Unified Hypothesis for the Origin of Aplastic Anemia and Periodic Hematopoiesis

By Michael C. Mackey

The clinical and laboratory data related to periodic hematopoiesis (PH) are briefly reviewed, and it is concluded that the dynamics of PH probably originate in the hematopoietic pluripotential stem cell (PPSC) population. A model for the PPSC population is developed and analyzed. Based on the model, the simplest hypothesis for the origin of aplastic anemia (AA) and PH is that they are both due to the irreversible loss of proliferative stem cells. In addition to offering estimates for normal PPSC parameters in man and dogs, the model offers an explanation for the rarity of PH and its observed dynamics. The hypothesis predicts that for subjects without proliferative PPSC loss and with a normal differentiation flux M^* out of the PPSC, the effect of cell loss from proliferation will be hypocellularity and pancytopenia. However, in both man and dogs with an M^* significantly less than normal, loss of proliferating PPSC at a low rate gives mild pancytopenia, loss at a higher rate gives PH, and at an even higher rate severe pancytopenia appears. The threshold M^* for the appearance of PH in man is predicted to be about 3.7×10^7 cells/kg/day at a minimal period of 17 days. For mongrel dogs, the M^* at which PH ensues is 6.6×10^7 cells/kg/day and the period is about 9 days. Finally, the hypothesis predicts that the PPSC in subjects with AA and PH will have an increased proliferating fraction, and that stem cells should display an increased doubling time during periods of exponential growth.

CYCLIC NEUTROPENIA (CN) is a disease characterized by an oscillation in circulating neutrophil numbers from normal to low values. In man the majority of cases display a period in the range of 17 to 28 days.[1-4] All grey collies have this disorder, apparently differing from human CN only in that the period is 11 12 days.[5-7] In CN in both man[1,2] and the grey collie[6,7] a concomitant oscillation of all of the formed elements of the blood is observed. These elements oscillate with the same period as the neutrophils, but with phase lags or leads consistent with the known differences in maturation times for each of the cell types.[1] Thus this disorder is more appropriately termed periodic hematopoiesis[2] (PH) or cyclic hematopoiesis[7] (CH).

The granulocyte turnover rate (GTR) in grey collies ranges from normal to low values, and the neutrophil half-life is normal.[6] A similar observation has been made in humans with CN,[8,9] implying the existence of a marrow production defect. In line with this possibility, it has been noted that in the collie normal hematopoiesis results when normal bone marrow is transplanted into an irradiated grey collie.[10] Conversely, the transplantation of marrow from grey collies with PH into irradiated normal collies is marked by the onset of PH in

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Submitted June 15, 1977; accepted December 19, 1977.

Supported by Grant NRC-A-0091 from the National Research Council of Canada. Computing services were made possible through a grant from the MacDonald-Stewart Foundation to the Biomedical Engineering Unit, Faculty of Medicine, McGill University.

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 ISSN 0006-4971/78/5105-0007$02.00/0.

the recipients.[11] Both of these results have been confirmed,[12] and they demonstrate that a component of the marrow, probably the pluripotential stem cell (PPSC), is the primary location of the defect responsible for the peculiar dynamics of periodic hematopoiesis.

Cyclic neutropenia has received some attention from theoreticians[13,14] who have examined the potential involvement of a long-range (circulating to stem cell) humoral control mechanism in the genesis of the disorder. It is known that colony-stimulating factor (CSF) and serum erythropoietin (ESF) levels in grey collies and humans with CN oscillate with a period identical to the circulating cellular period.[15,16,19] However, it is also known that CSF and ESF are not regulators of the PPSC population.[17,18] Thus a significant involvement of peripheral to stem cell humoral feedback mechanisms in periodic hematopoiesis is unlikely.

The available clinical and laboratory data seem most consistent with the view that periodic hematopoiesis is due to a short-range defect at the pluripotential stem cell level, manifested by a periodic failure of the production of all formed elements of the blood.[2,7,11,20]

Aplastic anemia (AA), by definition, is characterized by a mild to severe pancytopenia and hypocellular bone marrow.[21] It is frequently observed as a result of chemotherapy and radiotherapy. It has been argued that the primary locus of the defect in aplastic anemia is resident in the PPSC population, and that the defect may be either transient or permanent.[22]

In this paper the analysis of a model for the control of stem cell production is presented. Based on the analysis, it is demonstrated that a sufficient explanation for the origin of aplastic anemia and periodic hematopoiesis is the existence of irreversible cell loss from the proliferating phase of the cell cycle.

THE MODEL

A schematic representation of the pluripotential stem cell model is shown in Fig. 1. The equations describing the model, with heuristic arguments for their structure, and a list of symbols used are given in Appendix 1.

Stem cells are classified as proliferating-phase (population P, cells/kg) or resting-phase (G_0) cells (population N, cells/kg).[17,23,24] The distinction between the proliferating and resting states is as follows: Cells travel through proliferation as if in a pipeline to undergo mitosis at a fixed time $\tau(d)$ from their time of entry into the proliferative state. However, cells entering G_0 may exit randomly

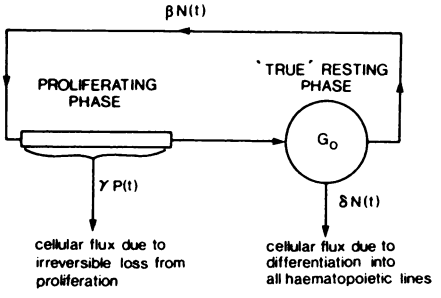

Fig. 1. Schematic representation of pluripotential stem cell population. Stem cells are located in either a proliferating phase or a true resting (G_0) phase. Cells travel through the proliferating phase as if in a "pipeline" in a time $\tau(d)$, and it is assumed that all cells completing mitosis reenter G_0. There is a flux βN (cells/kg/day) of cells from the resting to proliferating phase. The flux δN (cells/kg/day) represents the total differentiation out of G_0 into all hematopoietic lines, and the flux γP (cells/kg/day) represents the irreversible and pathologic loss of cells from proliferation. See text for further details.

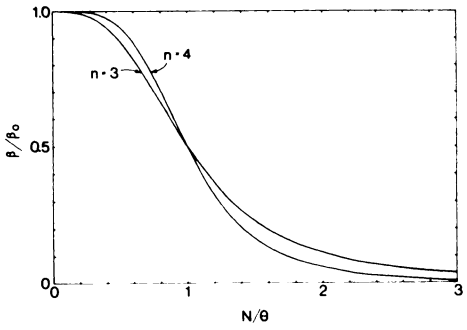

Fig. 2. Form of the mitotic regulatory function. Rate β of G_0 cellular reentry into proliferation, in units of the maximal rate β_0, shown as a function of G_0 cellular population numbers N, in units of θ [the G_0 population at which $\beta(N)$ is equal to $\frac{1}{2}\beta_0$]. See text and Appendix 1 for further details.

to either reenter proliferation at a rate $\beta(d^{-1})$ or to be irreversibly lost via differentiation into the hematopoietic lines at a rate $\delta(d^{-1})$. Although proliferating cells in the model may be irreversibly lost at a rate $\gamma(d^{-1})$ from any phase of the cell cycle, here a "normal" stem cell population will, by definition, be characterized by $\gamma = 0$. Assume throughout that γ, δ, and τ are constant with respect to time and cell population numbers.

Control mechanisms within stem cell populations are not well understood, although there exists strong evidence for potent short-range mechanisms (as opposed to long-range circulating regulators) acting to limit stem cell numbers.[17,25-28] Without specifying the exact nature of control, assume that the PPSC population is capable of recognizing the number of G_0 phase cells and changing the mitotic reentry rate β in response to changes in N (see Appendix 1). Specifically, when the G_0 population is small β approaches a maximum, and when N increases β decreases as shown in Fig. 2. In Fig. 2 the parameter $\beta_0(d^{-1})$ is the maximal rate of cellular entry into proliferation, and θ (cells/kg) is the number of G_0 cells at which β has its maximum rate of change with respect to N. The maximum slope of the β versus N relationship, which occurs at $N = \theta$, is $-n\beta_0/4\theta$, where n is a dimensionless number.

As outlined in Appendix 2, the stem cell model of Fig. 1 has steady-state proliferating and resting cellular populations P^* and N^* (cells/kg). The time-dependent behavior of the populations $P(t)$ and $N(t)$ falls into one of two broad classes that are of importance for understanding aplastic anemia and periodic hematopoiesis. Thus from numerical and analytical studies (see Appendix 2) the PPSC populations will either (1) approach the steady-state populations P^* and N^* [given by Eqs. (2.1)†] in a smooth or damped oscillatory fashion, or (2) oscillate in a stable fashion around the steady-state population values P^* and N^*. The oscillation may be either peroidic or aperiodic, depending on the choice of parameters.

Although the parameter θ is involved in determining the magnitude of the steady-state populations, it plays no role in determining which of these two behaviors the stem cell population will exhibit. That behavior is completely determined by the values of the kinetic parameters γ, δ, β_0, τ, and n. Generally speaking, if all five of these parameters are such that the populations approach a steady state, then an increase in any one of the parameters with the other four

†Equation (2.1), for example, is to be found in Appendix 2.

held constant may lead to the appearance of oscillations. Even in the case where $\gamma = 0$, increases in δ, β_0, τ, and/or n may lead to a transition between these two behaviors.

The notion that control systems with a time delay may become unstable and start to oscillate is one familiar to control engineers. A simple example of this phenomenon is available in the mundane workings of a home heating unit. If the delay is too long between times when changes in temperature are made and when they are sensed, the temperature may start to oscillate around the set point (roughly analogous to θ). Likewise, if the thermostat is too sensitive (β_0 or n too large), the temperature may begin to overshoot and undershoot the desired value. Finally, if heat loss from the house (loosely analogous to δ) or from the furnace (γ) is too great, oscillatory excursions about the desired temperature may ensue.

The determination of values for the set of parameters γ, δ, τ, β_0, n, and θ is difficult because of their number and the lack of any large body of data related to stem cell physiology and pathophysiology. A detailed treatment of the parameter determination is given in Appendix 3.

Normally, $\gamma = 0$, although a number of agents may cause the death of proliferating-phase stem cells.[29] To determine δ and τ, assume that[30,31] (1) the proliferating fraction F_p of the stem cell population is at most 0.10 and (2) the total stem cell population is 5×10^{10} cells/70 kg. These two assumptions allow unique values of δ and τ to be calculated for any value of the steady-state flux of cells, $M^* = \delta N^*$ (cells/kg/day), into the differentiated hematopoietic lines (myeloid, erythroid, and thrombocytic). Using these values for δ and τ, and further assuming that (3) the doubling time t_D for the stem cell population is 24 hr when the population undergoes exponential growth,[32] allows a unique value for β_0 to be calculated. The variation in δ, τ, and β_0 with M^* is shown in Fig. 3 for a range of differentiation fluxes.

There are few data on which to base a choice for n, which controls the sensitivity of the mitotic reentry rate (Fig. 2) to changes in the size of the G_0 phase. If the interpretation offered in Appendix 1 for the form of β is correct, it would be expected that n would be small (on the order of 5 or less). The response to one mitotic regulator, granulocytic chalone derived from rat granulocytes,[33] is described by the form for β adopted here [Eq. (1.3)], with $n = 3.15$ (coefficient of concordance $r^2 = 0.99$), lending further support to the notion that n is not

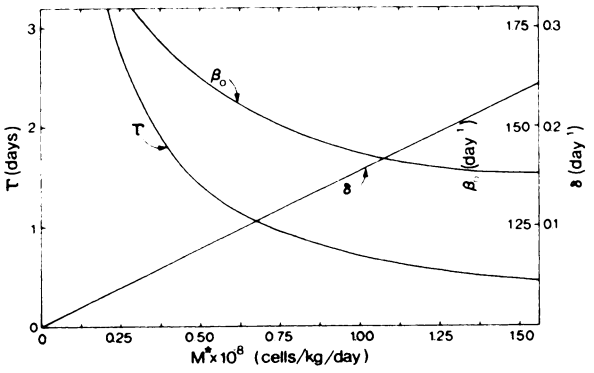

Fig. 3. PPSC parameter values. Variation δ in the rate of cellular differentiation out of G_0, cell cycle time τ, and maximal rate β_0 of G_0 cell movement into proliferation as a function of flux M^* of cells into differentiated hematopoietic cell lines. δ directly proportional to M^*; τ is inversely proportional to M^*. β_0 determined as a function of δ and τ. See text and Appendix 3 for details.

large. In point of fact, n is determined in this study by the peculiar dynamics of periodic hematopoiesis in man and dogs.

Once a value is assumed for n, the final parameter θ may be calculated with the previously determined values of β_0 and δ in conjunction with the estimate for N^* and Eqs. (2.1).

ORIGIN OF APLASTIC ANEMIA AND PERIODIC HEMATOPOIESIS

In examining this pluripotential stem cell model with respect to the origins of aplastic anemia and periodic hematopoiesis, I conclude that they may both have a common origin. More specifically, they may both be encountered as a single dynamic parameter within the stem cell population is changed.

Evidence has been recently reviewed[22] that tends to support the hypothesis that the PPSC in aplastic anemia is abnormal with respect to proliferative function. This concept is supported by the observation that the administration of a single large dose of busulfan in mice results in a permanent transformation in the proliferative capacity of marrow stem cells, manifested by mild to severe marrow hypoplasia and pancytopenia.[34]

Insight into the origin of periodic hematopoiesis is afforded by examining the effects of continuous cyclophosphamide and busulfan administration in normal dogs.[35,36] Usually there is a simple progressive pancytopenia that becomes more severe with higher drug doses. However, in some animals this pattern is altered to one in which low drug levels give simple pancytopenia, higher levels result in cyclic neutropenia (with a period of 11-17 days), and even higher dosages abolish the cyclic neutropenia, leaving the dogs severely pancytopenic. The onset of cyclic neutropenia, when it occurs, is at a circulating neutrophil level of one-half to one-third normal. Furthermore, the appearance of cycling granulocyte levels in a number of patients undergoing continuous hydroxyurea therapy,[37] and in one patient receiving cyclophosphamide,[38] has been noted.

Cyclophosphamide and busulfan are alkylating agents, while hydroxyurea is an antimetabolite.[29] Hydroxyurea is known to selectively kill proliferating pluripotential stem cells during DNA synthesis.[27,39] Likewise, cyclophosphamide kills stem cells,[25] and within the context of this study I interpret the mode of action of all these agents to be one resulting in $\gamma \neq 0$. If γ is not zero in the stem cell population, then it can be shown that the total steady-state PPSC population $P^* + N^*$ continuously decreases in size as the rate of irreversible cell loss from proliferation increases. The steady state flux of cells, $M^* = \delta N^*$ (cells/kg/day), out of G_0 into all of the hematopoietic lines will also decrease, resulting in pancytopenia.

Figure 4 deals with the implication of killing proliferating-phase stem cells in a previously normal PPSC population; therein is plotted the predicted steady-state cellular flux into the differentiated hematopoietic lines, M^*, as a function of the rate of irreversible cell loss from proliferation. I have assumed $n = 3$ and selected parameters corresponding to a normal differentiation flux for man of 1.05×10^8 cells/kg/day when $\gamma = 0$. As may be seen in this example, the total PPSC population numbers decrease with increasing γ, as does the total flux into the differentiated hematopoietic lines. Also, with increasing

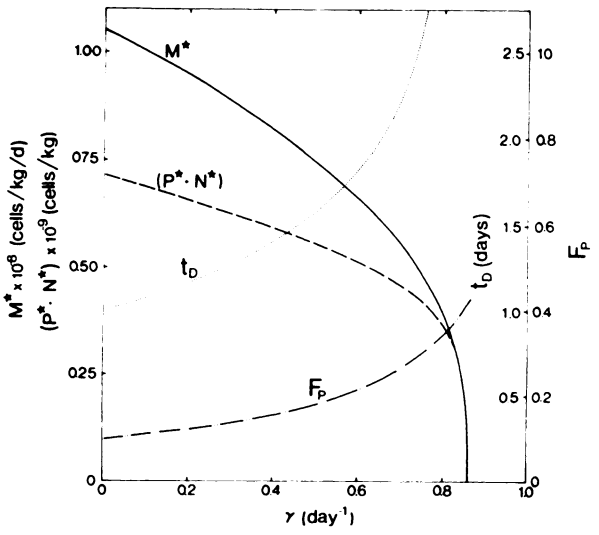

Fig. 4. Development of pancytopenia with increasing proliferative cellular loss from normal PPSC. Total stem cell population $P^* + N^*$ and flux out of the PPSC into the differentiated hematopoietic series, M^*, decrease as the rate of cellular loss from proliferation, γ, increases. Proliferating fraction F_p of the PPSC increases as γ increases, as does the exponential doubling time t_D. Parameter values: $n = 3$, $\delta = 0.16$/day, $\beta_0 = 1.43$/day, $\tau = 0.68$ days, and $\theta = 3.22 \times 10^8$ cells/kg. At $\gamma = 0$, $P = 0.71 \times 10^8$ and $N = 6.43 \times 10^8$ cells/kg, and $M^* = 1.05 \times 10^8$ cells/kg/day.

γ the proliferating fraction F_p continuously increases, as does the doubling time t_D during exponential growth. Thus increasing the rate at which the PPSC loses cells from proliferation results in a generalized pancytopenia and, presumably, a marrow hypocellularity.

Figure 5 indicates that for an initial ($\gamma = 0$) M^* less than the mean for man, the lower the initial M^*, the more profound will be the pancytopenia developed as γ increases. For an initial M^* less than 3.86×10^7 cells/kg/day, an abrupt change in the behavior of the stem cell population becomes apparent as γ is increased. Thus for an initial M^* of 3.81×10^7 cells/kg/day a progressive increase of γ leads to a progressive decrease in M^*. Based on the analysis of

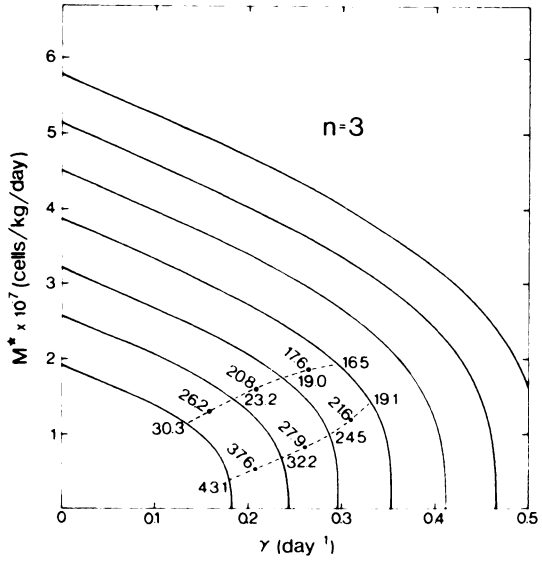

Fig. 5. Variation in cellular flux into differentiated lines as a function of the irreversible rate of loss, γ, of cells from proliferation for man. All parameters as in Fig. 3 (with $n = 3$ to reproduce the properties of aplastic anemia and periodic hematopoiesis in man). Dashed lines, boundaries within which stable periodic hematopoiesis is predicted; numbers along dashed lines, period (in days) of oscillation. See text for further details.

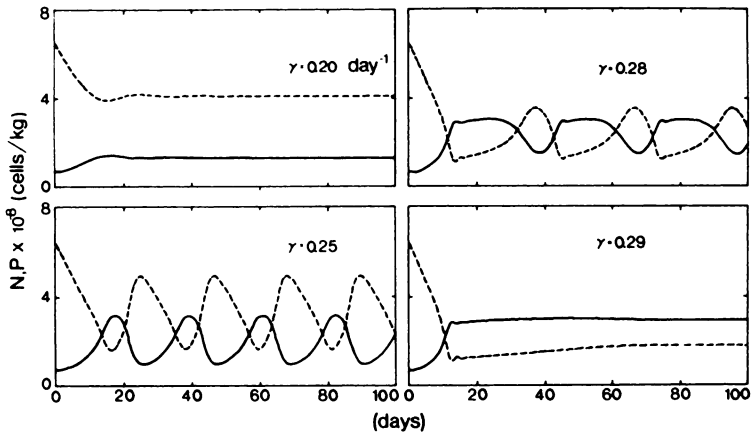

Fig. 6. Simulated population numbers of the G_0-phase (dashed line) and proliferating-phase (solid line) stem cells over a 100-day period, for four different values of γ, in man. Initial population numbers ($P_0 = 0.71 \times 10^8$ and $N_0 = 6.43 \times 10^8$ cells/kg) correspond to the stable stem cell population when $\gamma = 0$. Parameters used in the simulation: $\delta = 0.05$/day, $\beta_0 = 1.77$/day, $\tau = 2.22$ days, $\theta = 1.98 \times 10^8$ cells/kg, and $n = 3$. See text for discussion.

Appendix 2, it is predicted that when $\gamma = 0.30$/day M^* will no longer be depressed and constant but will start to oscillate with a period of 16.5 days, the onset of periodic hematopoiesis. Progressive increases in γ beyond this value lead to a progressive increase and then decrease in the amplitude of this oscillation, accompanied by an increase in the period, until at about $\gamma = 0.33$/day (with a period of 19 days) the periodic hematopoiesis ceases and a steady pancytopenia reappears.

Figure 6 shows a sequence of computed PPSC population numbers as a function of time, assuming an initial M^* of 3.22×10^7 cells/kg/day and all other parameters corresponding to this M^* as given in Fig. 3. The solutions shown in Fig. 6 start from the normal steady-state values for N^* and P^* and show the expected changes over a 100-day interval if, at $t = 0$, γ is suddenly changed from $\gamma = 0$ to the value indicated in each panel. The sequence of events is as follows: (1) For values of $\gamma < 0.24$/day there is a simple depression in P^* and N^* below their normal steady-state values and thus a decrease in M^*; (2) For values of γ in the range in which periodic hematopoiesis is predicted (see Fig. 5; $0.24 \leq \gamma \leq 0.29$/day), the G_0 cellular population numbers oscillate, initially with a period of approximately 19 days, which lengthens to 25 days for γ near 0.29/day; (3) For $\gamma > 0.29$/day the G_0 population numbers are even more depressed but constant.

The stem cell population dynamics displayed in Figures 4 through 6 adequately duplicate the qualitative and quantitative properties of aplastic anemia and periodic hematopoiesis in humans.

The same qualitative sequence of behaviors shown in Figs. 4–6 is found for higher values of the parameter n. The quantitative differences in this pattern as n is increased are twofold: the larger the value of n, the larger the initial flux into differentiation at which the periodic hematopoiesis will initially be found, and the larger the value of n, the shorter the period of the oscillation. With these points in mind, it is clear that an explanation of the patterns of periodic

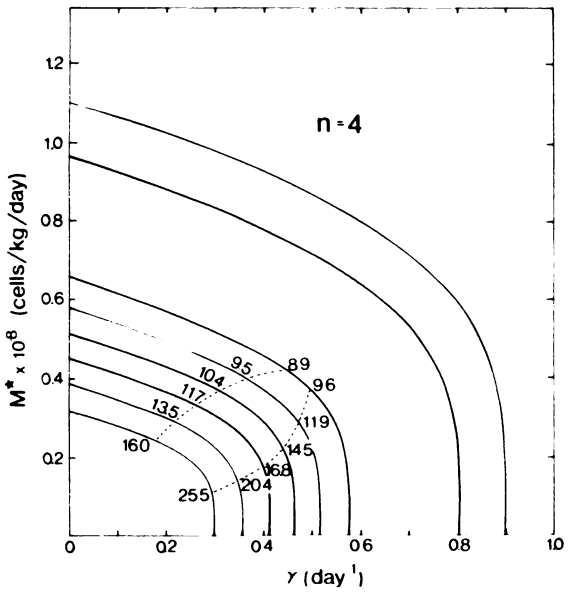

Fig. 7. Variation in the cellular differentiation flux in dogs as a function of the irreversible rate of loss of stem cells, γ, from proliferation; n = 4. See Fig. 5 and text for further details.

hematopoiesis in grey collies, and the results of continuous administration of cyclophosphamide and busulfan, will require a value of n greater than for man.

These points are illustrated in Fig. 7 for n = 4. As indicated, the transition from simple pancytopenia to periodic hematopoiesis requires an initial M^* of 6.56×10^7 cells/kg/day. The period of the periodic hematopoiesis, when it occurs, is about 9 days. Progressive decreases in the initial M^* lead to progressive increases in the period of the oscillation.

Fig. 8 shows, for n = 4 and an initial $M^* = 5.19 \times 10^7$ cells/kg/day, the sequence of behaviors encountered as γ is increased. The analysis of Appendix 2

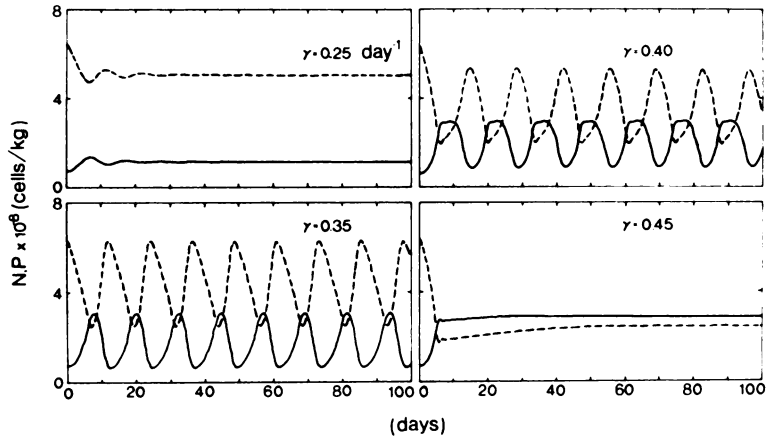

Fig. 8. Simulated variation in the proliferating and resting stem cell populations over a 100-day period in dogs with four levels of cell loss from proliferation. P_0 and N_0 as in Fig. 6. Other parameters: δ = 0.08/day, β_0 = 1.62/day, τ = 1.39 days, $\theta = 3.07 \times 10^8$ cells/kg, and n = 4. Proliferative loss rates indicated in each panel.

indicates that mild aplastic anemia should result for all values of $\gamma < 0.31$/day, that periodic hematopoiesis (with an initial period of 10.4 days) will be present for $0.31 \leq \gamma \leq 0.44$/day, and that severe hypocellularity and pancytopenia will appear for $\gamma > 0.44$/day.

DISCUSSION AND PREDICTIONS

Since the populations of the stem cell model are capable of sustained oscillations when $\gamma = 0$, the most parsimonious assumption would be that aplastic anemia and periodic hematopoiesis are due to the alteration of one of the parameters θ, β_0, δ, n, or τ. An analysis of the effects of changes in these parameters on the flux of cells out of G_0 reveals that it is only by an increase in n that pancytopenia and marrow hypoplasia followed by the appearance of periodic hematopoiesis, as observed in cyclophosphamide-treated dogs,[35,36] can be effected. It is common in aplastic anemia to find a pancytopenia of 50% or more.[21] A simple calculation indicates that within the estimated range of normal parameters n would have to be increased from a normal value of 3 to about 12 for man, or from a value of 4 to about 125 for dogs, in order to account for this reduction. This notion seems untenable on several grounds, not the least of which is that the analysis of Appendix 2 indicates that periodic hematopoiesis will occur long before any detectable pancytopenia occurs.

Thus in examining AA and PH, attention is sharply focused on the proliferative loss rate γ from the PPSC. The analysis in Appendix 2 of the PPSC population dynamics and the results of that analysis displayed in Figs. 3-8 indicate that increasing the proliferative loss rate will have one of two effects: (1) a decrease in the total PPSC population numbers, accompanied by a generalized and steady pancytopenia, or (2) a reduction in the PPSC and steady pancytopenia for low values of γ, PH for higher values of γ, and severe but steady pancytopenia for even larger γ. Therefore the prolonged marrow hypocellularity and pancytopenia associated with idiopathic AA, or AA due to chemotherapy or radiotherapy, is adequately accounted for by this model for the PPSC. Likewise, the dynamics of idiopathic PH and the pattern of induction of PH with cytocidal drugs are encompassed by a unified explanation.

Thus a sufficient explanation for the origin of aplastic anemia and periodic hematopoiesis is that they are both due to irreversible cellular loss from the proliferating pluripotential stem cell compartment.

In addition to offering estimates for the parameters characterizing the production and control of pluripotential stem cells, this hypothesis offers a satisfying explanation for two observations: (1) Spontaneous periodic hematopoiesis in man and dogs is rarely observed. This finding is a consequence of the fact that in the model PH is encountered only in subjects who initially ($\gamma = 0$) have an M^* far below the average value. (2) Spontaneous periodic hematopoiesis in man and dogs, when it occurs, displays a periodicity equal to or greater than about 17 and 9 days, respectively. In the model, this observation serves to place some constraints on the values of the parameter n.

With respect to pancytopenia and marrow hypoplasia the hypothesis offers the following quantitative predictions: (1) If it is desired to induce PH, then subjects with an initially low M^* will have a much higher probability of de-

veloping PH. The threshold M^* for the induction of PH should be on the order of 3.7×10^7 cells/kg/day in man and 6.6×10^7 cells/kg/day for mongrel dogs. (2) In man and dogs treated with cycle-active agents PH is not expected to occur until M^* has been depressed to about 30% and 40% of its mean values, respectively. Note, however, that this is a sufficient but not necessary requirement for the development of PH.[35,36]

Finally, the hypothesis predicts that the pluripotential stem cell population in aplastic anemia and periodic hematopoiesis will be characterized by an elevated proliferating fraction F_p and by an elevated doubling time t_D during exponential growth.

ACKNOWLEDGMENT

I would like to thank Professors B. Cooper, D. Dale, J. Dancey, and W. Hammond for their comments on this work, and I thank Christine Pamplin for her meticulous typing of the manuscript.

APPENDIX 1. MODEL EQUATIONS

In this appendix the equations describing the dynamics of the populations of proliferating- and G_0-phase stem cells are given without derivation, along with heuristic arguments for their structure. The derivation of the equations rests on a time-age formulation[40] and utilizes techniques illustrated elsewhere.[41] The parameters and functions appearing in the equations are defined below and discussed in the section entitled The Model.

Symbols

$P(t)$, density (cells/kg) of proliferating phase stem cells as a function of time (days).

$N(t)$, resting-phase (G_0) stem cell population density (cells/kg) as a function of time (days).

P^*, steady-state proliferating-phase stem cell density (cells/kg).

N^*, steady-state G_0-phase stem cell density (cells/kg).

τ, cell cycle time, or time spent by a cell in the proliferating phase (days).

δ_M, rate (days^{-1}) of differentiation out of the G_0 phase of the PPSC into the myeloid series. Subscript E or T denotes the analogous rates into the erythroid and thrombocyte series, respectively.

δ, total rate (days^{-1}) of differentiation out of the G_0 phase stem cell compartment into all of the hematopoietic lines. $\delta = \delta_M + \delta_E + \delta_T$.

M^*, total steady-state differentiation cellular flux (cells/kg/day) from the G_0 phase of the PPSC into all of the hematopoietic lines. $M^* = \delta N^*$.

γ, rate (days^{-1}) of irreversible cell loss from all portions of the proliferating-phase stem cell population.

β, mitotic reentry rate (days^{-1}), or rate of cell movement from G_0 into proliferation.

β_0, maximal rate (days^{-1}) of cellular movement from G_0 into proliferation.

θ, the G_0 stem cell population (cells/kg) at which the rate of cell movement from G_0 into proliferation is one-half of its maximal value (β_0).

n, dimensionless number characterizing the sensitivity of the mitotic reentry rate β to changes in the size of G_0.

Model Equations

The equation for the population $N(t)$ of resting G_0 phase cells is

$$\frac{dN}{dt} = -\delta N - \beta(N)N + 2\beta(N_\tau)N_\tau e^{-\gamma\tau}, \qquad \tau < t, \tag{1.1}$$

where $N_\tau = N(t - \tau)$. Equation (1.1) equates the total rate of change of $N(t)$ with the sum of three terms. The first term on the right-hand side of (1.1) accounts for irreversible loss from the G_0 population due to differentiation; the second corrects for cell loss due to the movement of cells into proliferation; and the last term represents a cellular gain due to the movement of proliferating

cells into G_0 one generation time ago; the factor of 2 accounts for mitosis, and $e^{-\gamma\tau}$ corrects for the probability of cellular loss from the proliferating population.

The dynamics of the population $P(t)$ of cells in proliferation is described by

$$\frac{dP}{dt} = -\gamma P + \beta(N)N - \beta(N_\tau)N_\tau e^{-\gamma\tau}, \qquad \tau < t. \tag{1.2}$$

As before, the first term on the right-hand side of (1.2) is an irreversible-loss term, the second represents cellular entry from the G_0 phase, and the last accounts for the flux of cells from the proliferating to the nonproliferating phase one generation time previously.

The specific functional form chosen for $\beta(N)$ is

$$\beta(N) = \beta_0\theta^n/(\theta^n + N^n), \tag{1.3}$$

where β_0 (days^{-1}), θ (cells/kg), and n are parameters. The qualitative predictions of this model are independent of the form β takes as long as (1) $\beta(N)$ is a smoothly decreasing function of increasing N, (2) β has a finite maximum (e.g., β_0), and (3) β has an adjustable inflection point (e.g., θ) and adjustable slope (e.g., $-n\beta_0/4\theta$).

The form for $\beta(N)$ can be argued for as follows: Suppose each cell contains a receptor for a mitotic regulatory molecule.[42] When the receptor is in the uncombined form it is active in the sense that mitosis proceeds in a regular fashion, but when combined it is inactive or capable of preventing mitosis. Assume the following: (1) the reaction between receptor and regulator molecule proceeds according to $M + nC \rightleftharpoons L$, where M and L denote the active and inactive forms of the receptor and C is the regulatory molecule; (2) the equilibrium constant for the reaction is K, so that $[M]\,[C]^n = K[L]$, where brackets denote a concentration; and (3) there are a fixed number of receptors $[T]$ per cell, $[T] = [M] + [L]$. Then the fraction of active receptors $[M]/[T]$, will be given by $K/(K + [C]^n)$. Assuming the number of regulatory molecules to be directly proportional to the number of G_0-phase cells, $[C] = dN$, and the maximal rate of entry into the cell cycle to be β_0 thus gives the form for β as shown in Eq. (1.3), where $\theta^{-1} = \alpha K^{1/n}$.

With this choice for the control function β, the model is completely specified, and Eqs. (1.1) and (1.2) become, respectively,

$$\frac{dN}{dt} = -\delta N - \frac{\beta_0\theta^n N}{\theta^n + N^n} + \frac{2\beta_0\theta^n N_\tau}{\theta^n + N_\tau^n}e^{-\gamma\tau}, \qquad \tau < t, \tag{1.4}$$

$$\frac{dP}{dt} = -\gamma P + \frac{\beta_0\theta^n N}{\theta^n + N^n} - \frac{\beta_0\theta^n N_\tau}{\theta^n + N_\tau^n}e^{-\gamma t}, \qquad \tau < t. \tag{1.5}$$

APPENDIX 2. PROPERTIES OF THE MODEL

Equations (1.4) and (1.5) have two sets of steady-state solutions P^* and N^* at which $dP/dt = dN/dt = 0$. The first is $P^* = N^* = 0$, and the second is

$$N^* = \theta[(\beta_0/\delta)(2e^{-\gamma\tau} - 1) - 1]^{1/n}, \qquad P^* = N^*(\delta/\gamma)(1 - e^{-\gamma\tau})/(2e^{-\gamma\tau} - 1). \tag{2.1}$$

P^* and N^* will be greater than zero only if

$$0 < \gamma\tau < \ln[2\beta_0/(\beta_0 + \delta)] < \ln 2. \tag{2.2}$$

In the "normal" state there is no loss of cells from proliferation ($\gamma = 0$). From (2.2) the limiting steady-state cellular populations in this case become

$$N^* = \theta(\beta_0/\delta - 1)^{1/n}, \qquad P^* = \delta\tau N^*, \tag{2.3}$$

while the condition (2.2) becomes $(\beta_0/\delta) > 1$.

The behavior of the model under mitotic regulatory control is not presently amenable to analytic description. What is possible is a discussion of the behavior near the steady-state cellular populations. Take the linear portion of (1.4) near any equilibrium point N^*, with $N(t) = z(t) + N^*$, $|z(t)/N^*| \ll 1$, to give the linear differential delay equation

$$\frac{dz}{dt} + Az(t) + Bz(t - \tau) = 0, \tag{2.4}$$

where $A = \delta + \beta_0 F(N^*, n)$, $B = -2\beta_0 F(N^*, n)e^{-\gamma\tau}$, and

$$F(N^*, n) = \theta^n \frac{\theta^n + (1 - n)(N^*)^n}{[\theta^n + (N^*)^n]^2}.$$

Assume that (2.4) has a solution of the form $e^{\lambda t}$, $\lambda = \mu + j\omega$, $j^2 = -1$, and substitute into (2.4) to give

$$\lambda + A + Be^{-\lambda\tau} = 0. \tag{2.5}$$

Equate the real and imaginary portions of (2.5) to find

$$\omega\tau < \cos^{-1}(-A/B), \tag{2.6}$$

where $\omega^2 = B^2 - A^2$, $|A/B| < 1$, is a necessary and sufficient condition for $\mu < 0$. Equation (2.6) gives the requirement that the stem cell parameters must satisfy in order that small changes $z(t)$ away from the steady state N^* will die out. When $\mu = 0$,

$$\omega\tau = \cos^{-1}(-A/B), \tag{2.7}$$

and periodic solutions, of period $T = 2\pi/\omega$, appear.

At the first steady state, $P^* = N^* = 0$, $F = 1$ and conditions for very small proliferating and resting cellular populations to approach zero may be obtained. However, numerical solutions to (1.4) and (1.5) indicate that the cellular populations will never be zero unless they are zero initially.

At the nonzero steady state defined by (2.1), F is a complicated function. Generally speaking, an increase in any one of the five parameters γ, δ, β_0, n, or τ with the other four held constant *may* lead to the loss of stability at a steady state and the appearance of oscillatory cellular populations.

For large deviations away from the steady state, as well as for values of the parameters that do not satisfy (2.6), the behavior of the populations cannot be predicted from this analysis. To investigate further the properties of the model under these conditions, numerical solutions to (1.4) and (1.5) were computed using a predictor-corrector integration scheme with an integration step size of 0.01. Further decreases in the step size led to no changes in behavior.

Briefly, the results of approximately 360 computed solutions to (1.4) and (1.5) with a range of parameters and initial conditions indicate that (1) for γ, δ, β_0, n, and τ that satisfy (2.6), the cellular populations always approach the steady states defined by (2.1); (2) Eq. (2.7) accurately predicts the onset of stable periodic variations in $P(t)$ and $N(t)$ (the period of the oscillation is accurately given by $T = 2\pi/\omega$, where $\omega^2 = B^2 - A^2$ and A and B are defined above); and (3) for values of the parameters such that $\omega\tau > \cos^{-1}(-A/B)$, periodic solutions of a complex nature and aperiodic solutions may occur.

The nature of these complicated bifurcations in the dynamics of Eqs. (1.1) and (1.2) will be considered elsewhere.

APPENDIX 3. PARAMETER ESTIMATION

The estimation of parameter values is made difficult by the lack of any body of common data on normal and abnormal stem cell properties. There are a number of parameters and thus many ways of arriving at estimates for them based on the existing data. The following assumptions have been made:

(1) In the normal state there is no irreversible cell loss from the proliferating phase ($\gamma = 0$). Note, however, that a number of pathologic situations may be characterized by loss from proliferation.[29]

(2) In the normal state the stem cell population is at a stable steady state defined by Eqs. (2.3).

With these two assumptions the expression for the proliferating fraction, $F_p = P^*/(P^* + N^*)$, becomes $F_p = \delta\tau/(1 + \delta\tau)$. Using triatiated thymidine (^{3}H-dt) suicide technique data on normal steady-state adult mouse marrow and spleen,[30] F_p is at most 0.10, from which $\delta\tau = \frac{1}{9} = 0.11$.

The rate of differentiation into the hematopoietic cells lines, δ, is made up of three components: δ_M, the rate of differentiation into the myeloid series; δ_E, the rate into the erythroid series; and δ_T, the rate into the thrombocyte series. To determine these individual contributions to δ, proceed as follows:

The granulocyte turnover rate (GTR) has been measured in man[43,45] and dogs,[6,46,47] using initially [^{32}P] diisopropylphosphofluoridate (DF^{32}P) and more recently ^{3}H-dt. The results obtained by the two methods are discrepant by a factor of approximately 0.5, and there is strong evidence that the ^{3}H-dt estimates of GTR are more accurate.[45,47] Assuming this to be the case, I have adopted the mean GTR estimates based on ^{3}H-dt measurements: 0.87×10^{9} cells/kg/day for man, 1.65×10^{9} cells/kg/day for mongrel dogs. If there is no significant cell loss from the maturing nonproliferating granulocyte precursor compartments[45,47] and from the proliferating compartments, then the steady-state GTR must be equal to the cellular flux into the myeloblast compartment ($\delta_M N^*$) times the amplification in cell numbers due to proliferation in the myeloblast, promyelocyte, and myelocyte states (2^m): GTR = $2^m(\delta_M N^*)$ cells/kg/day. Summarized data[41] on the relative frequencies of proliferating granulocyte precursor cells give m values ranging from 2.6 to 5.6, and I have adopted a value of $m = 4$.

The total PPSC compartment for a 70-kg human has been estimated at 5×10^{10} cells, or $P^* + N^* = 7.14 \times 10^{8}$ cells/kg.[31] With a proliferating fraction of 0.10, this gives $P^* = 0.71 \times 10^{8}$ and $N^* = 6.43 \times 10^{8}$ cells/kg, in agreement with a previously determined[43] lower bound on N^* of 5×10^{8} cells/kg.

With these considerations in mind, δ_M is given by $\delta_M = \text{GTR} \times 9.72 \times 10^{-11}$ (day^{-1}), where the GTR is given in units of cells/kg/day, to predict normal values of δ_M for humans of 0.09/day and 0.16/day for dogs.

To obtain an estimate for δ_E, note that there are on the order of 5×10^{9} nucleated red blood cells in the marrow.[48] The transit time through this compartment is about 180 hr,[49] and it is estimated that there are about four mitoses in the nucleated red blood cells,[50] so the cellular efflux from the normoblast compartment is $2^4\delta_E N^*$, and the influx into that compartment from the PPSC is $\delta_E N^*$. Thus in a steady-state situation in man δ_E is about 0.07/day. It is not known if the elevated GTR in dogs is reflected in an elevated erythrocyte production rate relative to man. However, I assume here that it is and take $\delta_E = 0.07$/day for normal humans and $\delta_E = 0.07 \times (1.65/0.87) = 0.13$/day for mongrel dogs.

The rate δ_T of differentiation into the thrombocyte line is derived from the observation that the megakaryocyte compartment in man is about 6.1×10^{6} cells/kg,[51,52] and that the mean time spent in the megakaryocyte compartment is approximately 2.5 days.[53] Thus the megakaryocyte production rate is $(6.1/2.5) \times 10^{6} = 2.44 \times 10^{6}$ cells/kg/day. This must be equal to $\delta_T N^*$, and thus in man $\delta_T = 3.81 \times 10^{-3}$/day. As before, a value for dogs is derived from the human value: $\delta_T = 7.23 \times 10^{-3}$/day.

These estimates predict a total rate of loss from G_0 into the differentiated hematopoietic lines of $\delta = 0.164$/day for normal humans and 0.297/day for normal dogs. Thus the total average cellular flux for each is $M^* = 1.05 \times 10^{8}$ and 1.91×10^{8} cells/kg/day, respectively.

Having obtained δ, the cell cycle time τ can be estimated directly from $\delta\tau = \frac{1}{9}$ (see Fig. 3). Independent measures for δ and τ do not exist in the literature. However, other workers have estimated the cell cycle time at between 12 and 36 hr.[54-57] The values for τ corresponding to the calculated mean flux into differentiation for both man and dogs are in agreement with the estimates of these other workers.

The stability analysis of Appendix 2 indicates that if N and P are started near the nonzero steady states [Eqs. (2.1)] they will always return to N^* and P^* when (2.6) is satisfied. For small initial values N_0 and P_0, $(N_0/N^*) \ll 1$ and $(P_0/P^*) \ll 1$, the solutions will also ultimately approach N^* and P^*. Computer solutions show that the growth characteristics of the stem cell populations under these conditions are typified by a period of exponential growth followed by a gradual slowing in the growth rate as the steady state is approached. These are the same growth properties noted in splenic colonies arising from the injection of marrow cells into irradiated recipients.[32] From these observations, a simple analysis for deriving estimates for β_0 from the exponential doubling time is possible.

For small values of N, $N \ll \theta$, the nonlinear equations (1.4) and (1.5) may be approximated (see Appendix 2) by the pair of linear time-delay differential equations

$$\frac{dN}{dt} = -(\delta + \beta_0)N + 2\beta_0 e^{-\gamma\tau}N_\tau, \qquad \tau > t, \tag{3.1}$$

$$\frac{dP}{dt} = -\gamma P + \beta_0 N - \beta_0 e^{-\gamma\tau}N_\tau, \qquad \tau < t. \tag{3.2}$$

In keeping with observations from computer simulations, I assume $N(t)$ to grow exponentially, $N(t) = N_0 e^{\lambda t}$, where λ is the growth rate. Thus Eq. (3.1) reduces to

$$\lambda + \delta + \beta_0 - 2\beta_0 e^{-(\gamma+\lambda)\tau} = 0. \tag{3.3}$$

With $N(t) = N_0 e^{\lambda t}$, Eq. (3.2) becomes a linear differential equation whose solution is

$$P(t) = P_0 e^{-\gamma t} + (N_0\beta_0/\lambda)(e^{\lambda t} - 1)(1 - e^{-(\gamma+\lambda)\tau}). \tag{3.4}$$

The doubling time for the cell population is defined as the time it takes $P + N$ to become twice its initial value, or

$$P(t_D) + N(t_D) = 2(P_0 + N_0). \tag{3.5}$$

Set $t = t_D$ in $N(t) = N_0 e^{\lambda t}$ and Eq. (3.4) and substitute the results into (3.5) to give

$$F_p(2 - e^{-\gamma t_D}) = (1 - F_p)[e^{\lambda t_D} + (\beta_0/\lambda)(e^{\lambda t_D} - 1)(1 - e^{-(\gamma+\lambda)\tau}) - 2], \tag{3.6}$$

where I have made use of the relation

$$P_0(1 - F_p) = N_0 F_p. \tag{3.7}$$

Equation (3.7) is a transcendental equation involving β_0, γ, t_D, F_p, τ, and λ. If values for t_D, F_p, γ, and τ are available, then (3.6) contains only two unknowns, β_0 and λ. Rewrite (3.3) as

$$\beta_0 = (\lambda + \delta)/(2e^{-(\gamma+\lambda)\tau} - 1) \tag{3.8}$$

and substitute the result into (3.6) to obtain a transcendental equation in λ alone, with δ known, that may be numerically solved. Having once obtained a value for λ by this procedure, β_0 is directly available from Eq. (3.8).

REFERENCES

1. Hoffman HJ, Guerry D, Dale DC: Analysis of cyclic neutropenia using digital band-pass filtering techniques. J Interdiscipl Cycle Res 5:1–18, 1974

2. Guerry D, Dale DC, Omine M, Perry S, Wolff SM: Periodic hematopoiesis in human cyclic neutropenia. J Clin Invest 52:3220–3230, 1973

3. Page AR, Good RA: Studies on cyclic neutropenia. Am J Dis Child 94:623–661, 1957

4. Morley AA, Carew JP, Baikie AG: Familial cyclical neutropaenia. Br J Haematol 13:719–738, 1967

5. Lund JE, Padgett GA, Ott RL: Cyclic neutropenia in grey collie dogs. Blood 29:425–461, 1967

6. Dale DC, Ward SB, Kimball HR, Wolff SM: Studies of neutrophil production and turnover in grey collie dogs with cyclic neutropenia. J Clin Invest 51:2190–2196, 1972

7. Dale DC, Alling DW, Wolff SM: Cyclic hematopoiesis: The mechanism of cyclic neutropenia in grey collie dogs. J Clin Invest 51:2197–2204, 1972

8. Deinard AS, Page AR: An improved method for performing neutrophil survival studies. Blood 36:98–110, 1970

9. Meuret G, Fliedner TM: Zellkinetik der Granuolopoese und des Neutrophilensystems bei einem fall von zyklischer Neutropenie. Acta Haematol 43:48–63, 1970

10. Dale DC, Graw, RG: Transplantation of allogenic bone marrow in canine cyclic neutropenia. Science 183:83–84, 1974

11. Weiden PL, Robinett B, Graham TC, Adamson J, Storb R: Canine cyclic neutropenia. A stem cell defect. J Clin Invest 53:950–953, 1974

12. Jones JB, Yang TJ, Dale JB, Lange RD: Canine cyclic haematopoiesis: Marrow transplantation between littermates. Br J Haematol 30:215–223, 1975

13. King-Smith EA, Morley A: Computer simulation of granulopoiesis: Normal and impaired granulopoiesis. Blood 36:254–262, 1970

14. Reeve, J: An analogue model of granulopoiesis for the analysis of isotopic and other data obtained in the non-steady state. Br J Haematol 25:15–32, 1973

15. Dale DC, Brown CH, Carbone P, Wolff SM: Cyclic urinary leukopoietic activity in grey collie dogs. Science 173:152–153, 1971

16. Guerry D, Adamson JW, Dale DC, Wolff SM: Human cyclic neutropenia: Urinary colony-stimulating factor and erythropoietin levels. Blood 44:257–262, 1974

17. McCulloch EA, Mak TW, Price GB, Till JE: Organization and communication in

populations of normal and leukemic hemopoietic cells. Biochim Biophys Acta 355:260–299, 1974

18. Stohlman F Jr, Quesenberry PJ: Colony stimulating factor and myelopoiesis. Blood 39:727–731, 1972

19. Adamson JW, Dale DC, Elin RJ: Hematopoiesis in the grey collie dog. Studies of the regulation of erythropoiesis. J Clin Invest 54:965–973, 1974

20. Patt HM, Lund JE, Maloney MA: Cyclic hematopoiesis in grey collie dogs: A stem cell problem. Blood 42:373–384, 1974.

21. Williams DM, Lynch RE, Cartwright GE: Drug-induced aplastic anemia. Semin Hematol 10:195–223, 1973

22. Boggs DR, Boggs SS: The pathogenesis of aplastic anemia: A defective pluripotential hematopoietic stem cell with inappropriate balance of differentiation and self-replication. Blood 48:71–76, 1976

23. Burns FJ, Tannock IF: On the existence of a G_0 phase in the cell cycle. Cell Tissue Kinet 3:321–334, 1970

24. Lajtha LG, Gilbert CW, Porteous DD, Alexanian R: Kinetics of bone-marrow stem-cell population. Ann NY Acad Sci 113:742–752, 1964

25. Fried W, Husseini S, Gregory S, Knopse WH, Trobaugh FE: Effect of cyclophosphamide on the hematopoietic microenvironmental factors which influence hematopoietic stem cell proliferation. Cell Tissue Kinet 6:155–163, 1973

26. Vassort F, Winterholer M, Frindel E, Tubiana M: Kinetic parameters of bone marrow stem cells using in vivo suicide by tritiated thymidine or by hydroxyurea. Blood 41:789–796, 1973

27. Patt HM, Maloney MA: Relationship of bone marrow cellularity and proliferative activity: A local regulatory mechanism. Cell Tissue Kinet 5:303–309, 1972

28. Vos O: Multiplication of haemopoietic colony forming units (CFU) in mice after x-irradiation and bone marrow transplantation. Cell Tissue Kinet 5:341–350, 1972

29. Wintrobe MM: Clinical Hematology (ed 7). Philadelphia, Lea & Febiger, 1976, p 1703

30. Becker AJ, McCulloch EA, Siminovitch L, Till JE: The effect of differing demands for blood cell production on DNA synthesis by hemopoietic colony-forming cells of mice. Blood 26:296–308, 1965

31. Lajtha LG, Oliver R: Studies on the kinetics of erythropoiesis. A model of the erythron, in Wolstenholme GEW, Cameron M (eds): Ciba Foundation Symposium on Haemopoiesis: Cell Production and its Regulation. London, Churchill, 1969, p 289

32. Sutherland DJA, Till JE, McCulloch EA: A kinetic study of the genetic control of hemopoietic progenitor cells assayed in culture and in vivo. J Cell Physiol 75:267–274, 1970

33. Rytomaa T, Kiviniemi K: Control of granulocyte production. II. Mode of action of chalone and antichalone. Cell Tissue Kinet 1: 341–350, 1968

34. Morley A, Trainor K, Blake J: A primary stem cell lesion in experimental chronic hypoplastic marrow failure. Blood 45:681–688, 1975

35. Morley A, King-Smith EA, Stohlman F Jr: The oscillatory nature of hemopoiesis, in Stohlman F Jr (ed): Hemopoietic Cellular Proliferation. New York, Grune & Stratton, 1969, p 3

36. Morley A, Stohlman F Jr: Cyclophosphamide induced cyclical neutropenia. N Engl J Med 282:643–646, 1970

37. Kennedy BJ: Cyclic leukocyte oscillations in chronic myelogenous leukemia during hydroxyurea therapy. Blood 35:751–760, 1970

38. Dale DC, Alling DW, Wolff SM: Application of time series analysis to serial blood neutrophil counts in normal individuals and patients receiving cyclophosphamide. Br J Haematol 24:57–64, 1973

39. Vassort F, Frindel E, Tubiana M: Effects of hydroxyurea on the kinetics of colony forming units of bone marrow in the mouse. Cell Tissue Kinet 4:423–431, 1971

40. Von Foerster H: Some remarks on changing populations, in Stohlman F Jr (ed): The Kinetics of Cellular Proliferation. New York, Grune & Stratton, 1959, p 382

41. Rubinow SI, Lebowitz JL: A mathematical model of neutrophil production and control in normal man. J Math Biol 1:187–225, 1975

42. Bullough WS: Mitotic control in adult mammalian tissues. Biol Rev 50:99–127, 1975

43. Cronkite EP, Vincent PC: Granulocytopoiesis, in Stohlman F Jr (ed): Hemopoietic Cellular Proliferation. New York, Grune & Stratton, 1969, p 211

44. Bishop CR, Rothstein G, Ashenbrucker HE, Athens JW: Leukokinetic studies. XIV. Blood neutrophil kinetics in chronic steady-state neutropenia. J Clin Invest 50:1678–1689, 1971

45. Dancey JT, Deubelbeiss KA, Harker LA, Finch CA: Neutrophil kinetics in man. J Clin Invest 58:705–715, 1976

46. Raab SO, Athens JW, Haab OP, Boggs

DR, Ashenbrucker H, Cartwright GE, Wintrobe MM: Granulokinetics in normal dogs. Am J Physiol 206:83 88, 1964

47. Deubelbeiss KA, Dancey JT, Harker LA, Finch CA: Neutrophil kinetics in the dog. J Clin Invest 55:833 839, 1975

48. Donohue DM, Reiff RH, Hanson ML, Betson Y, Finch CA: Quantitative measurement of erythrocytic and granulocytic cells of the marrow and blood. J Clin Invest 37:1571 1576, 1958

49. Killman SA, Cronkite EP, Fliedner TM, Bond VP: Mitotic indices of human bone marrow cells. I. Number and cytologic distribution of mitoses. Blood 19:743 750, 1962

50. Schumacher HR, Erslev AJ: Bone marrow kinetics, in Zirmai ES (ed): Nuclear Hematology. New York, Academic, 1956, p 89

51. Harker LA: Megakaryocyte quantitation. J Clin Invest 47:452 457, 1968

52. Harker LA, Finch CA: Thrombokinetics in man. J Clin Invest 48:963 974, 1969

53. Ebbe S, Stohlman F Jr: Megakaryocytopoiesis in the rat. Blood 26:20 35, 1965

54. Blumenson LE: A comprehensive modeling procedure for the human granulopoietic system: Overall view and summary of data. Blood 42:303 313, 1973

55. Blumenson LE: A comprehensive modeling procedure for the human granulopoietic system: Detailed description and application to cancer chemotherapy. Math Biosci 26:217 239, 1975

56. Korn AP, Henkelman RM, Ottensmeyer FP, Till JE: Investigations of a stochastic model of haemopoiesis. Exp Hematol 1:362 375, 1973

57. Vogel H, Niewisch H, Matioli G: Stochastic development of stem cells. J Theor Biol 22:249 270, 1969