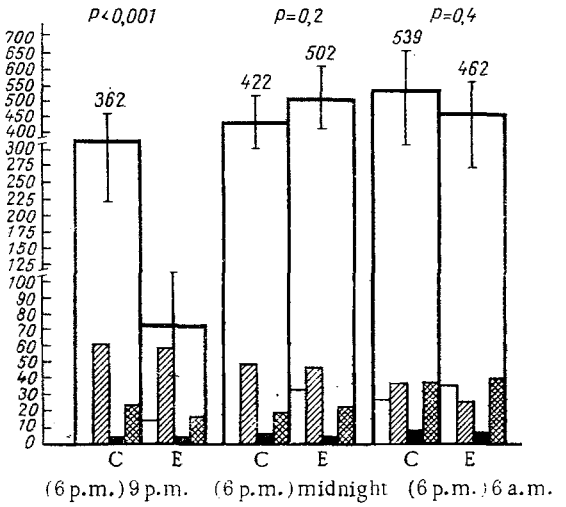

Fig. 2

Fig. 1. Changes in mitotic activity of corneal epithelium of albino rats cooled in the afternoon. C) Control, E) experiment. Large columns denote number of mitoses in 100 fields of vision; small columns: 1) prophases, 2) metaphases, 3) anaphases, 4) telophases (in % of total number of mitoses). Ordinate, number of mitoses; abscissa, time of cooling (in parentheses) and of sacrifice of animals.

Fig. 2. Changes in mitotic activity of corneal epithelium of albino rats cooled during the evening. Legend as in Fig. 1.

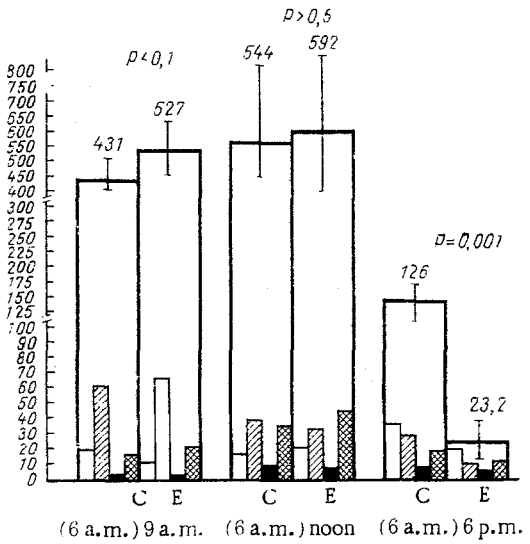

Fig. 3. Changes in mitotic activity of corneal epithelium of albino rats cooled in the morning. Legend as in Fig. 1.

hibition of cell division was observed 3 h after cooling in the afternoon (noon to 1 p.m.), when the number of mitoses in the experimental animals was reduced by 14 times (Fig. 1). A significant decrease was found in the relative number of prophases and metaphases. A tendency toward restoration of normal cell division was observed after 6 h, when the number of mitoses in the experimental animals was 70% of the corresponding control value. A statistically significant increase in the number of mitoses occurred 12 h after cooling.

In rats cooled during the evening (6-7 p.m.) inhibition of mitotic activity was observed only 3 h later, when the number of mitoses in the experimental animals was reduced

by 5 times (Fig. 2). No difference was observed between the values recorded in the experimental and control animals 6 and 12 h after cooling.

In animals cooled in the morning (6-7 a.m.) no changes were found in the number of mitoses 3 and 6 h later (Fig. 2). Depression of mitotic activity was observed only 12 h after cooling (the number of mitoses was reduced almost to one fifth of the control).

Changes in mitotic activity of the corneal epithelium in rats cooled at different times of day thus showed a definite circadian rhythm. The absence of inhibition of cell division during the morning peak of mitoses is interesting. It confirms the results of the writers' previous investigation [3] which showed that the inhibitory action even of such an active stressor as pyrogenal is not exhibited during the morning. This fact may perhaps be partly explained on the basis of experimental results obtained by Kolpakov et al. [1], who showed that the response of the adrenals to a stressor is modified in the morning. Like the epithelial cells in the present experiments, cells of the adrenal glands did not respond to the action of the stressor in the morning, immediately after the experiment, but did respond by increased secretion in the evening.

The possibility cannot be ruled out that the character of the cell response in the morning is also connected with the pattern of the cell cycle in the morning [4, 5] and, in particular, with the shortening of the G_2 period, the time when the inhibitory effect of stressor hormones is particularly strong. Perhaps the sensitivity of the cells themselves to the action of antimitotic agents is reduced in the morning. Further experiments are required to elucidate these problems.

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