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Research Article

QUANTITATIVE MEASUREMENT OF THE ERYTHROCYTIC AND GRANULOCYTIC CELLS OF THE MARROW AND BLOOD [1]

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In the preceding article, a method for measuring total marrow cellularity was described (1). The purpose of this report is to relate numerically (*a*) the erythroid marrow to the circulating red cell mass, and (*b*) the granulocytic marrow to the circulating granulocytic leukocytes. Results in three animal species and in man are reported, and are compared with data obtained by other methods.

MATERIALS AND METHODS

Studies were performed on Sprague-Dawley rats weighing between 200 to 300 Gm., New Zealand white rabbits weighing 2 to 3½ Kg., and 2 to 3 Kg. male Rhesus monkeys. All animals were given approximately 15 mg. per Kg. of iron as iron dextran [2] at least two weeks before the study to insure adequate iron stores. Only those animals with hemoglobin levels above 13 Gm. were used. Reticulocyte counts in rabbits were required to be below 4 per cent and those of monkeys and rats below 3 per cent before the animals were considered suitable for study.

Clinical studies were performed on male patients with controlled tuberculosis who were being subjected to thoracotomy. Prior to operation, these patients had normal leukocyte counts and were afebrile, but plasma iron values were usually subnormal. On the morning of operation their red counts were above 4.5×10^6; their reticulocyte counts were less than 2 per cent. They had not been transfused during the previous six months.

I. *Erythrocytic series*

A. Studies of the circulating erythrocytes. A red count was performed by enumerating a minimum of 1,000 cells from two or more pipettes in two counting chambers. Reticulocyte counts were performed on dried smears of blood stained with new methylene blue. One thousand cells were surveyed. Blood volume measurements were performed by the Cr^{51} or P^{32} techniques on only a few of the animals studied. In the remainder of the animal experiments and in clinical studies with patients, volumes were calculated from Kg. body weight according to normal values previously established in our laboratory. The value of 60 ml. per Kg. was used for rats, rabbits, monkeys and for the patients studied. While the volume for normal ambulatory man was previously found to be about 68 ml. per Kg. (2), additional studies indicated that the usual value for patients at bed rest was approximately 60 ml. per Kg.

B. Studies of the noncirculating erythropoietic tissue. In animals, a cell suspension of marrow was prepared from the central 60 per cent of the femur of the sacrificed animal. In human studies, a portion of rib removed at operation was the source of marrow. Dispersion of marrow cells was achieved by shaking and by filtering the suspension through a nylon mesh. Total cell counts, employing red cell pipettes and counting chambers, were performed with the enumeration of approximately 1,000 cells. The suspension was then concentrated by centrifugation. Smears were made directly from this concentrate, stained with Wright and Giemsa, and a 2,000 cell differential count was performed. In this study eosinophil and basophil forms were excluded from the granulocytic series. Additional smears were made of the marrow cells stained with new methylene blue, and 2,000 non-nucleated red cells were examined for reticulum. Details of this method are presented in the preceding article (1).

The quantitative relationship between the aliquot of marrow in the central shaft of the animal femurs or from the rib in man and the total marrow was established through the use of Fe^{59} as a marrow tag. Iron was injected either bound to the β_1 globulin of fresh plasma or in a manner to insure binding *in vivo* (3). In animals, 2 to 5 μc. per Kg. of Fe^{59} and in man ½ μg. per Kg. were employed. Marrow was obtained at the time of minimal circulating radioactivity for preparation of a cell suspension, *i.e.*, 5 hours in the rat, 6 hours in the rabbit, 12 hours in the monkey and 15 hours in man.

In animal studies the radioactivity, cell count and composition of the marrow suspension were determined. Losses in preparation of the suspension, as reflected in

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the residual radioactivity on bone fragments, filter, and in the supernate of the marrow suspension, were also determined as an index of the suitability of the preparation. In animals the total skeleton was isolated from the autoclaved carcass and its radioactive content determined.

From the specific activity of cells in the marrow suspension and the total marrow activity of the skeleton, it was possible to calculate the number of marrow hematopoietic cells. In man, it was necessary to assume that a certain percentage of the injected radioiron would be located in the marrow at the time of marrow sampling. On the basis of animal studies (1), this figure is taken to be 66 per cent.

C. Formulae employed—Calculations employed

1. *Circulating erythron. Total circulating erythrocytes* are calculated from the product of the red count per ml. and the total blood volume in ml. *Circulating reticulocytes* are represented by the product of the total circulating erythrocytes and the per cent of reticulocytes. *Circulating mature erythrocytes* equal the difference between total circulating erythrocytes and circulating reticulocytes.

2. *Noncirculating erythron.* In animals, total marrow cellularity (Ctm) is determined from the cells per ml. marrow suspension (Cs), the radioactivity of the centrifuged cells in 1 ml. marrow suspension (Rs), and total marrow radioactivity (Rtm) as follows:

$$\text{Ctm} = \frac{\text{Rtm} \times \text{Cs}}{\text{Rs}}. \qquad \text{(I)}$$

It should be noted that total marrow radioactivity includes, along with the activity of the isolated skeleton, the radioactivity of the marrow aliquot and the activity lost in its preparation (*i.e.*, on bones, in filter and in supernate).

Essentially the same formula is employed for man except that total marrow radioactivity may not be determined directly. In its place the injected dose (Ri) times a factor of 0.66 is substituted to allow for the per cent localized in the skeleton:

$$\text{Ctm} = \frac{\text{Ri} \times 0.66 \times \text{Cs}}{\text{Rs}}. \qquad \text{(II)}$$

In these studies the radioactivity in marrow attributable to the circulating blood was ignored, since such activity represented less than 2 per cent of the counts in the marrow. In the event of greater peripheral blood contamination this would be important, and appropriate corrections would be required in the calculation of results.

The percentages of nucleated red cells, reticulocytes, and adult erythrocytes were obtained from the differential cell counts in the marrow suspension. Knowing the total number of marrow cells, we could then calculate the absolute number of each of these. Other results (1) indicate that there is no appreciable number of mature nonreticulated erythrocytes in the fixed marrow, so the number of these cells was taken to reflect the amount of circulating blood mixed with marrow. From this figure and from the per cent of reticulocytes in circulation, a correction in the total marrow reticulocyte count was made as follows:

$$\text{True marrow reticulocytes} = \text{Rm} - \left(\text{Em} \times \frac{\text{Rb}}{100 - \text{Rb}}\right). \qquad \text{(III)}$$

When Rm is total marrow reticulocyte, Em is erythrocytes without reticulum in marrow suspension, and Rb is the per cent reticulocytes in the circulating blood. The erythron has thus been arbitrarily divided into four categories of cells: (*1*) nucleated red cells of the marrow, (*2*) reticulocytes of the marrow, (*3*) reticulocytes in circulation and (*4*) mature erythrocytes in circulation.

II. *Granulocytic series*

Total granulocytes in circulation were determined from the concentration of granulocytes and the estimated blood volume, as described for circulating red cell estimates. A total of 500 leukocytes was enumerated in the counting chamber in determining the leukocyte count per cu. mm. of blood, and a differential count of 200 white cells was performed on the Wright-stained peripheral blood smear. Differential cell counts on smears of the marrow suspensions were performed on 2,000 to 4,000 nucleated cells. *Total marrow granulocytic forms* were determined from the total marrow cellularity (Formulae I and II) times per cent granulocytic cells. Morphologic criteria employed arbitrarily to separate the granulocytic series into designated categories were those generally employed (4). From the differential counts of the granulocytic series, the numbers of myelocytes, metamyelocytes, bands and segmented forms were determined.

RESULTS

I. Erythron

Data for the number of erythroid forms in the marrow and blood of five rats, five rabbits and five monkeys are summarized in Table I, and comparable data in nine human subjects are shown in Table II. The number of nucleated red cells is comparable in three of the four species when expressed as cells per Kg. body weight.[3] Thus, in the rat there were 5.5×10^9 nucleated erythrocytes per Kg., in the rabbit there were 5.8, and in man, 5.4, while in the monkey a value of 9.2 was obtained. In these same four species the combined reticulocytes of marrow and blood were 14.5, 16.4,

[3] In all species the red cell hemoglobin concentration was similar, individual values varying between 31 and 34 per cent. Red cell volume, however, showed species' differences. In the rat, mean corpuscular volume averaged 55.5 cu. mm.; in the rabbit the volume was 67; in the monkey, 68; and in man, 86 cu. mm.

TABLE I

Animal erythroid cells

Species	Number of animals	Nucleated red blood cells/ Kg. body weight $\times 10^9$	Marrow reticulocytes/ Kg. body weight $\times 10^9$	Circulating reticulocytes/ Kg. body weight $\times 10^9$	Mature red blood cells/ Kg. body weight $\times 10^9$
Rat	5	5.55 ± 0.59*	6.05 ± 1.41	8.46 ± 3.55	493 ± 62
Rabbit	5	5.8 ± 2.3	4.8 ± 1.4	11.6 ± 2.6	370 ± 28
Monkey	5	9.2 ± 1.9	6.1 ± 1.6	4.9 ± 3.1	380 ± 15

* Standard deviation.

8.95 and 11.0 × 10^9 cells per Kg., respectively. Of this total compartment, the percentage in the marrow [4] was 42, 29, 65 and 56 per cent. Average figures for red cells in these four species were 493 × 10^9 Kg. in rats, 370 in rabbits, 309 in man and 380 in monkeys.

II. Granulocytic series

Summary data of measurements of the granulocyte series of marrow and blood are shown in Tables III and IV. Circulating granulocytes in rats, rabbits, monkey and man averaged 0.17, 0.24, 0.44 and 0.30 × 10^9 Kg. Total marrow granulocyte forms of these species were 6.7, 4.8, 15.4 and 11.4 × 10^9. The accompanying tables summarize further distribution of relative numbers of cells at various stages of their development.

[4] Other studies have indicated that a 20 per cent differential destruction of nucleated, as compared to non-nucleated, erythrocytes may occur in preparation of the marrow cell suspension (5). Thus, the actual value of marrow reticulocytes may be less than this recorded value by 0 to 20 per cent. Assuming the maximum difference, this would reduce the estimates summarized in Table I to 4.8, 3.8, 4.9 and 4.6 × 10^9 marrow reticulocytes per Kg.

DISCUSSION

Various experimental approaches have been employed to estimate the number of marrow precursors of the circulating blood elements. It is of interest to review these methods and the results obtained by their use (Table V). Kindred (6) carried out meticulous and detailed studies in the rat in which he counted both the number of cells in histologic sections and the volume of the marrow cavities. From these data he directly calculated total nucleated erythrocytes. Hudson and Yoffey used an experimentally determined marrow volume (7) and counted the cells in a given volume of femoral marrow (8). Osgood (9) used the known turnover rate of the mature erythrocyte and the estimated maturation time of immature erythrocytes derived from the time of reticulocyte response following alterations of marrow function

TABLE II

Human erythroid cells

Subject	Nucleated red cells (total cells/ Kg. × 10^9)	Marrow reticulocytes (total cells/ Kg. × 10^9)	Circulating reticulocytes (Cells/cu. mm. × 10^6)	Circulating reticulocytes (Total cells/ Kg. × 10^9)	Circulating mature erythrocytes (Cells/cu. mm. × 10^6)	Circulating mature erythrocytes (Total cells/ Kg. × $10)^9$
T. S.	8.51	10.92	0.042	2.50	4.69	281
K. A.	2.28	2.88	0.073	4.44	5.22	313
B. T.	3.75	7.03	0.064	3.83	5.32	319
R. B.	4.46	3.34	0.041	2.62	5.14	308
C. Ba.	5.63	5.11	0.035	2.13	5.91	354
P. M.	4.76	4.37	0.071	4.09	5.46	328
B. N.	6.48	4.10	0.066	4.00	5.11	307
C. Bo.	5.24	6.23	0.029	2.04	4.87	292
C. O.	7.15	7.63	0.056	3.35	4.66	279
Average	5.36	5.73	0.053	3.22	5.28	309
Geometric mean	5.04	5.25	0.052	3.10	5.14	308

TABLE III

Animal granulocytes per Kg. body weight

Species	Number of animals	Circulating granulocytes ($\times 10^9$)	Total marrow granulocyte forms ($\times 10^9$)	Segmented granulocytes ($\times 10^9$)	Marrow band ($\times 10^9$)	Metamyelocytes and myelocytes ($\times 10^9$)
Rat	4	0.17 ± 0.13	6.76 ± 1.76	0.45 ± 0.08	3.59 ± 0.91	2.72 ± 0.97
Rabbit	4	0.24 ± 0.06	4.84 ± 1.25	0.29 ± 0.15	2.28 ± 0.74	2.27 ± 0.87
Monkey	4	0.44 ± 0.06	15.40 ± 3.65	1.57 ± 0.80	7.11 ± 2.43	6.73 ± 1.09

in man. Patt (10) also used the known erythrocyte production rate and erythroid/myeloid ratio, but employed Japa's data (11) on frequency of mitosis in erythroid and granulocytic series, and data on duration of mitosis from other sources (12, 13). Suit (14) employed the radioiron method for his determinations. In surveying the results by these various approaches carried out in six different species, the amount of total hematopoietic tissue has been reported to range from 12 to 49 $\times$ 10^9 marrow cells per Kg. in all species.

To consider more specifically the red cell series, nucleated red cells have been reported to range from 3 to 22 $\times$ 10^9 cells per Kg. The present results fit within this range, values in rat, rabbit and man being between 5 and 6 $\times$ 10^9 per Kg. The only fraction of erythroid cells capable of cell division is within their nucleated cell population. Since the number of cells produced may be calculated from the number and life span of circulating erythrocytes, the doubling time of the nucleated red cell fraction may be estimated. In this calculation the red cell turnover is assumed to be 1 per cent per day in man and monkey (15) and 2 per cent per day in rat and rabbit (16). Figures for doubling time of nucleated red cells would be 0.56 day in rats, 0.76 in rabbits, 2.43 in monkeys and 1.7 days in man. It is recognized that these calculations are approximate, and the assumption is made that there is no great discrepancy between total erythropoietic activity by the marrow and circulating red cell turnover, *i.e.*, between total and effective erythropoiesis (17).

Seip was the first to attempt to express quantitatively the relation of marrow reticulocytes to nucleated cells of the marrow (18). He obtained a ratio of 1.7 : 1. In a similar study by us (5), the ratio of marrow reticulocytes to nucleated cells in man was found to be approximately 1 : 1. In the present study of four species the number of noncirculating reticulocytes was approximately equal to that in circulation. The rabbit showed the greatest disparity, having only 29 per cent of reticulocytes in the marrow. This could ex-

TABLE IV

Human granulocytic cells

Subject	Total marrow granulocytic forms (cells/Kg. $\times 10^9$)	Marrow myelocytes (cells/Kg. $\times 10^9$)	Marrow metamyelocytes (cells/Kg. $\times 10^9$)	Marrow band forms (cells/Kg. $\times 10^9$)	Marrow adult granulocytes (cells/Kg. $\times 10^9$)	Circulating mature granulocytes (cells/Kg. $\times 10^9$)
T. S.	17.3	2.8	3.8	6.0	4.7	0.31
K. A.	7.2	1.5	1.4	2.4	1.9	0.28
B. T.	9.5	2.4	1.5	3.5	2.2	0.19
R. B.	8.1	1.8	1.6	3.7	1.0	0.38
C. Ba.	10.3	3.8	1.7	3.1	1.7	0.28
P. M.	5.7	1.8	1.7	1.6	0.6	0.27
B. N.	10.3	2.5	1.8	4.8	1.2	0.37
C. Bo.	17.3	2.9	3.3	5.9	5.2	0.5
R. J.	21.6	4.9	8.1	3.6	4.9	0.35
G. R.	6.8	1.2	2.1	1.7	1.7	0.09
Average	11.4	2.6	2.7	3.6	2.5	0.30
Geometric mean	12.6	2.4	2.3	3.3	1.6	0.28

plain the higher circulating reticulocyte of the rabbit as compared to the rat, despite their similar red cell life span (16).

Measurements of the granulocyte series are essentially a by-product of those employed to estimate red cell precursors, since these may be related through the erythroid/myeloid ratio of the marrow. The number of marrow granulocytic forms in six species has been estimated or determined by others to be variously 4.8 to 27 $\times 10^9$ cells per Kg. In these species the circulating granulocytes range from 0.15 to 0.45 $\times 10^9$ cells per Kg. (4). Our own studies in four species indicate a range of 4.8 to 15.4 $\times 10^9$ marrow cells per Kg. and a range of circulating granulocytes of 0.17 to 0.44 $\times 10^9$ cells per Kg. Thus, in contrast to the distribution of erythroid cells where the bulk of the population is in circulation, granulocytic forms of the marrow far outnumber those in active circulation. The ratio between marrow and circulating granulocytic forms was 40:1 in the rabbit, 18:1 in the rat, 35:1 in the monkey and 38:1 in man.

In this marrow population of granulocyte precursors, it is likely that only myelocytes and their precursors are capable of cell division. In man, this represents 2.6 $\times 10^9$ cells. If about one-half of the nucleated erythrocytes are capable of cell division, *i.e.*, the basophilic and polychromatic normoblasts, then the number of mitotable red cell precursors would also represent 2.6 $\times 10^9$ cells per Kg. The presence of mitosis is almost identical in these two series (11). With similar numbers of mitosis in the two cell series, actual production rates will depend on the duration of the mitotic cycle in each.

The supply of mature and near mature granulocytes in the marrow is of interest in leukocyte physiology. Man appears to have a large reserve of mature granulocytes in the marrow—eight times the amount in circulation. However, if both granulocytes and stab forms are included, there are marrow reserves of about 20 times those in circulation. At present, depletion studies by leukophoresis (19) have not removed enough cells to exhaust a reservoir of this capacity; hence, there is no direct evidence that extra-marrow tissue leukocyte reserves exist. The differences obtained from measurements of leukocyte life span may be dependent on whether or not the methods employed include this marrow reservoir (20). Turnover of circulating leukocytes might be expected to be some 10 to 40 times greater than that of combined marrow and circulating leukocyte populations.

TABLE V

Quantitative estimates of hematopoietic tissue of marrow

Author	Species	Erythroid marrow (cells/Kg. $\times 10^9$)	Granulocytic marrow (cells/Kg. $\times 10^9$)
Kindred (6)	Rat	10.0	14.6
Yoffey (8)	Guinea pig	6.7	9.6
Osgood (9)	Man	8.6	25.7*
Patt (10)	Rat	22.0	27.0
	Dog	6.7	11.5
	Man	3.4	8.3
Suit (14)	Man	4.6	
Present report	Rat	5.6	6.7
	Rabbit	5.8	4.8
	Monkey	9.2	15.4
	Man	5.0	11.4

* Exclusive of adult granulocytes.

SUMMARY

Measurements have been made of the cellularity of the erythroid and granulocytic cells in the marrow and circulating blood in three animal species and in man. These data, together with knowledge of rate of red cell production, permit calculation of turnover time of nucleated red cell mass and of marrow reticulocyte pool. While leukocytic production rates and survival data are not available, the figures presented indicate the presence of an appreciable pool of leukocytes in the marrow.

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