CHANGES IN MITOTIC ACTIVITY IN RATS IN RELATION TO THE TIME OF DAY OR NIGHT

M. T. Gololobova

From the Laboratory of Histophysiology (Head – Candidate Biol. Sci. V. N. Dobrokhotov) of the Institute of Experimental Biology (Director – Prof. L. N. Maiskii) of the AMN SSSR, Moscow

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On the basis of reports in the literature [8-10, 12-19] it can be taken as established that there is a daily rhythm of mitotic activity in the body. This leads to the thought that in the normal animal there are conditions which regulate the process of mitotic division. In recent years data has been obtained in this direction by G. S. Strelin and his co-workers [7], L. A. Alov and his co-workers [1, 2], S. Ia. Zalkind [3] and others. It is natural to suggest that disturbance of these conditions may lead to abnormality of the daily rhythm of cell division.

The question arises whether these changes in the daily rhythm of mitotic activity in the body arise during various pathological states, and in particular in malignant growth. Very few investigations have been devoted to this problem [1, 8, 11, 20]. The scanty reports in the literature on the character and intensity of the changes in the daily rhythm of mitotic activity do not enable any definite opinion to be formed on the laws affecting this phenomenon.

In this connection it appeared desirable to carry out research in order to shed light on the following problems: 1) What is the daily periodicity of the processes of cellular proliferation in the different meristems* of the normal animal? 2) Is the mitotic activity changed in these meristems in the course of day and night when a focus of malignant growth is present in the body? 3) Is there a daily rhythm of cell division in the tumor itself?

EXPERIMENTAL METHOD

We carried out experiments on white rats (males). Altogether 80 animals were used in a control series and 64 rats in the experimental series, weighing from 90 to 110 g. The animals of the experimental series were inoculated with sarcoma M-1. The rats were killed by decapitation at intervals of 3 hours, i. e. 8 times in the course of 24 hours. The material was fixed in Zenker's solution; staining was with hematoxylin by Caracci's method. The mitotic activity was assessed by calculation of the mitotic coefficient – the ratio between the number of dividing cells and the total number. The whole of the results were treated statistically by the Fisher-Student method.

EXPERIMENTAL RESULTS

The purpose of the first control series of experiments was to establish the character of the daily periodicity of cellular proliferation in the cornea of the eye, the epidermis of the skin and the crypts of the small intestine in normal rats. In Fig. 1 the changes in the mitotic coefficient in the course of the 24 hours in the three structures indicated are expressed graphically.

It follows from the numerical data for the mitotic coefficients obtained for the corneal epithelium of normal rats that, starting at 12 noon the number of mitoses in this tissue gradually falls. The minimum number

* As in original.

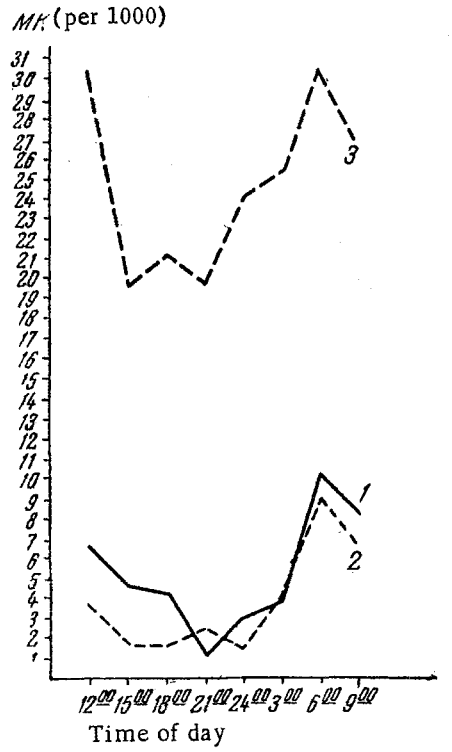

Fig. 1. The mitotic coefficient (MK) in different meristems of normal rats in the course of 24 hours. 1) corneal epithelium; 2) epidermis of the skin; 3) epithelium of the small intestine.

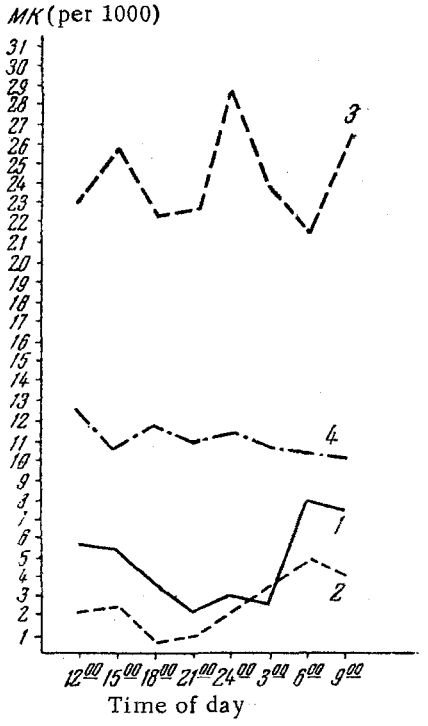

Fig. 2. The mitotic coefficient in different meristems of rats inoculated with a tumor, in the course of 24 hours. 1) corneal epithelium; 2) epidermis of the skin; 3) epithelium of the small intestine; 4) M-1 sarcoma.

of dividing cells is found at 9 p.m. The fall occurring in the interval between 6 p.m. and 9 p.m. is statistically significant (P=0.002). Next the number of dividing cells gradually increases and reaches a maximum value at 6 a.m. The increase in mitotic activity between 3 a.m. and 6 a.m. is statistically significant (P=0.005). Although the level of mitotic activity in the corneal epithelium is lower at 9 a.m. than at 6 a.m. (P=0.05), it remains relatively high and at approximately the same level as at 12 noon.

The results described demonstrate that in the corneal epithelium of normal rats there are daily changes in mitotic activity, and the curve of these changes is seen to be clearly unimodal in character.

The changes in the mean values of the mitotic coefficients in the epidermis of the skin at various times of day and night also demonstrate the presence in this meristem of a daily rhythm of cellular proliferation, and the curve of the changes in mitotic activity is unimodal in character. Starting at 12 noon a fall in the mitotic activity occurs (P=0.01), the subsequent variations of which are not statistically significant. The minimum number of divisions is observed at 12 midnight, after which a sharp rise takes place in mitotic activity, reaching a maximum value at 6 a.m. (in the interval between 3 a.m. and 6 a.m. P=0.01). The fall in the value of the mitotic coefficient to 9 a.m. and later is insignificant, and hence the period from 3 a.m. to 12 noon may be regarded as a time of relatively high mitotic activity in the epidermis of the skin.

As in the first two cases (cornea of the eye, epidermis of the skin), starting at 12 noon the value of the mitotic coefficient for the epithelium of the crypts of the small intestine falls sharply. By 3 p.m. the mitotic activity is already at a minimum and in the interval between 3 p.m. and 9 p.m. it remains practically at the same level. At 12 midnight and later the level of mitotic activity gradually rises; the maximum number of dividing cells is also found at 6 a.m. As also in the case of the epidermis of the skin, the period from 12 midnight to 12 noon must be regarded as a time of relatively high mitotic activity.

Thus in all the meristems of normal rats examined there exists during the course of the 24 hours a daily rhythm of cellular proliferation, and the curve of the changes in mitotic activity is unimodal in character. The mitotic activity is known to characterize the degree of renewal of cells in the particular tissue, i. e. the level of its physiological regeneration. The results obtained enable the conclusion to be made that the level of physiological regeneration is lower in the epidermis of the skin by comparison with the corneal epithelium and, more especially, with the epithelium

of the crypts of the small intestine, where the mitotic activity is 3-4 times higher than in the cornea.

In all the tissues studied we determined not only the number of dividing cells but also the phases of mitosis. We were unable to establish any essential changes in the relationships between the phases of mitosis at the different times in the 24 hours.

The purpose of the second series of experiments was to elucidate the problem of the character of the possible changes in the daily periodicity of cellular proliferation in the different meristems of the animal affected with a focus of malignant growth. The experimental rats were killed on the 15th day after inoculation of a M-1 sarcoma into the thigh muscles. At the moment of death, the weight of the tumors in the different animals varied between 10 and 40 g.

Comparison of cellular proliferation in the cornea of the control and experimental animals demonstrates the identity of the character of the daily rhythm of mitotic activity in normal animals and in those affected by a malignant growth (Fig. 2). The smallest number of divisions, like in the normal animal, is found at 9 p.m., and the maximum mitotic activity is observed at 6 a.m. However it should be pointed out that the extent of the variations of the mean values of the mitotic coefficients in the cornea is shown to a lesser degree in the experimental than in the control animals.

The same can be stated concerning comparison of the daily changes in mitotic activity in the epidermis of the skin of normal and experimental animals; in their character these changes are of the same type. The daily variations in the mitotic activity of the crypts of the small intestine are shown somewhat differently in the experimental animals. In animals inoculated with tumors the fall in the value of the mitotic coefficient in the crypts of the small intestine begins at 3 p.m. and continues at approximately the same level until 9 p.m. The maximum number of divisions is observed at 12 midnight, not at 6 a.m. as in normal animals. In the interval from 6 p.m. to 12 midnight the difference in the values of the mitotic coefficient is statistically significant, although the sharp fall in the level of mitotic activity at 6 a.m. is not significant in character, as is also the case with its increase which commences at 9 a. m. Consequently in an animal affected by a malignant growth the variations in the mitotic activity in the crypts of the small intestine demonstrate a disturbance of the character of the daily rhythm of cell division. In addition the maximum and minimum values of the mitotic coefficients in the experimental rats are smaller than in the controls.

How does the value of the mitotic coefficient change in the course of the 24 hours in the tumor itself?

Analysis of the available data leads us to the conclusion that a daily rhythm of cell division is absent from the transplanted M-1 sarcoma. The value of the mean mitotic coefficients is practically unchanged at different hours of the day or night; under these conditions it is slightly higher than the value of the maximum mitotic activity of the corneal epithelium and of the epidermis of the skin in normal rats, but significantly lower than that in the epithelium of the small intestine.

Thus the absence of a daily rhythm of mitotic activity in the tumor may be evidence of a definite peculiarity of the process of cellular proliferation.

The results which we obtained confirm the observations made by several authors [9-11, 16, 19] on the presence of a daily periodicity in cellular proliferation. The curve of mitotic activity has a unimodal character. At the same time our results do not agree with the findings of other workers [12, 15] who find that the curve of the daily rhythm of mitotic cell division is bimodal. This is evidently accounted for by the fact that in Bullough's [12] work a method was used in which material was repeatedly taken from the same animal.

From the data in the literature there is some doubt about the problem of the cynchrony of the daily changes in mitotic activity in the different meristems of the same animal. According to some authors [10] the daily changes in the different meristems are not of the same type, whereas other workers [12] maintain the opposite view. This divergence of opinion may be explained by the fact that each of these authors cited used different meristems of the animal for comparison.

The problem of the synchrony of the changes in mitotic activity in different tissues is of great importance in solution of the question of the degree of specificity of the factors regulating cell division in different meristems of the animal. The experimental findings which we obtained demonstrate that the daily changes in cell

division in the cornea, the epidermis of the skin and the epithelium of the crypts of the small intestine in normal rats belong to the same type.

So far as the changes in the daily periodicity of cellular proliferation in rats affected by an inoculated M-1 sarcoma are concerned, our findings suggest some disturbance with the rhythms established in normal animals. We point out the reduction in the range of variations of the mean values of the mitotic coefficients in the course of the 24 hours and the displacement of the maximum number of cell divisions in the crypts of the small intestine from daytime to nighttime.

The question of the effect of a malignant growth on the changes in the daily periodicity of cellular proliferation may be finally settled by investigations on spontaneous or indiced tumors.

SUMMARY

Mitotic activity in the corneal epithelium, cutaneous epidermis and cryptic epithelium of the small intestine of normal rats is characterized by a distinct 24-hour rhythm.

In all of these tissues the maximum number of cellular divisions was observed in the morning and the minimum in the evening. In rats with sarcoma M-1 (transplanted subcutaneously) no distinct changes were discovered in the 24-hour rhythm of the cellular division of the same tissues. The 24-hour rhythm of the cellular division is absent in the transplantable M-1 sarcoma.

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