MITOTIC INDICES OF INDIVIDUAL TYPES OF BONE MARROW CELLS OF NORMAL RATS

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The mitotic index of the erythroid and myeloid series of bone marrow cells collectively and of nine individual types of marrow cells was determined in normal Wistar rats.

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The need for a more accurate determination of the proliferative activity of hematopoietic tissue has led to the abandonment of determination of the mitotic index (MI) in the total number of hematopoietic cell by determination of the MI separately for the erythroid and myeloid series [2, 6] or simply for a group of cells capable of division [4, 5, 8]. However, determination of MI for each individual type of marrow cells is of the greatest importance when the level of proliferation is to be assessed and the kinetics of the cell populations to be calculated accurately. No such information is given in the literature.

The object of this investigation was to determine the mitotic index for each type of marrow cell capable of division in rats.

EXPERIMENTAL METHOD

Experiments were carried out on 20 normal male Wistar rats weighing 150 g. The animals were sacrificed between 10:00 and 10.30 A.M. by bloodless destruction of the medulla. Both femora were quickly removed, the marrow was exposed, and impressions were made by a stroke technique on slides. Myelograms of 20 rats and mitoses in 10 rats were calculated in specimens stained by Pappenheim's method. The total number of nucleated cells in the femoral marrow was determined for 10 animals, using the suspension obtained after flushing out the marrow from the epiphyses through a needle with 2 ml of 0.9% NaCl solution. The mitotic indices of cells of the erythroid and myeloid series capable of division and also of reticulum cells, promyelocytes, myelocytes, and basophilic and polychromatophilic normoblasts were determined after counting 1000 of the corresponding cells, including the number in mitosis, MI of the hemocytoblasts, myeloblasts, and erythroblasts was calculated after counting 200 cells, and MI of eosinophils after counting a total of 200 eosinophilic myelocytes and promyelocytes. When mitoses of polychromatophilic normoblasts were counted, cells with pycnotic nuclei were not included. The distribution of mitoses among the four phases (pro-, meta-, ana-, and telophase) was determined by means of the usual criteria [1, 3, 12]. Reconstructive nuclei of cells just divided were not included in the number of mitoses. Because of the presence of specific granules it was impossible to determine the stage of division accurately, and for this reason mitoses of the eosinophilic cells (promyelocytes and myelocytes) are given without separation into phases.

The high quality of the hematological procedures meant that it was easy to determine the type of cell in which the mitoses were found, especially the more mature stages (promyelocyte, myelocyte, polychromatophilic and basophilic normoblasts). It was more difficult to identify mitoses in young forms of cells, such as myeloblasts and hemocytoblasts. Some of the important features used when determining the nature of dividing cells in the early stages of maturation will therefore be described.

Mitosis in a myeloblast was identified from the general appearance of the cell, but the essential feature for the identification of these cells at various stages of division was the structural heterogeneity of the

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TABLE 1. MIs of Individual Bone Marrow Cells of Rats, Distribution of Mitoses by Phases, and Absolute Number of Mitoses

Type of cells	Mitotic index (in %)	Distribution of mitoses by phases and percentage of total number				Absolute number of mitoses in femoral bone marrow ($\times 10^3$)
		prophase	metaphase	anaphase	telophase	
Reticular	19,5± 0,92	6,3± 0,61 32%	7,8± 0,82 40%	3,3± 0,31 17%	2,1± 0,2 11%	186,3
Hemocytoblasts	83,0± 3,08	38,0± 1,54 46%	31,0± 2,06 37%	7,60± 1,54 8,5%	7,0± 1,54 8,5%	164,3
Erythroblasts	110± 5,63	55,0± 2,57 50%	33,0± 4,1 30%	11,0± 1,54 10%	11,0± 2,05 10%	204,0
Basophilic normoblasts	46,0± 1,75	16,6± 0,41 36%	16,2± 0,82 35,2%	8,8± 1,13 19,2%	4,4± 0,51 9,6%	348,0
Polychromatophilic	45,0± 4,22	16,9± 0,92 37,5%	16,020,92 35,5%	7,1± 0,82 16%	5,0± 0,51 11%	720,0
Myeloblasts	135,5± 11,3	58,5± 4,62 43%	41,5± 2,57 31%	21,5± 2,05 16%	14,0± 2,05 10%	301,0
Promyelocytes	56,4± 0,92	18,9± 0,61 33,5%	22,5± 0,41 40%	11,0± 0,62 19,5%	4,0± 0,31 7%	292,0
Myelocytes	26,4± 0,92	8,7± 0,72 33%	10,5± 0,61 40%	5,3± 0,51 20%	1,9± 0,51 7%	249,0
Eosinophils (promyelocytes + myelocytes)	56,0± 2,05	—	—	—	—	83,0

cytoplasm in rat myeloblasts, in which it consists of uniformly colored granules which persist until the end of division. Dividing hematocytoblasts were differentiated from myeloblasts by the greater smoothness and structurelessness of the cytoplasm, characteristic of cells of this type, which is especially important for the identification of the middle and late phases of mitosis. Mitosis in an erythroblast was identified from a group of signs, including the large size of the cell, the characteristic solidly dark blue (or dark blue with a violettinge) cytoplasm, and the rather coarser and darker appearance of the chromatin in prophase and the other phases. The method used to determine mitoses did not allow differentiation of dividing pronormoblasts, so that in most cases their mitoses were included among those of erythroblasts (since pronormoblasts are similar in size to erythroblasts).

EXPERIMENTAL RESULTS

The total number of nucleated cells in the femoral marrow of the rats was $(132.5 \pm 5.35) \cdot 10^6$. The mean myelogram ($n = 20$) was as follows ($X \pm x$): reticular cells 7.2±0.26%, hemocytoblasts 1.5±0.07%, erythroblasts 1.4±0.09%, pronormoblasts 2±0.11%, basophilic normoblasts 5.7±0.34%, polychromatophilic normoblasts 11.8±0.44%, oxyphilic normoblasts 1.6±0.12%, myeloblasts 1.6±0.09%, promyelocytes 3.9±0.27%, myelocytes 7.1± 0.4%, metamyelocytes 7.7±0.65%, stab cells 12.3±0.81%, polymorphs 3.6±0.27%, basophils 0.4±0.04%, eosinophils 2.8 ± 0.27%,* monocytes 0.9±0.08%, lymphocytes 27.8±1.18%, plasma cells 0.4± 0.07%, mast cells 0.2±0.02%, megakaryocytes 0.1±0.024%, total number of cells of erythroid series 22.5± 0.7%, total number of granulocytes 39.4±1.66%. The high percentage of lymphocytes in the myelogram is a special feature of rats of this age. The value of MI per 1000 cells of the erythroid series of the bone marrow capable of division was 62.8±1.64%, and MI for cells of the myeloid series capable of division was 41.3± 0.61%. These figures differ from those given by other workers who found that MI for cells of the myeloid series was higher than MI for cells of the erythroid series [5]. The reason for the higher value obtained for MI of cells of the erythroid series in the present experiments is that it was counted only for cells capable of division, and this number excluded all the oxyphilic cells and those polychromatophilic normoblasts which had a small, dense, pycnotic nucleus. Values of MI of individual types of cells, the distribution of mitoses by phases, and the absolute number of mitoses in the femoral marrow of the rat are all given in the table. These results show that the younger the cells in the erythroid and myeloid series, the higher

*Promyelocytes and myelocytes account on the average for 40% of 100 eosinophils in each rat. This figure was used to calculate the mean absolute number of mitoses (Table 1).

their MI. Within the erythroid series the ratio between the values of MI (starting with the erythroblast) was 2.5 : 1: 1, and within the myeloid series 5.1 : 2.1 : 1. The reason for the sharp decrease in the proportion of dividing cells during the transition to the next stage could be either a slowing of the rate of passage through the mitotic cycle, which has been confirmed for neutrophils [10, 11], or an increase in the number of differentiating cells. The distribution of mitoses by phases (Table 1) in general agrees with the results obtained by analysis of the cell populations of the bone marrow of other species of animals [1, 12, 13] and in man [2, 13].

The suggestion can be made that the relatively low MI of reticular cells, which are the chief source of regeneration of hematopoietic cells, is dependent on the fact that a certain proportion of them is withdrawn from the mitotic cycle and remains in the stage of a regenerative reserve (R_1 and R_2), to use the expression of Epifanov [9]. The possibility is not ruled out that the transition into subsequent stages of differentiation, in the case of reticular cells, is accompanied by shortening of the mitotic cycle of the cell. It likewise must not be forgotten that the population of reticular cells is heterogeneous, so that the value of the MI given reflects the proliferative activity of the group, and not of one type of cells.

Summation of the absolute numbers of mitoses (Table 1) shows that about 2% of cells in the bone marrow of rats of this age are dividing forms. Although this value is deduced on the basis of one measurement, and it cannot therefore characterize the proliferative activity of the hematopoietic activity of any time of day or night [5], it is much higher than the figure (about 1%), also deduced on the basis of single measurements, which some writers have used for evaluating the mitotic activity of the marrow and for calculating the kinetics of cell populations [7, 8, 11].

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