Morphological Aspects of the Kinetically Inactive Human Neutrophilic Granulocytopoiesis (35016)

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(Introduced by R. L. Bacon)

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From previous investigations we have concluded that in the multiplicative compartment of human granulocytopoiesis dormant cells must be present (1–5). The generation time of 30 hr observed by time lapse cinematography differs from the cell doubling time (t_d) of 91 hr, calculated from the mitotic index (MI) of the same bone marrow, and the observed mitotic time (t_m) of 1 hr in its cultures [$t_d = t_m/\mathrm{MI}$]. The difference between generation time and cell doubling time together with the exclusive occurrence of symmetrical mitoses is the basis of our hypothesis of the presence of granulocyte precursors inactive in regeneration in addition to the frequently dividing and quickly maturing cell-families observed *in vitro*. It is difficult to understand that culture conditions can accelerate biological processes like DNA-reduplication, mitoses, and maturation. However, the mitotic index *in vitro* is not increased to such a degree as to shorten the cell doubling time to the equal duration of the generation time. Another argument for the existence of nondividing or resting cells in human granulocytopoiesis is the arresting of no more than 20% of granuloblastic mitoses with colchicine or xanthopterine within 30 hr (6). If the resting pool is extensive, the mitotic index by colchicine blocking decreases. Therefore Astaldi and Cardinali (7) use colchicine blocking as a parameter to measure the proliferation. Flash labeling would not label all the cells in a population in which all the cells are proliferating and 100% labeling of mitoses is obtained only at specific times after labeling (8–10).

During mitosis the cell volume is halved and during the following interphase both daughter cells grow by two thirds of their mitotic volume (1–3). Therefore, throughout the maturation sequence of the granulocytes, every succeeding generation is smaller than the preceding one. In contrast to the maturing population the dormant population must have a very long generation time (5). In any event, because of the metabolic processes taking place in the dormant cells we expect that these long living precursors become larger than their short living maturing sister cells. Therefore, a bone marrow with a large proportion of inactive granuloblasts should have larger granuloblasts than one with relatively few inactive cells. This hypothesis can be confirmed by measuring the cells in smears of typical specimens of human bone marrow.

Methods. In equally spread Wright's stained smears of normal bone marrow and the bone marrow in several types of disease the cellular and nuclear diameter of at least 200 promyelocytes were measured with an ocular micrometer. The largest and the smallest diameters of each cell and of its nucleus were averaged. A binocular Zeiss microscope was used with 100× Noefluar Ap 1.30. The ocular micrometer was gauged with an object micrometer.

The size and shape of the promyelocytes and myelocytes vary in the Wright's stained smears. Therefore investigators have subdivided them into the following groups: promyelocyte I and II, and myelocyte I and II. We counted all the cells with an azurophilic

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granulation as promyelocytes, independent of the amount of basophilic substance in the cytoplasm. Only neutrophilic precursors, without any azurophilic granulation, were classified as myelocytes or, if their nuclei were more deformed, metamyelocytes. From phase contrast observation of short-term cultures we know that myelocytes and metamyelocytes are different aspects of the same individual cell, but with its movable nucleus turned laterally. In this investigation we were concerned only with the azurophilic granulated promyelocyte in the stained smear.

Since the size of the sample was very large (200 to 400 cells) the complete table showing the correlation between the cellular diameters has been omitted. A normal case is shown in Fig. 1 and will be discussed later.

We used a computer to measure the median nuclear as well as cellular diameters within the total distribution. These mean values correspond to the concentrations in the distributions of the counted and measured cell populations, which had been fitted by regression lines.

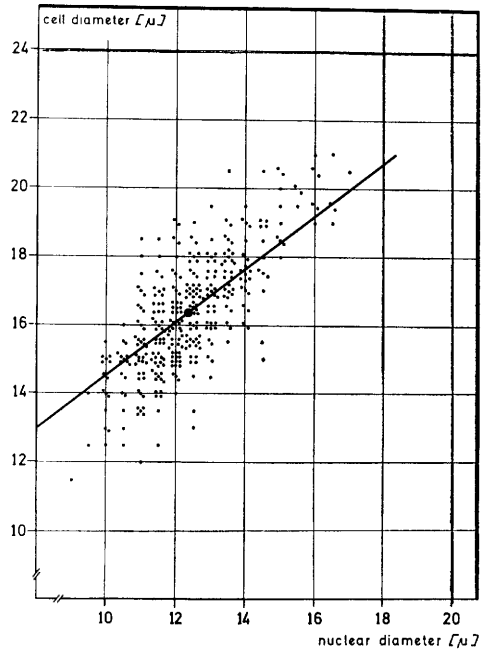

FIG. 1. Distribution of the promyelocytes of the normal granulocytopoiesis: $\bar{x} = 12.35$; $\bar{y} = 16.35$; regression: $y = 0.768\ x + 6.841$.

TABLE I. Measurement of the Diameters of the Promyelocytes and Their Nuclei in Wright's Stained Bone Marrow Smears of Normal Individuals, in Acute or Chronic Stimulation of the Granulocytopoiesis, in Agranulocytosis, or in Polycythemia Vera.

	Normal	Acute stimulation	Chronic stimulation	Promyelocytic phase of an agranulocytosis	Polycythemia vera
No. of cells measured	348	400	406	198	199
Av diam of the cell (μ)	16.35 (11.5–21.0)	15.10 (11.5–21.5)	14.45 (10.0–20.0)	13.45 (12.0–20.5)	14.62 (10.5–19.0)
Deviation of the mean cellular diameter	±0.45	±0.32	±0.23	±0.43	±0.21
Av diam of the nucleus (μ)	12.35 (9.0–17.0)	11.23 (8.5–15.5)	10.95 (8.5–15.0)	10.73 (8.0–14.0)	10.51 (8.0–13.0)
Equation of regression	$y = 0.768x + 6.841$	$y = 1.029x + 3.543$	$y = 1.387x - 0.737$	$y = 1.028x + 2.415$	$y = 2.012x - 6.545$
Deviation of the gradient of the regression line	±3.014	±1.940	±1.544	±3.009	±1.678
Median nuclear cytoplasmic ratio	0.57	0.54	0.58	0.64	0.54

Results (Table I). Bone marrow specimens from two young healthy men were investigated for normal granulocytopoiesis, and two patients with severe leukocytosis and pneumonia were studied for acutely stimulated granulocytopoiesis. One patient with tuberculosis of the pleura and one patient with chronic septic bacterial infection were studied for chronically stimulated granulocytopoiesis. A patient with severe agranulocytosis caused by intoxication from phenothiazine was investigated in the promyelocytic phase 9 days after the myeloblastic phase. The promyelocytes of a patient with polycythemia vera were also investigated since a previous investigation of the mitotic indices of the different granulocyte precursors in this disease revealed that no sleeping population of granuloblasts exists (11).

The promyelocyte diameters decrease in size from normal to acute, to chronic stimulation and to polycythemia vera. The smallest promyelocytes occur in agranulocytosis. The diameters of the nuclei behave correspondingly, but do not differ significantly in chronic stimulation, agranulocytosis, and polycythemia. The nuclear cytoplasmic ratio remains the same in all cases.

To represent the two-dimensional distribution of nuclear and cellular diameters of normal granulocytopoiesis, the nuclear diameters were entered as x-values on the abscissa, the cellular diameters as y-values on the ordinate of Fig. 1. The number of observed pairs is represented by dots grouped in squares of 0.5-μ length. Thus the difference in the intensity of black gradation gives a clue to frequency. The concentrated distribution is marked as a larger, filled circle halving the co-ordinates (12.35/16.35 μ). As a whole, the distribution of the nuclear cytoplasmic ratio is represented by a regression line with the equation $y = 0.768\,x + 6.841$.

The analyses of data for acutely and chronically stimulated granulocytopoiesis, for agranulocytosis, and for polycythemia vera, were done according to the analysis of cases of normal granulocytopoiesis. The distributions of these different cell populations are shown in Fig. 2 as mean values including

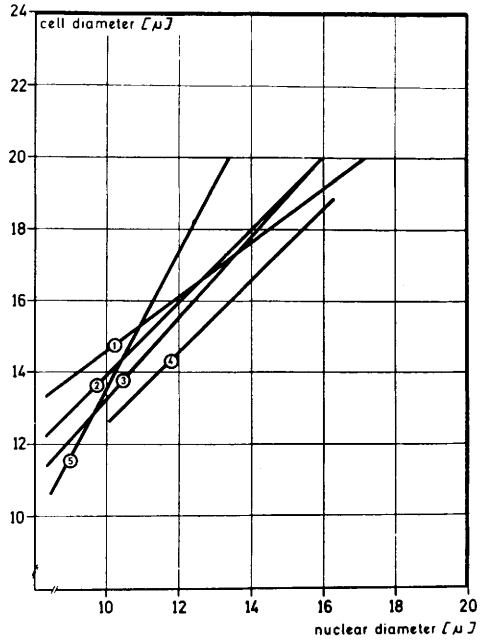

FIG. 2. Regression lines of (1) normal granulocytopoiesis ($y = 0.768x + 6.841$); (2) agranulocytosis ($y = 1.028x + 2.415$); (3) acute stimulated granulocytopoiesis ($y = 1.029x + 3.543$); (4) chronic stimulated granulocytopoiesis ($y = 1.387x - 0.737$); (5) polycythemia vera ($y = 2.012x - 6.545$).

their standard deviation, represented by regression lines and concentrations. It becomes evident that the median nuclear and cellular diameters of normal granulocytopoiesis are different from pathological forms, *i.e*, the concentrations of distribution for stimulated granulocytopoiesis, agranulocytosis, and polycythemia vera are shifted towards smaller sizes of cells and nuclei.

The different angles of the regression lines show that the distributions not only vary in regard to their concentration, but that for the different forms of granulocytopoiesis the nuclear and cellular diameters are correlated differently. The regression slope is the flattest in the cases of normal granulocytopoiesis, the steepest in the case of polycythemia vera. This shows that in the normal subject there are relatively more large cells with a high nuclear cytoplasmic ratio than in Case 5, the polycythemia vera, which has the largest number of cells with a low nuclear cytoplas-

mic ratio. Cases 3, 4, and 5 lie between the extremes of Cases 1 and 2.

When drawn upon "Gauss-paper," the nuclei of the patient with agranulocytosis lay on a straight line, *i.e.*, they are Gauss-distributed. On the other hand, average diameters of the nuclei of the promyelocytes in the normal subjects (650 measurements in 3 cases) form a slight curve with most nuclei of the promelocytes having the smallest diameters. This may be explained with the existence of two populations of nuclear sizes not sharply separated with nuclei in transition from the smaller to the bigger size.

Discussion. The average diameters of promyelocytes measured in stained bone marrow smears verify our hypothesis that in a bone marrow with a large portion of sleeping cells in the granulocytopoietic precursors the size of the promyelocytes and their nuclei should be bigger than in the bone marrow in an acute or still more in a chronic inflammation with severe leukocytosis. The diameters of the promyelocytes and their nuclei were the biggest in healthy men (Table I). Their median cell size is similar to the mean diameters of 15–16 μ found by Alder (12). During infectious diseases and in polycythemia vera with the missing inactive population of granuloblasts (11) the promyelocytes are significantly smaller (Table I), *i.e.*, belong only to the maturing population, while "normal" granulocytopoiesis consists of this maturing population of promyelocytes (and myelocytes), of cells in the process of becoming dormant ones and of cells already dormant. The regression slopes (Fig. 2, no. 1) illustrate that the nuclei in the larger, presumably dormant, cells are relatively large, consequently the nuclear cytoplasmic ratio is higher than in infectious diseases. The not-Gauss-distributed sizes of the promyelocyte nuclei in healthy men correlate with the high nuclear cytoplasmic ratio in the larger cells and point to a growth more in nuclear than in cytoplasm size in the dormant population.

In polycythemia vera the nuclear cytoplasmic ratio has the steepest slope (Fig. 2, no. 5), *i.e.*, in the large cells the nuclei stay relatively small. The regression line of the polycythemia shows that the cell size is not as good a parameter as the nuclear size. From living cell observation with phase contrast (1–3) it is evident that the different spreading of the cells causes their variable size.

In the case of agranulocytosis the promyelocytes and their nuclei are small, *i.e.*, they belong to the maturing population; 9 days before, none could be found and since that time they had had not enough time to form a dormant population. On the other hand, the leukocytosis of 35,000/mm^3 4 days later confirms that the whole population was maturing. The nuclei of the myeloblasts and of the basophilic cells as intermediates to the endothelial cells of 9 days before had a size (10.71 μ) equal to that of the nuclei of the promyelocytes. In these measurements of smeared cells in agranulocytosis the nuclear size does not increase in transition from the myeloblast to the promyelocyte. Only in the dormant population of normal granulocytopoiesis, however, the nuclear more than the overall cell size of the promyelocytes becomes significantly enlarged. In further maturing steps, the decrease of the volume from the promyelocyte to the myelocyte and metamyelocyte is confirmed by phase contrast observation (1–3).

Summary. The enlargement of the promyelocytes and their nuclei in unstimulated granulocytopoiesis of healthy men are compared to patients with infectious diseases, agranulocytosis, or polycythemia vera. The result is statistically evaluated and supports the hypothesis of the existence of a dormant population in normal granulocytopoiesis.

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