DIURNAL RHYTHM OF MITOTIC ACTIVITY, DNA SYNTHESIS, AND DURATION OF MITOSES IN MOUSE BONE MARROW CELLS

K. G. Moskalik

UDC 612.419.014.3:612.6"52"

Cells of the myeloid and erythroid series of mouse bone marrow are characterized by a well-defined diurnal rhythm of mitotic activity and of DNA synthesis, with coincidence between the times of maxima of the number of mitoses and of DNA-synthesizing cells (in the afternoon and night) and of their minima (in the morning and evening). The increase in mitotic activity is connected with the entry of large numbers of cells into mitosis and not with an increase in the duration of mitosis.

KEY WORDS: mitotic index; DNA synthesis; diurnal rhythm; bone marrow.

It has now been shown that most normal tissues have a well-defined rhythm of cell division and DNA synthesis. As yet, however, there are no sufficiently conclusive data on the presence of a diurnal rhythm of cell division in bone marrow [2, 10, 14, 16]. The role of the duration of mitosis in the diurnal rhythm of tissue mitotic activity likewise has not been finally explained [6, 8, 9, 13, 15].

Changes in mitotic activity, in the number of DNA-synthesizing cells, and in the duration of mitosis in mouse bone marrow were studied during the 24-h period.

EXPERIMENTAL METHOD

Experiments were carried out on male SHR mice weighing 18-20 g in the fall. The animals were kept under normal conditions of light and darkness and on a normal diet. The mice of experimental group 1 received an intraperitoneal injection of colchicine in a dose of 1 μg/g 6 h before sacrifice. The late phases of mitosis were not found in bone marrow preparations. The animals of experimental group 2 were injected intraperitoneally with thymidine-H^3 (specific activity 11.6 Ci/mmole) in a dose of 1 μCi/g body weight 1 h before sacrifice. The mice of the control and experimental groups were killed every 3 h during the 24 h. At each time of the experiment five or six animals were taken in each group. Squash preparations were obtained from the femoral marrow. The preparations obtained from mice receiving the isotope were coated with type M liquid emulsion and exposed for 2.5 months. The autoradiographs were prepared in the usual way. All films were stained by Pappenheim's method. The mitotic index (MI) was calculated in the films separately for 2000-3000 nucleated cells of the myeloid and erythroid series, and the labeling index (LI) determined in the same way for 1000 cells. The duration of mitosis was calculated by the equation

$$t_m = \frac{MI \cdot A}{MI_{colch}},$$

where MI is the mitotic index in the control group, MI_{colch} the mitotic index in the group of mice receiving colchicine, and A the time of action of the colchicine.

The numerical results were subjected to statistical analysis by the Student–Fisher method.

High Energy Laboratory, N. N. Petrov Institute of Oncology, Ministry of Health of the USSR, Leningrad.
(Presented by Academician of the Academy of Medical Sciences of the USSR A. I. Serebrov.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 81, No. 5, pp. 594-596, May, 1976. Original article submitted November 28, 1975.

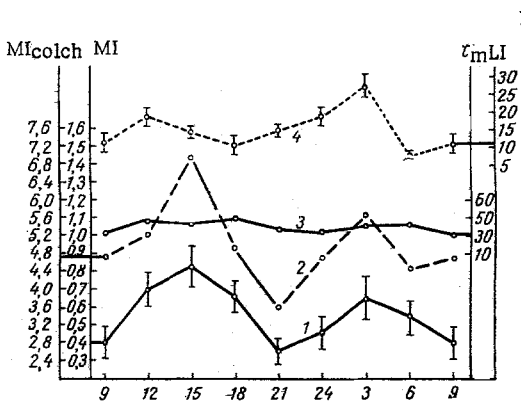

Fig. 1

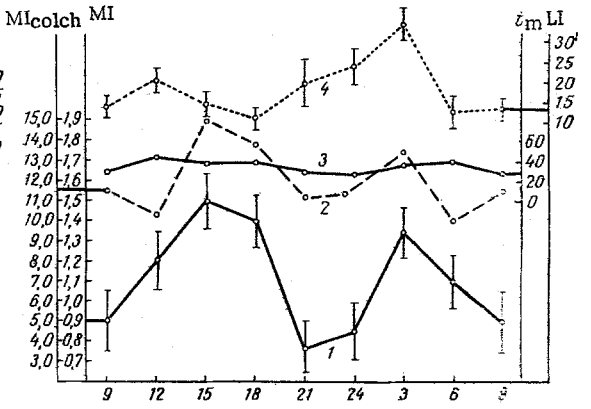

Fig. 2

Fig. 1. Changes in MI (1), MI_{colch} (2), the duration of mitosis (3), and LI (4) during the 24-h period in cells of the myeloid series. Here and in Fig. 2: abscissa, time of day (hours); ordinate: left, mitotic index and index of colchicine mitoses (in %); right, labeling index (in %), duration of mitosis (t_m, in min).

Fig. 2. Changes in MI (1), MI_{colch} (2), duration of mitosis (3), and LI (4) during 24-h period in cells of erythroid series.

EXPERIMENTAL RESULTS

Investigation of mitotic activity showed a diurnal rhythm of mitosis in the cells of the myeloid series in mouse bone marrow with a maximum at 3 p.m. and 3 a.m. and a minimum at 9 a.m. and 9 p.m. The mean diurnal MI was 0.6 ± 0.06% (Fig. 1). A similar diurnal rhythm of mitotic activity was found in the cells of the erythroid series. The number of mitoses also reached a maximum at 3 p.m. and 3 a.m. and a minimum at 9 a.m. and 9 p.m. to midnight. The mean diurnal MI was 1.1 ± 0.1% (Fig. 2). Differences between the maximal and minimal values of MI in the cells of the myeloid and erythroid series were statistically significant ($P < 0.02$–0.01). In both cases the increase in mitotic activity in the afternoon was greater than at night.

The study of incorporation of thymidine-H^3 into the dividing cells of the myeloid and erythroid series of the mouse bone marrow showed that they also have a characteristic diurnal rhythm of DNA synthesis. Maximal values of LI in cells of both the myeloid and erythroid series were recorded at noon and 3 a.m. and minimal values at 6-9 a.m. and 6 p.m. Differences between them were statistically significant ($P < 0.05$–0.01). The increase in LI was greater at night than in the afternoon, by contrast with the increase in MI. For instance, whereas the maximal number of labeled cells of the myeloid series at noon exceeded their minimal number by 1.8 times, the maximal value of LI at 3 a.m. was 2.4 times greater than the minimum. In cells of the erythroid series these differences were 1.7 and 3 times respectively. The mean diurnal LI was 14.3 ± 1.8% in cells of the myeloid series and 18.2 ± 2.2% in cells of the erythroid series (Figs. 1 and 2).

The duration of mitosis in the bone marrow cells at different times of the 24-h period was calculated from the accumulation of colchicine mitoses. Counting the blocked mitoses showed differences in the accumulation of C-mitoses during different 6-h time intervals in the course of the 24-h period. The maximal number of colchicine mitoses was observed in cells of both myeloid and erythroid series at the same times as for normal mitoses, namely at 3 p.m. and 3 a.m. The minimal number of C-mitoses in cells of the myeloid series occurred at 6-9 a.m. and 9 p.m., and in cells of the erythroid series at 6 a.m., noon, and 9 p.m. to midnight. The mean diurnal coefficient of blocked mitoses in the cells of the myeloid series was 5.0 ± 0.2% and in cells of the erythroid series 12.1 ± 0.8%. Calculation showed that the duration of mitosis in cells of the myeloid series varied during the 24-h period from 32 to 50 min and in cells of the erythroid series from 28 to 43 min. The mean diurnal duration of mitosis was 41 and 35 min, respectively. The fact should be noted that the duration of mitosis was not increased during the time of increased mitotic activity in the mouse bone marrow.

The principles governing the diurnal rhythm of mitosis have now been studied in detail in several tissues. Some tissues have a monophasic diurnal rhythm of proliferation, when the increase in mitotic activity in most cases occurs in the morning and a decrease in the evening [2, 5, 8]. In other tissues the curve of the change in MI during the 24-h period has two peaks [28, 8]. Information on the diurnal rhythm of cell division in the bone marrow is contradictory. Some workers found no variation in the level of mitotic activity in the

bone marrow during the 24-h period [2, 14]. Others likewise did not observe such a diurnal rhythm of mitosis in the thymus, which also plays the role of a hematopoietic organ. Meanwhile other workers found changes in the number of dividing cells both in the bone marrow [10, 16] and in the thymus [1, 11, 12] during the 24-h period. The present experiments showed that mouse bone marrow has a diurnal rhythm of cell division with two maxima of mitotic activity. They also showed that mouse bone marrow is characterized by changes in the number of DNA-synthesizing cells during the 24-h period, and this increase in LI was observed at approximately the same time of day or night as the increase in MI. As has been stated in the literature, the index of labeled cells usually increases a few hours before the increase in mitotic activity [9]. However, some workers, like the present writer, have found that the changes in MI and LI during the 24-h period coincided in time [8]. In these cases coincidence of the phases of the diurnal rhythm of cell division and incorporation of thymidine-H^3 can probably be explained on the grounds that the afternoon maximum of the number of DNA-synthesizing cells is responsible for the night increase in MI, and the night increase in the first of these indices is responsible for the afternoon increase in the second. The diurnal rhythm of LI is due to a change in the number of DNA-synthesizing cells and not to lengthening of the S period [8, 9]. As regards the role of the duration of mitosis in the diurnal rhythm of cell division, information on this point is ambiguous. Some workers have observed an increase in the duration of mitosis during the peak of mitotic activity [13, 15]. According to other workers, the diurnal peak of mitotic activity is connected with the entry of a larger number of cells into mitosis and not with an increase in the duration of mitosis itself [6, 8, 9]. According to the results of the present experiments the diurnal rhythm of cell division in the mouse bone marrow is the result of a change in the number of dividing cells during the 24-h period. In the present experiments the mean diurnal duration of mitosis in cells of the myeloid and erythroid series of mouse bone marrow, it will be noted, was close to the value given in the literature [4].

The results of this investigation thus indicate that mouse bone marrow cells have their own diurnal rhythm of division. During the 24-h period two maxima of cell division can be distinguished, during the afternoon and night, and two minima, during the morning and evening. Mouse bone marrow is also characterized by coincidence between the times of the changes in the diurnal rhythm of mitotic activity and changes in the number of DNA-synthesizing cells during the 24-h period. The duration of mitosis in cells of the erythroid and myeloid series of mouse bone marrow also changed during the 24-h period. However, the duration of mitosis did not correlate with the diurnal fluctuations of mitotic activity. Consequently, the diurnal rhythm of cell division in mouse bone marrow is due to differences in the number of cells starting on mitosis and not to changes in the duration of mitosis itself.

LITERATURE CITED

1. I. A. Alov, Byull. Éksp. Biol. Med., No. 11, 107 (1959).
2. I. A. Alov and N. V. Krasil'nikova, Dokl. Akad. Nauk SSSR, 142, 933 (1962).
3. G. P. Gruzdev, L. M. Rozhdestvenskii, et al., Byull. Éksp. Biol. Med., No. 2, 83 (1966).
4. O. I. Epifanova, A. I. Zosimovskaya, et al., Byull. Éksp. Biol. Med., No. 1, 96 (1963).
5. L. D. Liozner (editor), Cell Renewal [in Russian], Leningrad (1966).
6. L. D. Liozner and I. V. Markelova, Byull. Éksp. Biol. Med., No. 6, 89 (1975).
7. V. I. Malyuk, L. N. Kharchuk, et al., Tsitologiya, No. 8, 1106 (1972).
8. S. G. Mamontov, "Cell renewal in the epithelium of the mouse cornea and tongue," Author's Abstract of Candidate's Dissertation, Moscow (1969).
9. Yu. A. Romanov, in: Diurnal Rhythms of Physiological Processes of the Organism [in Russian], Moscow (1972), p. 48.
10. V. M. Seraya and G. P. Rodina, in: Current Problems in Space Biology and Medicine [in Russian], Moscow (1971), p. 241.
11. V. V. Sinel'shchikova, in: Diurnal Rhythms of Physiological Processes of the Organism [in Russian], Moscow (1972), pp. 33-34.
12. Yu. P. Khussar, Arkh. Anat., No. 10, 109 (1966).
13. E. Gyergyay Malatinszky and F. Gyergyay, Rev. Roum. Embryol. Cytol., Ser. Cytol., 4, 65 (1967).
14. S. A. Killman, E. P. Cronkite, et al., Blood, 19, 743 (1962).
15. L. Kreyberg et al., Acta Path. Microbiol. Scand., 64, 176 (1969).
16. A. Milleti, Arch. Ital. Anat. Embriol., 54, 339 (1950).