DURATION OF PERIODS OF THE MITOTIC CYCLE OF ERYTHROPOIETIC CELLS OF RAT BONE MARROW

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In experiments on Wistar rats weighing 140-150 g using thymidine-H^3, information on the duration of the phases of the mitotic cycle of particular types of bone marrow erythroid cells was obtained from the curve of labeled mitoses. The mitotic cycle was found to be lengthened during differentiation of erythronormoblasts. Its duration for erythroblasts and for basophilic and polychromatophilic normoblasts was 7.7, 8.20, and 12.2 h, respectively. The cycle for the more mature cells was lengthened mainly on account of an increase in the period of DNA synthesis.

Information on the duration of the mitotic cycle of bone marrow erythroid cells of different species of animals has been obtained experimentally [2, 4, 8, 12]. In recent years during the investigation of the kinetics of erythronormoblast proliferation in the bone marrow of dogs [11] and rats [10, 17] attempts have been made to study the cell cycle separately in less mature and more mature forms. Determination of the parameters of the cycle for individual types of bone marrow erythroid cells by the labeled-mitosis method is hampered by the difficulty of identifying division figures. Nevertheless, such information is essential for estimating the rate and character of proliferation in the erythropoietic system.

In the present investigation the duration of the mitotic cycle and of its periods was therefore carried out separately in erythroblasts and basophilic and polychromatophilic normoblasts in rat bone marrow.

EXPERIMENTAL METHOD

Male Wistar rats aged 8-10 weeks and weighing 140-150 g were used. All the animals received thymidine-H^3 (specific activity 2300 μCi/g) by intravenous injection in a dose of 0.6 μCi/g at the same time of day. Imprints were prepared from the femoral arrow 30 min and 1, 2, 4, 6, 8, 10, 12, 14, 16 and 18 h after injection of the isotope. Five animals were used at each time of the experiment. The imprints were fixed with methanol, coated with type "M" (NIIKhF) liquid emulsion and exposed in a lightproof cupboard at 4°C for 4 weeks. The autoradiographs were developed in amidol developer at 18°C and fixed in 20% hypo solution. Immediately after the photographic processing the imprints were stained by the Romanovsky–Giemsa method (pH of the water 5.2).

The division figures of the erythroblasts were identified from the characteristic saturated dark-blue cytoplasm of these cells, with intensely stained, dark chromatin. As a rule the dividing erythroblast is larger than the mitotic figures of other erythroid cells. During identification of mitoses of the basophilic and polychromatophilic normoblasts, the degree of basophilia of the cytoplasm, density of the chromatin, and size of the cell and nucleus were considered. Division figures of pronormoblasts could not be distinguished, and their mitoses were therefore counted mainly together with mitoses of erythroblasts, and some of them were included with mitotic figures of the basophilic normoblasts.

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In the imprints from each animal 100 mitotic figures were counted separately in basophilic and polychromatophilic normoblasts and 50 mitoses in erythroblasts. Labeled and unlabeled cells were distinguished among the division figures and distributed among the phases of mitosis. A cell was taken as labeled if at least five grains of silver were present above its nucleus. A curve of labeled mitoses of erythroblast and of basophilic and polychromatophilic normoblasts was plotted from the results.

The duration of the mitotic cycle (T) was taken as the time equal to the distance between the corresponding points on the ascending portions of the curve of labeled mitoses [3]. Since in these experiments the number of dividing labeled cells did not fall lower than 50%, the time of DNA synthesis (t_S) was defined as the interval between the middle of the ascending and descending parts of the first wave of mitoses. The distance from the time of injection of thymidine-H^3 to the middle point of the ascending part of the curve corresponded to the duration of the postsynthetic period (t_{G_2}) plus half of mitosis (t_M) [1]. The duration of t_M for the individual cells was determined from the time between the stage of middle prophase and the stage of middle telophase on the ascending parts of the mitosis curves plotted separately for each phase of division [9]. The duration of the presynthetic period (t_{G_1}) was calculated by the formula:

$$t_{G_1} = T - (t_S + t_{G_2} + t_M).$$

The mean duration of the mitotic cycle of individual erythropoietic cells was also determined from the results of their labeling with thymidine-H^3 by means of Quastler's equation [15] for populations in a steady state. To find the labeling index in the bone marrow autoradiographs, 1000 erythronormoblasts of each type were counted and the percentage of labeled cells determined. When the mitotic index of the polychromatophilic normoblasts was calculated, only their proliferating forms were considered [5, 11]. In this experiment the imprints of the bone marrow were prepared 30 min after a single injection of the isotope (0.6 μCi/g).

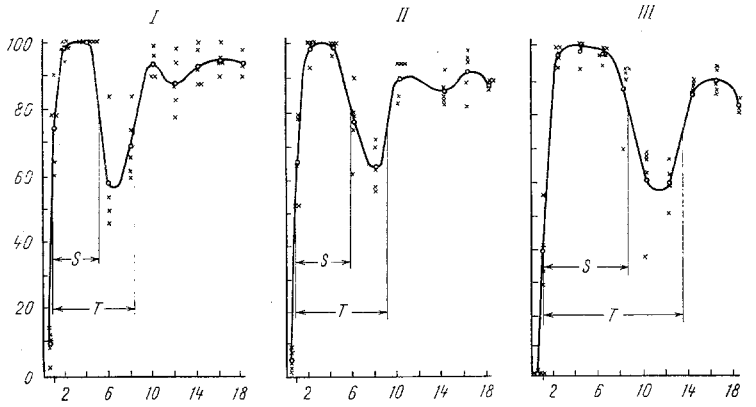

Fig. 1. Curves of labeled mitoses of erythroblasts (I) and basophilic (II) and polychromatophilic (III) normoblasts. Abscissa, time after injection of thymidine-H^3; ordinate, percentage of labeled division figures.

TABLE 1. Parameters of Mitotic Cycle of Erythropoietic Cells of Rat Bone Marrow

Type of cells	T (in h)	Period of cycle (in h)			
		G_1	S	G_2	M
Erythroblast	7,7	2,5	4,2	0,57	0,43
Basophilic normoblast.	8,2	2,2	4,8	0,53	0,67
Polychromatophilic normoblast	12,2	3,3	7,4	0,84	0,66

TABLE 2. Duration of Generation Period (T) of Bone Marrow Erythropoietic Cells Determined by Different Methods

Type of cells	T from curve of labeled mitoses (in h)	T from Quastler's equation (in h)	Labeling index (in percent)
Erythroblast	7,7	6,1	69,6±2,4
Basophilic normoblast.	8,2	7,0	68,2±2,2
Polychromatophilic normoblast	12,2	19,0	39,0±2,1

EXPERIMENTAL RESULTS

Data for the duration of the periods of the mitotic cycle of individual types of erythronormoblasts are given in Fig. 1 and Table 1. Most of the cycle in these cells was accounted for by the period of DNA synthesis, which for the erythronormoblasts was 55-60% of the duration of the cycle. About one-third of the cycle was occupied by the presynthetic period. The shortest period was that of mitosis itself. Erythroid cells of different degrees of maturity did not differ in their t_M value, which was 5.5-8% of the duration of the mitotic cycle. The periods of prophase, metaphase, anaphase, and telophase of these cells accounted for 25, 45, 20, and 10% of the duration of mitosis, respectively.

The lengthening of the cycle of the erythropoietic cells as they differentiated will be noted. The ratio between T for erythroblasts and basophilic and polychromatophilic normoblasts was 1:1.1:1.6. The increase in duration of the cycle of the more mature erythroid cells took place chiefly on account of t_S. The increase in the values of t_{G_1} and t_{G_2} during maturation of the erythronormoblasts was less marked.

The observed increase in duration of the mitotic cycle in the series from erythroblast to polychromatophilic normoblasts probably reflects slowing of proliferation during differentiation of the cells. Monette et al. [13] found that the rate of proliferation in young erythroid cells in rats is five times greater than in more mature cells, while t_S for less differentiated cells in 2.5 times shorter than for later stages. Some increase in the duration of t_S and, correspondingly, of T in the transition from less to more mature erythroid cells in rats was observed by Hanna et al. [10], but the difference which they found was much less than the difference observed by the previous investigators and in the experiments now described.

A marked increase in the duration of the mitotic cycle during maturation of cells has also been established for granulocytes of the bone marrow in rats [7] and dogs [14].

The character of the curves of labeled mitoses obtained for the erythroblasts and basophilic and polychromatophilic normoblasts indicates differences in the rates at which cells of the same type pass through the periods of the cycle. Variability of parameters of the cycle for bone marrow erythronormoblasts have also been mentioned by other workers [11]. In connection with differences in the rate of passage of cells of the same population through the mitotic cycle, the duration of the mitotic cycle of erythronormoblasts calculated by Quastler's equation is probably inaccurate. The more heterogeneous the cell population studied, the greater the possible error. Values of T for erythropoietic cells found by the labeled mitoses method, as well as its values calculated from the labeling index and t_S by means of Quastler's equation [15, 16], are given in Table 2 for comparison. The duration of the cycle determined by these methods differed by 25 and 17% for erythroblasts and basophilic normoblasts, respectively. The calculated duration of the cycle of the polychromatophilic normoblasts was 55% greater than the value of T obtained experimentally from the labeled mitosis curve. The fact that the proliferative pool of these normoblasts is 100% [6] suggests that the observed increase in T was not due to that part of the cell population which was outside the cycle.

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