CHANGES IN THE MITOTIC ACTIVITY IN THE INJURED CORNEAL EPITHELIUM OF RATS DURING DRUG-INDUCED SLEEP

L. V. Sokolova

Laboratory of Histophysiology (Head, Candidate of Biological Sciences V. N. Dobrokhotov), Institute of Experimental Biology (Director, Professor I. N. Maiskii) of the AMN SSSR, Moscow

Presented by Active Member AMN SSSR N. N. Zhukov-Verezhnikov
Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny,
Vol. 53, No. 6, pp. 77-80, June 1962
Original article submitted April 10, 1961

It has been shown that animals in a state of quiet have a higher level of mitotic activity than animals in a state of excitation. Bullough [10] observed an increase in mitotic activity in the epidermis of the ear in mice during drug-induced sleep. We have shown [7] that prolonged drug-induced sleep (10 hours) causes a considerable increase in mitotic activity in the corneal epithelium of rats. A similar phenomenon was observed in the cornea of mice by S. Ya. Zalkind [5].

There are reports in the literature that drug-induced sleep encourages the process of tissue regeneration, and that the administration of small doses of sedatives stimulates wound healing. We therefore thought it would be interesting to study the effect of drug-induced sleep on the changes in mitotic activity in the tissues after wounding of animals.

EXPERIMENTAL METHOD

As sedative we used sodium amytal, injected subcutaneously into the dorsal region of the rats in a dose of 0.5 ml/100 g body weight of the 2% solution. The animals usually fell asleep 5-10 minutes after the sodium amytal injection, and the sleep lasted 2-3$^1/_2$ hours.

In the first series of experiments, one hour after injection of the sedative a linear incision was made by means of a razor blade in the central part of the right cornea. At the same time a similar incision was made in the right cornea of a control animal in a waking state.

In the second series of experiments a similar incision was made in the cornea of one eye of the experimental rats by means of a razor blade; one hour after the operation the animals received an injection of the sedative. The object of this series of experiments was to discover the effect of drug-induced sleep on mitotic activity arising in early periods after infliction of a wound.

Each experimental group comprised 7-10 animals and the control group included 7 animals. The experiments were conducted on male rats weighing 150-170 g.

The experimental and control animals were sacrificed at the same times of day, 2, 6, 12, and 24 hours after infliction of the wound. The mitotic activity was studied in both corneas (injured and intact). The corneas were fixed in Zenker's fluid and stained with hematoxylin by Caracci's method; total specimens were prepared. The mitotic coefficient was determined by counting an average of 95 fields in vision in each preparation. The figures for the number of mitoses were calculated in terms of the total number of cells. The mitoses in the preparations of the intact corneas were counted along two perpendicular lines, intersecting at the center of the cornea, and in preparations of the injured corneas along the line of the incision to the edges of the specimen. The results were treated statistically by the Fisher-Student method.

EXPERIMENTAL RESULTS

In Table 1 we give the results of the first series of experiments in which the corneal wound was inflicted on the animals during sleep. The sedative was injected at 4 P.M., and at 5 P.M. the linear incisions were made in both experimental and control animals.

TABLE 1. Mitotic Coefficient (per 1000 cells) in the Corneal Epithelium of Rats After Wounding During Sleep

Time elapsing after wounding (in hours)	Experiment		Control	
	injured cornea	intact cornea	injured cornea	intact cornea
2	2.8	3.2	1.3	1.2
6	2.5	2.2	6.1	6.4
12	11.2	10.8	8.6	9.6
24	5.8	5.6	4.9	3.7

It may be seen in Table 1 that the values of the mitotic coefficient in the injured and intact corneas were very similar in each investigation in both the experimental and control animals. Nevertheless a difference was present between the results obtained in the experimental and control animals.

In the control animals, 2 hours after wounding, the number of mitoses in the intact and injured corneas was less ($P = 0.02$) than in the corresponding corneas of the experimental rats. Six hours after wounding, the mitotic coefficient in the control animals rose significantly, while that in the experimental animals remained at the same level. This difference was statistically significant (for the intact corneas $P = 0.001$ and for the injured $P = 0.004$). The considerable increase in the number of mitoses in the control animals at this period and thereafter may be regarded as the result of the diurnal periodicity of mitotic activity [1, 2]. In the experimental animals the diurnal periodicity of mitotic activity became manifest only 12 hours after wounding. Starting at this time, the differences in the number of mitoses in the experimental and control animals were almost completely eliminated (after 12 hours $P = 0.11$, after 24 hours $P = 0.03$).

These results are in agreement with the results of M. T. Gololobova's experiments, demonstrating the preservation of the diurnal periodicity of mitotic activity in the epidermis of rats after the infliction of skin wounds [3].

Thus after wounding of the cornea of animals in a state of drug-induced sleep the ordinary diurnal changes in mitotic activity were deranged, at least for the first 6 hours.

In the second series of experiments the cornea of the waking animals was incised at 5 P.M. and the sedative administered at 6 P.M. The results are shown in Table 2.

TABLE 2. Mitotic Coefficient (per 1000 cells) in the Corneal Epithelium of Rats Wounded Before the Onset of Sleep

Time elapsing after wounding (in hours)	Experiment		Control	
	injured cornea	intact cornea	injured cornea	intact cornea
2	2.1	3.8	1.3	1.2
6	3.6	5.6	6.1	6.4
12	10.7	10.8	8.6	9.6
24	5.6	5.3	4.9	3.7

Two hours after infliction of corneal injury on the rats, which were in a state of drug-induced sleep or awake, the mitotic coefficient was higher than in the corresponding corneas of the control rats ($P = 0.03$). These differences began to disappear 6 hours after wounding: the differences between the numbers of mitoses in the injured corneas of the experimental and control animals were statistically significant ($P = 0.02$), but in the uninjured corneas the difference between the numbers of mitoses (after 12 hours $P = 0.19$, and after 24 hours $P = 0.09$). The high values of the mitotic coefficient at these times of day reflected the usual diurnal periodicity in the number of mitoses in this tissue.

The following conclusions may be drawn from a comparison of the results of the two series of experiments.

A noteworthy feature was the very small number of mitoses in the corneas of the control animals, found 2 hours after wounding. This may evidently be attributed to the presence of reactive inhibition of mitotic cell division, arising as a result of the general excitation of the animal and the application of the painful stimulus [4, 8].

Meanwhile, in the animals whose corneas were wounded during drug-induced sleep, or which received the sedative very soon after wounding, reactive inhibition of mitosis developed to a weaker degree, as shown by the significant differences between the numbers of mitoses in the control and experimental animals in the early stages of the experiment. Six hours after wounding, the reactive inhibition in the control animals had completely disappeared, and the mitotic activity began to change to correspond to the diurnal rhythm of cell division typical of this tissue. At this time the mitotic activity in the first series of experimental animals remained as before on a low level, and in the animals of the second series it began to increase slightly, especially in the intact cornea.

A single injection of this particular dose of a sedative thus causes a change in the mitotic activity of the corneal epithelium in rats which may be traced for the next 6 hours. Without supplementary investigations it is difficult to give a more detailed explanation of this phenomenon. A possible cause of these changes is the relaxation of the regulating influence of the nervous system on the processes of cell proliferation. On the other hand, investigations by various workers suggest that during drug-induced sleep essential changes take place in metabolism, especially carbohydrate metabolism, playing an important role in determining the level of mitotic activity in the body tissues [11, 12]. Furthermore the experimental data of A. P. Krasil'nikova [6] and A. V. Fridman-Pogosova [9] have shown that during drug-induced sleep the intensity of incorporation of amino acids into the proteins of the blood serum and of other organs is increased.

LITERATURE CITED

1. M. T. Gololobova, Byull. Éksper. Biol., 9, 118 (1958).
2. M. T. Gololobova, Byull. Éksper. Biol., 3, 94 (1959).
3. M. T. Gololobova, Byull. Éksper. Biol., 10, 118 (1960).
4. S. Ya. Zalkind, Doklady Akad. Nauk SSSR, 99, 6, 1091 (1954).
5. S. Ya. Zalkind, Byull. Éksper. Biol., 7, 99 (1959).
6. A. P. Krasil'nikova, Trudy Khabarovsk. Med. Inst., 13, 48 (1954).
7. L. V. Sokolova, Byull. Éksper. Biol., 7, 95 (1959).
8. G. S. Strelin and V. V. Kozlov, Arkh. Anat., 2, 3 (1959).
9. A. V. Fridman-Pogosova, Doklady Akad. Nauk SSSR, 102, 6, 1227 (1955).
10. W. S. Bullough, Proc. roy. Soc. B., v. 135, p. 233 (1948).
11. Idem. Nature, v. 163, p. 680 (1949).
12. W. S. Bullough and E. A. Eisa, J. exp. Biol., v. 27, p. 257 (1950).
