Estimation of the Total Number of Nucleated Cells in the Bone Marrow of Young Adult Albino Rats*†

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(Received February 20, 1960)

In this laboratory we have been studying the quantitative aspects of the lymphocyte production in the thymolymphatic tissues of albino rats (OSOGOE et al., 1957). Along with such studies, detailed studies on the production of blood cells in the bone marrow are also being performed. In this paper we wish to present the results of some determinations on young adult albino rats conducted along this line of investigation.

MATERIALS AND METHODS

Forty-one male WISTAR strain albino rats weighing around 200 g served as the material. They were taken from the laboratory colony maintained on the standard stock diet[1]. In the present study it was of particular importance to employ test animals under comparable nutritional conditions and with almost identical body weights, for it is often observed that the cellular density or the rate of cell multiplication may vary, to a fairly considerable extent, depending upon the nutritional conditions and the monthly (or even weekly) age of the animal.

Immediately after stunning the animal, the bone marrow[2] was taken out from the main shaft of the right femur and weighed carefully. It is transferred into a small vial containg a known volume (1.0–4.0 cm^3) of homologous serum or human plasma and the tissues were finely dispersed in the plasma or serum by the aid of a PASTEUR pipette with a rubber bulb, to yield a homogeneous suspension of cellular material constituting the bone marrow. The volume of the serum or plasma used for diluting the cells was about 100 times the weight (or more correctly, the volume) of the bone marrow[3]. Aliquots of the bone marrow cell suspension so prepared were taken

* Aided by a grand to Professor Bunsuke Osogoe from the ministry of Education (Grant-in-Aid for Fundamental Scientific Research, Cooperative).

† Reproduced from the Acta Histologicum Japonicum, Vol. 19, No 1 (November 1959).

1 The stock diet consisted mainly of unpolished rice, pressed barley and dried small sardine, added with small amounts of cod liver oil, sodium chloride and calcuim carbonate.

2 About 40 mg of the bone marrow could be obtained from one of the femora, but the main shaft of the femur yielded only 30 mg of the bone marrow.

3 As the medium for diluting the bone marrow, commercial human plasma yielded results not inferior to those obtained with homologous serum. We have therefore employed mainly the human plasma in this study.

into 4 pipettes (checked for precision) for white blood cell counting, diluted with the TURK's fluid and used for estimating the number of nucleated cells per cubic millimeter of the suspension in a NEUBAUER hemocytometer. This method was a modification of the procedure of FRUHMAN and GORDON (1953). Since the specific gravity of human bone marrow is 1.028 according to MECHANIK (1926), the number of nucleated cells contained in 1 mm^3 of the fresh bone marrow (cellular density) may be obtained easily from the formula

$$\text{Cellular density} = \frac{\text{total number of nucleated cells in the specimen of bone marrow used}}{\text{weight of the specimen of bone marrow used}} \times 1.028.$$

In a pilot experiment, the total number of nucleated cells per cubic millimeter was compared between the right and left femoral marrows. No significant difference was noted between the two, $(1.78 \pm 0.08) \times 10^6$ and $(1.76 \pm 0.09) \times 10^6$ cells (average of 15 animals) being obtained from the bone marrows of the right and left femora, respectively. Also estimations with other 11 animals gave the following results: $(1.69 \pm 0.11) \times 10^6$ cells for the right femoral, $(1.70 \pm 0.07) \times 10^6$ cells for right tibial, and $(1.60 \pm 0.09) \times 10^6$ cells for the right humeral marrows and also $(1.60 \pm 0.06) \times 10^6$ cells for the left femoral, tibial and humeral marrows combined. Such results indicate that the cellular density of the bone marrows in the right and left long bones constituting the major part of the bone marrow of the whole body may be almost identical. It is for this reason that we estimated, for convenience' sake, the cellular density of the bone marrow of the right femur which served as the basis for estimating the total number of nucleated cells in the total bone marrow of the animal body.

As to the amount of the bone marrow, we used the value of 1.65 g per 100 g of body weight as given by HASHIMOTO (1953) and WATANABE (1955). The literature on the amount of the bone marrow of albino rats is scanty and only contributions by FAIRMAN and CORNER (1953) and KINDRED (1942) may be mentioned here. Values of 2.71–3.23 g were given by the former authors and a value of 1.80 g by the latter author as the weight of the bone marrow per 100 g body weight. Since the fat marrow was included in the estimation of HASHIMOTO and WATANABE, their published figures may require some correction. But the relative amount of the fat marrow being unknown, their material was tentatively assumed to contain the red marrow only.

RESULTS AND DISCUSSION

Results of the determination performed on the right femoral marrows of 41 young adult albino rats are shown in Table 1. The mean and the standard error of

Table 1. Total number of nucleated cells in the whole bone marrow of young adult albino rats (average 41 rats).*

Body weight (g)	Marrow volume (cm^3)	Total number of nucleated cells	
		per mm^3** ($\times 10^6$)	per organ ($\times 10^6$)
200.9 ±1.2	3.22 ±0.02	1.81 ±0.05	5,840 ±161

* Mean±standard error.

** The standard deviation was calculated to be 0.32×10^6 and the coefficient of variation to be 17.6%.

the number of nucleated cells per cubic millimter of fresh bone marrow[4] is $(1.81 \pm 0.05) \times 10^6$, from which the total number of nucleated cells in the bone marrow of the whole body was computed to be $(5{,}840 \pm 161) \times 10^6$, on the assumption that the weight of the total bone marrow was 1.65 per cent of the body weight and the specific gravity of the bone marrow 1.028. The number of nucleated cells of the right femoral and total bone marrow was subject to individual variation, the lowest

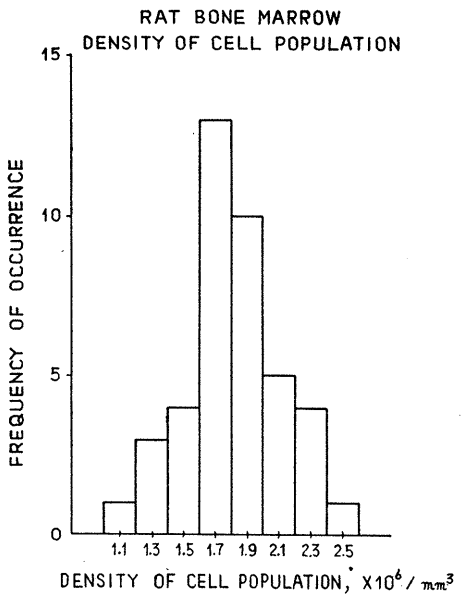

Fig. 1. Histogram of the frequency distribution of the density of cell population, expressed as the number of nucleated cells per $1mm^3$ of fresh tissue, in the femoral bone marrow of young mature albino rats weighing around 200 g.

[4] According to MECHANIK (1926), the specific gravity of the bone marrow is 1.028. The number of nucleated cells per mg of fresh bone marrow was thus calculated to be 1.76×10^6. This value can be regarded as almost equal to the value of 1.81×10^6 cells per cubic millimeter, so that the number of cells per mg tissue was practically the same as that per mm^3.

values being 1.11×10^6 and $3,420 \times 10^6$, respectively, and the highest values 2.49×10^6 and $8,330 \times 10^6$, respectively. The frequency distribution of the cellular density per 1 mm^3 is illustrated in Figure 1, its standard deviation and variation coefficient being 0.32×10^6 and 17.6 per cent, respectively.

Compared with the previously reported values on the bone marrow of albino rats, the above results fall about in the middle between the higher and lower values (Table 2). From the results of FRUHMAN and GORDON (1953, 1955) and of MEINEKE and CRAFT (1956), it can be seen that the cellular density of the bone marrow

Table 2. Number of nucleated cells per cubic millimeter of bone marrow tissue of rats estimated by different authors.

Author	Number of animals	Number of nucleated cells per mm^3 ($\times 10^6$)
Kindred (1942)	8	1.98
Mota (1951)	15	1.460±0.035
Fruhman a. Gordon (1953)	5	2.40 ±0.11
Fruhman a. Gordon (1955)	9	2.52 ±0.06
Meineke a. Crafts (1956)	9	1.75 ±0.17
Meineke a. Crafts (1956)	5	2.00 ±0.16
Table 1, this work	41	1.81 ±0.05

varies according to the strains of the albino rat used. Thus, the cellular density of the bone marrow may be varied to an appreciable extent by the difference in the strain and diet.

Further, the difference in cellular density according to the monthly or weekly age must be considered. According to the recent study of BURKE et al. (1959), the number of nucleated cells in the bone marrow of the albino rats is $(2.060 \pm 0.227) \times 10^6$ per cubic millimeter on the 7th day of postnatal growth, increases rapidly up to a value of $(3.062 \pm 0.368) \times 10^6$ in 6 weeks after birth, but thereafter declines sluggishly to level off at $(2.310 \pm 0.158) \times 10^6$ in 50 weeks. Thus, an appreciable variation in the cellular density is discernible in the bone marrow during the course of postnatal growth. Accordingly, we tried in this study to minimize such a variation by using animals of fixed body weight.

Finally mention should be made about the relationship between the amount of the bone marrow and the density of nucleated cells. It should be pointed out that only the density of nucleated cells decreases without any change in the amount of the marrow in the case of atrophy of the bone marrow. Conseqeuntly, the change in the total number of nucleated cells in the bone marrow is paralleled by the change in the density of the nucleated cells it contains. In contrast, this is not the case in the lymphoid tissues and thymus, because both the tissue weight and the cellular density decrease in the case of atrophy of such tissues.

Summary

Estimation of the number of nucleated cells in the bone marrows of 41 young adult albino rats, weighing around 200 g, gave a value of $(1.81 \pm 0.05) \times 10^6$ nucleated cells per cubic millimeter of the fresh marrow. From this value, the total number of nucleated cells in the total bone marrow of the whole body was calculated to be $(5{,}840 \pm 161) \times 10^6$ on the assumption that the weight of the bone marrow was 1.65 per cent of the body weight and its specific gravity 1.028.

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