DIURNAL FLUCTUATIONS IN PROLIFERATIVE ACTIVITY OF RAT BONE MARROW CELLS

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Definite changes in the mitotic index (MI) of bone marrow cells of Wistar rats were discovered during the 24-h period. The curve showing changes in the number of mitoses of the granulopoietic cells is bimodal in character (maxima at 9 a. m. and 8 p. m.), while that for the erythropoietic cells is unimodal (maximum at 9 a. m.). Correlation is observed between changes in MI and the labeling index (incorporation of thymidine-H^3) of individual bone marrow cells. The diurnal fluctuations in proliferative activity of the bone marrow cells are accompanied by corresponding changes in the total number of myelokaryocytes.

KEY WORDS: diurnal fluctuations of proliferation; bone marrow cells; labeling index.

The existence of diurnal fluctuations in the proliferative activity of various tissues is now firmly established. The diurnal rhythm of mitosis has been studied in most detail in the epithelial cells of various organs [1, 4-10]. Information on changes in the proliferative ability of the hematopoietic tissue during the 24-h period is limited and contradictory. Gruzdev et al. [3] found a constant rate of division of rat bone marrow cells by determining the mitotic activity for the whole population of bone marrow elements. According to Gololobova [2], the mitotic index (MI) of the dividing granulopoietic and erythropoietic mouse bone marrow cells changes appreciably in the course of the 24 hours. Diurnal fluctuations in proliferative activity of the bone marrow cells have also been found in man [12].

The dynamics of proliferative activity of rat bone marrow cells during the 24-h period was studied and at the same time the values of MI and the labeling index (LI) were determined for the erythropoietic and granulopoietic cells separately after administration of thymidine-H^3.

EXPERIMENTAL METHOD

Experiments were carried out on male Wistar rats aged 3-3.5 months (weight 180-190 g). The diet of the animals conformed to variant No. 2 on instruction No. 163 of the Ministry of Health of the USSR, "Norms for feeding laboratory animals and producers," dated March 10, 1966.

The proliferative activity of the bone marrow cells was expressed as MI and LI for the various types of myelokaryocytes after injection of thymidine-H^3.

MI was determined by counting the number of mitoses in a 1000 granulopoietic and erythropoietic cells, capable of dividing, separately. Squash preparations were made from the femoral marrow of animals killed in the course of the day (at 3, 6, and 9 a. m., 12 noon, 3, 6, and 8 p. m., and 12 midnight). At each time of investigation five rats were killed.

To determine IM the animals were given an intravenous injection of thymidine-H^3 (specific activity 2.3 Ci/g) in a dose of 0.6 μCi/g 30 min before sacrifice. The rats were killed every 6 h throughout the day and night (at 3 and 9 a. m. and 3 and 8 p. m.). Squash preparations were made from the femoral marrow of these animals, fixed in methanol, and coated with type M emulsion. The autoradiographs were ex-

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TABLE 1. Changes in MI (in %) of Granulopoietic and Erythropoietic Cells during the 24-h Period (M ± m)

Cells	Value of LI at different times of day (in h)								Mean diurnal value
	3	6	9	12	15	18	20	24	
Granulopoietic	32,0±3,08	32,6±1,5	51,0±2,08	31,0±1,6	25,6±1,7	30,6±1,4	44,0±1,8	29,8±0,97	34,6±1,76
Probability of random differences P	6—9 h 0,001		9—12 h 0,001		15—18 h 0,05		20—24 h 0,001		
Erythropoietic	16,8±1,2	24,4±2,6	46,2±5,3	33,0±1,6	31,8±1,5	30,2±3,0	30,6±1,3	28,6±1,5	30,2±2,2
Probability of random differences P	3—24 h 0,001		3—6 h 0,03		6—9 h 0,006	9—12 h 0,04	9—15 h 0,04		

posed for 36 days, then developed in amidol developer, fixed in 20% sodium hyposulfite solution, and stained by the Romanovsky–Giemsa method. The percentage of granulopoietic and erythropoietic cells labeled with thymidine-H^3 in the autoradiographs was determined separately. To determine IM for the granulopoietic cells, 200 myeloblasts, 200 promyelocytes, and 1000 myelocytes were counted. To determine IM for the erythropoietic cells, 300 erythroblasts and pronormoblasts, 500 basophilic normoblasts, and 1000 polychromatophilic normoblasts were counted, noting both labeled and unlabeled cells. The total number of cells in the animal's femur was counted at the same times of day and night. The experiments were carried out in October and November.

The numerical results were subjected to statistical analysis by Student's method.

EXPERIMENTAL RESULTS

Significant fluctuations in MI of the granulopoietic cells during the 24-h period were observed (Table 1). The number of mitoses was maximal (51 and 44 respectively) at 9 a. m. and 8 p. m. The number of dividing cells was minimal at 3 p. m. (25.6%). At other times of investigation MI remained at roughly the same level (29.8-32.6%) and approximated to the mean diurnal value (34.6%). Definite fluctuations in the value of MI for the erythropoietic cells also were found during the 24-h period (Table 1). The number of dividing cells of this series reached a maximum (46.2%) at 9 a. m. and a minimum (16.8%) at 3 p. m. At all other times of the investigation MI remained at or fluctuated very slightly around the mean diurnal value (30.2%).

In the course of the 24-h period the number of bone marrow cells labeled with thymidine-H^3 also varied (Table 2). The value of LI for cells of different levels of maturity varied unequally. The most marked diurnal fluctuations in LI were found in the myeloblasts. The number of labeled myeloblasts was minimal at 9 a. m. ($P=0.05$), but at other times of investigation LI was greater and reached a maximum at 3 a. m. ($P=0.005$). Diurnal fluctuations in LI of the promyelocytes were not statistically significant. The largest number of labeled myelocytes was observed at 3 p. m. ($P=0.04$) and the smallest at 9 a. m.

Changes in LI for the erythroid cells during the 24-h period were not statistically significant except for the erythroblasts. The number of labeled erythroblasts was appreciably higher at 3 a. m. ($P=0.02$), but at all other times of the investigation the LI of the erythroblasts was lower.

The results indicate dirurnal fluctuations in the proliferative activity of rat bone marrow cells. The character of the change in LI differs for granulopoietic and erythropoietic cells. Whereas the curve of mitotic activity of the granulopoietic cells with bimodal (maxima at 9 a. m. and 8 p. m.), the fluctuations in MI of the erythroid cells were less marked and had a single maximum at 9 a. m.

Comparison of the results with the dynamics of MI for human [12] and mouse [2] bone marrow cells shows that the diurnal changes in mitotic activity of the erythropoietic cells in different test objects are similar in character. Meanwhile the fluctuations in mitotic activity of the granulopoietic cells were more marked in rats.

A definite relationship was found between the changes in MI and LI for the various marrow cells. For instance, the maximum of the number of dividing granulopoietic cells at 9 a. m. was preceded by an increase in the percentage of labeled myeloblasts at 3 a. m. and the maximum of the

TABLE 2. Changes in LI (in %) of Rat Bone Marrow Cells during the 24-h Period (M±m)

Cells	Value of LI at different times of day (in h)			
	3	9	15	20
Granulopoietic:				
Myeloblasts	63,5±1,3	50,0±3,4	57,0±1,7	58,5±2,5
Promyelocytes	58,0±1,6	59,5±1,2	61,0±1,3	56,0±2,0
Myelocytes	32,8±1,4	30,4±1,6	35,0±1,0	34,4±2,0
Erythroid:				
Erythroblasts, pronormoblasts	77,0±0,6	69,0±2,6	72,0±1,1	66,5±2,5
Basophilic normoblasts	72,8±1,7	67,2±2,8	67,2±2,4	72,0±2,3
Polychromatophilic normoblasts	33,2±2,1	31,8±0,9	30,7±1,8	31,6±0,4

number of mitoses at 8 p. m. was preceded by an increase in IM of the myelocytes at 3 p. m. No such relationship could be found for promyelocytes, evidently because of differences in the duration of the period of DNA synthesis in granulocytes at differences stages of maturity [11]. The correlation between the changes in MI and LI of the erythroid cells in the course of the 24-h period was less marked.

These changes in the proliferative activity of the bone marrow cells during the 24-h period were accompanied by corresponding changes in the absolute number of myelokaryocytes. The largest number of cells in the femoral bone marrow of the rats – $(1.80 \pm 0.06) \cdot 10^8$ and $(1.71 \pm 0.06) \cdot 10^8$, respectively – was observed at 9 a. m. and 8 p. m., i.e., at the same times as the maxima for MI of the myelokaryocytes.

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