A Quantitative Study on the Rate of Cell Production in the Bone Marrow of Young Mature Albino Rats.*

By

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The present work is a continuation of the previous studies on the rate of cell production in the thymolymphatic organs (Osogoe et al., 1957; Monden, 1959a; and Ito, 1959), and deals with the rate of mitotic activity in the bone marrow. Its principal objective is to estimate the total number of blood cells newly produced by mitosis per day in the bone marrow of young mature albino rats, and, using the previously reported data on total cellular numbers in the circulating blood and bone marrow of similar rats (Monden, 1958b and Awaysa et al., 1960), to calculate the time taken for the replacement of a number of cells equal to that in the whole population (turnover time), with respect to each type of blood cells.

It is a very difficult task to make quantitative approaches along this line. So far as the writer is aware, Kindred is the first who made a series of studies of this type, using rats as material (Kindred, 1938, 1940 and 1942). These studies are chiefly based on cell counts and mitotic counts of given areas in sections of the hematopoietic organs. It should be borne in mind, therefore, that the tedious histologic counting difficulties may cause uncertainty in the results, and that extrapolation of the results from cell counts in limited areas of sections to whole organ can be misleading.

After Kindred, only fragmentary information has been reported concerning the quantitative aspects of cell production in the bone marrow.

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Material and Methods

Young mature male albino rats of a subline of the Wistar strain weighing around 200 g were chosen as the standard animals to be examined as in the earlier studies (Monden, 1959a, b; Ito, 1959 and A way a et al., 1960). They were raised on a standard laboratory diet, which consisted chiefly of unpolished rice, pressed barley and dried small sardine with a small amount of cod liver oil and minerals, supplemented once a week with green vegetables.

They were divided into 5 groups consisting of 5 or 4 animals each. The animals of the first group were killed at 10:00 a.m. and served as controls. Each animal of the other four groups received a single intracutaneous injection of 0.10 mg of colchicine per 100 g of body weight at 10:00 a.m. Thereafter, the animals were killed at successive intervals from 2 to 8 hours as shown in Table 1. All rats had been starved since the previous evening. Unlike in the previous experiments by Ito (1959b), adrenalectomy was not performed in the present research.

Table 1. Summary of animal number and body weight of the rats used.

Time in hours after colchicine injection	0	2	4	6	8
Animal No. and body weight in grams	N 1, 200	Col. 3, 189	Col. 4, 191.5	Col. 5, 191.5	Col. 9, 196
	N 2, 196	Col. 6, 189	Col. 7, 191	Col. 8, 189	Col. 12, 195
	N 3, 200	Col. 10, 193	Col. 13, 195.5	Col. 11, 191	Col. 20, 196
	N 4, 207	Col. 15, 194	Col. 14, 195.4	Col. 18, 192	Col. 21, 199
	N 5, 200	Col. 16, 192	Col. 17, 191	Col. 19, 198	—
Mean $\pm$ S. E.	200.6 $\pm$ 1.8	191.4 $\pm$ 1.0	192.9 $\pm$ 1.1	192.3 $\pm$ 1.5	196.5 $\pm$ 0.9

S. E.: standard error.

Immediately after the animals had been killed by cervical dislocation and bleeding, the tissues to be examined were quickly taken out. For preparing imprint and smear preparations, bone marrow from the distal part of diaphysis of the right femur was used. After that, the marrow was fixed in Zenker-formol to prepare in serial sections.

Differential bone marrow counts and mitotic counts were performed in stamp preparations stained with May-Giemsa or Giemsa. For differential cell counting, the imprint preparations

Table 2. Comparison of the values of the mitotic index obtained in imprints with those obtained in sections of bone marrow at successive intervals after colchicine injection. All figures in per mill.

Group	Time in hours Animal No. Cell type	0					2					4				
		N 1	N 2	N 3	N 5	Mean	3	10	15	16	Mean	4	7	13	17	Mean
Imprints	Erythrocytic series	19.4	19.8	11.2	13.2	15.9	25.8	46.8	49.0	40.6	40.6	88.4	85.0	104.2	77.2	88.7
	Granulocytic series	3.2	3.0	3.6	4.6	3.6	8.6	11.8	9.8	10.0	10.1	16.0	19.0	19.4	19.2	18.4
Sections	Erythrocytic series	18.4	18.8	12.6	10.6	15.1	46.0	46.0	49.4	56.8	46.9	115.4	80.2	86.2	83.8	91.3
	Granulocytic series	3.2	4.0	3.8	4.4	3.9	9.6	12.8	9.8	10.8	10.6	17.8	19.2	18.2	21.8	19.2

Group	Time in hours Animal No. Cell type	6					8				
		5	18	19	Mean	9	12	20	21	Mean	
Imprints	Erythrocytic series	138.2	157.4	89.8	128.5	161.2	169.0	180.6	174.0	171.2	
	Granulocytic series	28.2	27.6	26.2	27.3	37.2	34.2	35.8	37.8	36.3	
Sections	Erythrocytic series	142.8	141.2	125.6	136.5	146.0	148.6	188.0	163.2	162.5	
	Granuloblastic series	31.2	27.2	28.8	29.1	39.6	35.4	39.6	36.0	37.7	

* Determined after counting 5,000 cells.

were considered to be preferable to the smear preparations, since cell counting in the former generally gave higher values with respect to the percentage of the nucleated red cells than that in the latter, as will be described in detail elsewhere.

The relative percentage of different types of cells and the mitotic indices were determined after counting 5,000 nucleated cells. In every instance, the figures of the mitotic index obtained in imprint preparations were compared with those obtained in sections. The results are summarized in Table 2 and depicted in Figures 1 and 2. It was found that the results from both imprints and sections well coincided with each other.

Fig. 1. Comparison of the values of the mitotic index for the nucleated red cells obtained in imprints with those obtained in sections, at successive intervals after colchicine injection.

Fig. 2. Comparison of the values of the mitotic index for the myelocytes obtained in imprints with those obtained in sections, at successive intervals after colchicine injection.

Generally, the identification of cell type is difficult because dividing cells lose not only nuclear characteristics but also some cytoplasmic features. Therefore, special attention was directed to

the identification of dividing marrow cells.

The cells of the erythrocytic series in mitosis may easily be discriminated by their characteristic deep blue cytoplasm tinged with reddish tone and by the chromosomes which are broader and shorter than those of myeloid cells. The identification of the cells of the granulocytic series in mitosis is more easily done because of the presence of the special granules except for myeloblasts. According to the amount of the granules and the basophilic area of cytoplasm, myelocytes were classified into immature, half-mature and mature forms. While the amount of granules increases in this order, the basophilic area of cytoplasm decreases in the same order, and it disappears completely in the mature forms. Myeloblasts are generally smaller in size than myelocytes and have bluish nongranular cytoplasm. Promyeloblasts are slightly larger than myeloblasts and characterize by the presence of a few azurophil granules.

No attempt has been made to present all quantitative data in terms of the amount per 100 g of body weight. The variances accompanying the averages are the standard errors of the means. For multiplication of the two averages, the resultant variance was calculated by a conventional formula $\pm(Ba+Ab)$ instead of using the theoretical formula $\pm\sqrt{B^2a^2+A^2b^2}$, in which A and B are respectively the averages, and a and b their respective standard errors. Although the former formula gave generally greater values than the latter, this does not seriously affect the results, provided the variances are sufficiently small in contrast to the averages.

Results

1. *Differential bone marrow counts.*

The results of differential bone marrow counts in standard rats at successive intervals after colchicine injection are listed in Table 3. Myelogram of normal bone marrow of standard rats is shown in Figure 3. It is seen from the table that proportions of the cells of the erythrocytic series tended to decrease after the injection of colchicine in a dose of 0.10 mg per 100 g body weight. This resulted in an relative increase in proportions of the cells of the granulocytic series (that is, an increase in the granulocytic-erythrocytic ratios). As will be reported elsewhere, colchicine produced a marked reduction in number of the cells of the erythrocytic

Table 3. Differential bone marrow counts* in standard rats weighing around 200 g at successive intervals after colchicine injection in a dose of 0.10 mg per 100 g of body weight. All figures in per mill. Mean $\pm$ S.E.

Cell type	Time in hours		2		4		6		8	
	No.	of animals	5		5		5		4	
Erythrocytic series			420.9 $\pm$ 11.2		430.7 $\pm$ 15.3		392.6 $\pm$ 6.1		360.6 $\pm$ 16.5	
Granulocytic series			424.0 $\pm$ 19.2		447.2 $\pm$ 19.0		474.9 $\pm$ 8.1		432.3 $\pm$ 12.6	
(Neutrophils)			(373.7 $\pm$ 31.1)		(401.4 $\pm$ 14.5)		(426.6 $\pm$ 10.1)		(377.2 $\pm$ 13.6)	
(Eosinophils)			(42.6 $\pm$ 8.9)		(41.9 $\pm$ 6.8)		(43.7 $\pm$ 7.9)		(51.9 $\pm$ 3.6)	
(Basophils)			(7.6 $\pm$ 2.2)		(4.0 $\pm$ 0.5)		(4.6 $\pm$ 1.2)		(3.3 $\pm$ 1.9)	
Lymphocytes			116.9 $\pm$ 25.3		95.2 $\pm$ 7.5		79.7 $\pm$ 11.3		131.0 $\pm$ 12.3	
Monocytes			2.7 $\pm$ 1.0		1.2 $\pm$ 0.3		1.6 $\pm$ 0.5		1.6 $\pm$ 0.6	
Others			35.5 $\pm$ 9.3		25.7 $\pm$ 2.1		51.2 $\pm$ 7.0		74.4 $\pm$ 10.4	
Mitotic figures			19.5 $\pm$ 2.0		53.0 $\pm$ 5.1		107.6 $\pm$ 5.2		164.0 $\pm$ 13.8	
Nucleated red cells			15.7 $\pm$ 1.7		43.0 $\pm$ 4.7		88.6 $\pm$ 4.4		136.6 $\pm$ 12.8	
Granulocytes			3.8 $\pm$ 0.3		10.0 $\pm$ 0.5		19.0 $\pm$ 0.8		27.4 $\pm$ 1.0	
Neutrophils			3.4 $\pm$ 0.3		9.7 $\pm$ 0.6		18.5 $\pm$ 1.0		26.4 $\pm$ 0.8	
Eosinophils			0.3 $\pm$ 0.1		0.2 $\pm$ 0.1		0.5 $\pm$ 0.2		1.1 $\pm$ 0.3	

* The number of nucleated cells counted was 5,000 in each smear.

Fig. 3. Myelogram of normal bone marrow of standard rats.

series when injected in this dosage. There was no remarkable numerical changes in the other types of cells.

After colchicine injection there was a progressive accumulation of metaphases as shown in Table 4.

2. *The mitotic rates of the nucleated red cells and granulocytes.*

Normal values of the mitotic index were found to be: for the erythrocytic series 15.7 ± 1.7 per thousand nucleated marrow cells and 36.1 ± 2.8 per thousand nucleated red cells; and for the granulocytic series 3.8 ± 0.3 per thousand nucleated marrow cells and 8.9 ± 1.1 per thousand granulocytes (14.8 ± 2.4 per thousand non-segmented granulocytes).

The changes in values of the mitotic index obtained for both series of cells after colchicine injection are given in Table 5. It should first be noticed that there was an almost linear increase in the number of mitoses per thousand nucleated cells of the respective series (Figs. 4 and 5). Another finding upon which the author wishes to lay a great stress is that the most actively proliferating cells are polychromatic erythroblasts in the erythrocytic series and

Table 4. Number of different mitotic phases per 5,000 cells counted in bone marrow imprints at successive intervals after colchicine injection.

Cell type	Time in hours											
	0			2			4			6		
	P	M	A	T	P	M	A	T	P	M	A	T
Proerythroblast	0	0	0	0	0	1	0	0	0	4	0	0
Basophil Ebl.	18	6	0	4	7	21	0	0	6	102	0	0
Polychromatophil Ebl.	35	10	0	0	41	60	0	0	32	585	0	0
Orthochromatophil Ebl.	0	0	0	0	0	0	0	0	0	0	0	0
Myeloblast	0	0	0	0	0	3	0	0	0	13	0	0
Promyelocyte	0	1	1	0	1	6	0	0	0	0	0	0
Immature Mc.	0	8	2	0	1	8	2	1	0	48	0	0
Half-mature Mc.	1	2	1	0	0	17	0	0	2	61	0	0
Mature Mc.	0	3	1	0	0	3	0	0	1	10	0	0
Eosinophil Mc.	0	2	0	0	0	1	0	0	0	8	0	0
Total	54	32	5	4	50	120	2	1	41	831	0	0

Abbreviations: P: prophase; M: metaphase; A: anaphase; T: telophase; Ebl: erythroblast; Mc.: myelocyte

Table 3. MITOTIC RATES OF NUCLEATED RED CELLS AND GRANULOCYTES IN THE BONE MARROW after colchicine administration (0.10 mg/100 g). Mean of 5 rats weighing around 200 g each. Mean $\pm$ S.E.

Nucleated red cells					
Time after colchicine (hours)	Number of mitoses per thousand nucleated cells	Number of mitoses per thousand nucleated red cells	Differential count of erythroblasts in mitosis (%)		
			Baso.	Polychrom.	Orth.
0	15.7 ± 1.7	36.1 ± 2.8	30	68	2
2	43.0 ± 4.7	100.8 ± 9.8	27	73	0
4	88.6 ± 4.4	224.7 ± 11.3	22	78	0
6	136.6 ± 12.8	379.1 ± 33.5	18	82	0
8	171.2 ± 4.1	551.5 ± 50.6	19	81	0

Granulocytes											
Time after colchicine (hours)	Number of mitoses per thousand nucleated cells	Number of mitoses per thousand granulocytes	Differential count of granulocytes in mitosis (%)								
			Mbl.	Pro.	Myelocytes, neut.			Non-seg., neut.	Seg., neut.	Eos.	
					Imm. M.	Half-m. M.	Mat. M.				
0	3.8 ± 0.3	8.9 ± 1.1 (14.8 ± 2.4)*	10	7	37	27	10	0	0	0	9
2	10.0 ± 0.5	22.3 ± 2.0 (39.6 ± 3.6)*	14	6	35	36	7	0	0	0	2
4	19.0 ± 0.8	39.9 ± 2.4 (74.1 ± 6.9)*	16	4	33	35	9	0	0	0	3
6	27.4 ± 1.0	63.5 ± 4.1 (107.9 ± 6.9)*	6	3	33	38	6	0	0	0	4
8	36.3 ± 0.8	70.2 ± 5.3 (123.0 ± 4.9)*	17	4	34	38	3	0	0	0	4

* Values when mature granulocytes with filamented or segmented nucleus are excluded.

Abbreviations:

Baso.	Basophilic erythroblasts	Mat. M.	Mature myelocytes
Polychrom.	Polychromatic erythroblasts	Meta-M.	Metamyelocytes
Orth.	Orthochromatic erythroblasts	Non-seg., neut.	Neutrophilic non-segmented leucocytes, including band and ring-nucleated cells
Mbl.	Myeloblasts		
Pro.	Promyelocytes		
Myelocytes, neut.	Neutrophilic myelocytes	Seg., neut.	Segmented neutrophils
Imm. M.	Immature myelocytes	Eos.	Eosinophils, all stages
Half-m. M.	Half-mature myelocytes

Fig. 4. Number of mitotic figures per thousand nucleated red cells in the bone marrow of standard rats, at successive intervals after colchicine injection.

Fig. 5. Numbers of mitotic figures per thousand granulocytes in the bone marrow of standard rats, at successive intervals after colchicine injection.

immature and half-mature neutrophilic myelocytes in the granulocytic series (Figs. 6 and 7). Either orthochromatic erythroblasts or metamyelocytes do not appear to divide normally.

It is also noteworthy that at 8 hours after colchicine injection, many of the arrested metaphases, especially those of the erythrocytic series, were undergoing degeneration by nuclear pyknosis and a few of them by nuclear fragmentation (Fig. 8). In this connection, it is of interest to note that beyond a 6-hour period the effect of colchicine appeared to be weakened to a considerable extent, so far as the cells of the granulocytic series are concerned (see Fig. 5). This was not the case in the erythrocytic series, however, probably because colchicine produced a marked reduction in number of non-dividing cells of this series, as already stated.

Fig. 6. Mitotic activity of erythroblasts at different stages of maturation, from 0 to 8 hours after colchicine injection.

Fig. 7. Mitotic activity of myelocytes at different stages of maturation, from 0 to 8 hours after colchicine injection.

Taking these facts in consideration, it was decided to use the data of colchicine treatment for a 4-hour period and of those for a 6-hour period for the calculation of daily mitotic rates.

3. *The blood cell reserve in the bone marrow.*

It would be of interest to calculate the ratio of the total number of nucleated cells of each series to the total number of blood corpuscles of the corresponding series present in the general circulation, for such ratio appears to indicate the amount of blood cell reserve in the bone marrow. Using the data of total number of nucleated cells in the bone marrow of standard rats previously reported by A way a et al. (1960) and the results of bone marrow differential counts given in Table 4, the number of nucleated cells of each type of blood cells within the whole bone marrow was

Fig. 8. Accumulation of numerous mitotic figures (metaphases) in the bone marrow, 8 hours after colchicine injection. Section from the femoral marrow. Notice almost all the erythroblasts are in mitosis.

computed. When the values obtained for the bone marrow are contrasted with the corresponding values for the circulating blood estimated by Monden (1951),* the following figures may be obtained

Table 6. Blood cell reserve in bone marrow: ratio of the total nucleated cells of the respective series in marrow to the total circulating blood corpuscles in standard rats. Mean $\pm$ S.E.

Cell type	Total number of nucleated cells in marrow $\times 10^6$	Total number of corpuscle in blood* $\times 10^6$	Ratio Marrow: Blood
Total nucleated cells	5,840 $\pm$ 161**		
Erythrocytic series	2,458 $\pm$ 133	114,400 $\pm$ 9,380	1: 47
Granulocytic series (Total granulocytes)	2,476 $\pm$ 180	48.5 $\pm$ 6.1	51: 1
Neutrophils	2,183 $\pm$ 236	46.7 $\pm$ 5.6	47: 1
Eosinophils	249.0 $\pm$ 58.8	1.75 $\pm$ 0.45	142: 1
Lymphocytes	682.8 $\pm$ 16.7	132.0 $\pm$ 12.0	5: 1
Monocytes	15.7 $\pm$ 6.5	7.96 $\pm$ 1.06	2: 1

* Cited from the results of Monden (1959).

** Cited from the results of A way a et al. (1960).

* Mean of 50 standard rats.

as the ratios between bone marrow and blood: 1:47 for the erythrocytic series, 51:1 for the granulocytic series, 47:1 for the neutrophils, 142:1 for the eosinophils, 5:1 for the lymphocytes, and 2:1 for the monocytes (cf. Table 6).

It is of particular interest that the granulocytic cell reserve within the whole bone marrow was estimated to be about 50 times as great as the number of total granulocytes present in the circulating blood, whereas the total number of nucleated red cells within the whole marrow was found to be as small as about 1/50th of the total number of erythrocytes present in the circulating blood.

It has been pointed out that there is an abundant myeloid cell reserve within the bone marrow. According to Yoffey (1956) who made an extensive quantitative study of bone marrow of guinea-pigs, especially the lymphocyte content therein, the granulocyte reserve in the bone marrow is about 100 times the number of granulocytes present in the blood.

As regards the lymphocyte population of bone marrow, our data indicated that the total bone marrow of standard rats contain 5 times as many lymphocytes as are present in the circulating blood (cf. Table 6). Similar quantitative data by Yoffey (1956) on the lymphocyte content of bone marrow of guinea-pigs demonstrated that the lymphocyte population of normal marrow is 23 times as great as that of the blood.

4. The total number of mitoses per day of each type of blood cells in the whole marrow.

On the basis of the results obtained thus far, the total number of mitoses of each type of blood cells per 24 hours in the whole marrow may easily be calculated. The results of calculations based on colchicine treatment either for a 4-hour period or for a 6-hour period are summarized in Table 7.* Here, special attention was directed to the number of mitoses of the late forms of bone marrow elements, because only the late forms, such as polychromatic and orthochromatic erythroblasts in the erythrocytic series and half-mature and mature myelocytes in the granulocytic series, are considered to be the direct supply source of circulating blood cells. In contrast, the early forms, such as proerythroblasts and basophilic

* Diurnal variation in mitotic rates in the bone marrow was not taken into consideration in the present study. Concerning such variation further investigation is required.

Table 7. Total number of mitoses of each type of cells per day in the whole marrow in standard rats. Mean $\pm$ S. E. The total number of nucleated cells in the whole marrow is assumed to be $(5,840 \pm 161) \times 10^6$ according to A way a et al. (1960).

Cell type	Colchicine treatment	Percentage of mitoses after treatment		Total percentage of mitoses in the respective series		Total number of mitoses per day $\times 10^6$	
		Total	Late forms*	Total	Late forms*	Total	Late forms*
Erythrocytic series	4 hours	22.5 $\pm$ 1.2	17.6 $\pm$ 1.0	134.8 $\pm$ 6.9	105.4 $\pm$ 5.5	3,104 $\pm$ 239	2,418 $\pm$ 193
	6 hours	37.9 $\pm$ 3.4	30.6 $\pm$ 2.9	151.6 $\pm$ 13.4	122.5 $\pm$ 11.4	3,190 $\pm$ 354	2,579 $\pm$ 206
Granulocytic series	4 hours	4.0 $\pm$ 0.2 ¹⁾	1.8 $\pm$ 0.1	23.9 $\pm$ 1.4	10.6 $\pm$ 0.5	664 $\pm$ 47.4	294 $\pm$ 29.4
	6 hours	6.4 $\pm$ 0.4 ²⁾	2.8 $\pm$ 0.5	25.4 $\pm$ 1.6	11.3 $\pm$ 1.9	641 $\pm$ 41.6	282 $\pm$ 48.8
Neutrophils	4 hours	4.3 $\pm$ 0.3	1.9 $\pm$ 0.2	26.0 $\pm$ 2.0	11.6 $\pm$ 0.9	647 $\pm$ 52.8	291 $\pm$ 29.1
	6 hours	7.0 $\pm$ 0.5	3.1 $\pm$ 0.5	28.0 $\pm$ 1.8	12.3 $\pm$ 2.1	616 $\pm$ 40.0	271 $\pm$ 40.6
Eosinophils	4 hours	1.1 $\pm$ 0.8	—	6.6 $\pm$ 4.7	—	16.8 $\pm$ 9.6	—
	6 hours	2.1 $\pm$ 0.6	—	8.3 $\pm$ 2.5	—	25.2 $\pm$ 6.8	—

* The term "late form" denote polychromatic and orthochromatic erythroblasts in the erythrocytic series and half-mature and mature myelocytes in the granulocytic series, respectively.

^{1) 2)} These figures are shown in Table 5 to be 39.9 $\pm$ 2.4% and 63.5 $\pm$ 4.1%, respectively.

erythroblasts in the erythrocytic series and myeloblasts, promyelocytes and immature myelocytes in the granulocytic series, are thought to be the cells from which the late forms of bone marrow elements arise.

5. *The turnover time of each type of blood cells.*

The principal objective of the present study is to calculate the time taken for the replacement of a number of cells equal to that in the whole population (turnover time) with respect to each type of blood cells. Since the total cellular numbers in the blood and bone marrow and the total number of mitoses per day in the bone marrow are known, the turnover time of each type of cells may easily be calculated. The results of calculations are given in Table 8, together with the values for the "Daily Replacement Factor" of Yoffey (1954).*

One of the assumptions upon which our calculations are based is that all the blood cells newly produced by mitoses enter into the

Table 8. Estimated values for turnover time and "Daily Replacement Factor" of each type of blood cells in the blood and blood-forming tissues.

Cell population	Turnover time in days		Daily Replacement Factor	
	Total ¹	Late forms ²	Total ¹	Late forms ²
Blood				
Total granulocytes	0.073—0.076	0.165—0.169	13.2—13.6	5.9—6.1
Neutrophils	0.072—0.076	0.161—0.172	13.2—13.9	5.8—6.2
Eosinophils	0.069—0.104	0.125—0.250	9.0—14.4	4.0—8.0
Erythrocytes	36—37	44—47	0.026—0.028	0.021—0.022
Bone Marrow				
Total granulocytes	3.94 —4.19 (2.3)*			
Neutrophils	3.56 —3.84 (1.9)*			
Eosinophils	12—15			
Nucleated red cells	0.66 —0.74			

¹ Calculated on the basis of the total number of mitoses per day.

² Calculated on the basis of the number of mitoses of the late forms per day.

* Turnover time when mature granulocytes with filamented or segmented nucleus are excluded.

* This factor indicates how many times the cell population in the blood are daily replaced by the newly formed cells; it is expressed as the reciprocal of the turnover time in days.

general circulation through the normal process of cell maturation. Strictly speaking, this assumption is not correct. Since, as already mentioned, only the late forms of bone marrow elements may be regarded as being the direct supply source of the circulating blood cells, the calculation of the turnover times of cell populations in the circulating blood should be based on the number of mitoses per day of the late forms alone, subtracting the number of mitoses of the early forms (such as proerythroblasts, basophilic erythroblasts, myeloblasts, promyelocytes and immature myelocytes) from the total number of mitoses per day.

Thus, the turnover times of cell populations in the blood were estimated to be: 0.165-0.169 day for the total granulocytes; 0.161-0.172 day for the neutrophils; 0.125-0.250 day for the eosinophils; and 44-47 days for the erythrocytes. The values of the "Daily Replacement Factor" of Yoffey were as follows: 5.9-6.1 for the total granulocytes; 5.8-6.2 for the neutrophils; 4.0-8.0 for the eosinophils; and 0.021-0.022 for the erythrocytes. It should be emphasized here that the total number of granulocytes newly produced by mitoses per day in the whole marrow was estimated to be about 13 times, and that of granulocytes entering per day into the blood to be about 6 times, as great as the number of granulocytes present in the blood; whereas the daily replacement factor for the erythrocytes was exceedingly smaller and found to be in the order of 0.03 or 0.02.

The turnover times of cell populations in the bone marrow were calculated to be: 3.94-4.19 days for the total granulocytes; 3.56-3.84 days for the neutrophils; 12-15 days for the eosinophils; and 0.66-0.74 day for the nucleated red cells. When mature granulocytes with filamented or segmented nucleus are excluded, the turnover of the total granulocytes turns out to be 2.3 days and that of the neutrophils to be 1.9 day. From the results obtained, it is seen that the nucleated red cells is being most rapidly renewed. This deserves special attention.

6. *The mitotic time of dividing cells in the bone marrow.*

The duration time of mitosis may be estimated by the colchicine method, using Dustin's formula:

$$A = \frac{M \cdot t}{X}$$

where A equals the calculated duration of mitosis, M is the original

mitotic index, and X is the observed mitotic index after t hours of exposure to colchicine. The figures obtained are given in Table 9.

Table 9. Values of the mitotic time of dividing cells in the bone marrow estimated after the exposure to colchicine from 2 to 8 hours.

Time of exposure to colchicine in hours	Nucleated red cells (hour)	Granulocytes (hour)
2 (0—2)	0.72	0.75 (0.79*)
2 (2—4)	0.89	1.07 (1.12*)
4 (0—4)	0.64	0.80 (0.89*)
4 (2—6)	1.06	1.47 (1.42*)
6 (0—6)	0.57	0.82 (0.84*)
6 (2—8)	1.09	1.92 (1.90*)
8 (0—8)	0.52	0.96 (1.01*)

* Mature granulocytes with filamented or segmented nucleus are excluded.

As seen from the table, the mitotic time varies around one hour, with a relatively narrow range between 0.57 and 1.92 hour. There was no appreciable difference in the mitotic time between the cells of the erythrocytic series and those of the granulocytic ones. The colchicine method is originally based on the assumption that cells are permitted to enter mitosis but all mitoses are arrested at the metaphase, after the chemical is administered. Strictly speaking, however, this is not the case, probably due to the toxic action of the drug on resting and dividing cells. Namely, in most instances the rate of entrance of cells into mitosis is somewhat diminished and, in addition, not all the mitoses are blocked at the metaphase. Consequently, the values of the mitotic time estimated by the colchicine method tend to be greater than those obtained by other methods (Lushbaugh, 1956). Considering these facts, the author assumed the average mitotic time of dividing cells to be about one hour. In the preceding studies on the rate of cell production in the thymolymphatic organs, Ito (1959) also assumed the mitotic time of dividing cells of the lymphoid series to be about one hour. Besides, the author considered a mitotic cycle of 30-minute duration to be also possible for the cells of both the erythrocytic and the granulocytic series.

If the mitotic time (MT) and the mitotic index (MI) are known, the intermitotic time (IT) of tissue cells can be calculated from Hoffman's formula (Hoffman, 1953):

$$IT = MT/MI.$$

Using the figures for the mitotic index before colchicine injection that are given in Table 5 and assuming the mitotic time to be 1-hour or 30-minute duration, the intermitotic times of cell populations in the bone marrow were then calculated. The figures obtained are shown in Table 10, together with the corresponding values estimated by the colchicine method. Here, it should be noticed that the turnover time is approximately equal to the intermitotic time. Strictly speaking, the turnover time is the sum of of the intermitotic time plus the mitotic time. However, the mitotic time is only a small fraction of the intermitotic time and may therefore be neglected.

Table 10. Values of the turnover or intermitotic time of cell populations in the bone marrow.

Cell population	Direct method (colchicine method)	Indirect method*	
		1-hours duration of minosis	30-minute duration of mitosis
Nucleated red cells	0.66—0.74 day	1.17 day	0.58 day
Granulocytes	3.94—4.19 days (2.25—2.31)**	4.66 days (2.82 days)**	2.33 days (1.41 day)**

* Assumed that the mitotic time is of 1-hour and 30-minute duration.

** Values when mature granulocytes with filamented or segmented nucleus are excluded.

It is seen from the table that the figures obtained by both the direct and indirect methods fairly well agree with each other.

Discussion

1. *The rate of mitotic activity in the bone marrow.*

The present work is an attempt to calculate the total number of blood cells newly produced by mitosis per day in the bone marrow, on the basis of quantitative determinations of mitotic rates in this organ. Consequently, the reliability of our results depends upon whether the figures of the mitotic index used for calculations are quite reasonable or not.

It is therefore of interest to compare our figures with those published by other workers. It should be first emphasized that our figures for the mitotic index of bone marrow of normal standard

rats are in good agreement with the published figures. According to J a p a (1942), the mitotic index of normal human bone marrow is 1.5% for the total nucleated cells, 3.3% for the nucleated red cells, and 1.0% for the granulocytes; and according to R o h r (1949), it is 1.0-4.2% for the nucleated red cells and 0.6-1.1% for the granuloblasts.* In their recent study on normal human bone marrow, F l i e d n e r et al. (1959) estimated the mitotic index to be 0.9% for the total nucleated cells, 2.24% for the nucleated red cells (4% for the nucleated red cells capable of cell division**) and 2.0% for the granuloblasts.*** S a i t o and M i y a m o t o (1959) also reported similar figures for normal human bone marrow; namely, 4.18% for the erythroblasts and 1.03% for the granulocytes capable of cell division.**** On the other hand, our figures of the mitotic index of rat bone marrow were: $1.95 \pm 0.2\%$ for the total nucleated cells, $3.61 \pm 0.28\%$ for the nucleated red cells and $0.89 \pm 0.11\%$ for the granulocytes ($1.48 \pm 0.24\%$ for the non-segmented granulocytes). As mentioned above, the mitotic index of the nucleated red cells is of the order of 2 to 4 per cent and that of the granulocytes of the order of 0.6 to 1.5 per cent.

As regards the mitotic index of rat bone marrow, relatively low figures are reported by K i n d r e d (1942), who made an extensive quantitative study of bone marrow in sections, using young adult albino rats weighing approximately 200 g. His figures were 0.8-0.9% for the total nucleated cells, 0.93% for the nucleated red cells and 0.92% for the granulocytes. As far as the granulocytes are concerned, his figure well coincides with ours. However, his figure for the erythrocytic series is exceedingly smaller than ours. This is the crucial point upon which his view on transformation of lymphocytes into erythroblasts is based, as will be mentioned later.

2. *The turnover time of the granulocytes in the blood.*

We shall next consider the rate of renewal of the granulocytes and erythrocytes in the blood. A comparison of the values for the turnover time, or intervacular life span, of the granulocytes and erythrocytes in the circulating blood estimated by different methods is shown Table 11.

* Granulocytes capable of cell division.

** The orthochromatic normoblasts that do not divide normally are excluded.

*** Granulocytes capable of cell division.

**** Calculated from the data given in the paper of S a i t o and M i y a m o t o (1959).

Table 11. Comparison of the values for turnover time (intravascular life span or age) of the granulocytes and erythrocytes in the circulating blood estimated by different methods

Cell population	Species	Turnover time days	Method	Reference
Granulocytes	Rat	0.12	Parabiosis	Van Dyke & Huff, 1951
	"	0.033—0.071	Mitotic counts in sections	Kindred, 1942
	"	0.161—0.172	Mitotic counts by colchicine method	Table 8, this paper
	Human	0.021—0.062	Transfusion	White, 1954
	"	8.8	³² P incorporation in DNA	Kline & Cliffton, 1952
	"	2.7—3.4	"	Ottesen, 1954
	"	2	"	Osgood & Krippachne, 1955
	Human	120	Labelling, ⁵¹ Cr	Hyman et al., 1956
	Rabbit	64	Glycine ¹⁵ N incorporation in heme	Neuberger & Niven, 1951
	Rat	70	¹⁴ C incorporation in hemoglobin	Berlin & Lotz, 1951
Erythrocytes	"	59±2	Labelling, ⁵⁹ Fe and ⁵¹ Cr	Belcher & Harris, 1959
	"	382—790*	Mitotic counts in sections	Kindred, 1942
	"	44—47	Mitotic counts by colchicine method	Table 8, this paper

* Calculated from the data given in the tables of the paper of Kindred.

As regards the turnover time of the granulocytes in the blood, it should first be noticed that the values estimated by our method fairly well agree not only with the estimates by mitotic counts in sections (K i n d r e d, 1942), but also with those based on survival of granulocytes after either parabiosis (V a n D y k e and H u f f, 1951) or transfusion (W h i t e, 1954), and that the figures obtained are of the order of from 0.02 to 0.2 days (from thirty minutes to 5 hours). Since, however, the estimates based on mitotic counts are thought to be more reliable than those based on survival after parabiosis or transfusion, the author consider a renewal time of 0.161-0.172 day (about 4 hours) to be the most reasonable figure for the blood granulocytes.

Of particular interest in this connection is the fact that the values for the life span of the granulocytes* estimated by use of radioactive tracers are much greater than the above-mentioned figures. By measurement of the incorporation of ^{32}P into deoxy-pentose nucleic acids (DNA) of the granulocytes in man, K l i n e and C l i f f t o n (1952) estimated the life span of these cells in the circulating blood to be 8.8 days and the time they spend within the bone marrow as immature forms to be about 4 days. Using similar technique, O t t e s e n (1954) found the mean age of the granulocytes in the blood to be 8.7-9.4 days, and that the main part of the granulocytes enters the blood stream at an age of about 6 days. On the basis of *in-vitro* survival of human leukocytes and their DNA uptake of ^{32}P , O s g o o d and K r i p p a e h n (1955) indicated a life span of 2 day for the segmented neutrophils and a life span in the bone marrow alone of the order of 3 to 4 days; thus giving a total life span of 5-6 days for the cells of the neutrophilic series.

Similar discrepancy is also seen, with respect to the turnover time of the lymphocytes in the blood, between the values estimated by mitotic counts or cannulation of thoracic duct lymphocytes on the one hand, and the estimates based on DNA uptake of radioactive material and *in-vitro* survival of these cells on the other. Namely, the former methods gave a life span of about 0.3 day for the blood lymphocytes, whereas the life span of these cells estimated by the latter methods was longer than 3 days, and for the most lymphocytes

* The life span or average age of a type of cells is considered to be equal to the turnover time of the population of this type of cells.

it was estimated to be of the order of 100 to 200 days (Ottesen, 1954; Hamilton, 1954 and 1956).

The reason for such discrepancy is not clear. However, it may be interpreted as indicating an overproduction of both the granulocytes in the bone marrow and the lymphocytes in the thymolymphatic organs. In this connection, the claims of Osgood (1954) and Lissac (1956) are of particular interest that leukocytes are not primarily blood cells but are merely en route in the blood to the defense of the body, and that only a small portion of the total leukocytes outside the hematopoietic organs are present in the blood stream at any one time.

At this point, it should be recalled that the total number of granulocytes newly produced by mitosis per day in the whole marrow was estimated to be about 13 times as great as the total number of granulocytes present in the circulating blood. Similarly, the results of previous investigations on the thymolymphatic organs of rats have demonstrated that in these organs lymphocytes are being produced by mitosis in numbers sufficient to replace the blood lymphocytes about 2 to 3 times daily (Osogoe et al., 1957; Monden 1959b; Ito, 1959). In view of the fact that the true life span of the granulocyte and lymphocyte is hardly thought to be less than 24 hours, the above-mentioned findings seem to support the view that the length of the intravascular phase of the leukocytes does not represent their true life span. From such viewpoint, leukocytes cannot properly be called blood cells. It is generally believed that the extravascular pool of leukocytes is very great, far exceeding that within the vascular system.

3. *The turnover time of the erythrocytes in the blood.*

The results of estimation of the life span of the erythrocytes are in sharp contrast with those of the granulocytes. It is of essential importance that the values of the intravascular life span of the erythrocytes estimated by use of radioactive tracers are in good agreement with those estimated by mitotic counts, except for the results of mitotic counts in sections by Kindred (1942) upon which a brief mention will be made later. Among figures presented in Table 11, the life span of the rat erythrocytes is of especial interest; it was estimated on the basis of labelling with or incorporation of ^{14}C , ^{51}Cr or ^{59}Fe either to be 70 days (Berlin and Lotz, 1951) or to be 59 ± 2 days (Belcher and Harris, 1959),

while a life span of 44-47 days was obtained by mitotic counts in the present study (cf. Table 11). Since in our calculations the presence of a large amount of erythrocytes within the bone marrow was not taken into consideration, it can be stated that both methods give quite similar results. This seems to indicate that in erythrocytes the true life span well coincides with the intravascular life span. From this viewpoint, erythrocytes may be called blood cells in the true sense of the term.

The present findings also indicate that erythrocytes in the bone marrow of young mature rats may multiply themselves by mitosis in sufficiently great number to meet the demands of the blood, since the life span of erythrocytes estimated by mitotic counts is somewhat smaller than the estimates based on the labelling with or incorporation of ^{51}Cr , ^{59}Fe or ^{51}Cr . As already mentioned, Kindred (1942) who made an extensive quantitative study on the rate of cell production in the hematopoietic organs in sections, obtained exceedingly low values with respect to the mitotic index of the erythroblasts in the bone marrow, that is, of the order of less than 1%. This is as low as about 1/3rd of the figures obtained in man and rat by other workers including us, and the values of the turnover time of the erythrocytes calculated using the data given in the paper of Kindred are of the order of 400 to 800 days (cf. Table 11).

On the basis of the results mentioned above, Kindred maintains that the production of erythrocytes by mitosis in the whole marrow, when contrasted with the estimated needs of the circulating blood, is not sufficient to meet the demands of the blood, and supports the hypothesis of Jordan (1936, 1939) that lymphocytes produced in excess are filtered off in the bone marrow to become the stem cells for the development of erythrocytes. A similar view is also maintained by Yoffey (1954, 1955, 1956, 1958), who is making a close quantitative examination of the lymphocytes in the bone marrow of guinea-pigs. The present findings do not favor such view, however. It should again be emphasized here that in the bone marrow mitotic figures of erythroblasts occur in sufficiently great numbers when contrasted with the demands of the blood. There is no necessity for assuming that the erythroid population of the bone marrow must receive from some outside source in order to produce sufficient erythrocytes to meet the demands of the blood.

4. *The turnover times of the granulocytes and nucleated red cells in the bone marrow.*

As seen in Table 8, the turnover times of cell populations in the bone marrow were estimated to be of the order of 4 days for the granulocytes and less than 24 hours for the nucleated red cells. On the basis of measurements of incorporation of radioactive material (e.g., ^{32}P) into DNA of the granulocytes, on the other hand, the turnover time of these cells in the bone marrow was estimated by Kline and Clifton (1952) to be 4 days, by Osgood and Krippaehne (1955) to be 3-4 days and by Ottesen to be 5-6 days in man. It is of particular interest that the figure estimated by mitotic counts well coincides with the corresponding values estimated by use of radioactive material. As already stated, this was not the case as far as the turnover time of the granulocytes in the blood is concerned.

Summary

1. In an attempt to make possible a direct calculation of the total number of cells newly produced by mitosis per day in the bone marrow, a series of determinations have been made of the mitotic index of bone marrow of young mature male albino rats weighing around 200 g, before and at successive intervals after the subcutaneous injection of colchicine in a dose of 0.10 mg per 100 g of body weight.

2. Normal values of the mitotic index of bone marrow were found to be for the erythrocytic series 36.1 ± 2.8 per thousand nucleated red cells and for the granulocytic series 8.9 ± 1.1 per thousand granulocytes (14.8 ± 2.4 per thousand non-segmented granulocytes).

3. The total percentages of mitoses per day were estimated to be 134.8-151.6% for the erythrocytic series, 23.9-25.4% for the total granulocytes, 26.0-28.0% for the neutrophils and 6.6-8.3% for the eosinophils; whereas the corresponding figures for the late forms alone were 105.4-122.5% for the erythrocytic series, 10.6-11.3% for the total granulocytes, and 11.6-12.3% for the neutrophils.

4. Using the data of A way a et al. (1960) on the total number of nucleated cells in the whole marrow, the average numbers of mitoses per day were computed to be $(3,104-3,190) \times 10^6$ for the erythrocytic series, $(641-664) \times 10^6$ for the total granulocytes, $(614-$

$547) \times 10^6$ for the neutrophils, and $(16.8-25.2) \times 10^6$ for the eosinophils. When mitoses of the late forms alone were taken into account, the corresponding figures turned out to be $(2,418-2,579) \times 10^6$ for the erythrocytic series, $(287-294) \times 10^6$ for the total granulocytes, and $(271-291) \times 10^6$ for the neutrophils.

5. On the basis of an assumption that only the late forms of bone marrow elements are the direct supply source of the circulating blood cells and using the data of Monden (1959b) on total cellular numbers in the blood, the turnover times of cell populations in the blood were calculated to be 0.165-0.169 day (about 4 hours) for the total granulocytes, 0.161-0.172 day for the neutrophils, 0.125-0.250 day for the eosinophils, and 44-47 days for the erythrocytes.

6. By a direct calculation from the figures obtained for the daily mitotic rates, the turnover time of each type of blood cells in the bone marrow was estimated to be 3.94-4.19 days for the total granulocytes (2.3 day for the non-segmented granulocytes), 3.56-3.84 days for the neutrophils (1.9 days for the non-segmented neutrophils), 12-15 days for the eosinophils, and 0.66-0.74 day for the nucleated red cells.

7. Besides, an indirect calculation of the turnover times of cell populations in the bone marrow was also made from Hoffman's formula, assuming that the mitotic time of dividing cells is of 1-hour or of 30-minute duration.

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Table 12. Differential counts of non-dividing cells in the bone marrow of standard rats at successive intervals after colchicine injection. All figure expressed as the numbers of cells per thousand nucleated cells. Mean $\pm$ S.E.

Cell type	Time in hours		2		4		6		8	
	No. of animals	0	5		5		5		4	
Erythrocytic series		405.2 $\pm$ 9.9	387.7 $\pm$ 13.1		304.0 $\pm$ 7.6		224.0 $\pm$ 17.6		145.8 $\pm$ 33.5	
Proerythroblasts		0.5 $\pm$ 0.2	0.6 $\pm$ 0.4		0.4 $\pm$ 0.2		0.6 $\pm$ 0.2		0.9 $\pm$ 0.4	
Basophilic Ebl.		122.6 $\pm$ 29.0	120.3 $\pm$ 9.4		90.4 $\pm$ 5.7		76.8 $\pm$ 8.3		64.0 $\pm$ 22.5	
Polychromatic Ebl.		272.6 $\pm$ 7.6	266.2 $\pm$ 11.3		213.1 $\pm$ 6.3		144.0 $\pm$ 16.9		78.9 $\pm$ 10.5	
Orthochromatic Ebl.		9.5 $\pm$ 6.6	0.6 $\pm$ 0.6		0.2 $\pm$ 0.1		2.6 $\pm$ 1.2		2.0 $\pm$ 1.1	
Granulocytic series		420.2 $\pm$ 25.1	437.3 $\pm$ 14.0		456.0 $\pm$ 8.9		404.9 $\pm$ 14.1		480.3 $\pm$ 27.0	
Myeloblasts		1.9 $\pm$ 0.6	1.4 $\pm$ 0.3		2.6 $\pm$ 0.8		2.6 $\pm$ 0.8		2.2 $\pm$ 0.8	
Promyelocytes		15.4 $\pm$ 3.8	18.6 $\pm$ 3.6		21.6 $\pm$ 5.5		22.0 $\pm$ 1.1		24.3 $\pm$ 6.5	
Myelocytes, neut.		148.7 $\pm$ 22.6	152.9 $\pm$ 11.8		148.8 $\pm$ 12.8		136.2 $\pm$ 4.9		147.9 $\pm$ 2.9	
Immature Mc.		46.3 $\pm$ 18.0	43.4 $\pm$ 7.6		51.0 $\pm$ 10.3		48.0 $\pm$ 6.5		65.0 $\pm$ 12.0	
Half-mature Mc.		52.6 $\pm$ 9.6	57.2 $\pm$ 8.3		53.0 $\pm$ 5.8		50.0 $\pm$ 4.9		52.0 $\pm$ 4.9	
Mature Mc.		28.7 $\pm$ 5.1	31.6 $\pm$ 4.4		26.0 $\pm$ 6.9		22.8 $\pm$ 9.4		17.0 $\pm$ 6.7	
Meta-Mc.		21.2 $\pm$ 3.6	20.7 $\pm$ 2.9		18.7 $\pm$ 1.7		15.4 $\pm$ 1.6		13.5 $\pm$ 1.8	
Band forms, neut.		36.4 $\pm$ 13.1	24.6 $\pm$ 5.5		19.1 $\pm$ 6.1		15.4 $\pm$ 2.0		14.3 $\pm$ 4.9	
Segmented cells, neut.		167.8 $\pm$ 10.2	193.7 $\pm$ 16.3		217.2 $\pm$ 16.3		174.5 $\pm$ 17.5		219.7 $\pm$ 30.5	
Eosinophils, all stages		42.3 $\pm$ 8.8	41.6 $\pm$ 6.7		43.2 $\pm$ 7.3		50.8 $\pm$ 3.3		70.2 $\pm$ 5.2	
Basophils, all stages		7.6 $\pm$ 0.5	4.0 $\pm$ 0.5		4.6 $\pm$ 1.2		3.3 $\pm$ 1.9		1.8 $\pm$ 1.3	
Lymphocytes		116.9 $\pm$ 25.3	95.2 $\pm$ 7.5		79.7 $\pm$ 11.3		131.0 $\pm$ 12.3		102.2 $\pm$ 9.2	
Monocytes		2.7 $\pm$ 1.0	1.2 $\pm$ 0.3		1.6 $\pm$ 0.5		1.6 $\pm$ 0.6		1.0 $\pm$ 0.4	
Plasmocytes		8.5 $\pm$ 2.0	7.6 $\pm$ 0.6		13.8 $\pm$ 2.4		21.7 $\pm$ 2.9		26.9 $\pm$ 2.8	
Megakaryocytes		1.0 $\pm$ 0.2	0.6 $\pm$ 0.2		1.2 $\pm$ 0.3		1.1 $\pm$ 0.2		1.5 $\pm$ 0.3	
Reticulum cells		23.3 $\pm$ 8.9	14.3 $\pm$ 2.0		27.1 $\pm$ 7.0		40.2 $\pm$ 9.8		23.8 $\pm$ 5.7	
Unclassified cells		2.6 $\pm$ 0.9	3.1 $\pm$ 0.5		9.1 $\pm$ 2.4		11.5 $\pm$ 3.6		11.2 $\pm$ 3.7	

* The number of nucleated cells counted was 5,000 in each smear.

Abbreviations: Ebl., erythroblasts; neut., neutrophilic; Mc., myelocytes; Meta-Mc., metamyelocytes

Table 13. Differential counts of dividing cells in the bone marrow of standard rats after successive intervals after colchicine injection. All figures are expressed as the number of dividing cells per thousand nucleated cells. Mean $\pm$ S. E.

Cell type	Time in hours		No. of animals		2		4		6		8	
	0		5		5		5		5		5	
Erythrocytic series	15.7 $\pm$ 1.7		0.04 $\pm$ 0.04		43.0 $\pm$ 4.7		88.6 $\pm$ 4.4		136.6 $\pm$ 12.8		171.2 $\pm$ 4.1	
Proerythroblasts	0.04 $\pm$ 0.04		0.12 $\pm$ 0.11		0.12 $\pm$ 0.11		0.32 $\pm$ 0.25		0.40 $\pm$ 0.21		0.75 $\pm$ 0.11	
Basophilic Ebl.	4.64 $\pm$ 1.00		11.44 $\pm$ 1.76		11.44 $\pm$ 1.76		19.28 $\pm$ 1.74		25.72 $\pm$ 3.70		31.20 $\pm$ 3.60	
Polychromatic Ebl.	10.68 $\pm$ 1.42		31.40 $\pm$ 3.38		31.40 $\pm$ 3.38		69.00 $\pm$ 3.93		110.44 $\pm$ 13.38		139.25 $\pm$ 7.42	
Orthochromatic Ebl.	0.32 $\pm$ 0.32		—		—		—		—		—	
Granulocytic series	3.8 $\pm$ 0.3		10.0 $\pm$ 0.5		10.0 $\pm$ 0.5		19.0 $\pm$ 0.8		27.4 $\pm$ 1.0		36.3 $\pm$ 0.80	
Myeloblasts	0.36 $\pm$ 0.26		1.40 $\pm$ 0.40		1.40 $\pm$ 0.40		2.92 $\pm$ 0.38		4.68 $\pm$ 0.86		6.30 $\pm$ 1.06	
Promyelocytes	0.24 $\pm$ 0.05		0.64 $\pm$ 0.25		0.64 $\pm$ 0.25		1.04 $\pm$ 0.30		0.76 $\pm$ 0.34		1.55 $\pm$ 0.36	
Myelocytes, neut.	2.8 $\pm$ 0.5		7.7 $\pm$ 0.7		7.7 $\pm$ 0.7		14.5 $\pm$ 0.9		20.9 $\pm$ 1.8		27.1 $\pm$ 1.0	
Immature Mc.	1.40 $\pm$ 0.40		3.40 $\pm$ 0.73		3.40 $\pm$ 0.73		6.24 $\pm$ 0.78		9.32 $\pm$ 0.55		12.50 $\pm$ 1.01	
Half-mature Mc.	1.04 $\pm$ 0.65		3.60 $\pm$ 0.18		3.60 $\pm$ 0.18		6.64 $\pm$ 0.52		9.92 $\pm$ 1.36		13.55 $\pm$ 1.30	
Mature Mc.	0.40 $\pm$ 0.12		0.68 $\pm$ 0.21		0.68 $\pm$ 0.21		1.64 $\pm$ 0.54		1.68 $\pm$ 0.40		1.05 $\pm$ 0.22	
Meta-Mc.	—		—		—		—		—		—	
Eosinophils	0.32 $\pm$ 0.10		0.24 $\pm$ 0.07		0.24 $\pm$ 0.07		0.48 $\pm$ 0.26		1.08 $\pm$ 0.26		1.30 $\pm$ 0.37	

* The number of nucleated cells counted was 5,000 in each smears.

Abbreviations: Ebl., erythroblasts; neut., neutrophilic; Mc., myelocytes; Meta-Mc., metamyelocytes.