THE MITOTIC ACTIVITY OF THE EPIDERMIS BORDERING A WOUND AND OF THE CORNEAL EPITHELIUM OF RATS EXPOSED TO PROLONGED CONTINUOUS ILLUMINATION

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Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 54, No. 9, pp. 96-100, September, 1962
Original article submitted October 18, 1961

Among the factors influencing the diurnal rhythm of cell division, a special place is occupied by the photoperiodicity, for if the conditions of illumination under which animals are kept are changed, corresponding disturbances may take place in the rhythm of mitotic activity. According to Halberg and co-workers [11], for instance, inversion of illumination of mice (dark by day and light by night) leads to a corresponding change in the time of maximal and minimal indices of the rate of cell division in the liver and the epidermis of the ear.

The experiments of I. A. Alov [1] showed that inversion of illumination of mice for 40 days causes a change in the diurnal rhythm of mitosis in the corneal epithelium, the skin, the tongue, and the esophagus of experimental animals.

I. A. Utkin and L. P. Kosichenko [9] found a disturbance of the diurnal periodicity of mitosis of the cornea in mice when the animals were kept in the dark continuously for 2 and 4 weeks. L. P. Kosichenko [7] also showed that keeping mice in continuous artificial illumination leads to the appearance of a maximal number of cell divisions in the cornea in the evening, and a minimal number in the afternoon. Inversion of the illumination caused a modification of the diurnal rhythm of mitosis in the cornea.

In a previous investigation [4] we found that in the early stages of wound healing in the skin of rats (1st, 3rd, and 5th days of regeneration) the epidermis lying next to the wound preserved its mitotic rhythm, and that the maximal number of cell divisions was observed on the 3rd-4th day after wounding. In this connection it appeared interesting to study the character of the mitotic activity of the epidermis lying next to the wound in rats kept in conditions of continuous and prolonged illumination. Another object of our investigation was to study the diurnal changes in the mitotic activity of the corneal epithelium of experimental animals.

EXPERIMENTAL METHOD

Experiments were conducted on 60 male rats weighing 150 g; the 36 rats of the experimental group were placed in a special room where they were exposed to continuous electric lighting, so that all the cages containing the animals were illuminated comparatively uniformly for a period of 28 days. At the end of this period, at 9 A.M., a skin wound was inflicted on the rats in the dorsal region, measuring 3×8 mm. The skin was excised together with the subcutaneous cellular tissue. The animals of the experimental group were sacrificed on the third day after the operation at 9 A.M., 3 and 9 P.M., and 3 and 9 A.M.

Two groups of rats were used as controls. The first group (14 rats) were continuously illuminated for 28 days but had no wound inflicted (control I). The second group (10 rats) were kept in natural conditions of illumination and received a skin wound in the dorsal region at the same time as the experimental group (control II). The animals of both control groups were sacrificed on the same day as the experimental animals, but at two times of the day: 9 P.M. and 3 A.M. We considered that two times of fixation were sufficient in control series I and II because experiments of this type have been done previously by other workers [4, 7, 10].

In order to study the mitotic activity the following tissues were fixed: the epidermis bordering the wound, the epidermis of normal rats (not wounded), and the cornea. Zenker's fluid was used as fixing agent; sections of the epidermis and total preparations of the cornea were stained with Carazzi's hematoxylin.

EXPERIMENTAL RESULTS

In Fig. 1 we illustrate graphically the changes in the mitotic coefficient at different times of day in the epidermis bordering the wound (on the third day of healing), and in Fig. 2 we show the changes in the mitotic coefficient in the cornea of the same rats kept for 28 days in continuous illumination.

In the epidermis bordering the wound, as in the cornea of the rats kept in continuous illumination, the value of the mitotic coefficient at 9 A.M. and 3 P.M. on the third day lay almost on the same level. At 9 P.M. (the usual time of the minimal mitotic activity in rats) a slight elevation of the level of cell division was observed in both tissues, although these changes in the mitotic coefficients in the interval between 3 and 9 P.M. were not significant (P = 0.218 and 0.115 respectively). The variations in the mitotic coefficients both in the epidermis bordering the wound and in the cornea at other intervals of time (i.e., from 9 P.M. to 3 A.M. and from 3 to 9 A.M. were also not significant. Consequently, keeping rats in conditions of prolonged, continuous illumination leads to equalization of the indices of the mitotic coefficients during the 24 hour period both in the epidermis bordering the wound and in the corneal epithelium, i.e., to abolition of the diurnal mitotic rhythm in both tissues. The results obtained with the control animals shed light on the question whether the abolition of the diurnal rhythm of mitotic activity was the result of a change in the conditions of illumination of the animals or the result of the regeneration process (healing of the skin wounds).

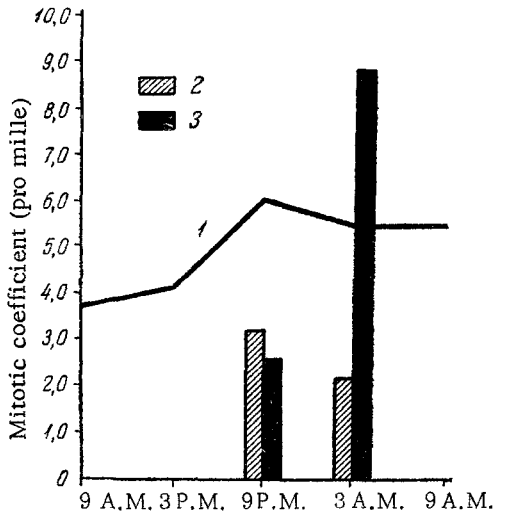

Fig. 1. Diurnal changes in the mitotic coefficients (MC) of the epidermis of rats kept in different conditions of illumination. 1) After wounding (continuous illumination); 2) without wounding (continuous illumination, control I); 3) after wounding (natural illumination, control II).

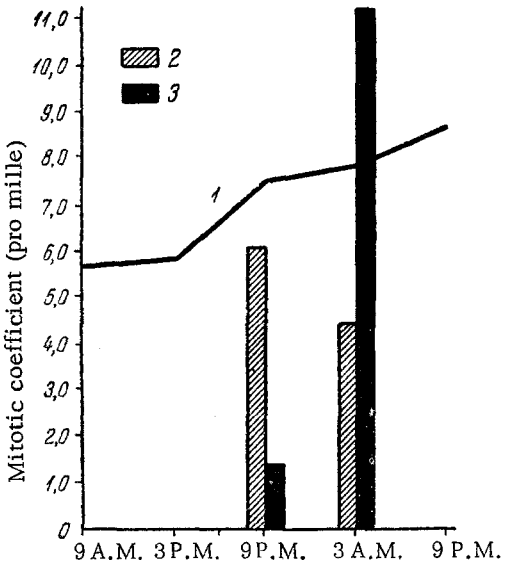

Fig. 2. Diurnal changes in the mitotic coefficients (MC) of the cornea of the same rats kept in different conditions of illumination. 1) After wounding (continuous illumination); 2) without wounding (continuous illumination, control I); 3) after wounding (natural illumination, control II).

In the animals of the first control group, kept in conditions of prolonged, continuous illumination, it was found that the mean value of the mitotic coefficient both in the epidermis and in the cornea during the evening (9 P.M.) did not differ significantly from the mitotic coefficients at the time of maximal cell division (3 A.M.). Consequently, keeping the animals in prolonged, continuous illumination led to the leveling out of the diurnal variations in the mitotic activity of the epidermis and cornea of the rats, i.e., to the abolition of the diurnal rhythm of cell proliferation.

In the animals of the second control group we studied the mitotic activity of the epidermis bordering the wound and of the corneal epithelium in rats kept in natural conditions of illumination. As in our previous communication [4], we found that clear diurnal variations in mitotic activity took place in the epidermis bordering the wound on the third day of healing, and that the mitotic coefficient at 3 A.M. was almost four times greater than at 9 P.M. The

probability that the difference between the mean values during this time interval was accidental was very small ($P = 0.001$). The cornea of the animals also showed clear diurnal variations in mitotic activity. Statistical treatment of the results showed that the difference between the number of mitoses at 9 P.M. and at 3 A.M. was significant ($P = 0.001$). Since the diurnal variations in the number of dividing cells were preserved in the epidermis bordering the wound and also in the cornea at a considerable distance from the site of the wound, it may be concluded that the presence of a focus of regeneration in the organism, when natural conditions of illumination held good, is not itself the reason for the disappearance of the diurnal variations in the indices of mitotic activity. It is interesting to note that the level of mitotic activity in the cornea of the rats with a skin wound differed from the level of cell division in the cornea of the normal animals [3]. This difference, as we have pointed out earlier [4], is found at the time of maximal cell division (at 3 A.M.). Whereas the level of mitotic activity in comparable animals at 9 P.M. was almost identical, at 3 A.M. the rate of cell division in the cornea of the wounded rats was 50% higher than in the cornea of the normal animals. As in other cases reported in the literature [2, 5, 6, 8], the effect of reparative regeneration of one part of the body on the intensity of the physiological regeneration of another part was observed here.

It was also discovered that at 9 P.M. the mitotic coefficient of the epidermis bordering the wound and of the corneal epithelium of animals kept in natural conditions of illumination was considerably lower than the mitotic coefficients of the corresponding tissues of rats kept in conditions of continuous illumination, and the difference was statistically significant ($P = 0.016$ and 0.001 respectively). Conversely, at 3 A.M. the value of the mitotic activity in both tissues in natural conditions of illumination was much higher than that in animals exposed to continuous illumination, and the difference was statistically significant ($P = 0.007$ and 0.001 respectively).

It may therefore be concluded from the experimental data that at the usual time of low mitotic activity of the tissues (9 P.M.), the continuous illumination of animals leads to a significant increase in the tempo of cell division, while at the usual time of high mitotic activity (3 A.M.) it causes a significant decrease in the tempo of cell division. Hence the diurnal fluctuations of mitotic activity characteristic of both tissues in natural conditions of illumination are leveled out in animals exposed to the continuous action of light.

Our results in respect to the action of continuous illumination on the epidermis and cornea of normal animals (rats) fully support the findings of those workers [7, 10] reporting the abolition of the diurnal rhythm of mitoses in mice not inflicted with wounds.

SUMMARY

A study was made of mitotic activity of epidermis adjacent to the wound (the 3rd day of regeneration) and of the epithelium of the rat corneum in conditions of 28-day constant illumination. As shown, prolonged constant illumination of animals led to elimination of the daily rhythm of cellular multiplication in both the body tissues studied. 28-Day constant illumination of intact animals also caused the leveling of the 24-hour differences in the number of mitoses in the epidermis and the epithelium of rat corneum. In animals with skin injury, kept in natural illumination conditions, both of the tissues studied retained the 24-hour regular variability of mitotic activity.

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