24-HOUR RHYTHM OF CELL MULTIPLICATION IN RAT EPIDERMIS DURING HEALING OF SKIN WOUNDS

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It is known from numerous observations that in the tissues around a wound defect the number of cell divisions increases greatly, to an extent which depends on the size of the wound inflicted and the species of experimental animal. But the question of the 24-hour rhythm of mitoses in tissues during wound healing has been very inadequately studied, and the available data are contradictory.

Blumenfeld [8] investigated the 24-hour rhythm of cell division in the epidermis during healing of skin wounds in rabbits. Each animal received six wounds at intervals of four hours. Mitotic activity was studied on the seventh day of wound healing. On the basis of the data so obtained Blumenfeld concluded that in the epidermis of a healing wound in the rabbit, there is a 24-hour rhythm in mitotic activity, the mean number of mitoses being twice as great in the regenerating epidermis as in the normal skin. Bullough and Laurence [9] showed that if six small radial wounds are made simultaneously on one ear of a mouse, mitotic activity in the epidermis next to the wounds is greatest on the third or fourth day of regeneration. They were not able to detect a 24-hour rhythm in the mitotic activity. The design of these experiments, which involved making several wounds in each animal, merits critical examination, since other data indicate that a regeneration process in one part of an organism has an effect on mitotic activity in other tissues—even those at a distance [3].

The disagreement in the published data concerning a 24-hour rhythm of mitotic cell division in regenerating tissues led us to carry out the present investigation. We set ourselves the problem of finding out, first, how the level of mitotic activity in the epidermis changes from one period to the next during the healing of skin wounds, and second, whether there is a 24-hour rhythm of cell multiplication in the epidermis adjacent to the wound defect, and in the regenerating epidermis – i.e., in the cell layer that is forming a cover over the wound surface.

METHODS

Experiments were performed on 100 rats (male) weighing 150 g. A wound (3 × 8 mm) was made on the skin of the back at 9:00 AM. The skin was removed together with the subcutaneous connective tissue. The first eight skin biopsies removed were fixed for the purpose of studying mitotic activity in the normal epidermis; a second group of biopsies (also eight in number) were fixed at 9:00 PM for the same purpose. For studies of mitotic activity during regeneration, skin biopsies were taken every six hours on the first, third, and fifth day, in such a manner that portions of both regenerating epidermis and epidermis adjacent to the wound were included (the dimensions of biopsies from each side of the wound were 5 – 7 mm). Mitotic activity was determined separately for each of these biopsies. Seven animals were used for each time period. The material was fixed in Zenker's solution and imbedded in paraffin. Sections 7 μ in thickness were stained with hematoxylin by Karazi's method.

RESULTS

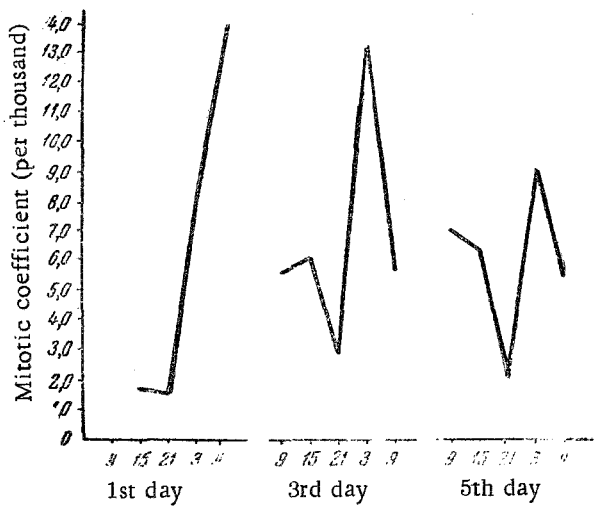
The figure shows the changes in the mitotic coefficient (MC) in epidermis adjacent to the wound at various times of the day. At 9 AM (before the wound was made) the mitotic coefficient had a value of 4.9 per thousand, and at 9 PM this coefficient was 2.1 per thousand.

Data obtained during the first day of the regeneration process show that at 3 PM, i.e., six hours after the wound was made, the number of cell divisions in the epidermis next to the wound had fallen considerably, to a level (MC = 1.6 per thousand) comparable to that observed in normal rat epidermis at the hours of lowest mitotic activity. At 9 PM, i.e., 12 hours after the wound

was made, the level of mitotic activity was almost the same as it was in control animals at the same time of day. At 3 AM the number of mitoses had risen greatly, and it continued to rise until it reached a maximum at 9 AM. The changes in the mitotic coefficient between 9 PM and 3 AM and between 3 AM and 9 AM were statistically significant (P= 0.0001 and P = 0.02).

We have shown previously [1] that in the normal rat epidermis the 24-hour rhythm of cell division can be expressed by a curve with a single peak, with the maximum number of mitoses in the early hours (6 AM) and the minimum at 9 PM. In our experiment the mitotic activity continued to increase during the first day of the regeneration process until 9 AM. This increase in the number of mitoses toward the end of the first 24 hours of the regeneration process can be explained by a compensatory increase in mitotic activity in response to inhibition of this activity.

At the beginning of the third day of regeneration (9 AM) mitotic activity in the epidermis adjacent to the wound was not significantly different from what was observed at 9 AM in the normal skin. The change in the mean MC during the interval between 9 AM and 3 PM also was not significant. After 3 PM we noted a reduction in the number of mitoses, but this reduction was not significant (P = 0.16). Then, after 9 PM the level of mitotic activity in the old epidermis increased so sharply that at 3 AM of the third day of regeneration we were able to establish the largest number of mitoses, differing significantly from the number at 9 PM (P = 0.0001). At 9 AM at the end of the third day of regeneration we observed a fall in the number of mitoses to a value, like that at 9 AM at the beginning of the same day, only a little greater than in the normal epidermis. The change in the MC between 3 AM and 9 AM was statistically significant (P = 0.001). Therefore, on the third day of



24-hour changes in the mitotic coefficients (MC) in epidermis during healing of skin wounds.

Change in the MC in Regenerating Epidermis on the Third and Fifth Days of Healing of Skin Wounds

Regeneration time	Time of day					Mean for the day
	9 AM	3 PM	9 PM	3 AM	9 AM	
3rd day	5,7	4,3	7,2	7,2	5,9	6,1
5th day	4,3	5,5	3,2	4,6	4,6	4,4

healing the epidermis adjacent to the wound is characterized by the presence of a 24-hour rhythm of cell multiplication that takes the form of a curve with a single maximum of the number of mitoses at 3 AM and a minimum at 9 AM.

At 9 AM, at the beginning of the fifth day, the level of mitotic activity in the epidermis was a little higher than at the same time in normal skin; but statistical analysis indicated that this difference was not significant (P = 0.04). Starting at 3 PM, the number of mitoses in the epidermis decreased, reaching a minimum at 9 PM. The difference in the number of mitoses between 3 PM and 9 PM was statistically significant (P = 0.002). At 3 AM the level of mitotic activity reached the highest point, and was significantly different from the level at 9 PM (P = 0.001). The reduction in the number of mitoses by 9 AM at the end of the fifth 24-hour period was considerable, but not significant (P = 0.04). It follows that on the fifth day of the regeneration process the epidermis next to the wound is also characterized by the presence of a 24-hour rhythm in cell multiplication, but the total level of mitotic activity is somewhat lower than on the third day of healing of skin wounds.

What sort of mitotic activity takes place in the regenerating epidermis? Since epithelization of the wound surface did not occur on the first day after the wounds were made, we began examining mitotic activity on the third day. On the third day of regeneration, we found tongues of regenerating epidermis under the scab, growing in from the margins of the old epidermis. But the regenerating epidermis covers only about one third of the whole surface of the wound at this time. On the fifth day the degree of epithelization of the wound surface is different: in some cases epithelium covers half the surface of the wound, while in others complete epithelization of the wound surface is seen. No consistent changes are seen in the number of mitoses in the regenerating epidermis at different hours of the day on the 3rd-5th day of regeneration. In other words, we have not been able to detect a 24-hour rhythm of cell multiplication in regenerating epidermis. We can only point out that the number of cell divisions on the third day of healing of skin wounds (mean MC = 6.1) is a little higher than on the fifth day (mean MC = 4.4; see table).

In view of work that has been done on reactive inhibition of mitoses [6] the impression may have been created that the level of mitotic activity in the animal organism is quite labile. It is true that in most cases such inhibition is of short duration, and the number of cell divisions quickly returns to normal. But the situation is somewhat different with the 24-hour rhythm of cell multiplication, in connection with which one might speak of a certain stability. To eliminate the 24-hour rhythm of mitoses or obtain an inversion of the rhythm, certain experimental conditions must be met [10].

We would like to call attention to the maintenance of the 24-hour rhythm of mitotic activity in the case of traumatic regeneration. Thus, for example, we have shown [3] that if a burn is produced on one cornea of the rat, the 24-hour rhythm of cell division is maintained in the other cornea. The 24-hour rhythm of mitotic activity also is seen distinctly in the liver of rats and mice after partial hepatectomy [5, 11].

Our results agree with those of Blumenfeld [8] concerning the presence of a 24-hour rhythm of mitotic activity in the epidermis during healing of skin wounds. But he examined the progress of regeneration in rabbit epidermis on the seventh day only, when the process of epithelization is over.

Our data on the absence of a 24-hour rhythm of mitotic activity in regenerating epidermis are of interest. V. N. Dobrokhotov [3] has also noted that in regenerating epidermis the level of mitotic activity at all periods during the healing of skin wound is almost the same as in the epidermis of normal skin, which agrees with the results of our experiments on the 3rd-5th days of regeneration.

Results similar to ours, though not directly concerned with the 24-hour rhythm, were obtained by G. A. Kovaleva [4], who showed that the response of regenerating tissue to stimulation of the animals with an electrical current is different from that in the adjacent epithelium: whereas a marked depression is seen in the rate of cell division in the epithelium adjacent to the wound (cornea, tongue), in the regenerating epithelial layer reactive inhibition is almost absent (tongue). At the end of the regeneration process the reactivity of the epithelium becomes normal.

The difference that we found between the levels of mitotic activity on the third and fifth days of regeneration appeared only at the time of the maximum number of cell divisions (3 AM), for the most part. A similar phenomenon has been reported by other authors [2, 7, 12]. For this reason, it is recommended that materials be fixed in the early morning hours to obtain more clear-cut differences between experimental and control groups.

SUMMARY

Observations revealed that cell multiplication follows a 24-hour rhythm in the epidermis adjacent to a skin wound on the rat's back. The greatest differences between the mitotic activities at different stages of wound healing (on the first, third, and fifth day of regeneration) were observed at the time when the number of cell divisions was maximal, i. e., in the early morning hours. No 24-hour rhythm was seen in the mitotic activity of regenerating epidermis directly over the wound.

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