DETERMINATION OF THE HALF-CIRCULATION TIME, GRANULOCYTE POOLS, AND MEDULLARY GRANULOCYTE PRODUCTION BY A FLUORESCENCE METHOD

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A method of studying the intravascular phase of the life cycle of granulocytes based on staining the leukocyte with mepacrine in vivo is described. The results obtained by this method are compared with data in the literature.

It is now known that the physiological life cycle of the leukocytes is composed of several phases: a phase of maturation (3-4 days), a phase of accumulation or medullary reserve (4-5 days), the phase of circulation (1-2 days), and the tissue phase [8, 9, 11-13]. The duration of stay of the leukocytes in the tissues has not been precisely established. With regard to the phase of circulation of the granulocytes, some special features must be mentioned. These are due, on the one hand, to the character of the circulation in the central and peripheral blood flow, and on the other hand, to the physiology of the cells themselves. Slowing of the blood flow in the peripheral circulation creates favorable conditions for the granulocytes to perform their physiological functions. The granulocytes adhere to the walls of the capillaries and form a temporary depot. Granulocytes which do not subsequently migrate into the tissues reenter the central circulation, i.e., they recirculate. Accordingly, the phase of circulation of the leukocytes consists of constantly interchanging periods of circulation and deposition [3, 7, 10].

Atheus et al. [4] and Raab et al. [14] developed methods of determining the number of granulocytes in a particular phase of the life cycle. The number of granulocytes constituting the phases of the cycle is described by these workers as the "granulocyte pool" or "reserve," and the following are distinguished: the medullary granulocyte reserve, the capillary pool, the circulating pool, and the total granulocyte pool of the blood. The quantitative method of determining the characteristics of the leukocyte reserves is based on the use of the radioactive indicator di-isopropylfluorophosphate (DFP^{32}). This compound gives sufficiently firm labeling of the cells and it is not reutilized. However, DFP^{32} labels all the blood cells, and to record the specific radioactivity of the granulocytes requires laborious centrifugation and washing off procedures. Even so, these measures do not guarantee complete removal of nonspecific radioactivity. Another disadvantage of the method is the large volume of blood (20 ml) needed for each determination. Because of these disadvantages it was decided to seek different methods for determining the circulation times of the granulocytes and the sizes of the granulocyte pools in vivo.

In the suggested method, the fluorescent dye mepacrine is used to label the granulocytes. The cells are labeled in vivo by White's method [17]. Samples, each consisting of 0.2-0.5 ml blood were taken from a vein on the side opposite to the injection of mepacrine, and 3-5 min after the injection and thereafter, further samples were taken every 30 min for 4-5 h. To each blood sample 20 times its volume of isotonic saponin solution in a concentration of 1 : 5000 was added. After hemolysis of the erythrocytes for 10-15 min, the blood samples were centrifuged (10 min, 1500 rpm), the supernatant was removed by a Pasteur pipet, and a drop of the leukocyte suspension was applied by means of the same pipet to a slide and covered with a cover slip. The granulocytes were counted under the ML-2 luminescence microscope (280×). In each blood sample 200 cells were counted. The number of luminescent granulocytes 3-5 min after injection

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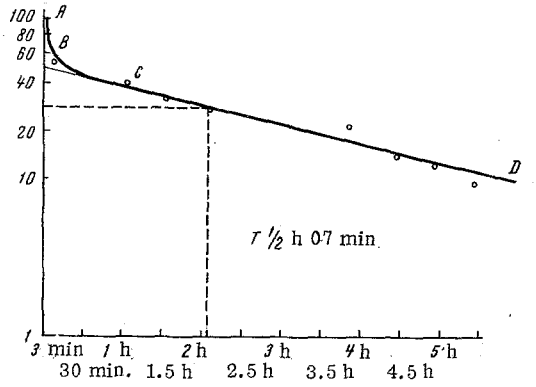

Fig. 1. Change in number of labeled granulocytes in peripheral blood. Abscissa, times of investigation (linear scale); ordinate, number of labeled granulocytes (in %, logarithmic scale). Remainder of legend in text.

of mepacrine was taken as 100%, and at all subsequent times of investigation the number of fluorescent cells was expressed as a percentage of the original. The total number of leukocytes in each blood sample was determined at the same time.

After plotting the experimental curve, the following parameters of the circulation phase of the granulocytes could be determined: the half-elimination time from the circulation ($T_{1/2}$) and the time of uniform mixing of granulocytes of the circulating and marginal pools. The interpretation of the results can best be explained by reference to a concrete example (results obtained with dog No. 7546, Fig. 1).

For convenience of analysis of the curve, it can be divided conventionally into segments. The initial segment AB reflects the rate of mixing of labeled leukocytes between the circulating and capillary pools. The steep slope of the segment indicates the intensity of the processes of deposition of labeled granulocytes. Since deposition and recirculation of the granulocytes in the normal organisms are known to take place constantly, it is natural to suppose that, as the number of deposited labeled leukocytes increases, their recirculation must also increase. This phenomenon reduces the rate of loss of labeled cells from the blood stream and is reflected in a change in slope of the curve in the segment BC. For the purposes of the present investigation, it was important to determine the time of completion of mixing of the circulating labeled granulocytes and of granulocytes deposited in the capillaries, i.e., the time when the number of labeled granulocytes entering the circulation corresponds to the number being deposited in the same time. When this time arrives, the decrease in number of fluorescent granulocytes from the circulation will be due entirely to their migration into the tissues. Some workers [6, 7] have shown that the rate of migration of granulocytes from the blood stream obeys a strict exponential law. The time of complete uniform mixing of the labeled and unlabeled granulocytes thus corresponds to the point on the curve starting from which it becomes exponential in character. To determine this time, the exponential segment CD is extrapolated to intersect the ordinate. The point where the line of extrapolation leaves the exponential line corresponds to the time of uniform distribution of the labeled granulocytes between the circulating and capillary pools. However, in the time from the beginning of labeling of the circulating granulocytes to their mixing in the blood stream, some labeled granulocytes must have migrated into the tissues. There are no grounds for supposing that the rate of their migration in the period of redistribution does not obey the exponential law. Accordingly, the point of intersection of the extrapolation line with the ordinate reflects the true number of labeled granulocytes (in %) in the blood stream at the time of uniform mixing of the circulating granulocytes and granulocytes deposited in the capillaries. The half-circulation time of the granulocytes ($T_{1/2}$) was determined graphically. The point of intersection of the extrapolation line with the ordinate was taken for this purpose as 100%, in which case the time during which 50% of the labeled cells leaves the circulation cycle corresponds to the half-circulation time of the granulocytes.

To calculate the sizes of the granulocyte pools, the principle suggested by E. N. Mosyagina [1] and by Atheus et al. [5] was used. The size of the circulating granulocyte pool (CGP) was found from the aver-

TABLE 1. Magnitudes of Granulocyte Pools, Medullary Production, and Half-Circulation Time of Granulocytes in Healthy Dogs (M±m)

Index	Units of measurement	Personal investigations	Results of Raab et al. [14]
		without correction for number of lymphocytes	
TGPB	No. of cells/kg body weight $\times 10^8$	9.62±0.83	10.2±3.48
CGP	The same	5.20±0.41	5.4±2.07
CapGP	" "	4.42±0.41	4.8±2.34
BMPG	10^8/day	70.15±9.52	30.5±11.1
$T^1/_2$	Hours	2.25±0.33	5.6±0.95

aged value of the absolute number of leukocytes per mm^3 of all the blood samples (M) and the blood volume (V) per kg body weight:

$$CGP = M \times V \times K,$$

where K is the coefficient of correction for the lymphocytes contained in the blood. Since the percentages of granulocytes and lymphocytes in the blood of healthy dogs are relatively stable, in all cases the coefficient of correction was taken as 0.76. The results obtained by Rapaport et al. [15] were used to determine the blood volume.

The total granulocyte pool of the blood (TGPB) was determined by the expression:

$$TGPB = \frac{CGP \times 100}{P},$$

where P is the percentage of labeled leukocytes in the blood at the moment of mixing of the circulating and capillary pools.

The number of granulocytes deposited in the capillaries, or the capillary granulocyte pool (CapGP), was found from the difference between the total and circulating granulocyte pools:

$$CapGP = TGPB - CGP.$$

From the resulting value of the half-elimination period of granulocytes from the blood stream ($T^1/_2$), the rate of circulation of the granulocytes (RCG) was determined [5]:

$$RCG = \frac{0.693}{T^1/_2} \times TGPB.$$

The term "rate of circulation of the granulocytes" reflects the number of cells leaving the circulation per unit time or the number of cells entering the circulation during the same time. Since the number of granulocytes leaving the circulation is made good purely by the entry of granulocytes from the bone marrow, it was decided to introduce a parameter defined as the "medullary production of granulocytes" (BMPG) in 24 h:

$$BMPG = \frac{0.693}{T^1/_2} \times TGPB \times 24.$$

Using the supervital dye mepacrine, the indices described above were determined in 10 healthy mongrel dogs weighing 12-15 kg and aged from 4 to 6 years. By means of the methods of calculation described above, the sizes of the granulocyte pools, the medullary production of granulocytes, and the half-circulation time of the granulocytes were determined. The results of these investigations were compared with those obtained by Raab et al. [14] on 30 healthy dogs using DFP^{32} (Table 1).

Comparison of these results with those obtained by Raab shows considerable agreement as regards the sizes of the pools, but the difference between the values of the half-circulation time and the medullary production of granulocytes can possibly be attributed to the use of different methods of investigation, each with different relative errors. It seems highly likely that the personal results are nearer the true values,

because Patt and Maloney [11] calculated theoretically that the intravascular phase of life of the granulocytes cannot exceed 2-3 h.

The results thus indicate that this method is suitable for experimental research purposes.

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