NORMAL BLOOD INDICES, BONE MARROW IMPRINTS, AND THE SPLEEN OF WHITE RATS

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M. F. Sbitneva, T. V. Kalyaeva, and I. A. Rudakov

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The few published reports of hemopoiesis in white rats are contradictory; the investigations were carried out as a rule on a small number of animals, and chiefly concern the morphology of the circulating blood. We have found no description of any complete study of the morphological indices of the circulating blood and of the hemopoietic organs (with a quantitative investigation of the latter).

The object of the present investigation has been to make a study of the indices of the circulating blood as well as of the hemopoietic organs (bone marrow and spleen) of white rats, and to use some new methods.

## EXPERIMENTAL METHOD

The work was carried out on 200 healthy white male rats weighing 170-220 g. In 100 rats blood for complete morphological analysis was taken from the tail vein; in 100 rats killed by decapitation, besides a study of the indices of the circulating blood we also measured the volume of the bone marrow of the femur and determined the number of the hemopoietic cells per mm<sup>3</sup>, using the method described by Mantz [5]. We then calculated the total number of myelokaryocytes in the bone marrow of the whole femur. We determined the weight of the spleen and determined the ratio of the weight of the spleen to that of the body in g, we calculated the myelogram and the lienocytogram from smear preparations.

## EXPERIMENTAL RESULTS

From the results obtained, which were treated statistically, it can be seen that the blood indices of the bone marrow and spleen of healthy rats normally show a considerable variation (Tables 1, 2, and 3).

The total number of erythrocytes was 6,690,000-7,110,000, and the mean diameter, as determined by a "cello-scope" was  $6.52\pm0.2\mu$ , in agreement with the results obtained by A. A. Kudryavtsev we used an oculomicrometer[1].

Individual variations in the number of leucocytes were significant (variation from 5,000 to 37,500 in blood taken from the tail vein, and from 5,700 to 17,600 in blood taken from the corpse). However, the mean values differed little from those obtained by other authors [3, 4, 6, 7].

In the leucocytic formula of rats lymphocytes predominated. Granulocytes consisted mostly of polymorphonuclear neutrophils. The percentage of neutrophils with rod-shaped nuclei and of eosinophils was small. The number of eosinophils as determined by a direct count in a Fuchs-Rosenthal chamber was  $580 \pm 66$  per mm<sup>3</sup> of blood.

We found a noticeable difference in the total number of leucocytes in the amount of hemoglobin and in certain other indices as between blood taken from the tail or from the corpse. In the first place the number of leucocytes and reticulocytes was almost twice as high as in blood from the corpse; there was a greater difference (of 3.6 g%) in the amount of hemoglobin, and in the total number of thrombocytes.

The results obtained on the morphological composition of blood were close to published findings. Only the mean value for the amount of hemoglobin in blood from the corpse was lower than has been found by most other investigators, but it is close to the values found and published by V. A. Pokrovskii [2].

TABLE 1. Blood Indices of Normal White Rats

	Blood from corpse				Blood from	Blood from tail vein			
Indices	M <u>+</u> m	X <sub>min</sub>	x max	迚	M±m	X min	X max	σ±	
Morphological composition									
Erythrocytes · 10 <sup>6</sup> Hemoglobin (g%)	$\begin{bmatrix} 6,69 \pm 0,056 \\ 12,985 \pm 0,11 \end{bmatrix}$	5,8 10,0	8,4 18,6	0,56 1,1	$\begin{bmatrix} 7,11\pm0,076 \\ 16,62\pm0,22 \end{bmatrix}$	5,14 11,5	8,99 22,7	0,76 2,23	
Reticulocytes (%)	17,06±1,21	4	44	12,06	31,6±1,63	6	88	16,3	
Thrombocytes · 10 <sup>3</sup> Leucocytes · 10 <sup>3</sup> Lymphocytes · 10 <sup>3</sup>	$\begin{array}{c} 271,18 \pm 8,94 \\ 10,0 \pm 0,28 \\ 7,3 \pm 0,17 \end{array}$		504,0 17,6 14,6	89,4 2,75 1,67	$397\pm15,4$ $16,0\pm0,52$ $11,23\pm0,51$	72 5,0 3,5	846 37,2 29,1	154,3 5,22 5,1	
Neutrophils · 10 <sup>3</sup> Lymphocytes (%) Neutrophils (%) Monocytes (%) Eosinophils (%)	2,02±0,09 74,02±0,57 20,71±0,54 3,11±0,17 1,12±0,1	0,6 59,5 9,0 0	3,7 88,0 36,0 7,0 3,5	0,93 5,66 5,37 1,7 1,0	$\begin{array}{c} 3,67\pm0,18\\ 70,16\pm0,83\\ 19,55\pm1,49\\ 5,73\pm0,23\\ 1,94\pm0,15 \end{array}$	1,0 48,0 4,5 1,0	12,3 89,5 37,5 12,5 7,5	1,83 8,26 7,45 2,29 1,48	
Reticuloendothelial cells (%)	0,94 <u>+</u> 0,06	0	2,5	0,55	0,8±0,03	0	2,0	0,3	
Plasma cells (%) Sedimentation rate (mm per h)	0,25±0,003 2,96±0,19	0 1,0	1,5 8,0	0,03	0,63±0,03	0	2,0	0,3	
Cytological changes									
Pyknosis of nuclei for the lymphocytes	1,65±0,12	0	6,0	1,2	Processing and a second and a s				
Fragmented neutro- phils (%)	1,25±0,11	0	5,5	1,1					
Hypersegmentation of neutrophil nuclei (%)	$0.2\pm0.002$	0	1,0	0,02					
Cytolysis of the granulocytes (%)	$0,42\pm0,04$	0	2,0	0,4					
Cytolysis of the agranulocytes (%)	$3,27\pm0,18$	0	9,0	1,8	ļ				

In our investigations the total bone marrow volume of the femur was  $27.8\pm0.84~\text{mm}^3$ . The number of cells per mm<sup>3</sup> of bone marrow was  $2.125\pm0.039$  million, and the total number of myelokaryocytes in the bone marrow of the whole femur was  $60.6\pm1.38$  million. In imprints of bone marrow of rats, normally the myeloid elements preponderated over the erythroblastic, i.e., the mature were more numerous than the young. According to our findings the erythroblastic elements formed  $23.6\pm0.45\%$ , total granulocytes  $59.1\pm0.66\%$ , and lymphocytes made up  $8.3\pm0.55\%$ . From the features of the morphological composition of bone marrow imprints we must draw attention to the characteristic structure of the nucleus of the metamyelocytes (they have a rounded form with an aperture in the center, so that they resemble a "bublik" (a thick ring-shaped roll of bread); they have a paler shade of protoplasm than do other species of laboratory animals and the myelocytes and metamyelocytes have a marked granularity.

We found the weight of the rat spleen to be  $1,060\pm33.3$  mg, and the ratio of the weight of the spleen in mg to body weight in g was  $5.25\pm0.14$ . In spleen imprints most of the cellular elements were lymphoid and comprised  $84\pm0.53\%$  of the total. The other cell forms were not numerous. Granulocytes comprised only  $5.2\pm0.29\%$ , and erythroblasts  $1.6\pm0.14\%$ .

TABLE 2. Morphological Constitution of Imprints of Bone Marrow of Normal White Rats (%), n = 100

		,	,	
Indices	M · · m	x <sub>min</sub>	x <sub>max</sub>	主
Morphological constitution				
Hemohistioblasts and hemocytoblasts	0,6±0,04	0	1,8	0,43
Total no. of erythroblastic cells Erythroblasts Pronormoblasts Basophillic normoblasts Polychromatophil normoblasts Oxyphil normoblasts Mitosis of red blood Megacaryoblasts and megacaryocytes Total number of granulocytes Myeloblasts Promyelocytes Myelocytes Metamyelocytes Metamyelocytes Rod-shaped nuclei Polymorphonuclear Basophils Eosinophils Lymphocytes Monocytes Reticulo-endothelial cells Plasma cells Cells of ferrata Mitosis in white cells	$\begin{array}{c} 23,6\pm0,45\\ 1,4\pm0,07\\ 1,7\pm0,1\\ 6,5\pm0,27\\ 13,2\pm0,44\\ 1,0\pm0,09\\ 0,3\pm0,001\\ 0,4\pm0,03\\ 59,1\pm0,66\\ 1,5\pm0,03\\ 2,4\pm0,11\\ 4,2\pm0,14\\ 13,9\pm0,42\\ 17,0\pm0,5\\ 15,0\pm0,62\\ 0,05\pm0,002\\ 4,7\pm0,27\\ 8,3\pm0,55\\ 1,2\pm0,09\\ 5,2\pm0,24\\ 0,3\pm0,03\\ 0,02\pm0,001\\ 0,54\pm0,02\\ \end{array}$	0 45,4 0,2 0,8 1,2 3,6 9,2 3,5	34,0 2,0 3,2 12,4 23,0 7,6 1,8 1,8 71,4 2,8 5,2 7,2 24,0 28,0 26,6 0,4 12,2 23,2 3,2 10,6 1,2 0,2 1,2	4,5 0,74 1,0 2,7 4,4 0,87 0,01 0,34 6,56 0,3 1,14 1,36 4,2 5,03 6,2 0,02 2,7 5,5 0,90 2,4 0,01 0,2
Cytological changes				
Giant cells Fragmentation Pyknosis of the nuclei of normoblasts Chromatinolysis Rexis Lysis Vacuolization Cytolysis	$\begin{array}{c} 0,4\pm0,01\\ 1,7\pm0,12\\ 2,5\pm0,18\\ 0,5\pm0,05\\ 0,2\pm0,03\\ 0,1\pm0,001\\ 0,3\pm0,02\\ 8,9\pm0,3 \end{array}$	0 ,2 ,2 0 0 0 5,0	1,6 5,6 5,6 1,8 1,2 0,4 1,2 16,0	0,1 1,2 1,8 0,5 0,34 0,01 0,2 3,0

## SUMMARY

Morphological composition of the blood and hemopoietic organs was studied in 200 healthy albino male rats. New hematological methods previously never applied by Soviet investigators were used (determination of erythrocytometric curve with the aid of celloscope study of the quantitative bone marrow composition).

A significant difference was noted in the total leucocyte, reticulocyte, and thrombocyte count, as well as in the hemoglobin content of blood obtained from the caudal vein and from the corpse. Higher indices were obtained in the first case.

The average diameter of rat erythrocytes determined with the aid of a celloscope was 6.52+0.02.

The figures of morphological blood composition obtained approached mostly those given in literature sources. It was only the average hemoglobin figures of the blood flowing from the corpse that proved lower than those recorded by the majority of investigators. Bone marrow volume in the rat femur was  $27.8\pm0.84$  mm<sup>3</sup>; bone marrow cell count per 1 mm<sup>3</sup> was  $2.125\pm0.039$  million.

Just as in other species of laboratory animals, in the bone marrow imprint of rats there were more myeloid elements than erythroblasts, and more mature cells than young cells. There were  $23.6\pm0.45$  erythroblastic elements, and  $59\pm0.66$  myeloid elements. Lymphoid elements preponderated in splenic imprints, forming  $84.0\pm0.53\%$  of the total; granulocytes accounted for only  $5.2\pm0.29\%$ , and erythroblastic elements  $1.6\pm0.14\%$ .

TABLE 3. Morphological Composition of Imprints of Normal White Rat Spleens (%),  $n\!=\!100$ 

Indices	<u>М</u> ±т	x <sub>min</sub>	x max	<u>o+</u>
Morphological constitution				
Reticulo-endothelial cells Total number of lymphoid elements Prolymphoblasts Prolymphocytes Medium-sized lymphocytes Small lymphocytes Cells in state of division Monocytes Plasma cells Granulocytes (total number)	$\begin{bmatrix} 5,6\pm0,25\\ 84,0\pm0,53\\ 1,5\pm0,07\\ 8,5\pm0,37\\ 62,5\pm0,74\\ 11,4\pm0,47\\ 0,3\pm0,02\\ 1,5\pm0,07\\ 0,2\pm0,002\\ 5,2\pm0,29 \end{bmatrix}$	1,6 7,2 0,6 2,8 47,2 2,0 0 0,4 0 0,2	14,4 88,0 3,2 16,4 77,0 20,0 1,2 3,2 0,8 7,0	2,5 5,3. 0,71 3,7 7,4 4,7 0,2 0,7 0,02 2,9
Erythroblastic cells (total number) Fibroblasts	$\begin{array}{c} 1.6 \pm 0.14 \\ 1.5 \pm 0.1 \end{array}$	$0,2 \\ 0$	11,8 4,4	1,4 1,0
Cytological changes Cytolysis Vacuolization	8,1±0,28 0,2±0,01	3,8 0	13,0 1,2	2,8 0,1

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