AN ANALYSIS OF GRANULOCYTE KINETICS IN BLOOD AND BONE MARROW*

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Technics for labeling granulocytes in human blood and bone marrow with tritiated thymidine (H^3-Th), P^{32}-labeled sodium phosphate (radiophosphate) and diisopropylfluorophosphate (DFP^{32}) have now been developed to the point where further analysis of the experimental results in terms of a model of granulocyte dynamics seems justified. The purpose of this report is to present a mathematical model of granulocyte kinetics which will describe the time course of radioactivity in blood granulocytes obtained in human subjects following the administration of these labels.

Description of the Model

The model is shown diagrammatically in FIGURE 1. In this model, cell kinetics are considered to be governed by certain laws of probability and thus it is a stochastic model. Cells in the bone marrow are divided into two populations, labeled A and B. Population A consists of cells which will divide at least once before entering the blood, while Population B consists of metamyelocytes and more mature granulocytes which are not capable of cell division. It is assumed that cells move through Population A in a sequential fashion to a point M where cell division occurs. When a cell in Population A divides, there is a given probability that one or both of the daughter cells may enter Population B or may reenter Population A. This latter possibility is illustrated by the recirculation arrow. Population A is considered to contain myelocytes only, because earlier forms than myelocytes are not labeled significantly (if at all) by DFP^{32}.[1] In the steady state the probability that the daughter cells will enter Population B is 0.5. The time for a cell to complete one cycle through Population A is referred to as Time A. This is the turnover time for this population. The time for a cell entering Population B to complete the maturation process and enter the blood is a random variable normally distributed around an average value, Time B. The standard deviation, σ, around this mean transit time is the other variable which describes this population.

In contrast to the sequential movement of cells in Populations A and B, the probability of a cell disappearing from the blood is not a function of the time elapsed since that cell entered the blood from the bone marrow. The studies of several groups of investigators[2,3,4] support this postulate. Thus, the rate of disappearance of cells from the blood (R) and the total number of granulocytes in blood (V) define this population ($\frac{R}{V}=\frac{.693}{T\,1/2}$, the fractional turnover rate of cells in the blood where T ½ is the half-disappearance time of granulocytes from the circulation). The previously demonstrated equilibrium between cells in the circulating blood and in marginal sites[5] is depicted by the two subcompart-

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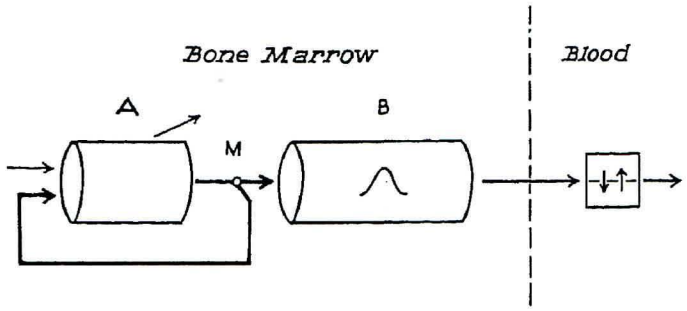

FIGURE 1. A "semiclosed" model of granulocyte kinetics. This model describes the time course of radioactivity in blood granulocytes following the administration of DFP^{32}, H^3-Th, or radiophosphate. For details, see text.

ments and the arrows in the blood population (FIGURE 1). The parameters in this model of granulocyte kinetics are Time A, Time B, σ, R, and V, and are assumed to remain constant during the course of each experiment. As with any labeling experiment, it is assumed that labeling of the cells does not disturb the steady state.

It should be emphasized that in this model it is postulated that the re-entry of myelocytes is the prime source of new cells for the myelocyte population. It seems obvious that some cells do enter Population A from populations of cells which are less mature (promyelocytes and myeloblasts). This source of cells is indicated by the smaller arrow entering Population A in FIGURE 1. However, in this analysis it was found necessary to minimize such a source of cells in order to describe the experimental observations. This type of population is referred to as "semiclosed" in that there is relatively insignificant inflow from other populations and the population is essentially self-regenerating. Population B is an "open" population in that its inflow comes from an earlier population; thus an "open" population is not self-regenerating. The definition of a population as "open" or "semiclosed" is not concerned with the presence or site of cell division. A loss of cells from the bone marrow due to cell death is represented by the arrow at the top leaving Population A and in this model is considered to be minimal. The effect of significant cell death will be discussed later.

Mathematical Representation of the Model

If CA represents the concentration of label in granulocytes in cell Population A as they complete division, then the time course of label concentration at this site is described by Equation 1. It is assumed that all cells take the same time to complete one cycle through A.

$$CA_{(t)} = CA_{(t=0)}\, 2^{-(N+1)} \quad , \tag{1}$$

where t is time after injection of label, and N is defined by

$$N = t/\text{Time } A \quad . \tag{2}$$

N is rounded to the last integer value (fractional term is discarded from this division operation) and represents the number of *complete* cycles through Population A that have occurred by t time units since injection of the label.

The concentration (CB) of label in cells leaving Population B is given by

$$(CB_{(t)} = CB_{(t=0)}\ (1 - {}_0\int^t H_{(t)}\ dt) + {}_0\int^t H_{(\lambda)}\ CA_{(t-\lambda)}\ d\lambda\ , \qquad (3)$$

where $H_{(\lambda)}$ is the distribution of times that cells spend in Population B. This distribution function is convolved with CA (the time course of granulocyte specific activity entering Population B), using $d\lambda$ as the interval of time between each iteration of the convolution. The use of the convolution principle here requires that $H_{(\lambda)}$ is stationary during the course of the measurement and that the system is linear; that is, the time course of radioactivity in blood has the same form regardless of the dose of isotope administered. That the latter condition is true has been demonstrated experimentally for several concentrations of DFP ^{32} [7] and radiophosphate.[8]

The distribution function $H_{(\lambda)}$ is assumed to take the normal form as shown in

$$H_{(\lambda)} = \frac{1}{\sigma\sqrt{2\pi}}\, e^{-0.5\left(\frac{\lambda - \text{Time } B}{\sigma}\right)^2}, \qquad (4)$$

where Time B is the average time a cell remains in Population B, and σ is the standard deviation around this average. $H_{(\lambda)}$ is the probability of a cell leaving Population B at λ time units after it entered this population.

The concentration (C) of label in granulocytes in the blood, is given by

$$\frac{dC}{dt} = \frac{R}{V}\,(CB_{(t)} - C_{(t)}), \qquad (5)$$

where V is the total number of granulocytes in the blood population and R is the rate at which cells enter or leave the blood. Thus $\frac{R}{V}$ is the fractional turnover rate of the blood granulocyte pool.

Solution of the Model

A program has been written for a general purpose digital computer which permits solution of these equations and thus predicts the blood granulocyte radioactivity curve that would evolve for a given set of model parameters (Time A, Time B, σ, R, and V) and a given set of initial labeling conditions, *i.e.*, the level of labeling in A, B, and C at $t = 0$. After the first solution with the appropriate set of initial labeling conditions and an arbitrary set of parameters, the program automatically compares the solution with the particular experimental data being analyzed and adjusts the parameters to better approximate the experimental data on the next solution. By such an iterative approach, the program converges uniquely on the optimal parameters for each set of experimental observations.

Experimental Observations

Three sets of experimental data are available for examination and comparison with the output from the above model and variations on this model to be described below.

DFP^{32}. Blood granulocyte radioactivity curves were obtained from 18 normal male subjects studied after their blood and bone marrow granulocytes were labeled by the intravenous injection of DFP^{32}.[6] A curve representing the average specific activity for 18 normal subjects at intervals of one hour to two days is shown in FIGURE 2. The initial high level of blood granulocyte radioactivity falls off rapidly during the first one to two days (Phase I) and then remains relatively constant (Phase II) for a period of from eight to 14 days. The radioactivity curve

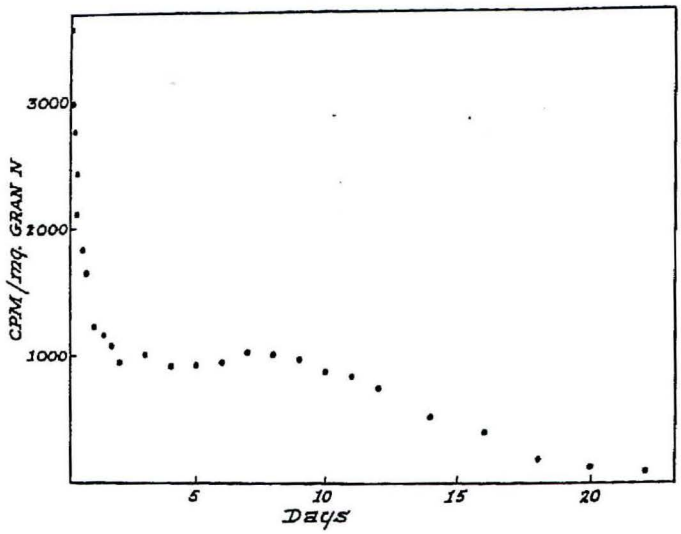

FIGURE 2. The configuration of the blood granulocyte radioactivity curve after the intravenous injection of DFP^{32}. Mean data curve for 18 subjects.

then falls relatively slowly to background levels over the next ten to 14 days (Phase III). The total evolution of the curve requires 20 to 24 days.

In autoradiographs prepared from one subject given tritiated DFP, myelocytes (Population *A*) contained twice as much label as postmitotic neutrophils (Population *B*).[1] Myeloblasts and promyelocytes were not labeled significantly. It was, therefore, assumed in the 18 subjects evaluated here that Population *A* was initially labeled twice as heavily as Population *B*.

Tritiated thymidine. Bond and colleagues[9] have studied a single human subject after the administration of tritiated thymidine. The blood granulocyte data as published (per cent cells labeled and average grains per cell) were not suitable for use in our stochastic model. However, the product of these two values for any given cell type converts the data into average radioactivity per cell in that population, and in this form the data are suitable for the present analysis. The blood granulocyte radioactivity curve calculated in this manner for their patient is shown in FIGURE 3.

The initial labeling conditions in this experiment have been described in detail.[10] It was found that neither PMN neutrophils in the blood nor PMN neutrophils and metamyelocytes in the bone marrow were labeled initially, whereas 70 per cent of myelocytes were labeled as were 35 to 93 per cent of the more immature forms in the granulocyte series.

P^{32}-labeled phosphate. The third set of available data consists of blood granulocyte radioactivity curves resulting from the administration of radioactive sodium phosphate to three subjects expressed as CPM/μg. DNA-phosphate.*[11] Initial labeling conditions produced with this label have not been studied autoradiographically. However, since the configuration of these curves is similar to that obtained with tritiated thymidine and since both agents are incorporated into

* We are indebted to Dr. C. G. Craddock for supplying us with the data on these subjects.

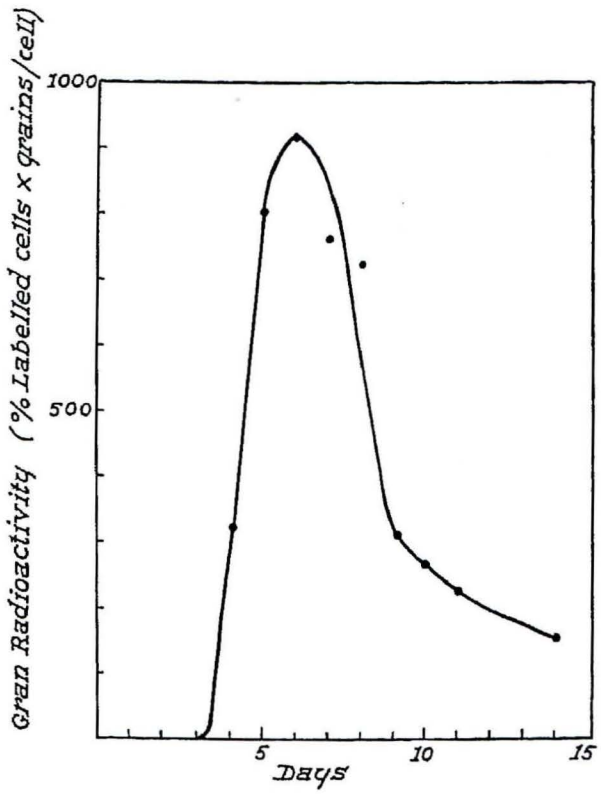

FIGURE 3. The blood granulocyte radioactivity curve after the intravenous injection of H^3-Th as calculated from Bond *et al.*[9]

DNA, it was assumed that the initial labeling conditions were the same as that produced with thymidine; namely, no label in marrow metamyelocytes and PMN neutrophils or in blood neutrophils initially but 70 per cent of cells in the myelocyte population labeled.

Comparison of Model Output and Experimental Observations

To obtain a solution of the mathematical model for simulation of the DFP^{32} curves, the initial labeling conditions produced by the intravenous injection of DFP^{32} were inserted in the model equations. As stated earlier, initial label concentration in A was assumed to be twice that in B, and initial concentration in B was automatically set equal to the average observed concentration in blood granulocytes between the second and seventh days in each experiment (Phase II). The initial value for C was equated to the measured concentration in blood granulocytes at one hour after injection of the label. Initial C was about three times initial B in these experiments. The model parameters required to match each of the 18 experimental DFP^{32} blood-granulocyte radioactivity curves were then determined by means of the computer program described above. A comparison of the experimental curve and the optimal model solution for two such curves

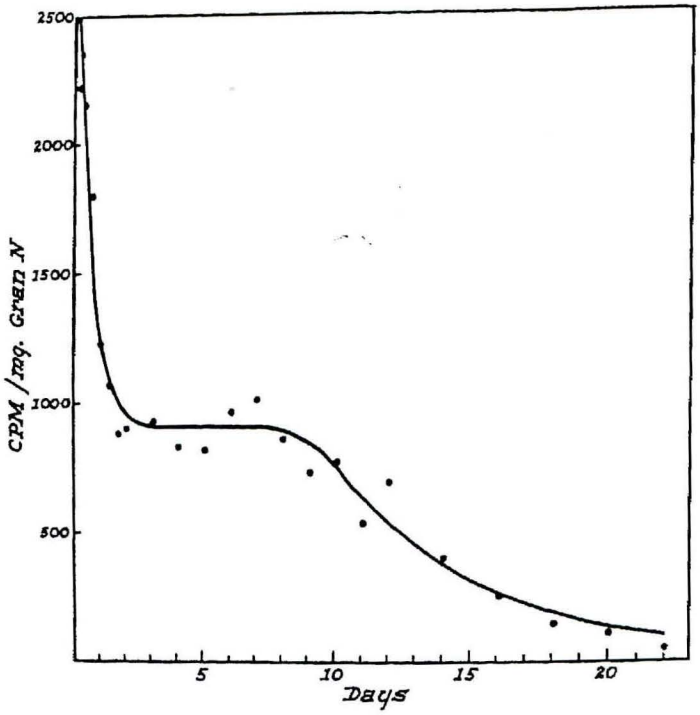

FIGURE 4. A comparison of the time course of the blood granulocyte radioactivity in DFP^{32} subject II-165 (*solid circles*) and the best solution predicted by the "semiclosed" model (*solid line*). The parameters used to achieve this match were: Time A of 2.97 days; Time B of 8.03 days, σ of 1.47 days; and $\frac{R}{V}$ of 1.88.

are shown in FIGURES 4 and 5. The optimal parameters required to match the blood granulocyte radioactivity curve of each of these subjects are given in the respective legends.

The parameter values were quite critical in achieving a good match between the model and data curves. That is, relatively small changes in Time A, Time B, σ, and $\frac{R}{V}$ resulted in obvious differences between the curve predicted by the model and the experimental curve. By achieving a good match between model and experimental curves and then altering a single model variable at a time, the effect of each variable was examined. Thus, small changes in $\frac{R}{V}$ ratio resulted in a poor match between model and data curves during Phase I. However, even large changes in $\frac{R}{V}$ had almost no effect on the remainder of the curve. The sum of Time A and Time B is determined by the duration of Phase II. Altering this sum by as little as one day produced an obvious discrepancy between model and experimental curves. σ is determined by the sharpness of the break between Phases II and III and to a lesser extent is affected by the rate of fall-off during Phase III. Thus, a large value for σ will result if the transition from Phase II to Phase III is very gradual. The probable error in the estimate of σ is less than 0.2

days. Time A, in addition to contributing to the duration of Phase II, was the critical parameter in determining the rate of fall-off in Phase III. Variations in Time A of as little as 0.3 days changed the model solution from the optimal solution by an amount that was readily detectable even by visual inspection.

The optimal parameters for each of the 18 subjects are given in TABLE 1. It is evident that there is considerable variation even among apparently normal subjects.

When this model was programmed with each set of parameters from TABLE 1 and with the initial labeling conditions produced by DNA labels, (i.e., label in 70 per cent of A but none in B and C, see FIGURE 6) the model output then gives the blood curve which each of the 18 subjects studied with DFP would be expected to have if he were given a DNA label instead of DFP^{32}. A comparison of the actual tritiated thymidine blood granulocyte curve of Bond and co-workers,[9] the P^{32}-labeled phosphate curve of Perry and co-workers,[8] and the two extremes (Cases III-14 and II-179) of the predicted curves for the 18 DFP subjects are shown in FIGURE 7. It is apparent that the configuration of these curves is similar and that the curves differ mainly in the time of onset of the initial upswing, a factor influenced almost entirely by Time B.

Other models. Other investigators have presented models of granulocyte proliferation in the bone marrow to explain the results of DNA labeling experiments.[8,9] In these models it was postulated that a population of primitive stem cells exists from which the cell line originates. This stem cell population is completely "closed" in that there is no cell entry into it other than re-entry (FIGURE

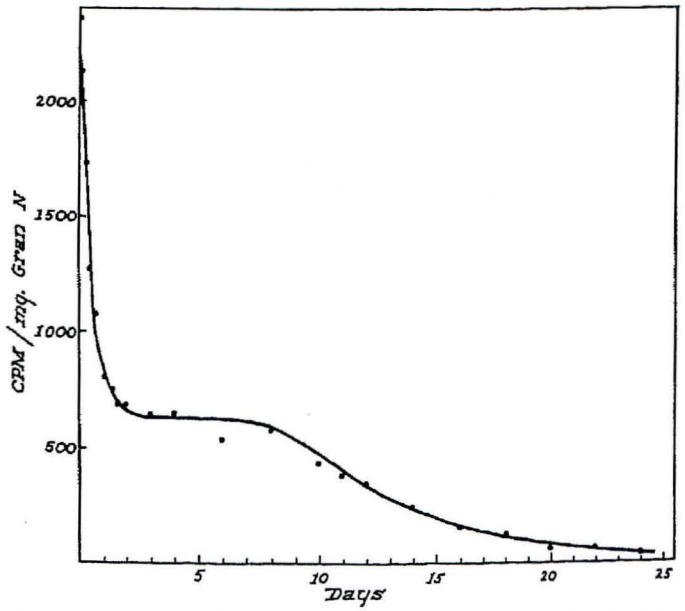

FIGURE 5. A comparison of the time course of the blood granulocyte radioactivity in DFP^{32} subject II-99 (*solid circles*) and the best solution predicted by the "semiclosed" model (*solid line*). The parameters used to achieve this match were: Time A of 3.05 days; Time B of 6.95 days; σ of 1.5 days; and $\frac{R}{V}$ 1.83.

TABLE 1

OPTIMAL MODEL PARAMETERS REQUIRED TO MATCH THE 18 DFP^{32} BLOOD GRANULOCYTE RADIOACTIVITY CURVES

Subject	Time A (Days)	Time B (Days)	σ (Days)	$\frac{R}{V}$
II–99	3.05	6.95	1.50	1.83
II–133	2.46	8.54	1.43	1.62
II–165	2.97	8.03	1.47	1.88
II–169	2.64	8.36	1.40	1.83
II–173	3.25	7.75	.82	1.77
II–179	1.17	12.83	1.50	3.48
II–183	2.12	8.88	1.20	1.86
II–187	3.00	9.00	1.50	3.45
II–219	2.21	11.79	1.50	1.72
III–2	2.00	12.00	1.50	1.38
III–10	2.44	11.56	1.50	1.85
III–14	4.00	6.00	1.50	1.43
III–18	1.64	12.36	1.15	1.57
III–22	2.88	9.12	1.05	1.96
III–30	3.42	8.58	1.50	1.42
IV–179	1.93	10.07	1.50	2.03
IV–183	2.29	12.00	1.16	3.21
IV–191	3.80	11.00	1.30	2.50
Mean of 18	2.63	9.71	1.36	2.04
Range	(1.17–4.00)	(6.00–12.83)	(0.82–1.50)	(1.62–3.48)

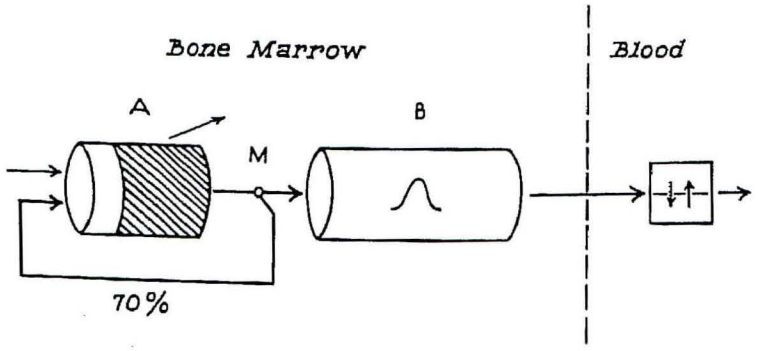

FIGURE 6. The "semiclosed" model of granulocyte kinetics with the initial labeling conditions produced by the DNA labels, H^3-Th, and radiophosphate, indicated by the shading.

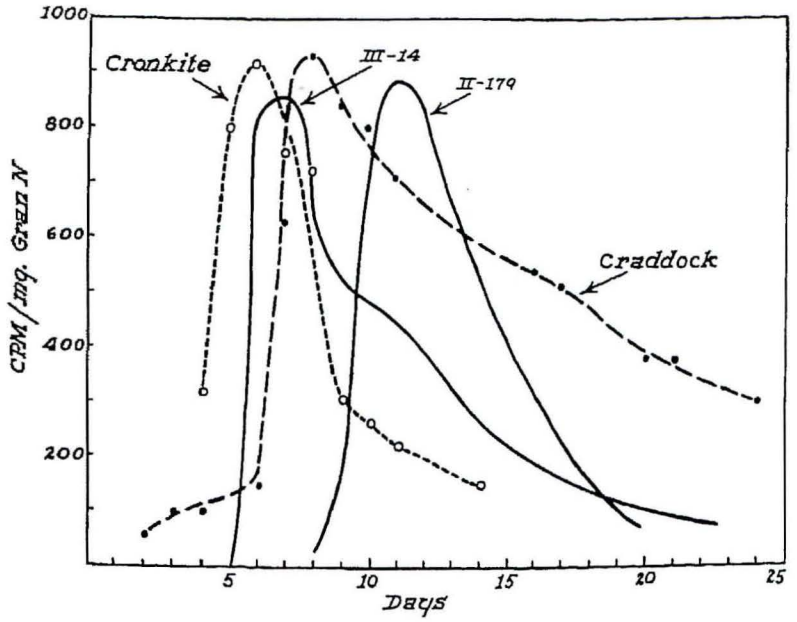

FIGURE 7. A comparison of the blood granulocyte radioactivity curves actually measured after the administration of H^3-Th by Cronkite and co-workers[9] and after the administration of radiophosphate by Craddock and co-workers,[8] with the curves predicted by the "semiclosed" model for the DFP^{32} subjects (III-14 and II-179) with the largest and smallest marrow granulocyte populations respectively.

8). All subsequent populations are "open"; that is, cells are entering from earlier populations as well as leaving, but no re-entry occurs. In the dividing populations, cells progress after division to the next more mature form.

To explore the possibility that such a scheme might also quantitatively describe the results of the DFP^{32} studies, solutions to such a model were obtained by replacing Equation 1 with

$$CA\ (I, t) = CA\ (I + N, t)\ 2^{-(N+1)}. \qquad (6)$$

In this Equation, I is the index for each subpopulation of the labeled myelocyte population. For any given subpopulation, I is equal to the number of subsequent divisions each cell in the Ith subpopulation will undergo before entering Population B. Since only myelocytes and more mature granulocytes are labeled with

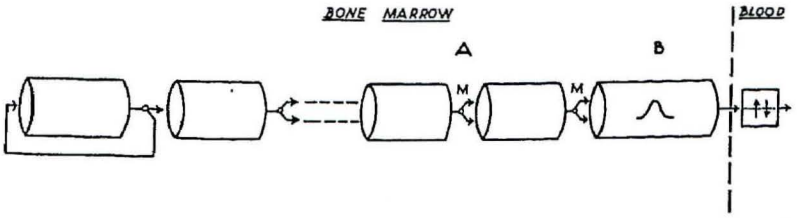

FIGURE 8. The "open" model of granulocyte kinetics. In this model, a single population of stem cells is self-regenerating. In all subsequent populations, cells move out and are replaced by cells from an earlier population. M is the site of cell division.

DFP^{32} only myelocyte populations need be considered here. The concentration of label in cells entering B at time t will be $CA\ (I, t)$, since $I = 1$ in the subpopulation which feeds cells directly into B. The variable N is defined as in Equation 2. If J is the total number of subpopulations of myelocytes (maximum number of divisions a cell may undergo as a myelocyte), cells in the $J + 1$ population are not labeled with DFP^{32},[1] since these are promyelocytes.

Solutions of the model equations for $J = 1, 2, 3$, and 4 are shown in FIGURE 9 and can be compared with the solution using the "semi-closed" model and with the experimental data. It can be seen that this "open" model will not describe the DFP^{32} data unless it is postulated that there are at least three and probably four myelocyte subpopulations with cell division in each and a Time A (generation time) of 2.6 days for each subpopulation.

Cell Death

It has been postulated, quite logically, that cells may be lost from one or more marrow populations through cell death. Therefore an analysis of the effect of cell death on the output of the "semiclosed" model (FIGURE 1) was undertaken.

The assumption of cell death in Population A has no effect on the model output (predicted blood curve) if adjustment of the probability of re-entry to a value greater than 0.5 is made. This adjustment means that cells are reentering A at a rate (R') greater than the rate cells enter the blood (R). Thus, R' minus R is the rate of cell death in Population A. If myelocytes do die at a significant rate, the size of Population A must be greater than the product of R and Time A and less than or equal to the product of R' and Time A depending on when, during the

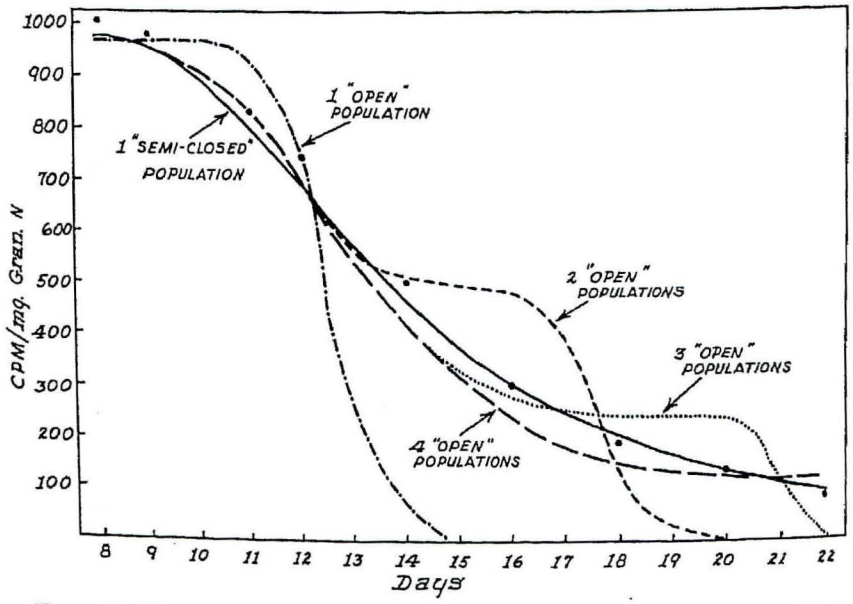

FIGURE 9. The DFP^{32} data (*solid dots*), "semiclosed" model output (*heavy solid line*), and "open" model output are compared. Only when at least three and preferably four subpopulations of myelocytes with one division in each subpopulation are used, can the "open" model approximate the data.

TABLE 2

RELATIVE SIZE OF THE MARROW NEUTROPHILIC MYELOCYTE AND METAMYELOCYTE PLUS ADULT NEUTROPHIL POPULATIONS AS ESTIMATED BY SEVERAL METHODS

Study	*A* Myelocyte population	*B* Metamyelocyte plus adult neutrophils	Ratio *A/B*
Present model analysis ($\times 10^9$ G./kg.)	4.7	17.4	0.270
Donohue *et al.*[12] ($\times 10^9$ G./kg.)	2.6	8.8	0.295
Wintrobe[13] (%)	12	42	0.285
Killman *et al.*[14]*	16.3	36.14	0.451

* The relative size of the myeloblast population was considered to be unity in this study and all other populations are given a size related to it.

generation time, the cells die. When estimates of the size of A made from the product of Time A and R (the mean granulocyte turnover rate as measured from Phase I), are compared with the size of the myelocyte population as measured by Donohue[12] using a completely different method, the former values are somewhat larger (TABLE 2). If cell death were included in the model, still larger values for calculated size of the myelocyte population would result.

Cell death in Population B can be handled in the "semiclosed" model by decreasing the recirculation fraction in Population A to less than 0.5, just the reverse of the mechanism for adjusting for cell death in Population A.

The effect of cell death can be introduced into the "open" model of Population A (which uses Equation 6) by assuming that the rate (R') at which cells leave the $I = 1$ subpopulation is greater than the rate (R) at which cells enter Population B. The rate of cell death in this subpopulation then is $R' - R$ and the rate of cell death in the Ith subpopulation is $(R' - R)\ 2^{1-I}$ if the rate of cell death per cell division remains constant among these subpopulations of myelocytes. Thus, in the "open" model also, the presence of cell death would necessitate a larger Population A to describe the experimental observations.

Since both models can describe the blood granulocyte radioactivity curves when no cell death is assumed and since the introduction of cell death into the models requires alterations in population size that seem less compatible with the available information on this point (TABLE 2), a more detailed analysis has been deferred until more specific information on the site and quantitative significance of cell death becomes available.

Discussion

It is evident that the blood granulocyte radioactivity curves which result from the administration of DFP^{32}, H^3-Th, or radiophosphate, can be described by the "semiclosed" model suggested here (FIGURE 1). In addition, several of the parameters necessary to achieve a good match between the experimental data and the model output correspond well with estimates of these parameters made by other means. Thus, Time A, the turnover time of the myelocyte population, averaged 2.63 days with a range of 1.2 to 4 days in these 18 normal males (TABLE 1). This

agrees well with the estimate of 2.43–2.67 days derived from mitotic index data obtained in 9 normal males by Cronkite and co-workers.[10] The single human subject studied with autoradiographs and H^3-Th also appears to have a myelocyte turnover time of 4.65 days,[10] only slightly outside the above range.

It is of further interest that with the "semiclosed" model, the relative size of Population *A* (myelocytes) as calculated from the product of Time *A* and *R*, and Population *B* similarly calculated from Time *B* and *R*, is in agreement with the relative size of these populations as measured by Donohue *et al.*[12] and with the normal bone marrow differential count as reported by Wintrobe[13] (ratio of *A*/*B* in TABLE 2). The values reported by Killman *et al.*[14] do not agree quite as well, however. Thus, the "semiclosed" model appears compatible with much of the available data. Of course, no theoretical model could describe data from both of two sources when the data are different from each source.

The "open" model will also describe the DFP^{32}, H^3-Th, and radiophosphate data. However, the match between the "open" model and the experimental DFP^{32} curves is not as good as with the "semiclosed" model (FIGURE 9). Furthermore, at least four cell divisions within the morphologically defined myelocyte pool are required to match the "open" model with the observed DFP^{32} curves. This number of sequential myelocyte divisions does not appear compatible with the total number of cell divisions calculated to occur in the neutrophil series. This calculation of the total number of cell divisions is based on the ratio of the total number of granulocytes in the marrow which are capable of division to the number of myeloblasts as shown in

$$M = \log_2 \left(\frac{X}{(MB)} \right), \tag{7}$$

where *M* is the number of cell divisions a cell undergoes between the blast stage and the metamyelocyte stage, *X* is the sum of the number of myeloblasts, promyelocytes, and myelocytes, 2 is the number of cells resulting from each division, and *MB* is the number of myeloblasts. Estimates of this ratio $\left(\frac{X}{(MB)}\right)$ from marrow differential counts[13] are about 10 (range 7.4 to 23) to 1, and thus three and at most four divisions occur from myeloblast through the myelocyte. If one stem cell division is added, four or five divisions must occur in the entire neutrophilic series. Similar calculations have been made by Killman *et al.*[15] These calculations do not appear compatible with the postulate of three or four divisions at the myelocyte stage.

An analysis of the "open" model in respect to data other than that obtained by the DFP^{32} technic may be made from published values for differential counting of bone marrow smears. From these technics it is apparent that the size of Population *A* is about 0.27 times the size of Population *B* (TABLE 2). If the generation time, *Tg*, for myelocytes is 2.6 days as reported by Cronkite *et al.*,[10] the "open" model yields a value for size of Population *A* compatible with the differential counting data, only if a cell undergoes three or four divisions as a myelocyte. That is, if only one division occurred in Population *A*, its size would be $\frac{1}{2}(R \times Tg)$, while if three divisions took place in this population, its size would be $\frac{R \times Tg}{2} + \frac{R \times Tg}{4} + \frac{R \times Tg}{8} = \frac{7}{8}(R \times Tg)$ thus approaching the value obtained by differential counting $(R \times Tg)$. Since three or more cell divisions must occur at the myelocyte level for the size of Population *A* to approach

($R \times Tg$), the total number of granulocyte cell divisions (M in Equation 7) must be at least five (three or more myelocyte divisions and at least one division at the promyelocyte and myeloblast level plus a stem cell division). Too many granulocyte divisions must be postulated for the "open" model to yield the population sizes observed and the probable number of granulocyte divisions as calculated in Equation 7 and by Killman.[15] Thus, the "open" model has defects beyond its inability to match the DFP^{32} data with the same precision as the "semiclosed" model.

The "semiclosed" model adequately describes the blood granulocyte radioactivity curves obtained with the several granulocyte labels available, and the parameters required to do so seem compatible with other estimates of myelocyte generation time and the relative size of the myelocyte and more mature granulocyte populations in the marrow. This postulate that myelocytes are self-regenerating is compatible with the observations by Osgood[16] that myelocytes from chronic myelocytic leukemia are capable of perpetuating a granulocyte population in cell cultures. However, the reasons for prefering the "semiclosed" to the "open" model are largely based on data obtained by marrow differential counts. Hence, the need for improving the accuracy of this technic is evident from the model studies. Only then can a choice be made with confidence between the alternate hypotheses presented here.

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