DURATION OF PERIODS OF THE MITOTIC CYCLE FOR INDIVIDUAL RAT GRANULOPOIETIC CELLS

L. E. Sil'vestrova

UDC 612.119.014.3:612.6"5"

The curve of labeled lipids (administration of thymidine-H^3) was used to determine the duration of the mitotic cycle and its periods for individual types of granulopoietic cells in male Wistar rats weighing 150 g. In the myeloblast–promyelocyte–myelocyte series the duration of the cycle increased from 11-12 h to 14.5 h, the duration of the S period from 6.0-7.0 h to 8.5 h, while the M periods were unchanged.

Measurement of the temporal parameters of the mitotic cycle of granulopoietic cells gives a more complete picture of the kinetics of cell populations of the hematopoietic tissue. The use of autoradiography has provided much greater opportunities for the investigation of hematopoiesis. This method can be used to determine periods of the mitotic cycle not only for the whole bone marrow population [7, 9] or of its individual series [2, 4], but also for each type of cell within the series. Only sporadic and incomplete information is available on the total duration of the mitotic cycle of individual human and mammalian granulocytes [5, 7, 9, 10].

With regard for the importance of the study of the proliferation kinetics of hematopoietic cells, it was decided to determine the duration of the mitotic cycle and its periods for individual types of granulopoietic cells in the rat.

EXPERIMENTAL METHOD

Male Wistar rats weighing 150 g received a single intravenous injection of tritiated thymidine (specific activity 2.3 Ci/g) in a dose of 0.6 μCi/g body weight. The animals were sacrificed five at a time at definite time intervals during the 24-h period. Impression films of the marrow were obtained from both femora, fixed with methyl alcohol, and coated with type M (NIIKhIMFOTO) fine-grain emulsion. Exposure lasted six weeks. After development in amidol developer and fixation in 20% sodium thiosulfate, the specimens were stained by the Romanovsky–Giemsa method. To obtain the curve of labeled mitoses 100 mitotic figures were counted separately for myeloblasts, promyelocytes, and myelocytes in the autoradiographs of the bone marrow of each animal, and their phase distribution was determined. A cell undergoing mitosis was considered to be labeled if it had above it more than four or five grains of silver.

It is not particularly difficult to identify dividing granulopoietic cells. Their chief morphological features are preserved during mitosis. The dividing myeloblast has a delicately stained chromatin, a nonhomogeneous blue cytoplasm, and its volume is small compared with the nuclear mass. The promyelocyte in any stage of division is the largest cell of the granulopoietic series, but its size is inconstant. Its morphology, on the other hand, is very typical, for its nuclear chromatin is more deeply stained than that of the myeloblast, its cytoplasm is of considerable volume and moderately basophilic, and it contains the characteristic promyelocyte granulation. In autoradiographs of the dividing myelocyte the chromatin is deeply

Institute of Biophysics, Ministry of Health of the USSR, Moscow. (Presented by Academician of the Academy of Medical Sciences of the USSR P. D. Gorizontov.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 73, No. 1, pp. 87-90, January, 1972. Original article submitted July 20, 1971.

©1972 Consultants Bureau, a division of Plenum Publishing Corporation, 227 West 17th Street, New York, N. Y. 10011. All rights reserved. This article cannot be reproduced for any purpose whatsoever without permission of the publisher. A copy of this article is available from the publisher for $15.00.

TABLE 1. Duration of Periods of Mitotic Cycle for Rat Granulopoietic Cells

	M (min)	G_2 (min)	S (h)	G_1 (h)	T (h)	$\frac{S}{T} \cdot 100$ (%)
Myeloblast	45	45	6,0—7,0	3,5—4,0	11,0—12,0	55—56
Promyelocyte	45	45	8,0—8,5	2,5	12,0—12,5	67—68
Myelocyte	45	45	8,5	4,5	14,5	61

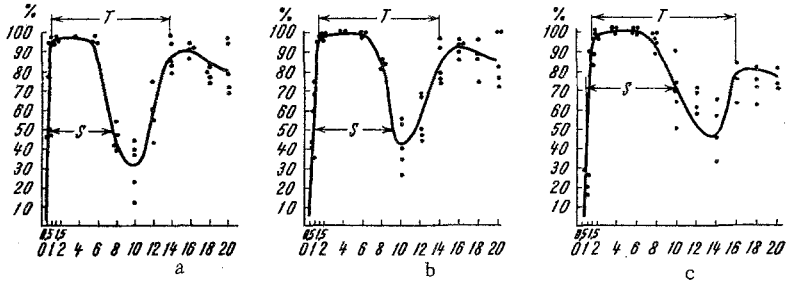

Fig. 1. Curves of labeled mitoses of myeloblasts (a), promyelocytes (b), and myelocytes (c). Abscissa, time after administration of thymidine (in h); ordinate, number of labeled mitoses (in %).

stained, and the weakly basophilic cytoplasm contains clear, fine myelocyte granules. The dividing granulopoietic cell is slightly larger than the interphase cell.

The duration of the periods of the mitotic cycle was determined graphically [11]. Curves of labeled mitoses were drawn for each type of cell (Fig. 1). In these experiments the duration of mitosis M corresponded to the time from the appearance of labeled prophases to the appearance of label in the cells at the reconstruction stage, i.e., in cells just completing cytokinesis; the premitotic or G_2-period corresponded to the interval from administration of thymidine-H^3 to the appearance of the first labeled prophases. The mean duration of the synthetic period S was taken as the time during which over 50% of labeled mitoses was observed. Since the curve of mitoses for the myelocytes in these experiments differed considerably from Quastler's classical curve, the method used was unsuitable for estimation of the S-period of these cells. Accordingly, besides Quastler's method [11], the time from the middle of the rising curve to the middle of the falling curve of the first wave of labeled mitoses was also taken as the mean duration of the synthetic phase. The duration of the mitotic cycle was estimated from the time interval between the two maxima of labeled mitoses and also from the time between the middle of the rising slope of the first wave of mitoses to the middle of the rising slope of the second wave. The duration of S and T for the myeloblasts and promyelocytes was determined by both methods, and S and T for the myelocytes by one method. The duration of the presynthetic period G_1 was determined from the formula: $G_1 = T - (M + G_2 + S)$.

EXPERIMENTAL RESULTS

In these experiments labeled prophases of all granulopoietic cells were found 45 min, and labeled cells in the stage of reconstruction 1.5 h after administration of thymidine. Consequently, the shortest duration of mitoses for all three types of cells was 45 min. Knowing the relative proportions between the phases of mitoses it was easy to determine the duration of each phase of mitotic division of the myeloblasts, promyelocytes, and myelocytes. The duration of prophase for these cells was 30, 25, and 17% of the total duration of mitosis respectively, and the corresponding figures for metaphase were 44, 52, and 58%, anaphase 20, 17, and 20%, and telophase 6, 6, and 4%. If the duration of mitosis was the same for all three types of cells, in the course of their maturation the duration of prophase was shortened while that of metaphase was lengthened. The same pattern has been found by other workers [1].

The temporal parameters of mitotic cycles of the rat granulopoietic cells are given in Table 1. The numbers reflect results obtained by the two methods described above.

Comparison of the mitotic cycles of the granulopoietic cells showed certain regular features: approximately equal durations of the M- and G_2-periods for all three types of cells; lengthening of the S-phase

and the whole cycle T as maturation of the granulocytes took place. The synthetic period occupied the largest part of the whole cycle of the promyelocytes. The shortest duration of the G_1-phase was observed in these same cells. The synthetic period in granulocytes (in the present experiments) and in erythroid cells of rats [13] is the longest part of the mitotic cycle (over 50% of its duration). Similar proportions have been observed for human and dog granulocytes [3, 9, 10]. By comparison with figures given by Cronkite et al. [5, 12], the following conclusion can be drawn: the ratio S/T for hemocytoblasts–myelocytes decreases during maturation of the cells. Zosimovskaya, who investigated the myeloid series as a whole [2], and Ohka Koiti [8], who studied human bone marrow, obtained results indicating that the greater part of the mitotic cycle is taken up by the G period and that only 30% of its duration corresponds to the S-phase. The results obtained for the individual hematopoietic cells are unquestionably more exact. However, the parameters of the mitotic cycles for hematopoietic cells may differ in different species, and this may be responsible for differences in the sensitivity of the hematopoietic system to the action of various factors.

LITERATURE CITED

1. L. V. Bogatov, Byull. Éksperim. Biol. i Med., No. 2, 103 (1970).
2. A. I. Zosimovskaya, Dokl. Akad. Nauk SSSR, 151, No. 3, 687 (1963).
3. J. Boll and G. Fuchs, Exp. Cell Res., 61, 147 (1970).
4. V. P. Bond, T. M. Fliedner, E. P. Cronkite, et al., in: F. Stohlman (editor), The Kinetics of Cellular Proliferation, New York (1959), p. 188.
5. E. P. Cronkite, V. P. Bond, and T. M. Fliedner, et al., in: G. E. W. Wolstenholme et al. (editors), Ciba Foundation Symposium on Haemopoiesis, London (1960), p. 70.
6. E. Frindel and M. Tubiana, Nature, 214, 1017 (1967).
7. B. I. Lord, Brit. J. Haemat., 11, 130 (1965).
8. Ohka Koiti, Shikoku Acta Med., 24, 603 (1968).
9. Ohtita Takeshi, Acta Haemat. Jap., 30, 507 (1967).
10. H. M. Patt and M. A. Maloney, in: L. F. Lamerton et al. (editors), Cell Proliferation, Oxford (1963), p. 157.
11. H. Quastler and F. G. Sherman, Exp. Cell Res., 17, 420 (1959).
12. P. Strychmans, E. P. Cronkite, I. Fache, et al., Nature, 211, 717 (1966).
13. R. G. Tarbutt, Exp. Cell Res., 48, 473 (1967).