EDITORIAL REVIEW

Kinetics of the Formed Elements of Human Blood

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The cells of the blood and marrow consist of different species whose functions of oxygen transport, hemostasis, phagocytosis, and immune defense are independent of one another. This paper will deal with erythrocytes, granulocytes, and platelets but excludes lymphocytes. While some of the kinetic characteristics of each cell type are likewise unique and particularly adapted to specific needs, all of these cells are irrevocably bound together through a common progenitor that proliferates and differentiates in a common space. Hence all of the marrow elements are affected by stem cell injury or by alterations in the marrow environment.

Although quantitation of circulating elements provides useful information concerning the adequacy of each cell system, it does not always reveal production abnormalities. Regulatory mechanisms exist whereby blood levels may be maintained through increased stem cell participation despite inefficiency in production or increased cell destruction.

In this review the turnover of red cells, granulocytes, and platelets is examined under basal and stimulated conditions. Each of these cell-generating systems has a developmental phase within the marrow and a circulating life span. Attention is given to the number of cells and time spent in each developmental stage and the techniques whereby these may be measured. The application of such physiologic knowledge is helpful in the recognition and management of hematologic disorders.

CIRCULATING CELL KINETICS

Measurements of intravascular elements are carried out with isotopic labels that permit the determination of total cell number, distribution, and turnover. Radionuclides currently used for red cell labeling include $^{51}$Cr (hexavalent), $^{32}$P-diisopropylfluorophosphate (DF$^{32}$P), and $^{14}$C-cyanate. $^{1-3}$ Properly washed $^{51}$Cr-tagged and $^{14}$C-cyanate-tagged red cells show less than 5% immediate elution during the first 24 hr, and therefore permit an accurate initial measurement of red cell numbers and distribution within different parts of the vascular system. The subsequent rate of $^{51}$Cr elution of about 1%/day makes it more difficult to detect a minor increase in red cell breakdown with this tag as compared to DF$^{32}$P and $^{14}$C-cyanate.$^{1-3}$ The finite red cell life span is about 120 days,
equivalent to a turnover rate of about 0.83%/day (Table 1 and Fig. 1). Variations in this turnover rate among normal subjects appear relatively small, with an estimated 95% of cells dying within 14 days of the mean life span.3,4

Currently the distribution and turnover of circulating platelets are derived from the use of platelets labeled in vitro with hexavalent 51Cr.5,6 51Cr disappearance in normal subjects is nearly linear (Fig. 1), with a finite platelet life span of about 9.5 ± 0.6 days when analyzed by least squares fitting to a non-linear gamma function.7,8 Neither 14C-serotonin nor DF32P are recommended for platelet survival measurements since the disappearance curves of both appear exponential due to initial elution and later reutilization.9,11 Daily platelet turnover is then 10% of the blood platelet mass (Table 1).

The kinetics of circulating neutrophils present more of a problem because of less adequate methods, the very short transit time through the circulating blood, and the highly unstable distribution between circulating cells and cells temporarily lodged in the microvasculature. A satisfactory label for clinical studies of circulating neutrophils has not yet been developed.12,13 3H-thymidine is the most reliable label, showing an immediate recovery of about 58% and an exponential disappearance from the circulation in man with a $t_\frac{1}{2}$ of 7.6 hr.14 Un-
fortunately, ³H-thymidine is not practical for clinical purposes because the labeling must be carried out in vivo and cells from one subject must be transfused to another. DF³²P, which has been used most extensively, shows an immediate recovery of about 46% and a t½ of 5–7 hr with a turnover of twice that observed with ³H-thymidine.¹⁴¹⁵ Simultaneous tagging with both isotopes indicates that the DF³²P tag elutes from the cells.¹⁴¹⁶ Since the degree of DF³²P elution appears to increase in the presence of inflammation, an empiric correction for elution is not dependable. Although ⁵¹Cr has been employed as a white cell tag, it shows a lower and more erratic recovery and longer and less consistent t½.¹⁷

In comparing the intravascular distribution of these three cell species, certain differences are apparent. All injected red cells are in active circulation. While there are differences in the normal blood hematocrit of various organs, a factor of 0.9 usually corrects the venous hematocrit to the lower mean body hematocrit.¹⁴ Only an enlarged spleen has been shown to affect this relationship significantly and even then the ratio 6 min after injection may be normal.¹⁸ With splenomegaly, as long as an hour may be required for complete mixing and as much as 50% of the red cell mass may be sequestered in that organ.¹⁹ Approximately one-third of the blood platelets are reversibly sequestered in the normal spleen.²⁰²¹ In the splenectomized individual the initial loss of labeled platelets is 10% or less, while in patients with marked splenomegaly 80%–90% may be sequestered from active circulation.²²²³ Indeed, the immediate splenic pooling appears to be a sensitive indicator of enlargement of the “red pulp” of the spleen. Platelets in this splenic pool are in dynamic equilibrium with the peripheral circulation and are therefore available for hemostasis elsewhere in the body. Studies with tagged granulocytes also show an immediate loss from the circulation of nearly 50%. This loss is believed to represent margination of cells within the microvasculature.¹⁵ Although not yet established, the spleen may contribute to this, since with splenomegaly there is an increase in the proportion of “sequestered” neutrophils.

The turnover of these three cell species in the circulating blood is calculated on the basis of the total number of blood cells, whether circulating or sequestered, and their disappearance rate. Such cell turnover rates are considered to represent “effective” production, implying that the cell has survived maturation and has entered the blood as a functional element. Normal values for “effective” turnover are shown in Table I.

MARROW CELLULARITY AND KINETICS

A more challenging problem has been the determination of the absolute number and turnover of precursor cells in the marrow. Attempts have been made in animals to estimate marrow cellularity by the principle of isotope dilution. The active marrow is labeled with an injectable isotope, such as ⁵⁹Fe, that is taken up by the immature erythroid cells. Cellularity is calculated by relating the number of cells and radioactivity of a marrow aliquot to the total marrow ⁵⁹Fe activity.²²²⁵ The accurate enumeration of marrow cells and the relation of an aliquot to the total marrow have presented methodologic problems. When a suspension of marrow cells is employed for counting, cells are broken in prep-
eration; in the study of Donohue et al.\textsuperscript{23} as much as 40\% of the radioiron tag was found in the supernatant as free hemoglobin, indicating that a large portion of immature red cells had been disrupted. This problem has been overcome by the use of marrow sections in which virtually all cells are intact and identifiable.\textsuperscript{14}

When cell counts on marrow section are corrected for cell size and section thickness, and when this procedure is combined with the marrow \textsuperscript{59}Fe isotope dilution technique, erythroid and neutrophil cellularity may be directly determined in the dog.\textsuperscript{25} A clinically applicable measurement of marrow cellularity has been made in normal man by relating the turnover of circulating red cells to the turnover of marrow erythroid cells. Thus, an equation has been derived wherein the number of circulating red cells in normal man (known) divided by their transit time (known) equals marrow reticulocytes (unknown) divided by their maturation time (which has been determined by Labardini et al.\textsuperscript{26}). Marrow reticulocytes may then be converted to normoblasts by the ratio of 1.5:1 between the two cell species in the marrow. In this fashion the normoblast population of man is calculated to be $5.3 \times 10^9$ cells/kg.\textsuperscript{14}

A similar approach to the determination of marrow neutrophil number has been carried out.\textsuperscript{14} The turnover of circulating neutrophils as determined by \textsuperscript{3}H-thymidine in normal subjects is $0.85 \times 10^9$ cells/kg/day. The transit time through the postmitotic neutrophil pool of the marrow has been measured by \textsuperscript{3}H-thymidine to be 6.6 days. With the assumption that the production of neutrophils by the marrow is equivalent to their turnover in the circulation, the number of postmitotic neutrophils in the marrow has been determined by dividing the daily turnover of circulating neutrophils by the \textsuperscript{3}H-thymidine marrow transit time. This calculation gives a postmitotic neutrophil pool of $5.7 \times 10^9$ cells/kg/day. These two measurements can then be interrelated through the ratio between postmitotic neutrophils and nucleated red cells. With a mean ratio as determined in marrow sections of 1:1.5, the number of nucleated red cells derived from the granulocyte calculations is $5.2 \times 10^9$. This agreement between the independent red blood cell and neutrophil population calculations based on the turnover of circulating red cells implies that there is a similar amount of ineffective production in each series. Since ineffective erythropoiesis has been estimated at 10\%-15\%, ineffective production may occur in a similar percentage in the neutrophil series.

While marrow megakaryocyte number and cytoplasmic mass have also been determined by marrow section techniques,\textsuperscript{8,24} there has been no practical method of determining marrow megakaryocyte turnover. Indirect calculations suggest that the megakaryocyte transit time in normal subjects is about 7 days (Table 1).

In various clinical disorders, the total number of red cell precursors can be determined indirectly by ferrokinetic measurements. The plasma iron turnover (PIT) correlates well with the number of erythroid precursors at increased levels of erythropoiesis.\textsuperscript{27} A correction, however, should be applied for the plasma iron concentration, since the erythron iron turnover measurement (EIT) has been shown to be affected by changes in plasma iron independent of actual changes in red cell production.\textsuperscript{28,29} Analysis of plasma iron kinetics indicates that this correction for the EIT may be made by the following formula:
EIT (mg/kg/day) = PIT - (plasma iron × plasmatocrit × 0.0035).

Employing this formula the erythron iron turnover may in turn be related to the number of erythroid cells according to the formula

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\text{erythroid cells (per kg)} = \frac{5.4 \times 10^9 \times \text{EIT}}{0.56},
\]

where \(5.4 \times 10^9\) is the mean number of nucleated red cells in the normal individual and \(0.56\) mg of iron/100 ml blood is the basal EIT. There are certain limitations to this approach. For example, it is assumed that the iron uptake/cell is always the same, whereas this is not true in iron deficiency. Empiric data, however, speak for the practical utility of the method.\(^{27,28,30}\)

The number of megakaryocytes can be derived from the number of erythroid cells and the megakaryocyte:erythroid ratio. In normal man this amounts to \(15 \times 10^6\) megakaryocytes/kg ± 3 \(\times 10^6\).\(^9\) The total megakaryocyte cytoplasmic mass available for platelet production has been calculated to be \(1.8 \times 10^{11}\) cu \(\mu\)m/kg, and the cytoplasmic mass released as platelets is \(2.5 \times 10^{10}\) cu \(\mu\)m/kg/day. It therefore follows that the marrow contains sufficient cytoplasmic mass to supply about a 7-day requirement for circulating platelets.

Given the number of erythroid cells, both neutrophil and megakaryocyte number can be determined by relating the erythroid cells to those other cell lines through marrow ratios. This calculation has been done employing marrow sections, and normal data are shown in Table 1. From a clinical standpoint the most useful form of expression is a production ratio which relates the production rate in the patient to that of the normal individual under basal conditions. Production ratios are determined by the number of the cell species in the marrow as compared to the basal norm or, in the case of red cells, by erythron iron turnover comparisons. Granulocyte production rates must be related to the mitotic pool only, since the number of postmitotic granulocytes will vary depending on the duration of their storage in the marrow (see below).

**MARROW STIMULATION**

Stimulation of cellular production in the subject with normal marrow function is accomplished through the action of “poietin” hormones that recruit stem cells leading to the proliferation of marrow precursors and a parallel increase in the number of cells entering the circulation. Red cell mitotic cycles are somewhat shortened, but the number of mitoses in the maturation sequence is unaffected.\(^{31}\) Granulocytosis appears to behave similarly, although information is limited. On the other hand, increased thrombocytosis is achieved by both an increase in the number of megakaryocytes found and an increase in megakaryocyte size through additional endomitoses.\(^{21}\) Cytoplasmic labeling suggests some shortening in maturation time when thrombocytosis is stimulated.\(^{32}\) The inverse relationship between megakaryocyte ploidy and the num-

\(^{9}\)Initial megakaryocyte:erythroid ratios reported by Harker\(^{24}\) require revision when improved non-shrinkage techniques of fixation and staining are applied. Using acrolein fixation, methacrylate embedded sections cut at 2 \(\mu\) thickness from 13 normal subjects, megakaryocyte diameter was 30.2 \(\mu\). After size correction the mega:erythroid ratio was calculated to be 1:350.
ber of circulating platelets is useful in distinguishing physiologic from pathologic thrombocytopoiesis. Therefore, in the thrombocytosis of iron deficiency and inflammation, megakaryocytes increase in number but are of smaller size, while megakaryocytopoiesis in thrombocythemia is characterized by an increase in both number and size.

An additional feature of stimulated hematopoiesis is the change in cell delivery from marrow to blood. Normally there is a 3-day store of reticulocytes in the marrow; with increased erythropoietin stimulation, a progressively larger portion of this pool appears in the circulating blood. "Shift" reticulocytes which are basophilic and macrocytic may be recognized in the Wright-stained blood film and serve as a simple indicator of erythropoietin stimulation, provided abnormalities of marrow stroma are excluded. Changes in circulating platelets reflecting marrow stimulation and premature platelet release are limited to increased spreading on smear, due to increased surface adhesiveness rather than to increased platelet volume. Neutrophils have the greatest potential for change due to shift, since the marrow contains a reserve of some 15 times the granulocytes present in the circulation. These "storage cells" may be rapidly discharged through the sinusoidal walls, and the presence of an increase in band forms in the blood has been assumed to reflect the shift. While this "shift to the left" in the circulating neutrophils may indicate granulocyte stimulation in acute infection, it is not a prominent feature in chronic neutropenia.

The maximum production capacity of the normal marrow for its various components is probably about five times basal, suggesting a reserve of stem cells of that magnitude. Given sufficient iron, the nucleated red cell population will increase four to five times within 1 wk, and further expansion to between six and eight times normal occurs with the extension of the marrow into the centrifugal skeleton. These red cell production estimates by ferrokinetic techniques have been validated in phlebotomized subjects, in whom the erythron iron turnover has been correlated with red cell balance. A good correlation has also been shown between erythron iron turnover and the number of red cells estimated from the marrow megakaryocytes:erythroid precursors ratio and platelet turnover in patients with effective thrombocytopoiesis. The megakaryocyte population has been shown to increase four- to eightfold with thrombocytopenia. While animal studies suggest a similar potential for neutrophil production, no quantitative data are yet available for man.

For the clinician the erythroid:neutrophil ratio of the marrow aspirate has been useful in the evaluation of red cell proliferation, provided neutrophil production is normal. Thus, a ratio of 1:1 indicates a moderate increase in erythropoiesis and a ratio of 3:1, marked red cell hyperplasia. The ratio has not been so helpful in evaluating granulocytopoiesis. One reason for this inability is that only about one-third of neutrophil forms in the marrow are involved in cellular multiplication, while the major portion consists of more mature cells held in a maturation storage pool. In neutropenic states it is quite possible that a significant increase in mitosing neutrophils may be masked by a shift of the storage pool into the circulation, and the marrow will be characterized as having an increased immaturity of marrow granulocytes.
IMPLICATIONS OF KINETIC MEASUREMENTS

The major use of kinetic measurements has been to separate production abnormalities from states of increased destruction. This application has been most directly accomplished by measuring survival of tagged cells in the circulation. Another important use has been the detection of ineffective production by comparing measurements of marrow and blood kinetics. Here, studies of the marrow have indicated adequate proliferation but provided further evidence that many of the cells produced in the marrow are so defective as to be destroyed before entering the circulation. An important use of marrow kinetic measurements has been to quantitate the production capacity of the marrow, and it is probable that this category will be the one to receive the greatest attention in the future.

It would be useful if the marrow capacity to proliferate, presumably related to the size of the active stem cell pool, could be evaluated. Assuming proper marrow stimulation, kinetic measurements should provide this quantitation much as the functional potential of endocrine glands may be evaluated after stimulation by trophic hormones. In experimental animals it has been possible through phlebotomy, plateletpheresis, and leukapheresis to stimulate independently each of the three cell series. In man advantage has been taken of disease states characterized by increased red cell and platelet destruction to establish maximum production rates, but purified trophic hormones suitable for intravenous injection are needed to provide stimuli if individuals whose marrows are not already challenged are to be evaluated. Even then the evaluation of red cell production potential will present a problem, since an augmented iron supply is required to support maximal erythropoiesis.38,39

Minimal requirements for life are better considered in terms of maximal normal production capacity. The critical red cell concentration in the blood is about one-fourth of basal, for neutrophils and platelets it is about one-tenth of basal. This amount, when matched against a normal production capacity of about five times basal, would suggest that minimal stem cell requirements for red cells may be about 4% of the normal red cell mass, and for neutrophils and platelets about 2%. From such considerations it seems likely that 5% of normal marrow functional capacity is quite compatible with survival, and at that level a change of as little as 1% might represent the difference between life and death.

Limited quantitative data are available concerning the critical level of marrow function in aplastic anemia. The success of marrow transplantation in severely affected patients makes it essential to distinguish those patients in whom the degree of marrow damage will be lethal. Alexanian and Alfrey have suggested that those patients who respond to androgens may be detected by the degree of erythropoiesis demonstrable by ferrokinetics.40 Further efforts would seem to be indicated to define the amount of functional marrow, particularly the granulocyte fraction, since this cannot be easily provided by transfusion. Circulating cell levels are not reliable in predicting marrow capacity when production is low, for the number of neutrophils in the circulation is subject to severe fluctuations according to altered distribution in the circulation and altered utilization. Marrow cells, on the other hand, represent a more stable
index and should correspond to the number of functional stem cells. The application of quantitative techniques of marrow functional capacity may provide better prognostic criteria for such problems.

REFERENCES

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