

# Parameters of the Bone Marrow and Evaluation of Blood Indexes Obtained in Automated Analysis in Healthy Rabbits

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We present the mean parameters of rabbit bone marrow and their physiological variations and blood cell counts obtained by routine manual methods or using hematological analyzers with consideration for the age, gender, and season.

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**Key Words:** *rabbits; bone marrow; peripheral blood; automated hematological analysis*

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Rabbits together with other laboratory animals are widely used in experimental hematology, in preclinical studies of pharmacological substances. There are published data on the main parameters of the peripheral blood (PB) cells obtained by manual methods and on parameters of the bone marrow (BM) in rabbits [1,2].

Here we present the results of evaluation of the parameters of PB formed elements using a hematological analyzer (HA) with consideration for the age, gender, and season, and BM parameters in healthy rabbits.

## MATERIALS AND METHODS

The study was carried out on 120 Chinchilla rabbits (males and females) weighing 2-4 kg (age 2-11 months) obtained from Rassvet nursery (Tomsk). The animals were kept in accordance with European Convention for the Protection of Vertebrate Animals used for Experimental and Other Scientific Purposes (Strasburg, 1986). Before and after the experiment the animals were kept in a vivarium at 20-23°C, humidity <50%, air exchange 8:10 (exhaust: input), at 1:1 day:night regimen, in metal cages 58×47×38 cm (one animal per cage), in an open system, and were fed a standard ration.

The animals were divided by the age and gender. Parameters of formed elements in a sample of PB (25 µl) drawn from the marginal ear vein were measured on an Abacus automated HA (Diatron) in a veterinarian regimen [3,5]. The data were compared with PB parameters of healthy rabbits evaluated by standard manual methods. Differential blood leukocyte count and the relative content of neutrophils and lymphocytes were evaluated by microscopic examination of blood smears stained with May-Grunwald stain, azure II, and eosin by the method of Nocht, reticulocytes were counted in blood smears after supravital staining with brilliant cresyl blue, hematocrit was determined after blood centrifugation in glass capillaries on a MTsG-8 microcentrifuge [3,4].

The state of hemopoiesis in BM was evaluated by the analysis of smears prepared from homogenized fragments of the myeloid tissue from the sternum and autologous serum (1:1) stained with May-Grunwald stain, azure II, and eosin by the method of Nocht [4].

The results were processed statistically using parametric Student *t* test.

## RESULTS

It was found that the mean parameters of BM and PB in healthy rabbits obtained by manual methods

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**TABLE 1.** Blood Parameters in Healthy Rabbits Measured by Manual Methods ( $X \pm m$ )

Parameter	Published data		Our data (in different seasons in 2002-2003)
	[2]	[3]	
Hemoglobin, g/liter	123 $\pm$ 1	From 72 to 142	From 97 $\pm$ 2 to 127 $\pm$ 1
Erythrocytes, 10 <sup>12</sup> /liter	5.44 $\pm$ 0.05	From 4.0 to 6.4-8.9	From 4.53 $\pm$ 0.15 to 6.36 $\pm$ 0.06
Hematocrit, %	31 $\pm$ 1	—	From 33 $\pm$ 1 to 39 $\pm$ 1
Reticulocytes, ‰	31 $\pm$ 1	To 8% (80‰)	From 28 $\pm$ 2 to 79 $\pm$ 4
Leukocytes, 10 <sup>9</sup> /liter	6.9 $\pm$ 0.4	From 3.8 to 12.0	From 6.3 $\pm$ 0.7 to 8.4 $\pm$ 0.7
Neutrophils, %	27.0 $\pm$ 0.1	From 8 to 50	From 10 $\pm$ 2 to 48 $\pm$ 4
Lymphocytes, %	64.0 $\pm$ 0.1	From 20 to 90	From 42 $\pm$ 3 to 77 $\pm$ 4
Platelets, 10 <sup>9</sup> /liter	266 $\pm$ 12	From 126 to 300	From 211 $\pm$ 11 to 455 $\pm$ 38

(Tables 1 and 2) did not considerably differ from published data [1,2].

Manual measurement of PB parameters showed that males had higher mean content of hemoglobin (at the age of 10-11 months) and erythrocyte count (at the age of 4-5 and 7-8 months) compared to the corresponding parameters in females (Table 3). The

content of platelets, neutrophilic and eosinophilic granulocytes was lower and lymphocyte count was higher in 7-8-month-old males compared to females. Young animals (4-5 months) had the lowest red blood parameters (erythrocyte count, hemoglobin content and hematocrit) and neutrophil and platelet counts and the highest lymphocyte count,

**TABLE 2.** BM Parameters in Healthy Rabbits

Parameter, %	Our measurements ( $n=44$ )			Published data [2]
	$X \pm m$	$\pm \delta$	$X \pm \delta$	$X \pm \delta$
Nondifferentiated blasts	0.20 $\pm$ 0.02	0.1	0.1-0.3	0.3-1.8
Myeloblasts	1.5 $\pm$ 0.1	0.7	0.8-2.3	0.7-1.4
Neutrophilic promyelocytes	1.2 $\pm$ 0.2	0.8	0.4-2.0	0.2-1.7
Neutrophilic myelocytes	3.7 $\pm$ 0.3	1.2	2.5-4.9	1.0-2.0
Neutrophilic metamyelocytes	4.7 $\pm$ 0.6	2.3	2.4-7.0	1.6-2.8
Stab neutrophils	6.1 $\pm$ 0.5	2.0	4.1-8.1	3.6-8.4
Segmented neutrophils	24.4 $\pm$ 1.3	5.1	19.4-29.5	29.0-39.0
Eosinophils	1.2 $\pm$ 0.1	0.7	0.5-1.9	0.4-1.5
Basophils	0.9 $\pm$ 0.1	0.7	0.2-1.6	0.2-1.0
Granulocyte mitoses	0.6 $\pm$ 0.1	0.7	0.0-1.3	0.1-0.2
Lymphocytes	11.8 $\pm$ 0.7	6.2	5.6-18.0	4.0-7.0
Monocytes	2.3 $\pm$ 0.1	0.7	1.6-3.0	1.5-2.8
Plasma cells	0.9 $\pm$ 0.1	0.7	0.2-1.6	0.5-1.3
Macrophages	0.30 $\pm$ 0.04	0.3	0.0-0.6	—
Megakaryocytes	0.30 $\pm$ 0.02	0.1	0.2-0.4	—
Erythroblasts	1.2 $\pm$ 0.1	0.4	0.8-1.6	1.8-3.2
Basophilic normoblasts	2.5 $\pm$ 0.3	1.2	1.3-3.7	6.2-9.4
Polychromatophilic normoblasts	32.8 $\pm$ 1.1	4.3	28.5-37.1	21.8-36.1
Oxyphilic normoblasts	2.2 $\pm$ 0.1	0.4	1.8-2.6	0.1-0.6
Erythroid mitoses	1.2 $\pm$ 0.1	0.7	0.5-1.9	0.1-0.4
Leukoblast-erythroblast ratio	1.1 $\pm$ 0.1	0.4	0.7-1.5	1.0-1.9

**Note.**  $\delta$ : mean square deviation.

**TABLE 3.** PB Parameters in Healthy Rabbits Measured by Manual Methods ( $X \pm m$ )

Parameter	Age, months					
	4-5		7-8		10-11	
	males (n=15)	females (n=15)	males (n=15)	females (n=14)	males (n=15)	females (n=15)
Body weight, g	2869±96	2927±124	3559±117	3941±180	3631±83	4178±116
Hemoglobin, g/liter	101±3	97±2	132±13	115±4	127±1	112±4*
Erythrocytes, $10^{12}$ /liter	4.93±0.14	4.53±0.15*	5.98±0.06	5.80±0.16	6.36±0.06	5.84±0.11*
Reticulocytes, ‰	49±8	47±7	40±3	38±3	28±2	30±2
Hematocrit, %	33±2	33±1	39±1	38±2	38±1	37±1
Platelets, $10^9$ /liter	211±11	213±12	395±23	484±20*	379±25	404±15
Leukocytes, $10^9$ /liter	7.8±0.4	6.3±0.7	7.4±0.6	6.4±0.5	8.4±0.7	7.5±0.6
Neutrophils, %	16±4	10±2	32±4	48±4*	25±3	23±4
Eosinophils, %	2.0±0.6	2.0±0.3	2.0±0.5	5.0±1.4*	2.0±0.6	2.0±0.4
Basophils, %	3.0±0.5	2.0±0.3	1.0±0.3	1.0±0.3	1.0±0.3	1.0±0.2
Monocytes, %	2.0±0.3	2.0±0.3	4±1	4±2	5±1	8±2
Lymphocytes, %	77±4	84±2	61±4	42±3*	67±5	66±5
ESR, mm/h	1.5±0.2	1.3±0.2	1.3±0.2	1.3±0.2	1±0	1.0±0.2

**Note.** \* $p < 0.05$  compared to males.

while 10-11-month-old animals had lower reticulocyte count.

Mean hemoglobin content, erythrocyte and platelet counts, and hematocrit determined using HA

were higher than the corresponding parameters obtained by manual methods. The total number of leukocytes determined on HA and counted using a Goryaev chamber did not differ significantly.

**TABLE 4.** PB Parameters in Healthy Rabbits Measured on HA ( $X \pm m$ )

Parameter	Age, months							
	males (n=7)				females (n=8)			
	2-3 (summer)	4-5 (fall)	6-8 (winter)	9-10 (spring)	2-3 (summer)	4-5 (fall)	6-9 (winter)	10-11 (spring)
Body weight, g	2104±72	1964±15	3499±112	3925±266	2235±104	1960±43	3546±109	3936±205
Hemoglobin, g/liter	138±4 <sup>+</sup>	104.4±2.4	149±5	150±4	137±5	99.2±2.7	137±3	140±5
Erythrocytes, $10^{12}$ /liter	6.57±0.25	5.70±0.10	7.10±0.27	6.78±0.19	6.29±0.23	5.27±0.15	6.44±0.14	6.39±0.15
Hematocrit, %	37±1*	30.0±0.4	42±2	41±2	38±1	27.9±0.7	38±1	38±1
Mean erythrocyte volume, fl	59±1	53±1	59±1	60±1	59±1	53±1	59±1	60±1
Degree of erythrocyte anisocytosis, %	18.4±0.3**	19.7±0.6	17.1±0.2	16.4±0.2	18.4±0.5**	19.3±0.7	16.6±0.3	15.9±0.3
Mean hemoglobin content, pg	21.2±0.5	18.3±0.3	21.0±0.4	21.8±0.3	21.8±0.5	18.9±0.5	21.3±0.3	21.8±0.4
Cell hemoglobin concentration, g/liter	360±5	347±5	359±2	362±2	371±6	354±4	363±3	365±3
Platelets, $10^9$ /liter	340±41	398±52	334±16	321±43	455±38	453±93	313±46	263±22
Thrombocrit, %	0.17±0.03	0.20±0.03	0.17±0.01	0.16±0.02	0.23±0.02 <sup>+</sup>	0.22±0.05	0.16±0.03	0.14±0.01
Mean platelet volume, fl	5.1±0.1	5.1±0.1	5.0±0.1	5.3±0.2	5.0±0.1	4.9±0.1	5.2±0.1	5.3±0.2
Degree of platelet anisocytosis, %	31.2±0.4	31.6±0.6	31.4±0.6	31.4±0.7	30.4±0.4*	30.6±0.5	32.1±0.5	31.3±0.7
Leukocytes, $10^9$ /liter	8.3±0.7	9.93±1.04	8.0±0.7	8.8±1.2	9.2±0.7*	9.74±1.26	7.4±0.4	10.5±1.4
Reticulocytes, ‰	79±4**		65±3	57±2	74±5 <sup>+</sup>		61±5	52±3
ESR, mm/h	1.9±0.3	1.9±0.3	1.6±0.3	1.8±0.3	1.9±0.4	1.8±0.3	2.0±0.2	1.4±0.2

**Note.** \* $p < 0.05$  compared to winter, \*\* $p < 0.05$  compared to spring.

**TABLE 5.** Parameters of White Blood in Healthy Rabbits Measured by Manual Method and on HA ( $X \pm m$ )

Parameter	Manual		HA	
	males	females	males	females
Neutrophils, %	27.2±3.3*	23.3±2.7*	50.4±4.3	54.2±5.0
Eosinophils, %	1.0±0.0	1.5±0.3	—	—
Basophils, %	3.7±1.3	3.0±0.7	—	—
Lymphocytes, %	63.1±2.8*	67.2±2.9*	41.3±3.3	39.1±4.3
Monocytes, %	5.0±0.4	5.0±0.7	—	—

**Note.** \* $p < 0.05$  compared to HA.

Measurement of PB parameters on HA revealed some sex-, age-, and season-related differences (Table 4). Mean hemoglobin content during winter time and erythrocyte count and hematocrit in fall-winter period were significantly higher in males than in females ( $p < 0.05$ ). Red blood parameters (hemoglobin content, erythrocyte count, and hematocrit) increased with age, while reticulocyte count decreased with age. Hemoglobin content, erythrocyte count and hematocrit were lower in fall and higher in winter and spring, while reticulocyte count was higher in summer. Other changes were less pronounced (females had lower leukocyte count in winter and platelet count in spring).

Comparison of the mean neutrophilic granulocyte count (with admixture of eosinophils and some monocyte forms) and leukocyte count measured on HA with the counts obtained by counting cells under a microscope revealed significant differences between these values (Table 5). The percent of MID (monocytes, eosinophilic granulocytes) in differen-

tial white blood count measured on HA ( $8.3 \pm 1.4\%$  in males and  $6.7 \pm 1.8\%$  in females) did not significantly differ from their count in blood smear ( $9.7 \pm 1.0\%$  in males and  $9.5 \pm 0.7\%$  in females).

Thus, comparison of hematological parameters obtained by different methods revealed advantage of HA: high rate of analysis and simplicity; it was also shown that HA cannot completely replace blood smear microscopy [5].

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