THE DAILY PERIODICITY OF THE MITOTIC ACTIVITY IN THE CORNEAL EPITHELIUM OF STARVING RATS AND MICE

O. T. Movchan

Laboratory of Biology, Institute of Experimental Pathology and Therapy, Academy of Medical Sciences, USSR (Director, Doctor of Medical Science B. A. Lapin), Sukhumi

(Presented by Active Member Academy of Medical Sciences, USSR, A. V. Lebedinskii)
Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 52, No. 7, pp. 103-106, July, 1961
Original article submitted November 9, 1960

In the literature devoted to problems of cell multiplication numerous facts have been described which suggest the existence of a marked daily periodicity in the mitotic activity of a number of mammalian tissues [1,2,3,10]. Some papers report a relation between the daily periodicity of cell multiplication and lighting [1,7,10], or the feeding habits [1,9]. It is, however, not quite clear which of these factors is decisive in determining the character of the daily periodicity of cell multiplication. For example numerous authors have produced evidence for the influence of lighting upon the daily periodicity [1,7,10] but only a few studies were devoted to the effect of alimentary stimuli upon the daily periodicity and the conclusions of the authors were of contradictory character [1,9].

To elucidate the factors which determine the daily periodicity of cell multiplication in the corneal epithelium we carried out experiments on starving rats and mice in which the influence of the alimentary factor had been excluded for a prolonged period. Our previous experiments served as the starting point for the present study. In these [4,5] we had shown that cell multiplication in the corneal epithelium of starving animals can for a considerable time be kept at a normal level.

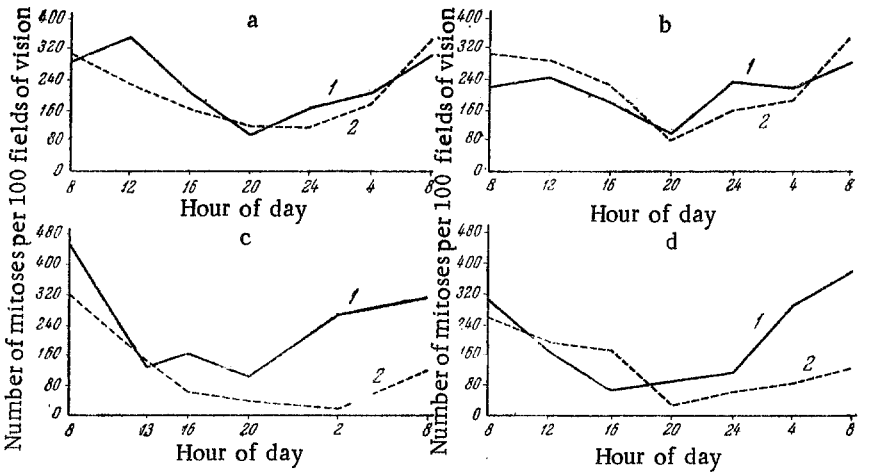
METHOD

The experiments were carried out on male white mice and white rats. The average weight of the experimental mice was 20 g. In the experiments on rats three weight groups were used: 1) heavyweight animals: 250-260 g; 2) animals of medium weight: 150-160 g; 3) animals of light weight: 50-60 g. The heavyweight rats were killed between the second and third day of starvation. This period was chosen in view of the fact that in our previous experiments no decrease in the mitotic activity had been observed in animals of that weight group after a similar period of starvation [5]. Rats of medium weight were killed on the fourth-fifth day of starvation, assuming that by that time possible changes in the mitotic activity would be found [5]. Lightweight rats were killed on the third-fourth day and mice on the first-second day of starvation: in the earlier experiments we had observed a marked decrease in the mitotic activity in lightweight rats and mice by that time [4,5]. Throughout the period of starvation the experimental animals received no food at all but were given unlimited quantities of water. The control animals received the normal quantities of food. In all other respects the conditions under which the experimental and controlanimals were kept were completely identical. The animals were killed every four hours beginning from 8 a.m. to 8 a.m. on the next day. Five experimental and five control animals were killed simultaneously. Altogether 205 white rats and 70 white mice were used for the present study.

The mitoses were counted on total preparations of the corneal epithelium after staining with hematoxylin. The various phases of the mitotic cycle were counted separately. The whole cornea was reviewed. The mitotic index was defined as the number of cells found in a state of division in a hundred fields of vision, which corresponded to 1 mm^2 area within the corneal epithelium.

RESULTS

Heavyweight rats. Experiments carried out on heavyweight rats show that the mitotic activity of the corneal



The daily periodicity in the mitotic activity of the corneal epithelium of starving rats and mice: a) heavyweight rats; b) medium-weight rats; c) lightweight rats; d) mice; 1) control animals; 2) starving animals.

epithelium underwent in both the fed animals and the starving animals considerable changes in the course of the day (see Fig. a). The maximum number of mitoses was found in the morning hours; in the course of the day the number of cells in a state of division decreased and the lowest number of mitoses was found at 8 p.m. (in both the starving and the fed rats the difference between the maximum and the minimum was found to be significant; p=0.000). Beginning from midnight the number of mitoses gradually increased and practically reached by 8 a.m. of the next day the original level. In no period could a statistically significant difference in the number of cells in a state of division be found between the control rats and the experimental rats respectively. The mean mitotic index for the day reached in the control animals 220 and in the starving animals 207 (this difference is statistically insignificant).

Rats of medium weight. In this series of experiments the daily periodicity was studied after periods of starvation ranging from 96 to 120 hours. The average values of the mitotic index are set forth in the Fig. b. The pattern of the mitotic activity in animals of this weight group was hardly different from the picture of changes described in the previous experiment. In both the starving and the fed animals the maximum number of mitoses was found in the morning hours (between 8 and 12) and the minimum activity in the eveninghours (8 p.m.). The mean daily mitotic index reached in the starving animals 244 and in the control animals 215 (the difference was statistically insignificant). It thus appears that in this weight group too we failed to find any changes in the character of the daily periodicity during starvation, changes which could have been regarded as a decrease in the average daily number of mitoses or as a disruption of the daily rhythm.

Lightweight rats. The mitotic activity was studied after periods of starvation ranging between 72 and 96 hours. The curves characterizing the mitotic activity set forth in Fig. c show that in the control animals the daily periodicity was the same as in animals of the former weight groups: the maximum number of mitoses was found in the morning hours (8 a.m), a minimum in the evening (8 p.m.).

Almost throughout the whole period of experiment a considerable decrease in the number of cells in state of division could be found in the starving animals compared with the fed animals. Besides it is a fact deserving attention that the minimum of the mitotic activity was shifted from 8 p.m. to 2 a.m. The difference in the number of mitoses between the peak and the bottom of the curve proved to be statistically significant (p=0.002).

Toward the end of the experiment (8 a.m. next day) the mitotic activity showed a slight increase. This increase was found not to be statistically significant (p=0.454). The mean daily mitotic index reached in the control rats 244 mitoses per 1 mm^2 cornea and in the starving rats 120 mitoses. This difference was found to be statistically significant (p=0.01). It thus appears that in the described series of experiments we were able to find an appreciable decrease in the number of cells in a state of division in starving animals compared with the corresponding control experiment almost throughout the day. There was, however, no complete levelling of the indices characterizing the mitotic activity, as a certain similarity to the usual rhythm of cell multiplication was preserved.

Mice. Throughout the first half of the experiment (starvation from 24 to 48 hours) no statistically significant difference in the number of mitoses was found in the starving and control animals respectively. The difference between the maximum and the minimum was found to be statistically significant (p=0.002 for the control animals and p=0.0000 for the starving animals). In the second half of the experiment beginning from 8 p.m. a general decrease in the mitotic activity could be observed in the starving animals but a statistically significant difference was found only toward the end of the experiment at 4 a.m. and 8 a.m. ($p = 0.005$ and $p = 0.006$ respectively). At the same time the starving mice preserved a tendency toward an increased number of mitoses in the morning hours. The difference between the maximum and minimum number of mitoses was characterized by a considerable degree of significance (p=0.037). The mean daily mitotic index reached in the control mice 209 and in the starving mice 135 ($p = 0.013$).

The results of four series of experiments quoted in the present paper concerning the daily periodicity of the mitotic activity show that a marked rhythm of cell multiplication exists not only in the control animals, which were fed, but also in the starving animals. In heavyweight and medium weight rats no statistically significant difference can be found in either the character of the curves of the daily rhythm of cell multiplication or the average daily number of mitoses compared with the control animals even after prolonged starvation (for three-five days). In both the starving and the fed animals the daily curves characterizing the mitotic activity have a peak in the morning hours (between 8 a.m. and noon) and the deepest point in the evening (8 p.m.).

In our opinion this fact can be explained with the fact that in the starving rats the content of protein in the eye, including the cornea, which represents the main plastic material, does not change compared with the corresponding control animals [8]. Consequently the presence of a certain amount of energetic reserves in the animal body makes it possible to maintain cell multiplication in the corneal epithelium of starving animals at normal levels. Only in lightweight rats and mice, which animals have a higher intensity of metabolism than heavyweight rats, could a decrease in the mitotic activity be observed. But even in these animals the daily periodicity is preserved notwithstanding the considerable fall in the average daily number of mitoses.

We were thus unable to confirm on the example of the corneal epithelium of white rats and white mice the conclusions of Blumenthal [9] obtained in experiments on guinea pigs concerning the daily rhythm of cell multiplication in starving animals.

As far as the factors determining the daily periodicity of the mitotic activity are concerned the results of a number of authors [1,7,10] must be emphasized who showed that a marked interrelation exists between artificial changes of sequence of day and night and a full reversion in the daily periodicity of the mitotic activity in the corneal epithelium as well as in the epithelium of the skin, the tongue and the esophagus. The influence of prolonged changes in the feeding habits [1] became manifest only in changes in the daily periodicity of the mitotic activity in the intestinal epithelium and the epithelium of the tongue, i. e., the tissues of those organs directly connected with the process of digestion, and did not become manifest in the mitoses found in the corneal epithelium and the epithelium of the skin.

Summing up the findings discussed in this paper and the investigations of the authors quoted above [1,7,10] we come to the conclusion that apparently it is not the feeding habits but the light factor which determines the regulation of the daily periodicity in the mitotic activity of the corneal epithelium. Light possibly acts as signal correlating the behavior reaction of the animals and thus changing the relative proportion between plastic and energetic forms of metabolism and thus determining one or the other level of cell multiplication at various times of the day.

SUMMARY

The author studied the 24 hour periodicity of mitotic activity in rats belonging to three different weight groups and mice during starvation. In heavy rats (250-260 g) and in those of moderate weight (150-160 g) starved for 2-5 days no statistically authentic differences were noted either in the 24 hour rhythm of mitosis, or in the mean 24 hour number of the latter (in comparison with control). Only in rats of low weight (50-60 g) and in mice the mitotic activity decreased considerably at the end of the experiment with prolonged starvation. However, the 24 hour rhythm of mitosis was retained to some extent. The 24 hour mitotic curves were characterized by a rise during the morning and reduction in the evening both in the animals on normal diet and in the starving ones.

LITERATURE CITED

1. I. A. Alov, Byull. Éksper. Biol. i Med., 48, 11 (1959) p. 107.
2. M. T. Gololobova, Byull. Éksper. Biol. i Med., 46, 9 (1958) p. 118.
3. L. P. Kosichenko, Byull. Éksper. Biol. i Med., 49, 6 (1960) p. 98.
4. O. T. Movchan, Byull. Éksper. Biol. i Med., 50, 7 (1960) p. 90.
5. O. T. Movchan, Byull. Éksper. Biol. i Med., 50, 12 (1960) p. 86.
6. E. Svetozarov and G. Shtraikh, Uspekhi Sovr. Biol., 12, 1 (1940) p. 25.
7. I. A. Utkin and L. P. Kosichenko, Dokl. AN SSSR., 134, 1 (1960) p. 191.
8. T. Addis, L. J. Poo, and W. Lew, J. Biol. Chem., 115 (1936) p. 11.
9. H. Blumenthal, Anat. Rec., 101 (1948) p. 679.
10. F. Halberg, J. J. Bittner, and D. Smith, Z. Vitamin-, Hormon- u. Fermentforsch. 9, 69 (1957).

All abbreviations of periodicals in the above bibliography are letter-by-letter transliterations of the abbreviations as given in the original Russian journal. *Some or all of this periodical literature may well be available in English translation.* A complete list of the cover-to-cover English translations appears at the back of this issue.
