THE MITOTIC ACTIVITY OF THE CORNEA IN MICE AFTER INFLICTION OF A BURN AT DIFFERENT TIMES OF DAY

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There are reports in the literature that the infliction of an injury or burn leads to reactive changes in the surrounding tissues. It is also known that injury to one of a pair of symmetrical organs causes changes in the other. The infliction of a burn to one cornea in rats, for instance, leads to a persistent fall in the number of dividing cells in both the damaged and the intact (symmetrical) cornea [2, 3].

The existence of a diurnal periodicity of cell proliferation has recently been proved in the cornea of rats [1] and mice [4]. There are certain indications of possible changes in the diurnal periodicity of mitosis under the influence of trauma in the work of Jaffe [5], who studied regeneration of the liver in rats.

In the present investigation we studied the changes in the diurnal periodicity of cell division after the infliction of a burn, in the damaged and the symmetrical cornea, and also the changes in the diurnal periodicity in relation to the time of day at which the burn was inflicted.

EXPERIMENTAL METHOD

For the experiment we used 160 male mice, weighing from 20 to 25 g (110 mice in the experimental group and 50 in the control group). The diurnal rhythm of cell division in the cornea was studied after fixation of the material at intervals of 6 hours.

After decapitation of the animals the material was fixed in Zenker's fluid and stained with hematoxylin by Carazzi's method.

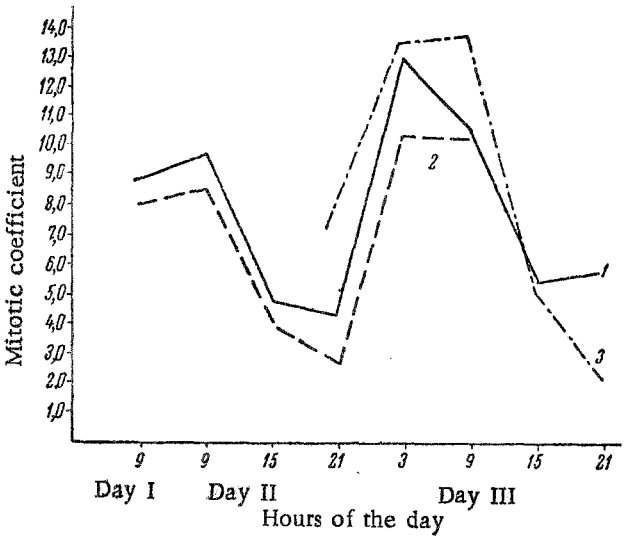
A burn was inflicted on the cornea of the right eye by means of a red-hot dissecting needle, in the first series of experiments this operation being carried out at 9 A. M. and in the second series at 9 P. M.

In order to count the dividing cells in the cornea, two lines were drawn at right angles to each other on the cover glass, and on each side of these lines all the fields of vision were examined in which mitoses were counted. On the average the number of fields of vision in one cornea was 145. In addition, in certain fields of vision the average number of cells was counted. The mitotic coefficient – i.e., the ratio of the number of dividing cells to the total number of cells – was calculated.

The whole of the numerical results were treated statistically by the Fisher-Student method.

EXPERIMENTAL RESULTS

Diurnal periodicity of cell proliferation in the normal cornea. The Figure shows graphically the changes in the mitotic coefficient in the course of 24 hours in the normal cornea and after burning. The results obtained



Changes in the mitotic coefficient in the cornea of a mouse in the course of 24 hours after infliction of a burn.
1) In the normal cornea; 2) after infliction of a burn in the morning (series I); 3) after infliction of a burn in the evening (series II).

indicate that in the epithelium of the cornea of normal mice there was a diurnal rhythm of cell proliferation. Starting at 9 A.M. the number of dividing cells gradually decreased, and reached a minimum at 9 P.M. The fall in the level of mitotic activity from 9 A.M. to 9 P. M. was statistically significant ($P = 0.001$).

At 3 A.M. a sharp rise in mitotic activity began, and this also was statistically significant ($P = 0.0001$).

At 9 A.M. on the following days a fall was observed in the number of dividing cells, but the difference in the intensity of cell division between 3 and 9 A.M. was not statistically significant.

These findings showed that there is a diurnal rhythm of mitotic activity in the cornea of normal mice: the curve was clearly unimodal in character.

Similar results have also been obtained by I. A. Utkin [4], but the diurnal rhythm of mitotic activity was examined by this worker in material fixed at only two times of day. Furthermore, we ourselves had previously shown the existence of the same sort of curve of diurnal rhythm of cell proliferation in the epithelium of the cornea, the epidermis and the small intestine of normal rats [1].

The level of mitotic activity in the cornea after burning. After infliction of a burn on the cornea the tempo of cell division there fell sharply after only 24 hours. The depression of mitotic activity 24 hours after infliction of the burn was statistically significant both in comparison with the normal cornea and in comparison with the opposite cornea to the one injured ($P = 0.0001$ and $P = 0.016$). Three days after burning the intensity of cell division in the injured cornea increased somewhat, but it remained at only half the level of that in the cornea of normal animals.

The fall in the value of the mitotic activity was statistically significant ($P = 0.017$ and $P = 0.027$).

Hence we could speak about a marked depression of the intensity of cell division in the cornea even three days after a burn had been inflicted on it.

Unfortunately it was not possible to study the changes in the cornea at other times during the experiment, since in the majority of cases the infliction of a severe burn led to a discharge of fluid from the eye and to shrinking of the cornea.

The diurnal rhythm of mitotic activity in the opposite cornea to the one injured, the burn being inflicted during the morning. The changes in the mitotic coefficient found in the 1st series of experiments showed that

the diurnal rhythm of cell division in the opposite cornea to the one damaged was preserved (see Figure). However, in material fixed at any time of day, the intensity of cell division here was rather below that in the cornea of the control animals.

The fall in the level of mitotic activity in the opposite cornea began, as it did in the normal cornea, at 9 A. M. The minimum number of divisions was observed at 9 P. M. The fall in the tempo of cell division at this time was statistically significant ($P = 0.0001$).

As in the cornea of normal mice, a sharp rise in mitotic activity was observed at 3 A.M. ($P = 0.0001$). At 9 A.M. on the third day after infliction of the burn, the number of dividing cells in the opposite cornea was approximately the same as that characteristic of the mitotic activity of normal mice.

Thus after infliction of a burn on the cornea of mice, during the morning hours, the diurnal rhythm of mitotic activity in the opposite cornea was preserved, although the intensity of cell division here was rather lower than in the cornea of the control mice.

The diurnal rhythm of mitotic activity in the opposite cornea to the one damaged, the burn being inflicted in the evening. In the 2nd series of experiments a burn was inflicted on the cornea in the evening (at 9 P.M.), i.e. at a time when under ordinary conditions the number of cell divisions in the cornea was minimal. Two days after infliction of the burn, the mitotic activity in the opposite cornea to the one injured was considerably higher than that in the cornea of the control animals and in the opposite cornea to the one injured when the burn was inflicted in the morning. The difference in the intensity of cell division in the corneas of the mice in the 1st and 2nd series of experiments (9 P.M.) was statistically significant ($P = 0.003$). At 3 A.M. a further increase in mitotic activity was observed ($P = 0.01$). However, in consequence of the considerable individual variations, the difference in level of the mitotic activity in the corneas of the mice in the 1st and 2nd series of this experiment at 3 A.M. was not statistically significant ($P = 0.05$). A sharp fall in the mitotic activity was then observed, continuing until 9 P.M. which indicated the possible compensation for the rise in mitotic activity.

Thus on the basis of the results of the 2nd series of experiments it could be concluded that after infliction of a burn to the cornea of mice during the evening hours, the diurnal rhythm of mitotic activity was preserved, although the intensity of cell division in this case was higher than when the burn was inflicted during the morning.

It is difficult at present to find the cause of this phenomenon. It can only be stated that the differences in the changes of mitotic activity after infliction of an injury to an animal during the morning or evening hours are not a chance happening, since our results are in agreement with those described in the paper by Jaffe [5].

It may thus be concluded from our results that the time of infliction of a burn has a bearing on the tempo of cell proliferation.

SUMMARY

The 24-hour rhythm of cellular division is preserved in symmetrical cornea by inflicting corneal burns in the morning. However, the average values of mitotic coefficients during the whole experiment appear to be lower than in the cornea of normal animals.

The infliction of burn in the evening has no effect on the character of the 24 hour rhythm. However the general level of mitotic activity is much higher. A stable depression of the tempo of cellular division was noted in the injured cornea.

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