MORPHOLOGY AND PATHOMORPHOLOGY

THE ABSOLUTE NUMBER OF CELLS IN BONE MARROW AND MYELOGRAMS OF NORMAL RABBITS

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In order to evaluate correctly the functional state of bone marrow it is necessary, in addition to appraisal of a myelogram, to know the absolute number of nucleated cells in unit volume or weight of bone marrow.

The absolute number of nucleated cells is usually determined by counts on bone marrow obtained by puncture. However, when small laboratory animals are used for experiments it is not always possible to make a puncture. In those experiments in which animals are sacrificed there is opportunity to take any part of bone marrow for analysis. The present work is concerned with determination of the absolute number of cells in bone narrow of experimental animals.

EXPERIMENTAL METHODS

Experiments were carried out on rabbits aged 4-6 months and weighing 2-2.4 kg. When the animals were sacrificed, bone marrow was withdrawn from the long bones; it was placed on a watch-glass and crushed to a semi-fluid consistency, after which samples of 200 mg were taken. From each tissue sample diluted with 5% acetic acid, a suspension of cells was prepared, using the following methods: 1) titration of the tissue with a pestle in a mortar with gradual addition of the diluting fluid; 2) treatment of the tissue sample mixed with the diluent in a Waring Blender apparatus fitted with a special blunt blade; 3) preparation of suspension in a glass homogenizer; 4) taking up of bone marrow into mixing vessel and shaking with the diluent.

The cells were counted in a Goryaev chamber after a homogeneous suspension was obtained. Multiple examinations of bone marrow from 6 normal rabbits were carried out, using the methods cited for the preparation of the suspension.

The results obtained showed that the highest absolute number of cells and closest values (in parallel examinations) were obtained when the suspension was prepared in a glass homogenizer and in the Waring Blender type of apparatus.

The suspension, when prepared by these methods, is more homogeneous, the cells are evenly distributed, the time taken to prepare the suspension is very much shorter (2 minutes) than that required for its preparation by hand, when the resultant suspension is less homogeneous and contains fewer cells. When the bone marrow is taken up in a mixing vessel the figures are often close to those obtained with preparation in a homogenizer, but in some cases this method tends to give results which are too low.

Using suspensions prepared in a homogenizer and the Waring Blender type of apparatus, bone marrow cell counts (femur) were made on 22 normal rabbits. The number of cells equals, on the average, 596,000 per 1 mg tissue from 393,000 to 900,000). Since it is known that bone marrow composition varies in different bones the cell content of the following bones was determined as well on 6 rabbits: ribs, femur, humerus and tibia (Table 1); the suspension was prepared in glass homogenizer.

EXPERIMENTAL RESULTS

Table 1 shows that the highest cell content is in the rib and the lowest in the tibia. Evidently this is connected with the smaller content of fat and higher content of dry non-fatty residue in rib marrow [1].

TABLE 1

No. of rabbit	Rib	Femur	Humerus	Tibia
1	983.2	787.6	835.6	796.2
2	1031.2	576.9	641.2	629.4
3	636.9	353.7	359.4	260.0
4	656.2	388.4	335.0	269.4
5	839.4	608.1	555.0	356.2
6	945.6	521.9	573.9	487.5
Average	848.8	539.4	550.0	466.4

In order to evaluate correctly bone marrow changes in experimental animals it is essential to know the limits of normal as regards myelograms and absolute content of various types of cells in the bone marrow, but data available in the literature are inadequate in this respect. Normal myelograms for rabbits are described by G. G. Karavanov [3]; other authors [4, 5, 6] only give normal values for peripheral blood in rabbits.

To determine the norm for myelograms, bone marrow from the femur was examined using 26 normal rabbits aged 4-6 months and weighing 2-2.4 kg (the animals were sacrificed by means of air emboli). Bone marrow smears were stained by the Pappenheim method; 1000 cells were counted on each preparation.

Data on percentage and absolute content of cells in the bone marrow of normal rabbits are presented in Table 2.

It can be seen that the main bulk of bone marrow cells is made up of cells belonging to the erythroid and granulocytic series; these are present in approximately equal proportions: total erythroblasts – 41.9%, total granulocytes – 42.4%. Neutrophils make up the greater part of the granulocytes (39.6%); the remainder (2.8%) is made up of myeloblasts (0.7%), basophils (0.7%) and eosinophils (1.4%).

According to our data cells of each succeeding generation are present in larger numbers than cells of the previous generation (with the exception of segmented neutrophils); this corresponds to the "rule of harmonic development and distribution of bone marrow cells" [2].

Among the erythroid cells are many hemoglobin-containing forms – polychromatophil and oxyphil erythroblasts. Young forms of erythroblasts constitute a total of 6.3%, the content of more mature ones being higher than that of less mature cells.

When changes in the percentage content of various generations of erythroblasts and neutrophils are to be evaluated, figures showing the percentage ratio of all the cells of a given series, the so-called fractional erythrograms and granulocytograms, are the most significant (Tables 3 and 4).

In 16 of 26 normal rabbits the absolute number of cells in 1 mg bone marrow was counted. Using myelogram data for these animals the absolute content of all types of generations of cells were calculated (Table 2). As can be seen from Table 2 the absolute number of all types of cells is given to considerable fluctuations. It appears to be impossible to determine the cause of these fluctuations on our relatively limited material or to demonstrate the relation between the absolute content of cells and their percentage ratios in the myelograms.

TABLE 2

Cellular Composition of Bone Marrow in Normal Rabbits

Name of cells		Percentage (26 rabbits examined)			Absolute numbers in thousands (16 rabbits examined)		
		minimal	maximal	average	minimal	maximal	average
	Reticular cells	0.2	1.7	1.0	1.8	12.6	6.1
	Hemohistoblasts	0.1	0.8	0.2			
Erythroblast generations	Pterythroblasts	0.2	0.8	0.2	0.8	4.9	1.3
	Basophil macroblasts	0.2	2.0	0 6	1.6	18.0	3.7
	Basophil normoblasts	0.4	10.8	5.5	10.4	58.6	33.1
	Polychromatophil erythroblasts	10.9	26.6	18.9	49.1	218.1	114.4
	Oxyphil erythroblasts	6.6	24.3	16.7	57.8	154.2	100.7
	Total erythroblasts	33.6	54.0	41.9	137.6	378.5	253.2
	Megakaryocytes	0.1	0.3	0.1	0.5	1.8	0.8
	Basophils	0.1	2.4	0.7	0.5	21.6	3.1
	Eosinophils	0.2	2.4	1.4	2.4	15.6	8.1
	Myeloblasts	0.2	1.6	0.7	1.8	8.6	4.4
Neutrophil generations	Premyelocytes	0.1	1.6	0.6	1.6	16.0	3.9
	Myelocytes	1.1	6.1	3.1	6.7	46.8	16.9
	Young	2.8	10.0	7.4	25.2	79.6	44.8
	Rod-nucleated	10.8	33.6	23.2	78.0	254.9	145.2
	Segmented	2.0	9.0	5.3	9.5	81.0	33.0
	Total neutrophils	23.0	51.6	39.6	143.9	408.9	240.8
	Total granulocytes	25.0	52.8	42.4	156.3	428.3	256.4
	Lymphocytes	4.1	21.3	12.6	22.1	149.4	68.3
	Monocytes	0.4	3.6	1.6	2.9	32.4	9.0
	Plasma cells	0.1	1.2	0.2	0.6	5.9	1.8

TABLE 3

Fractional Erythrogram for Normal Rabbits

Name of cells	Erythroblast content in %		
	minimal	maximal	average
Young erythroblasts (not containing haemoglobin)	2.6	32.7	14.9
Polychromatophil erythroblasts	27.9	64.9	44.8
Oxyphil erythroblasts	16.6	62.3	40.3

TABLE 4

Fractional Granulocytogram for Normal Rabbits

Name of cells	E Erythroblast content in %		
	minimal	maximal	average
Young neutrophils (pre-myelocytes, myelocytes, young forms)	11.0	40.7	28.2
Rod-nucleated neutrophils	41.8	70.3	58.3
Segment-nucleated neutrophils	3.9	26.2	13.5

SUMMARY

Absolute number of cells containing nuclei was determined in the bone marrow of rabbits and the ratio of various forms was calculated on myelograms. The content of cells in the bone marrow of various bones was different. The cells of erythroid and granulocytic series were in the same proportion (erythroblasts 41.9%, granulocytes 42.4%). The content of various generations of erythrocytes and neutrophils was in coordination with the "rule of harmonic development and distribution of cells, according to degree of their differentiation."

LITERATURE CITED

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