From: BIOPHYSICAL AND BIOCHEMICAL INFORMATION TRANSFER IN RECOGNITION
Edited by Julia G. Vassileva-Popova and Elwood V. Jensen
(Plenum Publishing Corporation, 1979)

DYNAMIC HAEMATOLOGICAL DISORDERS OF STEM CELL ORIGIN

Michael C. Mackey

Department of Physiology, McGill University

Montreal, Quebec, Canada

1. INTRODUCTION

From one point of view physiological systems may be considered as biological control systems, while from another vantage point they are nonlinear dynamical systems. Whatever the bias, these systems are involved in the laying down and maintenance of structure (cellular proliferation and differentiation) and/or function (e.g., the cardiovascular, respiratory, endocrine, and central nervous systems). Global understanding of system functioning is complicated by the strong interdependence between structure and function, as well as the interdependence between systems subserving apparently different functions.

In normal healthy mammals the outputs of these systems accessible to observation and/or manipulation generally show a relatively constant behavior (e.g., tissue mass) or predictable periodicities (e.g., respiratory and cardiac rate, menstrual frequency). However, in a number of chronic and acute disease states these output variables display periodic behavior in place of constant behavior, or show a modified periodicity, or even periodicity eliminated in favour of aperiodic or "chaotic" behavior, such as dysmenorrhea, cardiac arryhthmias, various respiratory anomalies (Cheyne-Stokes respiration, apneustic breathing, Biot breathing) and some haematological disorders.

Elsewhere it has been argued that "...there is a large class of dynamical diseases...characterized by the operation of a basically normal control system in a region of physiological parameters that produces pathological behavior" (56). Clinical haematology offers an astonishing array of such examples in which pathology is

marked by periodic oscillation or chaos. Here I focus my attention on several of these dynamic haematological disorders, which have their origin at the pluripotential stem cell (PPSC) level, to demonstrate how modeling can offer unifying and simplifying hypotheses for the etiology of disease.

Section 2 offers a short outline of the organization and control of normal haematopoiesis as currently defined. Section 3 discusses several dynamic haematological diseases, and reviews the evidence that establishes their origin at the PPSC level. Section 4 develops a physiologically realistic model for the PPSC, the equations governing the dynamics of the cells in the PPSC, and details the general types of behavior that may be expected from the PPSC. Section 5 utilizes the formulation and properties of the PPSC model to offer a sufficient explanation for the dynamics of aplastic anemia (AA) and periodic haematopoiesis (PH). In the final section I discuss the dynamics of chronic myelogenous leukemia (CML) and its periodic variant in light of the simplest hypothesis for their origins, based on clinical evidence and the modeling of the PPSC.

2. ORGANIZATION AND CONTROL OF NORMAL HAEMATOPOIESIS

From a sufficiently removed position the organization of normal haematopoiesis can be viewed as follows. Based on several lines of evidence it is generally believed that there exists a self-maintaining pluripotential stem cell population (PPSC) capable of producing committed stem cells (CSC) for the erythroid, myeloid, or thromboid lines (51,62-64,97). The CSC populations are not self-maintaining and depend on a cellular flux from the PPSC for their continued integrity (94,97). Cells at the CSC level are induced to enter a proliferative phase for the myeloid and erythroid series where they customarily undergo about four divisions before losing their nucleus to enter a marrow maturational compartment (5,8,13,15,16,24,28,29,50,82,98,99,117). In the myeloid series, cells are then released from this marrow maturational compartment to enter the blood as a mature leukocyte (5,8,13,15,16,82, 98,99,117). For the erythroid series this last step is different in that there exists a sizeable maturational population of blood reticulocytes which preceed the mature erythrocyte (24,28,29,50, 117). In the thromboid series the process of nuclear division described above has cytoplasmic division as its analog in which the DNA of the recognizable megakaryocytes duplicates to a maximum value of 32 N (12,26,27,73,74,117). Megakaryocytes produce platelets at all ploidy values above 8 N, with the majority of the production occuring at 16 N.

There is a well established long range hormonal control operating in the erythroid series between the circulating

erythrocyte and the CSC (28,29,50,96). In its simplest outline, decreases in hemoglobin levels lead to a decrease in oxygen tension, which then stimulates the juxtaglomerular cells of the kidney to release a substance called erythropoietin (EP). EP acts at the level of the CSC to induce an increase in cellular flux from the PPSC to the erythroid series CSC. Ultimately, the erythrocyte mass and thus the oxygen carrying capacity of the blood are increased. Analogous reasoning has lead a number of investigators to look for similar regulators in the myeloid and thromboid series, and to date the existence of putative granulopoietins (GP) (84,85) and thrombopoietins (TP) (12) have been claimed. However, their mode and site of production and action remains to be elucidated. Colony stimulating factor (CSF) may be the granulopoietin (85).

There have been numerous reports concerning the partial isolation of mitotic inhibitory substances in the myeloid and erythroid lines, termed granulocyte chalone and erythrocyte chalone (49, 87, 89,90). Both of these substances appear to be produced by mature leukocytes and erythrocytes respectively and they inhibit the transition from the CSC to the earliest recognizable precurrsor cell (myeloblast and erythroblast).

In addition to the long range peripheral to marrow feedback mechanisms discussed above, short range control mechanisms exist within the PPSC acting to control cell population numbers. Although the details have not been elucidated, it seems that the PPSC regulates its size by adjusting the rate at which cells enter an active mitotic phase on the basis of the number of resting (G_0 phase) PPSC cells (4,10,25,33,38,52,64,78,103,105,109).

3. SEVERAL DYNAMIC HAEMATOLOGICAL DISEASES OF PPSC ORIGIN

In this section I briefly discuss the clinical and laboratory findings related to several haematological diseases which fall into one of two classes. Aplastic anemia and periodic haematopoiesis form the core of the discussion related to underproduction anomalies, while in the class of over production diseases, I discuss chronic myelogenous leukemia in both its typical and periodic form.

Periodic haematopoiesis (PH) (also known as cyclical neutropenia CN), is a disease characterized by an oscillation in circulating neutrophil numbers from normal to low values (41,43,19,77). In addition to the neutrophil oscillation, a concomitant oscillation of all the formed elements of the blood is observed (18,21,41,43). In humans these elements oscillate with a period of 17 to 28 days, and the phase differences between various cell lines are consistent with the differences in maturation times for each line (41,43,69, 77). All grey collies have this disorder, and the only apparent

difference between human PH and canine PH is the 11 to 12 day period of the latter (18,21,54).

In humans with PH and in grey collies the granulocyte turnover rate ranges from normal to low values (22,65) and the neutrophil half life is normal (21).

It has been demonstrated that normal haematopoiesis results when normal collie bone marrow is transplanted into an irradiated grey collie (17). Conversely, the transplantation of marrow from grey collies into irradiated normal collies is marked by the onset of PH in the recipients (44,110). These results demonstrate that a component of the marrow, probably the PPSC, is the primary location of the defect responsible for the peculiar dynamics of PH.

Cyclical neutropenia has received some attention from theoretricians who have examined the potential involvement of a long range (circulating to stem cell) humoral control mechanism in the genesis of the disorder (48,81). It is known that colony stimulating factor (CSF) levels in grey collies and man with PH oscillate with a period identical to the circulating cellular period (20,40). However, it is also known that CSF is not a regulator of the PPSC population (64,98). In the grey collie and in man with PH, serum EP levels display the same periodicity (1,40). However, in the grey collie phlebotomy or hypertransfusion, which respectively increase and decrease the EP levels, have no effect on the circulating neutrophil periodicity (1), implying the EP is not involved in the generation of PH. Thus, a significant involvement of peripherial to stem cell humoral feedback mechanisms in PH is unlikely.

The available clinical and laboratory data seems most consistent with the view that periodic haematopoiesis 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 (18,41,79,110).

Insight into the origin of PH is afforded by examining the effects of continuous cyclophosphamide and busulfan administration in normal dogs (67,70). Usually there is a simple progressive pancytopenia that becomes more severe the higher the drug dose. However, in some animals this pattern was altered to one in which low drug levels gave simple pancytopenia, higher levels resulted in PH (with a period of 11 to 17 days), and even higher dosages abolished the PH, leaving the dogs severely pancytopenia. The onset of PH, when it occurred, was at a circulating neutrophil level of one-half to one-third normal. Further, the appearance of PH in a number of patients undergoing continuous hydroxyurea therapy has been noted (45), as well as the existence of PH in one patient receiving cyclophosphamide (18).

Cyclophosphamide and busulfan are alkylating agents, while hydroxyurea is an antimetabolite (117). Hydroxyurea is known to selectively kill proliferating PPSC cells during DNA synthesis (78, 104). Likewise, cyclophosphamide kills stem cells (33), and thus the mode of action of all these agents may be interpreted as the selective destruction of actively proliferating PPSC cells.

Aplastic anemia (AA) is characterized by a mild to severe pancytopenia and hypocellular bone marrow, and is frequently observed as a result of chemotherapy and radiotherapy (116). It has been argued that the primary locus of the defect in AA is resident in the PSCC population, and that the PPSC in AA is abnormal with respect to proliferative function (6). This 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 (71).

Chronic myelogenous leukemia (CML) is a neoplastic disorder of the haematopoietic system generally characterized by an increase in circulating cells of the myeloid and thromboid series, and normal or decreased erythroid elements (105,117,118). Approximately 80 - 90% of all CML patients carry the abnormal Philadelphia (Ph) chromosome in their nucleated myeloid, erythroid and thrombocyte precurrsors (30,93,102,112,113). These chromosomal abnormalities, along with cell isozyme studies (31,32), establish a strong circumstantial case for the clonal origin of CML, probably in the PPSC.

The initial phase of CML is marked by a simple overproduction of cells which mature and function normally, and are eliminated from the circulation in a normal fashion (72,75). The rate of production of cells in this phase is estimated at 5 to 25 times normal (2,60). A secondary phase of variable length is characterized by overproduction and increased peripheral lifespan, resulting in further increases in circulating levels of leukocytes (34). During this secondary phase, granulocyte half life ranges from normal (4-10 hours) to 89 hours (2,8,60,61). There is a gradual transformation in the nature of the disease which usually terminates in a blast crisis similar to the typical clinical picture of acute myelogenous leukemia (AML) (117).

In CML, it has been a consistent finding that the labeling index (MI) of the earliest granulocyte precurrsor cells, the myeloblasts, are less than normal (36,36,47,75,106). Their grain count halving time is prolonged, while mitotic and DNA synthesis times appear to be normal (14-16,100). Estimates for the generation time in the myeloblast compartments range as high as 80 hours (36, 39,47,75), in contrast to the 18-20 hour estimate for normal myeloblasts (14-16).

Little is known of the operation of control processes in leukemic patients. However, utilizing granulocytes from rats with Shays myelocytic chloroleukemia (CHL) and from humans with CML it has been found that the leukemic cell concentration of granulocytic chalone is 0.1 to 0.025 times the normal value (88,91,92). The implication of these results is considered later.

In the past decade reports in the clinical literature indicate that CML has an interesting and provocative periodic variant, periodic CML (PCML). In 16 PCML patients it has been reported that the peripheral leukocyte and thrombocyte counts are not simply elevated but oscillate around elevated levels with a period of 35 to 70 days in the absence of any clinical intervention (11,23,35,37, 45,58,59,68,76,83,95,107). (The variation in the period of the oscillation is between patients). It has also been noted that periodic AML is sometimes found (59). Of these 16 PCML patients, 15 were in the chronic phase of their disease, while one was in blast crisis (58). Two actually showed a small oscillation in the erythroid series (11,83), while one also displayed a lymphocyte oscillation (35). The characteristics of PCML are similar to PH in that the peripheral oscillatory cell counts have the same period in a given patient but are out of phase. Peripheral leukocyte counts range from normal to high values.

There have been several attempts to explain PCML on theoretical grounds by considering a defective long range feedback control from the periphery to the PPSC compartment (48,114,115). The reports of cycling CSF levels in three PCML patients (11,35,83), out of phase with the leukocyte oscillation, have been taken as evidence for such a defect. However, the lack of correlation between CSF activity and peripheral leukocyte counts (111), the known action of CSF on the myeloid CSC rather than the PPSC (64, 98), and the fact that leukophoresis in one patient had no effect on the phase of the oscillation (13) discounts this theory.

Rather, as in PH, the largely circumstantial evidence points to the PPSC as the origin of the defect giving rise to CML and PCML. The view that the defect is not due to an alteration in long range humoral feedback mechanisms is given substantial support by the recent observation that oscillations in the number of S-phase CFU-C in CML patients can occur in the absence or in the presence of peripheral leukocyte oscillations (76). Thus it must be concluded not only that PCML originates in the PPSC, but also that oscillations at the PPSC level may be more common than demonstrated by peripheral blood studies.

4. THE MODEL

A schematic representation of the PPSC model is shown in Figure 1. A list of symbols used in defining the model is given in Appendix 1, and the equations describing the model are derived in Appendix 2. In what follows I offer heuristic arguments for the structure of these equations, and describe the general properties expected from the PPSC model.

Cells in the PPSC are either proliferating (population P, cells/kg) or resting (G_0) phase cells (population N, cells/kg) (7, 53,64). Cells travel through proliferation to undergo mitosis at a fixed time τ (d) from their time of entry into the proliferative state. Cells entering G_0 exit randomly to either re-enter proli-

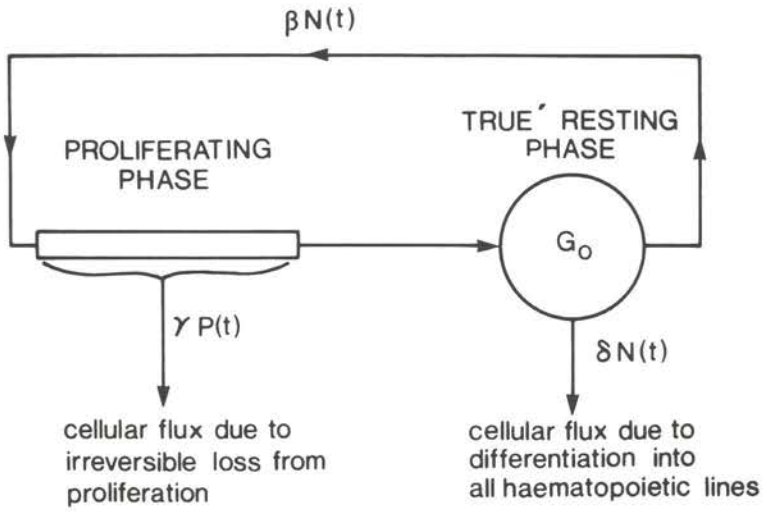

FIGURE 1: A schematic representation of the pluripotential stem cell population. Cells in the PPSC are in either a proliferating or a resting (G_0) phase. Cells travel through the proliferating phase in a fixed time, and all cells completing mitosis re-enter G_0. Out of G_0 there is a flux of cells to the proliferating phase, and a flux which represents the total differentiation out of G_0 into the myeloid, erythroid, and thromboid series. The flux γP represents an abnormal, irreversible, loss of cells from proliferation.

feration at a rate β (d^{-1}) or to irreversibly differentiate into the haematopoietic lines at a rate δ (d^{-1}). Although proliferating cells in the model may be irreversibly lost at a rate γ (d^{-1}) from any phase of the cell cycle, here a "normal" stem cell population will, by definition, be characterized by $\gamma = 0$. It is assumed throughout that γ, δ , and τ are constant with respect to time and cell population numbers.

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

$$\frac{dN}{dt} = -\delta N - \beta(N)N + 2\beta(N_\tau)\, N_\tau \exp(-\gamma\tau), \qquad \tau < t \qquad (1)$$

where $N_\tau = N(t - \tau)$. In Equation 1 the total rate of change of N (t) is equated with the sum of three terms. The first term on the right hand side of (1) accounts for irreversible loss from the G_0 population due to differentiation, and the second corrects for cell loss due to the movement of cells into proliferation. The last term represents cellular gain due to the movement of proliferating cells into G_0 one generation time ago; the factor 2 accounts for mitosis, and exp $(-\gamma\tau)$ corrects for the probability of irreversible cellular loss from the proliferating population before the completion of mitosis.

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

$$\frac{dP}{dt} = -\gamma P + \beta(N)N - \beta(N_\tau)N_\tau \exp(-\gamma\tau), \qquad \tau < t \qquad (2)$$

The first term on the right hand side of (2) is an irreversible loss term, while the second represents cellular entry from the G_0 phase. The flux of cells from the proliferating to the non-proliferating phase one generation time previously is accounted for by the last term.

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 (4,10,25,33,38,52,64,78,103-105, 109). Without specifying the exact nature of control I assume that the PPSC population is capable of recognizing the number of G_0 phase cells and changing the mitotic re-entry rate β in response to changes in N. Specifically, the functional form chosen for β (N) is

$$\beta(N) = \frac{\beta_0 \theta^n}{\theta^n + N^n} \qquad (3)$$

where β_0 (d^{-1}), θ (cells/kg), and n are parameters. When the G_0 population is small β approaches a maximum, and when N increases β decreases. The parameter β_0 is the maximal rate of cellular entry into proliferation, and θ 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 relation, which occurs at $N = \theta$, is $(-n\beta_0/4\theta)$ where n is a dimensionless number. The qualitative predictions of this model are independent of the form β as long as $\beta(N)$ is a monotone decreasing function of N with a finite maximum.

The form for $\beta(N)$ can be argued for in a number of ways, two of which are as follows. First assume each cell contains a receptor for a mitotic regulatory molecule (90). 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 further that: 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 the regulatory molecule; 2) The equilibrium constant for the reaction is K, so $[M]\,[C]^n = K[L]$, where $[\ ]$ denotes 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, $[C] = \alpha N$, and the maximal rate of entry into the cell cycle to be β_0 thus gives the form for β given in equation 3, where

$$\theta^{-1} = \alpha \sqrt[n]{K}$$

Alternately one could think of each cell containing a switch like mechanism which allows the cell to enter the proliferative phase as long as the number of inactive receptors does not exceed a threshold number of ϕ. Once the threshold ϕ is exceeded, the cell must remain in G_0. Further assume that for all of the cells in the population the threshold numbers ϕ are distributed with a probability density function $f(\phi) = n\theta^n\phi^{n-1}/(\theta^n + \phi^n)^2$, so $\beta = \beta_0 \left[1 - \int_0^N f(\phi)d\phi\right]$ is as given in (3).

With this choice for the control function β, equations 1 and 2 become:

$$\frac{dN}{dt} = -\delta N - \frac{\beta_0\theta^n N}{\theta^n + N^n} + \frac{2\beta_0\theta^n N_\tau}{\theta^n + N_\tau^n}\exp(-\gamma\tau), \qquad \tau < t \tag{4}$$

and

$$\frac{dP}{dt} = -\gamma P + \frac{\beta_0\theta^n N}{\theta^n + N^n} - \frac{\beta_0\theta^n N_\tau}{\theta^n + N_\tau^n}\exp(-\gamma\tau), \qquad \tau < t \tag{5}$$

respectively.

Equations 4 and 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:

$$N^* = \theta\left[\frac{\beta_0}{\delta}\ [2\exp(-\gamma\tau)-1] \quad -1\right]^{1/n} \tag{6}$$

$$P^* = N^*(\delta/\gamma)\ \ \frac{1 - \exp(-\gamma\tau)}{2\exp(-\gamma\tau)-1}$$

P^* and N^* will be physiologically meaningful (non-negative) only if

$$0 < \gamma\tau < \ln\left[\frac{2\beta_0}{\beta_0 + \delta}\right] < \ln 2 \tag{7}$$

In the "normal" state there is no loss of cells from proliferation ($\gamma = 0$). From (6) the limiting steady state cellular populations in this case become

$$N^* = \theta\left[\frac{\beta_0}{\delta} - 1\right]^{1/n} \tag{8}$$

$$P^* = \delta\tau N^*$$

while the condition (7) becomes $(\beta_0/\delta) > 1$.

In an attempt to understand the dynamics of PH and PCML, first it is necessary to have a clear idea of the behavior of the hypothetical PPSC population described by (4) and (5). Although it is not possible to completely characterize the dynamics of P(t) and N(t) analytically, it is possible to make restricted comments about the nature of P(t) and N(t) near their steady state values P^* and N^* as given by (6) or (8).

From the considerations of Appendix 3, the behavior of the cellular populations P(t), N(t) near their steady state values P^* and N^* falls into one of two categories. Thus for initial population values P_0, N_0 sufficiently close to P^*, N^* (i.e., such that $|(P_0 - P^*)/P^*| \ll 1$, $|(N_0 - N^*)/N^*| \ll 1$), P(t), N(t) will approach P^*, N^* in a smooth or damped oscillatory fashion or they will oscillate about P^*, N^* with a period T.

Rather than present the analytic formulation of these results (c.f., Appendix 3) I have presented them graphically in Figure 2a,

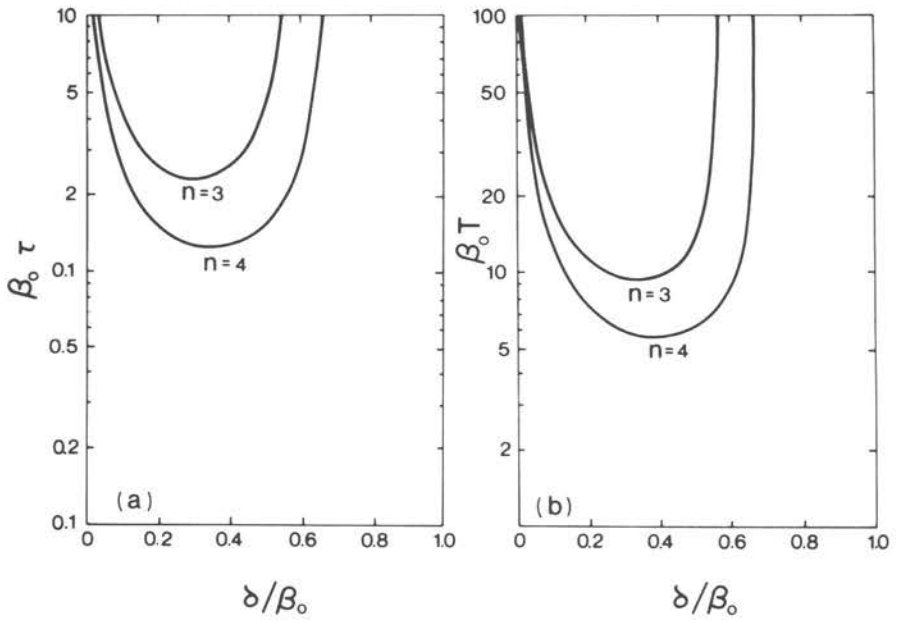

FIGURE 2: A graphical presentation of the stability analysis of equations 4 and 5 when $\gamma = 0$. See the text for details.

drawn for $\gamma = 0$. The concave up curves in that figure give, for representative n values of 3 and 4, the boundaries between these behaviors. Thus, for a given n, all values of $(\delta/\beta_0, \beta_0\tau)$ below the concave up curve ensure that, for small deviations away from the steady states (8), the cellular populations obtained as solutions to (4) and (5) will return to their steady state values. For values of $(\delta/\beta_0, \beta_0\tau)$ on one of the concave up curves, periodic solutions with period T, as shown in Figure 2b, occur. Similar conclusions hold for $\gamma \neq 0$. Generally speaking, an increase in any one of the five parameters $(\gamma,\delta,\beta_0,n,\tau)$ with the other four held constant may lead to the loss of stability at a steady state and the appearance of oscillatory solutions.

For large deviations away from the steady state, as well as for values of $(\delta/\beta_0, \beta_0\tau)$ which lie above one of the curves of Figure 2a, the behavior of the cellular populations cannot be predicted from this analysis. To investigate the properties of the model under these conditions, numerical solutions to (4) and (5) were obtained using a predictor-corrector integration scheme with an integration step size of 0.01.

Briefly, the results of many computed solutions to (4) and (5) with a range of parameters and initial conditions indicate that for all points $(\delta/\beta_0, \beta_0\tau)$ which are below one of the concave up curves of Figure 2a, the cellular populations always approach the steady states defined by equation 8 (or equation 6 for $\gamma \neq 0$). Furthermore, the concave up lines in Figure 2a accurately predict the onset of periodic variations in P(t) and N(t). At the onset of the periodic population variations, the period T of the oscillation is given by the relations shown in Figure 2b. Finally, for values of the parameters $(\delta/\beta_0, \beta_0\tau)$ above one of the lines in Figure 2a, complex periodic or aperiodic dynamic behavior in P(t) and N(t) may be found.

In order to examine the possible origins of PPSC derived pathologies within the context of this model, realistic estimates of the parameter values for the PPSC are needed. The determination of these parameters is tedious but straightforward, and has been considered in detail elsewhere (55). These values are summarized in Table 1 for a normal man and a normal dog.

5. A HYPOTHESIS FOR THE ORIGIN OF APLASTIC ANEMIA AND PERIODIC HAEMATOPOISIS

In light of the findings discussed in Section 3 on the effects of mitogenic agents, it is likely that AA and PH may both have a common origin within the PPSC. More specifically they both might be encountered as a single dynamic parameter within the PPSC population is varied. Since the populations of the stem cell model are capable of sustained oscillations when $\gamma = 0$, the most parsimonious

TABLE 1: PPSC parameter values for a normal man and a mongrel dog (55). These values are derived on the assumption that $\gamma = 0$ (116), the proliferating fraction $F_p = P^*/(P^* + N^*) = 0.10$ (4), $P^* + N^* = 7.14 \times 10^8$ cells/kg (16), the doubling time for the PPSC during exponential growth is 24 hours (101), and that the cellular differentiation flux out of G_0 is $M^* = 1.05 \times 10^8$ cells/kg/d for humans and 1.91×10^8 cells/kg/d for dogs (55).

Parameter	Units	Man	Dog
δ	d^{-1}	0.16	0.30
β_0	d^{-1}	1.43	1.39
τ	d	0.68	0.37
θ	cells/kg	3.22×10^8	4.66×10^8
n	-	3	4

assumption would be that AA and PH are due to the alteration of one of the parameters β_0, δ, n or τ.

In the normal case ($\gamma = 0$), the total steady state cellular flux, M* (cells/kg/d), out of the G_0 stem cell compartment into the differentiated haematopoietic lines is δN^*, or

$$M^* = \delta N^* = \delta\theta\left[\frac{\beta_0}{\delta} - 1\right]^{1/n} \qquad (9)$$

from equation 8. If we ask what single parameter to vary in order to decrease M* a number of possibilities appear. It is clear that a decrease in θ will be effective. However (Appendix 3) this will never lead to an oscillation in M*. From (9), a decrease in β_0 from its normal value (but never such that $\beta_0 < \delta$) will also decrease M*. However, a stable oscillatory state can never be reached with the ranges of other normal parameters (δ,τ,n) given in section 4. Also, decreases in δ from the normal value will lead to a decreased M* but, again, an oscillatory state cannot be reached in this fashion. Finally, M* may be decreased by increasing n. It is common in aplastic anemia to find a pancytopenia of 50% or more (116). 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 humans to account for the pancytopenia noted in AA. This seems an untenable notion on several grounds, not the least of which is that the analysis of Appendix 3 indicates that PH will occur long before any detectable pancytopenia occurs.

Based on the above considerations, I conclude that there is no way in which AA and PH can be explained in a single, unified way within the context of the normally operating stem cell populations. It is to be noted, however, that AA alone could be due to a decrease in any one or all of the parameters θ, δ, and β_0.

Thus in searching for a unified basis for AA and PH, attention is sharply focused on the proliferative loss rate, γ, from the PPSC. This is consistent with the effects of mitogenic drugs discussed in Section 3, and within the context of this study I interpret the mode of action of all of these agents to be one which results in $\gamma \neq 0$. If γ is not zero in the stem cell population, then the total steady state cellular flux out of the G_0 compartment into the differentiated haematopoietic lines is given from equation 6 by

$$M^* = \delta\theta\left[\frac{\beta_0}{\delta}\left[2\exp(-\gamma\tau)-1\right] - 1\right]^{1/n} \qquad (10)$$

It can be shown that M*, as given by (10), continuously decreases as γ increases up to the limiting value given by equation 7. Thus, increasing the rate at which the PPSC loses cells from proliferation results in a generalized pancytopenia and, presumably, a marrow hypocellularity. With the normal parameter values for humans given in Table 1, and with increasing γ the proliferating fraction, $F_D = P^*/(P^* + M^*)$, continuously increases as does the doubling time during exponential growth, t_D.

For an initial ($\gamma = 0$) M* less than the mean for humans, Figure 3 indicates that 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/d, a change in the qualitative behavior of the PPSC becomes apparent as γ is increased. Thus, for an initial M* of 3.81×10^7 cells/kg/d a progressive increase of γ leads to a progressive decrease in the M*. Based on the analysis of Appendix 3, it is predicted that when $\gamma = 0.30$ d^{-1} the M* will no longer be depressed and constant, but will start to oscillate with a period of 16.5 days which I interpret as the onset of PH. 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 $\gamma = 0.33$ d^{-1} (with a period of 19 days) PH ceases and a steady pancytopenia reappears.

In Figure 4 I show a sequence of computed differentiation fluxes as a function of time assuming an initial M* of 3.22×10^7 cells/kg/d and all other parameters corresponding to this M*, as given in Figure 4. The solutions shown in Figure 4 start with the normal steady state values for N* and P* and show the expected changes in M* over a 100 day interval if, at $t = 0$, γ is suddenly changed from $\gamma = 0$ to the values indicated. For values of γ less than 0.24 d^{-1} there is a simple depression in M* below the normal steady state values. For values of γ in the range in which periodic haematopoiesis is predicted (c.f. Figure 3, γ between 0.24 and 0.29 d^{-1}) the total differentiation flux oscillates, initially with a period of approximately 19 days which lengthens to 25 days for γ near 0.29 d^{-1}. For γ greater than 0.29 d^{-1}, M* is even more depressed but constant.

The PPSC population dynamics displayed in Figure 4 adequately duplicate the qualitative and quantitative properties of aplastic anemia and periodic haematopoiesis in humans.

The same qualitative sequence of behaviors shown in Figures 3 and 4 is found for higher values of the parameter n. The quantitative differences in this pattern as n is increased are two fold. First, the larger the value of n, the larger the initial flux into differentiation at which the periodic haematopoiesis will initially be found; and secondly, the larger the value of n, the shorter the

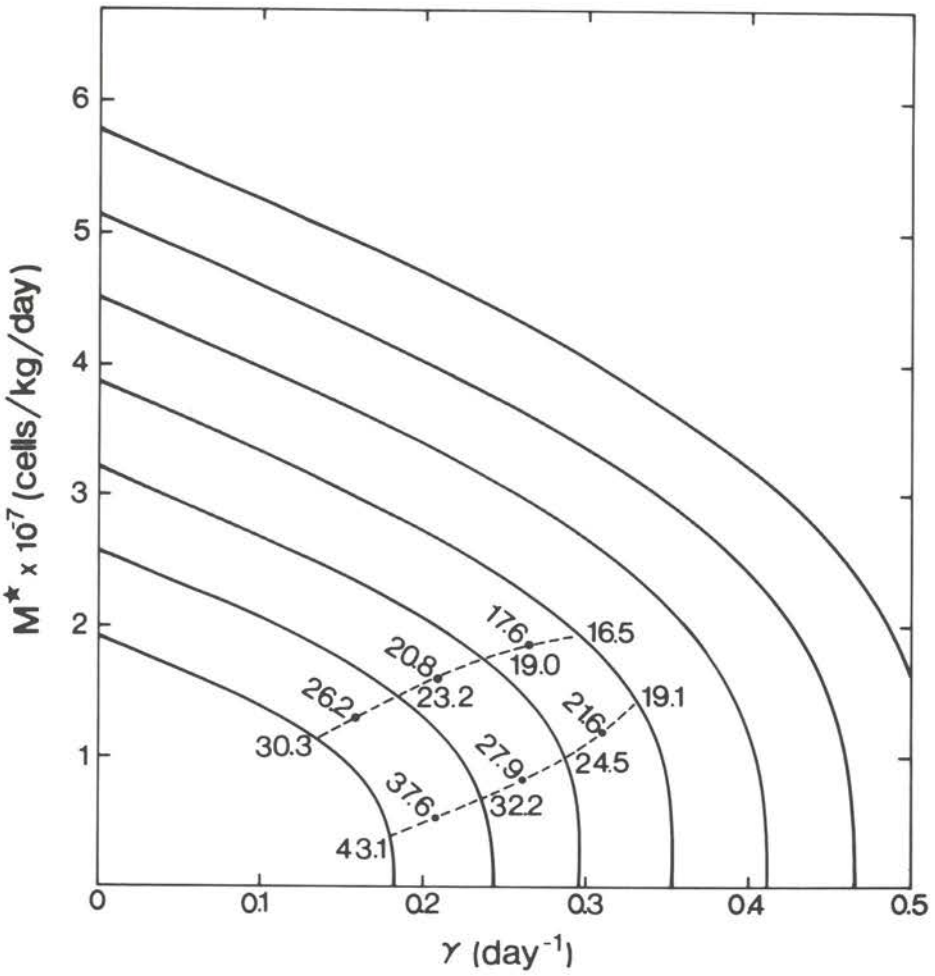

FIGURE 3: Variation in the total cellular differentiation flux as a function of the irreversible loss rate (γ) of cells from proliferation for humans (n = 3). The PPSC parameter values corresponding to each curve, from the top down, are: (δ, β_0, τ, $\theta \times 10^{-8}$) = (0.09, 1.58, 1.23, 2.52); (0.08, 1.62, 1.39, 2.40); (0.07, 1.66, 1.59, 2.27); (0.06, 1.71, 1.85, 2.13); (0.05, 1.77, 2.22, 1.98); (0.04, 1.84, 2.78, 1.81); and (0.03, 1.91, 3.70, 1.62), (d-1, d^{-1}, d, cells/kg). The dashed lines indicate the boundaries within which stable periodic haematopoiesis is predicted; the numbers along the dashed lines indicate the period (in days) of the oscillation.

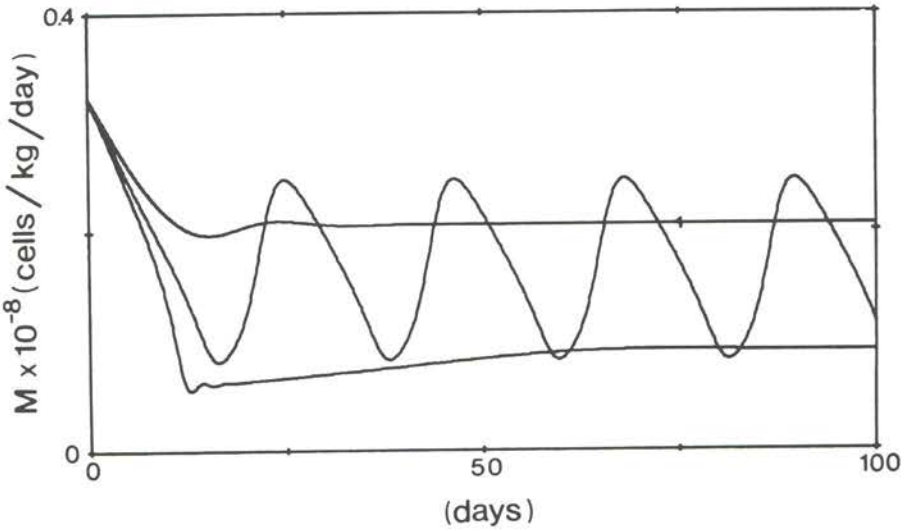

FIGURE 4: The computed cellular efflux from G_0 over a 100 day period for three different values of γ in humans. The parameters used in the simulation were: $\delta = 0.05\ d^{-1}$, $\beta = 1.77\ d^{-1}$, $\tau = 2.22$ d, $\theta = 1.98 \times 10^8$, $P_0 = 0.71 \times 10^8$, $N_0 = 6.43 \times 10^8$ cells/kg, and $n = 3$. The top curve which reaches a steady state corresponds to $\gamma = 0.20\ d^{-1}$; the middle oscillatory flux is for $\gamma = 0.25\ d^{-1}$; and the bottom curve is based on $\gamma = 0.29\ d^{-1}$.

period of the oscillation. Thus, it is clear than an explanation of the patterns of PH in grey collies, and the results of continuous administration of cyclophosphamide and busulfan, will require a value of n greater than for humans.

These points are illustrated in Figure 5 where, for $n = 4$ and an initial M^* of 5.19×10^7 cells/kg/d, I show the sequence of behaviors encountered in M^* as γ is increased. The analysis of Appendix 3 indicates that mild AA should result for all values of γ less than $0.31\ d^{-1}$, that PH (with an initial period of 10.4 days) will be present for γ between 0.31 and $0.44\ d^{-1}$, and that severe hypocellularity and pancytopenia will appear for γ greater than $0.44\ d^{-1}$.

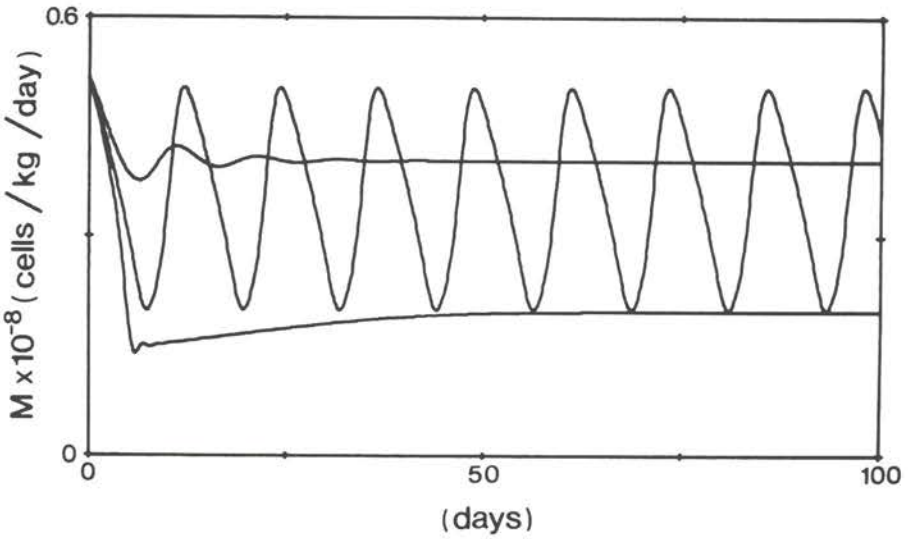

FIGURE 5: The simulated variation in the cellular efflux out of G_0 into differentiation over a 100 day period in dogs with three levels of cell loss from proliferation. P_0 and N_0 as in Figure 4, and the other parameters were: $\delta = 0.08\ d^{-1}$, $\beta_0 = 1.62\ d^{-1}$, $\tau = 1.39$ d, $\theta = 3.07 \times 10^8$ cells/kg, and $n = 4$. The proliferative loss rates are: $\gamma = 0.25\ d^{-1}$ for the top flux which reaches a steady state; $\gamma = 0.35\ d^{-1}$ for the oscillatory flux; and $\gamma = 0.45$ for the greatly depressed but eventually constant cellular efflux.

The analysis in Appendix 3 of the PPSC population dynamics, and the results of that analysis displayed in Figures 3 through 5, indicates that increasing the proliferative loss rate will either result in a decrease in the total PPSC population numbers, accompanied by a generalized and steady pancytopenia; or in 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 haematopoiesis is that they are both due to irreversible cellular loss from the proliferating pluripotential stem cell compartment.

6. THE DYNAMICS OF NORMAL AND PERIODIC CHRONIC MYELOGENOUS LEUKEMIA

In the searching for potential explanations for the kinetic behavior observed in CML and PCML, a few facts appear to be significant. First, the enormous increase in CFU-C cells found in CML patients (72,76) must, within the context of the present formulation, represent an increase in CSC numbers. This, in turn, is to be interpreted as an increase in the PPSC G_0 cell population numbers, N^*. Secondly, the elevation in the flux of cells, M^*, into differentiation in all but the erythroid series could represent an increase in N^* and/or the rate of differentiation out of G_0. Finally, the observations on the LI and MI of CML myeloblasts have been interpreted as indicating an increase in the generation time in the myeloblast compartment. I assume that the increase in myeloblast generation time is a reflection of a corresponding increase in the cell cycle time of the PPSC proliferating cells.

An increase in δ from its normal value with all other parameters normal will give an initial increase in M^* up to $\delta = 2\beta_0/3$ for $n = 3$. Thus, increases in δ are capable of increasing M^* to a maximum of 2.30 of normal. However this would, at the same time, result in a reduction in N^* to 0.40 of normal. Therefore, changes in δ lead to changes inconsistent with the observations in CML. A simple increase in δ with other PPSC parameters normal cannot lead to oscillatory PPSC dynamics.

Although increases in β_0 are capable of increasing both M^* and N^*, these two quantities are relatively insensitive to changes in β_0 due to their cube root dependence on β_0. To elevate M^* and N^* to five times normal with all other parameters normal would require an increase in β_0 by a factor of 1.15×10^2. Similarly, an increase by a factor of 25 in M^* and N^* would require a multiplicative increase in β_0 by 1.43×10^4. Thus, massive increases in β_0 could account for the elevated M^* and N^* of CML. Note, however, that an increase in β_0 alone with all other PPSC parameters at their normal value cannot give rise to oscillatory PPSC dynamics.

A somewhat simpler interpretation of the increases in N^* and M^* seen in CML is available if it is assumed that θ is increased. The quantities M^* and N^* are directly proportional to θ and thus proportional increases in θ will give exactly the same multiplica-

tion in P^*, N^*, and M^*. As pointed out in section 3, the per cell concentration of granulocyte chalone in CHL rat granulocytes is 0.10 to 0.025 times normal. If the mechanism of chalone action in CHL is interpreted as control within the PPSC was rationalized (mitotic regulatory molecule), and it is assumed that this is simply a reflection of a more basic control defect within the PPSC, then these results would correspond to an α of 0.10 to 0.025 normal value. Since (section 4) $\theta^{-1} = \alpha \sqrt[n]{K}$, this would imply an elevation in θ by a factor of 10 to 40. Recall however, that alterations in θ are incapable of qualitatively changing the PPSC dynamics, e.g., inducing oscillatory behavior.

On this basis I assume, as have others in modeling AML (86), that in CML there coexist within the PPSC two populations of cells. The normal cells have dynamics described by equations 1 and 2, while the leukemic cells are described by equations analogous to (1) and (2) with different parameter values and a different control function

$$\beta_L = \beta_{0L} \frac{\theta_L^n}{\theta_L^n + N_L^n}$$

where N_L is the population of G_0 leukemic cells.

The dynamics of such a population of PPSC cells is reflected in the total flux out of the PPSC into differentiation as shown in Figure 6 for the curve labeled "a" which corresponds to $\theta_L = 25\theta$ and all other PPSC parameters set at the normal values of Table 1. It is assumed that at $t = 0$ some transformation gives rise to one leukemic cell in a G_0 state. As may be seen in Figure 6, there is no apparent effect of this leukemic induction for about 40 days. For this initial period the leukemic cell population is growing exponentially, but the total leukemic population numbers are so low that the leukemic flux M^* is negligible. At about 40 days a dramatic exponential increase of the cellular flux M^* becomes apparent. M^* increases beyond this point in time to finally approach a steady state value of 26.25×10^8 cells/kg/day, 25 times the normal value.

Thus, in my opinion the simplest explanation available for the large increases in PPSC numbers and the overproduction noted in the myeloid and thrombocyte series of CML patients is that the parameter θ in the proposed control function (3) is greatly elevated from its normal value. This notion is qualitatively consistent with known properties of mature granulocytes in CHL rats.

It must be supposed that whatever control, if any, that operates between the periphery and the CSC for the myeloid and thromboid series elements is unable to compensate for this massive increase in PPSC numbers. On the other hand, erythroid series

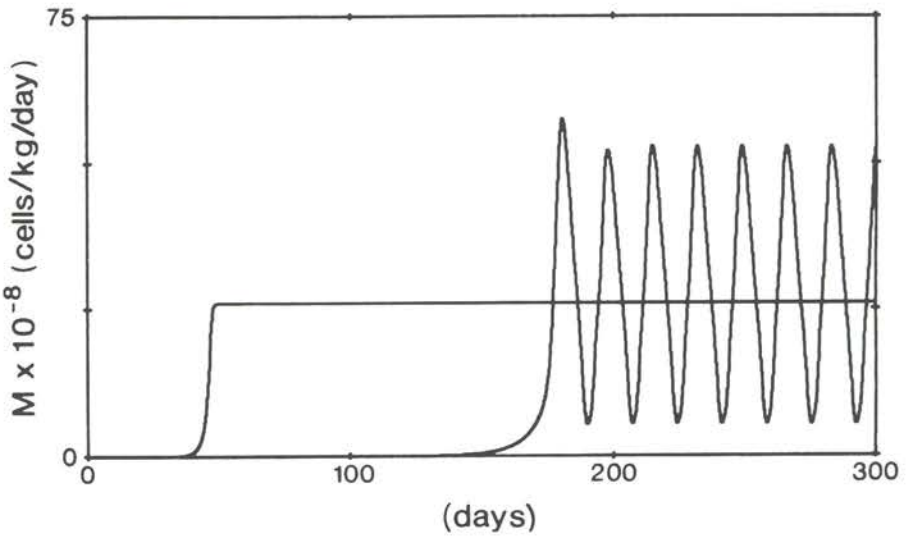

FIGURE 6: The onset of CML and PCML. The computed flux out of G_0, shown as a function of time over 300 days in curve a, was based on a normal set of PPSC parameters except for $\theta = 8.05 \times 10^9$ cells/kg. A parallel increase in the cell cycle time to four days, curve b, leads to a stable oscillatory cellular differentiation efflux with a period of about 17 days. In both cases, $P_0 = 0$, $N_0 = 1$ cell/kg.

control via erythropoietin is apparently capable of dealing with such massive influxes into the erythroid CSC and reducing δ_E appropriately to maintain normal erythropoiesis.

Although this picture a offers a qualitatively satisfying explanation for the early phases of CML, it offers no interpretation for the gradual transformation of CML to the blastic crisis characteristic of AML. Further, though an increase in θ seems the most likely candidate to explain the inferred increases in M* and N* in CML, it sheds no light on the dynamics of PCML. Nor, apparently, do possible changes in δ and/or β_0. It is from the increases in myeloblast cell cycle time, and the assumption that this reflects a corresponding change in the PPSC, that a clue to the origin of the dynamics of PCML comes.

From the analysis of Appendix 3, it is predicted that in humans the steady states N*, P* will be stable for all generation times $\tau < 2.66$ d with all other dynamic PPSC parameters held at their normal values. At a $\tau \simeq 2.66$ d, a Hopf bifurcation (57) occurs in the dynamics of the PPSC, and it is predicted that periodic variations in N(t), P(t) about their steady state values will ensue. The predicted period of these oscillatory excursions is 12.21 days.

The consequences of an elevation in the cell cycle time within the PPSC are illustrated in Figure 6. There, I show the total computed flux $M = \delta N(t)$ out of the PPSC into differentiated cells lines as a function of time. In the part of the figure labeled "a", the cell cycle time is at its normal value of $\tau = 0.64$ d, while in the "b" curve $\tau = 4$ d. All other PPSC parameters were set at their normal values given in Table 1, except for $\theta = 8.05 \times 10^8$ cell/kg, and for both computations it was assumed that there was initially one cell in the G_0 phase. As pointed out above, there is an approximate 40 day induction phase, followed by a rapid approach to the steady state $M^* = 26.25 \times 10^8$ cells/kg/d in the $\tau = 0.64$ d case. The increase to $\tau = 4$ d has two expected consequences. First, there is a dramatic prolongation in the induction period to more than 100 days because of the elevated doubling time associated with increased cell cycle time. Secondly, as predicted in Appendix 3, the steady state is no longer stable but rather shows stable oscillatory excursions in M(t) about the formerly stable steady state, M*. The period of the oscillation is about 17 days, and the ratio of the maximum to the minimum is 10.3.

If, indeed, the defect responsible for the oscillatory dynamics in some CML patients is simply an increase in the cell cycle time τ above the critical value of about 2.66 d with all other dynamic PPSC parameters near their normal values, then the oscillatory efflux would be expected to have a period in the range from about 12 days to about 17 days. This, at first glance, is at variance with the findings in PCML. However, there is good reason to believe that the reported periods of neutrophil and platelet oscillations in PCML are not accurate, and indeed are spurious because of sampling errors.

To see the influence of the sampling period on the apparent dynamics, refer to Figure 7. All three computed cellular differentiation fluxes shown there are based on $\tau = 4$ d, $\theta = 8.05 \times 10^9$ cells/kg, all other PPSC parameters as given in Table 1 for humans, and $P_0 = 0.71 \times 10^8$, $N_0 = 6.43 \times 10^8$ cells/kg. Figure 7a shows the cellular flux M(t) out of G_0 based on samples taken at ten equally spaced times per day (i.e., every 2.4 hours), and the flux has a period of approximately 17 days as in Figure 6b. The patterns shown in Figures 7b and 7c are examples of the well known anomalies that can appear when periodic processes are sampled at a frequency

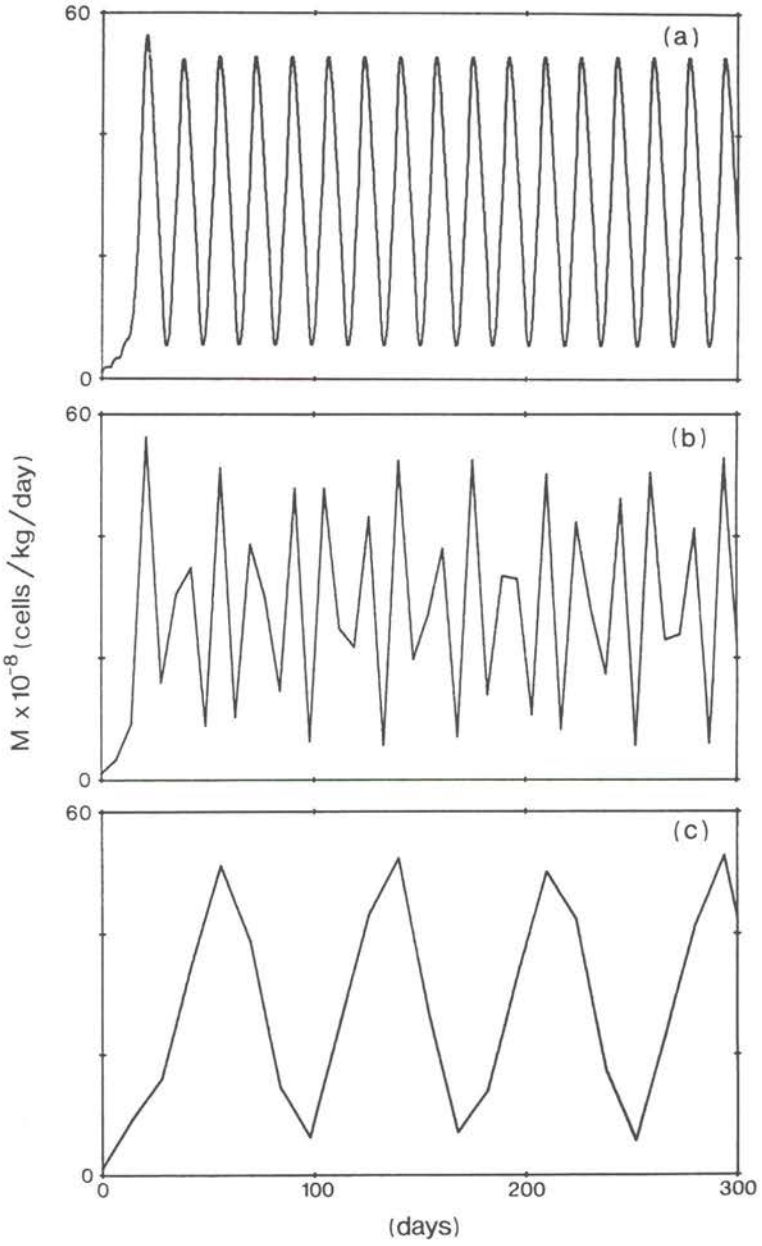

FIGURE 7: The effect of sampling frequency on the apparent dynamics of simulated PCML. See text for details.

too low compared with the frequency of the process. Thus, in Figure 7b a sampling interval of seven days produces a record of cellular efflux with an almost random or noisy pattern. In Figure 7c, constructed from an assumed biweekly sampling period, a strong "beat" phenomenon is noted between the underlying cyclical flux and the sampling procedure to produce an apparent periodicity of about 5 x 17 = 85 days.

For obvious limitations related to patient comfort and/or the availability of clinical facilities, obtaining samples from PCML patients may not be indicated more frequently than once weekly. However, from the simulation results of Figure 6, and the phenomena related to sampling frequency shown in Figure 7, it seems that in some instances PCML dynamics may be occuring on a time scale more rapid than previously thought. In order to obtain a more complete picture of this unusual and potentially instructive disease, more frequent peripheral blood determinations may be warranted when they do not compromise the total care of CML patients.

APPENDIX 1: LIST OF ABBREVIATIONS, PARAMETERS, AND VARIABLES

AA	Aplastic anemia
CN	Cyclical neutropenia
PH	Periodic haematopoiesis
AML	Acute myelogenous leukemia
CML	Chronic myelogenous leukemia
PCML	Periodic chronic myelogenous leukemia
CHL	Shay's myelocytic chloroleukemia
PPSC	Pluripotential stem cell
CSC	Committed stem cell
EP	Erythropoietin
TP	Thrombopoietin
GP	Granulopoietin
CSF	Colony stimulating factor
CFU-S	Splenic colony forming unit
CFU-C	Culture colony forming unit
d	Days
h	Hours
kg	Kilogram
P (t)	The density (cells/kg) of proliferating phase stem cells as a function of time
N (t)	The resting (G_0) phase stem cell population density (cells/kg) as a function of time
P*	The steady state proliferating phase stem cell density (cells/kg)
N*	The steady state G_0 phase stem cell density (cells/kg)
τ	The cell cycle time, or time spent by a cell in the proliferating phase (d)

δ_M The rate (d^{-1}) of differentiation out of the G_0 phase of the PPSC into the myeloid series. A subscript E or T denotes the analogous rate into the erythroid and thrombocyte series respectively.

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

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

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

β The mitotic re-entry rate (d^{-1}), or rate of cell movement from G_0 into proliferation.

β_0 The maximal rate (d^{-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 A dimensionless number characterizing the sensitivity of the mitotic re-entry rate β to changes in the size of G_0.

APPENDIX 2: DERIVATION OF THE EQUATIONS DESCRIBING THE PPSC DYNAMICS

To describe the variation in the numbers of cells in the PPSC model (refer to Figure 1 and Section 4), I pick time and maturity as independent variables for the proliferating cells, and time and age for the non-proliferating cells (108). If $p(m,t)$ and $n(a,t)$ are the cell density functions for the proliferating and non-proliferating cells, then it can be shown (57) that they satisfy the equations

$$\frac{\partial p}{\partial t} + \frac{\partial p}{\partial m} = -\gamma p(m,t), \qquad 0 \leq m \leq \tau \tag{2.1}$$

and

$$\frac{\partial n}{\partial t} + \frac{\partial n}{\partial a} = -(\beta + \delta)n(a,t), \qquad 0 \leq a \tag{2.2}$$

where maturity and age are denoted by m and a respectively.

The total populations at any point in time are obtained by integrating over the maturity or age variable. Thus

$$P(t) = \int_0^{\tau} p(m,t)\, dm \tag{2.3a}$$

and

$$N(t) = \int_0^{\infty} n(a,t)\, da \tag{2.3b}$$

To complete the description of the model, initial and boundary conditions must be set, and I take

$$p(m,0) = g(m)$$

$$n(a,0) = h(a) \tag{2.4}$$

and

$$p(0,t) = \beta N(t)$$

$$n(0,t) = 2p(\tau,t) \tag{2.5}$$

Using (2.1) through (2.5) we may derive differential equations for $P(t)$ and $N(t)$. Integrate equation 2.1 from 0 to τ, using (2.3a), to give

$$\frac{dP}{dt} + p(\tau,t) - p(0,t) = -\gamma P(t) \tag{2.6}$$

Since the general solution of $(\partial z/\partial x) + (\partial z/\partial y) = bz$ is $z(x,y) = \mu(x - y)\exp(by)$, where μ is an arbitrary function,

$$p(m,t) = \begin{cases} p(m-t,0) & 0 \leq t \leq m \\ p(0,t - m)\exp(-m\gamma_m) & m < t \end{cases} \tag{2.7}$$

where $\gamma_m = \gamma(t - m)$. However, from the initial condition $p(m,t) = p(m - t, 0) = g(m - t)$ for $0 \leq t \leq m$, and thus

$$p(\tau,t) = \begin{cases} g(\tau - t) & 0 \leq t \leq \tau \\ p(0,t - \tau)\exp(-\tau\gamma_\tau), & \tau < t \end{cases} \tag{2.8}$$

Therefore, using (2.8) in conjunction with the boundary condition on $p(m,t)$ gives the final form for equation 2.6:

$$\frac{dP}{dt} = -\gamma P(t) + \beta N(t) - \begin{cases} g(\tau - t), & 0 \leq t \leq \tau \\ \beta_\tau N_\tau \exp(-\tau\gamma_\tau), & \tau < t \end{cases} \tag{2.9}$$

To obtain an analogous equation for N(t), equation 2.2 is integrated to give

$$\frac{dN}{dt} + \lim_{a \to \infty} n(a,t) - n(0,t) = -(\beta + \delta)N(t) \tag{2.10}$$

As before,

$$n(a,t) = \begin{cases} n(a-t,0), & 0 \leq t \leq a \\ n(0,t-a)\exp[-a(\beta_a + \delta_a)], & a < t \end{cases} \tag{2.11}$$

so $\lim_{a \to \infty} n(a,t) = 0$ and from the boundary condition

$$n(0,t) = 2 \begin{cases} h(\tau - t), & 0 \leq t \leq \tau \\ \beta_\tau N_\tau \exp(-\tau\gamma_\tau), & \tau < t \end{cases}$$

Thus, the final form of equation 2.10 becomes

$$\frac{dN}{dt} = -(\beta + \delta)N(t) + 2 \begin{cases} h(\tau - t) & 0 \leq t \leq \tau \\ \beta_\tau N_\tau \exp(-\tau\gamma_\tau) & \tau < t \end{cases} \tag{2.12}$$

Equations (2.9) and (2.12), in conjunction with initial conditions and a specification of the time and/or density dependence of δ, β, and γ, offer a complete description of the dynamics of the PPSC.

APPENDIX 3: STABILITY OF THE PPSC POPULATIONS NEAR A STEADY STATE

Although it would be ideal if the full range of dynamical behavior of the PPSC model could be determined, the equations are sufficiently complex to make such an analysis presently impossible. As with ordinary non-linear differential equations without a time delay, it is possible to analyze the behavior of solutions near the steady state P*, N* defined by equations 6 or 8.

Take the linear portion of (4) near any equilibrium point N*, with $N(t) = z(t) + N^*$, $|z(t)/N^*| \ll 1$, to give the linear differential-delay equation

$$\frac{dz}{dt} + Az(t) + Bz(t - \tau) = 0 \qquad (3.1)$$

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

$$F(N^*,n) = \theta^n \frac{\theta^n + (1 - n)N^{*n}}{(\theta^n + N^{*n})^2} \qquad (3.2)$$

Assume that (3.1) has a solution of the form $\exp(\lambda t)$, $\lambda = \mu + j\omega$, $j^2 = -1$, and substitute into (3.1) to give

$$\lambda + A + B\exp(-\lambda\tau) = 0 \qquad (3.3)$$

Equate the real and imaginary portions of (3.3) to find

$$\omega\tau < \cos^{-1}(-A/B) \qquad (3.4)$$

where $\omega^2 = B^2 - A^2$, $|A/B| < 1$, is a necessary and sufficient condition for $\mu < 0$ (42). Equation 3.4 gives the requirement that the PPSC parameters must satisfy in order that small changes, $z(t)$, away from the steady state N^* will die out. When $\mu = 0$,

$$\omega\tau = \cos^{-1}(-A/B) \quad , \qquad (3.5)$$

a Hopf bifurcation (57) occurs 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 (4) and (5) indicate that the cellular populations will never be zero unless they are zero initially.

ACKNOWLEDGEMENTS

I would like to thank Professor Leon Glass for many stimulating discussions and comments, Mrs. Christine Pamplin for her typing assistance, Mr. Burt Gavin for his renditions of the figures, and Mr. Robert Thompson for photographic services.

This research was supported through a grant (NRC-A-0091) from the National Research Council of Canada. Computing facilities in the Biomedical Engineering Unit, Faculty of Medicine, McGill University were provided by a grant from the MacDonald-Stewart Foundation.

REFERENCES

1. Adamson, J.W., Dale, D.C., and Elin, R.J. Hematopoiesis in the grey collie dog. Studies of the regulation of erythropoiesis. J. Clin. Invest. 54:965-973, 1974.

2. Athens, J.W., Raab, S.O., Haab, O.P., Boggs, D.R., Ashenbrucker, H., Cartwright, G.E. and Wintrobe, M.M. Leukokinetic studies. X. Blood granulocyte kinetics in chronic myelocytic leukemia. J. Clin. Invest., 44:765-777, 1965.

3. Auslander, D.M., Oster, G.F. and Huffaker, C.B. Dynamics of interacting populations. J. Frank. Inst., 297:345-376, 1974.

4. Becker, A.J., McCulloch, E.A., Siminovitch, L. and Till, J.E. The effect of differing demands for blood cell production on DNA synthesis by hemopoietic colony-forming cells of mice. Blood, 26:296-308, 1965.

5. Boggs, D.R. Homeostatic regulatory mechanisms of hematopoiesis. Ann. Rev. Physiol., 28:39-53, 1966.

6. Boggs, D.R., Boggs, S.S. The pathogenesis of aplastic anemia: A defective pluripotential hematopoietic stem cell with inappropriate balance of differentiation and self-replication. Blood, 48:71-76, 1976.

7. Burns, F.J. and Tannock, I.F. On the existence of a G_0 phase in the cell cycle. Cell Tissue Kinet., 3:321-334, 1970.

8. Cartwright, G.E., Athens, J.W. and Wintrobe, M.M. The kinetics of granulopoiesis in normal man. Blood, 24:780-803, 1964.

9. Chervenick, P.A. and Boggs, D.R. Granulocyte kinetics in chronic myelocytic leukemia. Ser. Haemat. 1:24-37, 1968.

10. Chervenick, P.A. and Boggs, D.R. Patterns of proliferation and differentiation of hematopoietic stem cells after compartment depletion. Blood, 37:568-580, 1971.

11. Chikkappa, G., Borner, G., Burlington, H., Chanana, A.D., Cronkite, E.P., Ohl, S., Pavelec, M. and Robertson, J.S. Periodic oscillation of blood leukocytes, platelets, and reticulocytes in a patient with chronic myelocytic leukemia. Blood, 47:1023-1030, 1976.

12. Cooper, G.W. The regulation of thrombopoiesis in Gordon, A.S. (ed): Regulation of Hematopoiesis, vol. 2, New York, Appelton-Crofts, 1970, p 1611-1629.

13. Craddock, C.G. Control mechanisms for granulopoiesis in Williams, W.J. Eutler, E., Erslev, A.J. and Rundles, R.W. (ed): Hematology, New York, McGraw Hill, 1972, p 618-622.

14. Cronkite, E.P. and Fliedner, T.M. Granulocytopoiesis. N. Eng. J. Med., 270:1347-1352, 1964.

15. Cronkite, E.P. and Vincent, P.E. Granulocytopoiesis. Ser. Haemat., 4:3-43, 1969.

16. Cronkite, E.P. and Vincent, P.C. Granulocytopoiesis in Stohlman Jr., F. (ed): Hemopoietic Cellular Proliferation, New York, Grune and Stratton, 1970, p 211-228.

17. Dale, D.C. and Graw, R.G. Transplantation of allogenic bone marrow in canine cyclic neutropenia. Science, 183:83-84, 1974.

18. Dale, D.C., Alling, D.W. and Wolff, S.M. Cyclic hematopoiesis: The mechanism of cyclic neutropenia in grey collie dogs. J. Clin. Invest., 51:2197-2204, 1972.

19. Dale, D.C., Alling, D.W. and Wolff, S.M. Application of time series analysis to serial blood neutrophil counts in normal individuals and patients receiving cyclophosphamide. Brit. J. Haemat., 24:57-64, 1973.

20. Dale, D.C., Brown, C.H., Carbone, P. and Wolff, S.M. Cyclic urinary leukopoietic activity in grey collie dogs. Science, 173:152-153, 1971.

21. Dale, D.C., Ward, S.B., Kimball, H.R. and Wolff, S.M. Studies of neutrophil production and turnover in grey collie dogs with cyclic neutropenia. J. Clin. Invest., 51:2190-2196, 1972.

22. Deinard, A.S. and Page, A.R. An improved method for performing neutrophil survival studies. Blood, 36:98-110, 1970.

23. Delobel, J., Charbord, P., Passa, P. and Bernard, J. Evolution cyclique spontanee de la leucocytose dans un cas de leucemie myeloide chronique. Nouv. Rev. Fr. Hematol., 13:221-228, 1973.

24. Dormer, P. Kinetics of erythropoietic cell proliferation in normal and anemic man: A new approach using quantitative ^{14}C-autoradiography. Prog. Histochem. Cytochem., 6:1-83, 1973.

25. Dunn, C.D.R. The proliferative capacity of haemopoietic colony forming units in the rat. Cell Tissue Kinet., 6:55-67, 1973.

26. Ebbe, S. Megakaryocytopoiesis in Gordon, A.S. (ed): Regulation of Hematopoiesis, vol. 2, New York, Appleton Century Crofts, 1970, p 1587-1610.

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

28. Erslev, A.J. Feedback circuits in the control of stem cell differentiation. Am. J. Path., 65:629-639, 1971.

29. Erslev, A.J. Production of erythrocytes in Williams, W.J., Beutler, E., Erslev, A.J. and Rundles, R.W. (eds): Hematology, New York, McGraw Hill, 1972, p 162-177.

30. Ezdinli, E.Z., Sokal, J.E., Crosswhite, L. and Sandberg, A.A. Philadelphia-chromosome-positive and negative chronic myelocytic leukemia. Ann. Intern. Med., 72:175-182, 1970.

31. Fialkow, P.J., Lisker, R., Detter, J., Giblett, E.R. and Zavala, C. 6-Phosphogluconate dehydrogenase: Hemizygous manifestation in a patient with leukemia. Science, 163:194-195, 1969.

32. Fialkow, P.J., Lisker, R., Giblett, E.R., Zavala, C., Cobo, A. and Detter, J. Genetic markers in chronic myelocytic leukemia: Evidence opposing autosomal inactivation and favouring 6-PGD-RH linkage. Ann. Hum. Genet., 35:321, 1972.

33. Fried, W., Husseini, S., Gregory, S., Knospe, W.H. and Trobaugh, F.E. Effect of cyclophosphamide on the hematopoietic microenvironmental factors which influence hematopoietic stem cell proliferation. Cell Tissue Kinet., 6:155-163, 1973.

34. Galbraith, P.R. and Abu-Zahra, H.T. Granulopoiesis in chronic granulocytic leukemia. Br. J. Haematol., 22:135-143, 1972.

35. Gatti, R.A., Robinson, W.A., Deinare, A.S., Nesbit, M., McCullough, J.J., Ballow, M. and Good, R.A. Cyclic leukocytosis in chronic myelogenous leukemia. Blood, 41:771-782, 1973.

36. Gavosto, F. Granulopoiesis and cell kinetics in chronic myeloid leukemia. Cell Tissue Kinet., 7:151-163, 1974.

37. German, H.J., Smith, J.A. and Lindenbaum, J. Chronic intravascular coagulation associated with chronic myelocytic leukemia. Am. J. Med., 61:547-552, 1976.

38. Gidali, J. and Lajtha, L.G. Regulation of haemopoietic stem cell turnover in partially irradiated mice. Cell Tissue Kinet., 5:147-157, 1972.

39. Greenberg, M.L., Chanana, A.D., Cronkite, E.P., Giacomelli, G., Rai, K.R., Schiffer, L.M., Stryckmons, P.A. and Vincent, P.D. The generation time of human leukemic myeloblasts. Lab. Invest., 26:245-252, 1972.

40. Guerry, D., Adamson, J.W., Dale, D.C. and Wolff, S.M. Human cyclic neutropenia: Urinary colony-stimulating factor and erythropoietin levels. Blood, 44:257-262, 1974.

41. Guerry, D., Dale, D.C., Omine, M., Perry, S. and Wolff, S.M. Periodic hematopoiesis in human cyclic neutropenia. J. Clin. Invest., 52:3220-3230, 1973.

42. Hayes, N.D. Roots of the trancendental equation associated with a certain difference - differential equation. J. Lond. Math. Soc., 25:226-232, 1950.

43. Hoffman, H.J., Guerry, D. and Dale, D.C. Analysis of cyclic neutropenia using digital band-pass filtering techniques. J. Interdiscipl. Cycle Res., 5:1-18, 1974.

44. Jones, J.B., Yang, T.J., Dale, J.B. and Lange, R.D. Canine cyclic haematopoiesis: Marrow transplantation between littermates. Br. J. Haematol., 30:215-223, 1975.

45. Kennedy, B.J. Cyclic leukocyte oscillations in chronic myelogenous leukemia during hydroxurea therapy. Blood, 35:751-760, 1970.

46. Killman, S.A. Acute leukemia: The kinetics of leukemic blast cells in man. Ser. Haemat., 1:38-128, 1968.

47. Killman, S.A., Cronkite, E.P., Robertson, J.S., Fliedner, T.M. and Bond, V.P. Estimation of phases of the life cycle of leukemic cells from labeling in human beings *in vivo* with trikiated thymidine. Lab. Invest., 12:671-684, 1963.

48. King-Smith, E.A. and Morley, A. Computer simulation of granulopoieisis: normal and impaired granulopoiesis. Blood, 36: 254-262, 1970.

49. Kivilaakso, E. and Rytomaa, T. Erythrocytic chalone, a tissue specific inhibitor of cell proliferation in the erythron. Cell Tissue Kinet., 4:1-9, 1971.

50. Lajtha, L.G. Cellular mechanism of red cell production. Ser. Haemat., 2:26-33, 1965.

51. Lajtha, L.G. Kinetics of proliferation and differentiation in haemopoiesis in Gordon, A.S., Condorelli, M. and Peschle, C. (eds): Regulation of Erythropoiesis, Il Ponte, Milano, 1971, p 47-51.

52. Lajtha, L.G., Gilbert, C.W. and Guzman, E. Kinetics of haemopoietic colony growth. Br. J. Haemat., 20:343-354, 1971.

53. Lajtha, L.G., Gilbert, C.W., Porteous, D.D. and Alexanian, R. Kinetics of bone marrow stem cell population. Ann. N.Y. Acad. Sci., 113:742-752, 1964.

54. Lund, J.E., Padgeh, G.A. and Oh, R.L. Cyclic neutropenia in grey collie dogs. Blood, 29:452-461, 1967.

55. Mackey, M.C. A unified hypothesis for the origin of aplastic anemia and periodic haematopoiesis. Submitted for publication to Blood.

56. Mackey, M.C. and Glass, L. Oscillation and chaos in physiological control systems. Science, 197:287-289, 1977.

57. Marsden, J.E. and McCracken, M. The Hopf Bifurcation and Its Applications, New York, Springer-Verlag, 1976.

58. Mastrangelo, R., Stabile, A., Parenti, D. and Segni, G. Spontaneous leukocyte oscillation during blastic crisis of chronic myeloid leukemia. Cancer, 33:1610-1614, 1974.

59. Mastrangelo, R., Stabile, A., Parenti, D., Cimatti, G., Pesaresi, M.A., Arpaia, E. and Serra, A. A specific spontaneous leukocyte cycle in chronic myelogenous leukemia. Tumori, 62:197-204, 1976.

60. Mauer, A.M. and Jarrold, T. Granulocyte kinetic studies in patients with proliferative disorders of the bone marrow. Blood, 22:125-138, 1963.

61. Mauer, A.M., Athens, J.W., Ashenbrucker, H., Cartwright, G.E. and Wintrobe, M.M. Leukokinetic studies. II. A method for labeling granulocytes in vitro with radioactive diisopropyl-flurophosphate (DFP^{32}). J. Clin. Invest., 39:1481-1486, 1960.

62. McCulloch, E.A. and Till, J.E. Cellular interactions in the control of hemopoiesis in Stohlman, F. Jr. (ed): Hemopoietic Cellular Proliferation, New York, Grune and Stratton, 1970, p 15-25.

63. McCulloch, E.A. and Till, J.E. Regulatory mechanisms acting on hemopoietic stem cells. Am. J. Path., 65:601-619, 1971.

64. McCulloch, E.A., Mak, T.W., Price, G.B. and Till, J.E. Organization and communication in populations of normal and leukemic hemopoietic cells. Biochem. Biophys. Acta., 355: 260-299, 1974.

65. Meuret, G. and Fliedner, T.M. Zell kinetik der Granulopoese und des Neutrophilensystems bei einem fall von zyklischner Neutropenie. Acta. Haemat., 43:48-63, 1970.

66. Morley, A. and Stohlman, F. Studies on the regulation of granulopoiesis. I. The response to neutropenia. Blood, 35:312-321, 1970.

67. Morley, A. and Stohlman, F. Cyclophosphamide induced cyclical neutropenia. New Eng. J. Med., 282:643-646, 1970.

68. Morley, A.A., Baikie, A.G. and Galton, D.A.G. Cyclic leucocytosis as evidence for retention of normal homoeostatic control in chronic granulocytic leukaemia. Lancet, 1320-1322, 1967.

69. Morley, A.A., Carew, J.P. and Baikie, A.G. Familial cyclical neutropenia. Br. J. Haemato., 13:719-738, 1967.

70. Morley, A., King-Smith, E.A. and Stohlman, F. Jr. The oscillatory nature of hemopoiesis in Stohlman, F. Jr. (ed): Hemopoietic Cellular Proliferation, New York, Grune and Stratton, 1970, p 3-14.

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

72. Odeberg, H., Olofsson, T., and Olsson, I. Granulocyte function in chronic granulocytic leukemia. I. Bactericidal and metabolic capabilities during phagocytosis in isolated granulocytes. Br. J. Haemat., 29:427-441, 1975.

73. Odell, T.T. Megakaryocytopoiesis and its response to stimulation and suppression in Baldini, M.G. and Ebbe, S. (eds): Platelets: Production, Function, Transfusion and Storage, New York, Grune and Stratton, 1973, p 11-20.

74. Odell, T.T., Jackson, C.W. and Friday, T.J. Megakaryocytopoiesis in rats with special reference to polyploidy. Blood, 35:775-782, 1970.

75. Ogawa, M., Fried, J., Sakai, Y., Strife, A. and Clarkson, B.D. Studies of cellular proliferation in human leukemia. VI. The proliferative activity, generation time and emergence time of

neutrophillic granulocytes in chronic granulocytic leukemia. Cancer, 25:1031-1049, 1970.

76. Olofsson, T. and Olsson, I. Granulopoiesis in chronic myeloid leukemia. II. Serial cloning of blood and bone marrow cells in agar culture. Blood, 48:351-360, 1976.

77. Page, A.R. and Good, R.A. Studies on cyclic neutropenia. A.M.A. J. Dis. Child., 94:623-661, 1957.

78. Patt, H.M. and Maloney, M.A. Relationship of bone marrow cellularity and proliferative activity: A local regulatory mechanism. Cell Tissue Kinet., 5:303-309, 1972.

79. Patt, H.M., Lund, J.E. and Maloney, M.A. Cyclic hematopoiesis in grey collie dogs: a stem cell problem. Blood, 42:873-884, 1974.

80. Perry, S., Moxley, J.H., Weiss, G.H. and Zelen, M. Studies of leukocyte kinetics by liquid scintillation counting in normal individuals and in patients with chronic myelocytic leukemia. J. Clin. Invest., 45:1388-1399, 1966.

81. 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.

82. Robinson, W.A. and Mangalik, A. The kinetics and regulation of granulopoiesis. Sem. Hemat., 12:7-25, 1975.

83. Rodriguez, A.R. and Lutcher, C.L. Marked cyclic leukocytosis leukopenia in chronic myelogenous leukemia. Am. J. Med., 60: 1041-1047, 1976.

84. Rothstein, G., Hügl, E.H., Bishop, C.R., Athens, J.W. and Ashenbrucker, H.E. Stimulation of granulocytopoiesis by a diffusible factor *in vivo*. J. Clin. Invest., 50:2004-2007, 1971.

85. Rothstein, G., Hügl, E.H., Chervenick, P.A., Athens, J.W. and Macfarlane, J. Humoral stimulators of granulocyte production. Blood, 41:73-78, 1973.

86. Rubinow, S.I. and Lebowitz, J.L. A mathematical model of the acute myeloblastic leukemic state in man. Biophys. J., 16: 897-910, 1976.

87. Rytomaa, T. and Kiviniemi, K. Regulation system of blood cell production in Teir, H. and Rytomaa, T. (eds): Control of Cellular Growth In Adult Organisms, London, Academic Press, 1967, p 106-139.

88. Rytomaa, T. and Kiviniemi, K. Control of cell production in rat chloroleukaemia by means of the granulocytic chalone. Nature, 220:136-137, 1968.

89. Rytomaa, T. and Kiviniemi, K. Control of granulocyte production. I. Chalone and antichalone, two specific humoral regulators. Cell Tissue Kinet., 1:329-340, 1968.

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

91. Rytomaa, T. and Kiviniemi, K. Control of DNA duplication in rat chloroleukaemia by means of the granulocytic chalone. Eur. J. Can., 4:595-606, 1968.

92. Rytomaa, T. and Kiviniemi, K. Regression of generalized leukaemia in rat induced by the granulocytic chalone. Eur. J. Can., 6:401-410, 1970.

93. Sanberg, E.A. and Hossfield, D.K. Chromosomal abnormalities in human neoplasia. Ann. Rev. Med., 21:379-408, 1970.

94. Schooley, J.C. and Lin, D.H.Y. Hematopoiesis and the colony forming unit in Gordon, A.S., Condorelli, M. and Peschle, C. (eds): Regulation of Erythropoiesis, Il Ponte, Milano, 1971, p 52-70.

95. Shadduck, R.K., Winkelstein, A. and Nunna, N.G. Cyclic leukemia cell production in CML. Cancer, 29:399-401, 1972.

96. Stohlman, F. Control mechanisms in erythropoiesis in Gordon, A.S., Condorelli, M. and Peschle, C. (eds): Regulation of Erythropoiesis, Il Ponte, Milano, 1971, p 71-88.

97. Stohlman, F. Stem cell regulation in Baldini, M.G., and Ebbe, S. (eds): Platelets: Production, Function, Transfusion, and Storage, New York, Grune and Stratton, 1973, p 1-9.

98. Stohlman, F. Jr. and Queensberry, P.J. Colony stimulating factor and myelopoiesis. Blood, 39:727-731, 1972.

99. Stohlman, F. and Queensberry, P.J. and Tyler, W.S. in Brown, E.B. (ed): Progress In Hematology, vol. VIII, New York, Grune and Stratton, 1973, p 259-297.

100. Stryckmans, P.A., Cronkite, E.P., Fache, J., Fliedner, T.M. and Ramos, J. Deoxyribonucleic acid synthesis time of erythropoietic and granulopoietic cells in human beings. Nature, 211: 717-720, 1966.

101. Sutherland, D.J.A., Till, J.E. and McCulloch, E.A. A kinetic study of the genetic control of hemopoietic progenitor cells assayed in culture and in vivo. J. Cell Physiol., 75:267-274, 1970.

102. Tough, I.M., Jacobs, P.A., Court-Brown, W.M., Baikie, A.G. and Williamson, E.R.D. Cytogenetic studies on bone marrow in chronic myeloid leukaemia. Lancet, 1:844-846, 1963.

103. Trentin, J.J. Determination of bone marrow stem cell differentiation by stromal hemopoietic inductive microenvironments (HIM). Am. J. Path., 65:621-628, 1971.

104. Vassort, F., Frindel, E. and 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.

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

106. Vincent, P.D. Cell kinetics of the leukemias in Gunz, F. and Baikie, A.G. (eds): Leukemia, New York, Grune and Stratton, 1974, p 189-221.

107. Vodopick, H., Rupp, E.M., Edwards, C.L., Goswitt, F.A. and Beauchamp, J.J. Spontaneous cyclic leukocytosis and thrombocytosis in chronic granulocytic leukemia. New Eng. J. Med., 286:284-290, 1972.

108. VonFoerster, H. Some remarks on changing populations in Stohlman, F. Jr. (ed): The Kinetics of Cellular Proliferation, New York, Grune and Stratton, 1959, p 382-407.

109. 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.

110. Weiden, P.L., Robinett, B., Graham, T.C., Adamson, J. and Storb, R. Canine cyclic neutropenia. A stem cell defect. J. Clin. Invest., 53:950-953, 1974.

111. Wewerka, J.R. and Dale, D.C. Colony-stimulating factor in patients with chronic neutropenia. Blood, 47:861-867, 1976.

112. Whang-Peng, J., Canellos, G.P., Carbone, P.P. and Tjio, J.H. Clinical implications of cytogenetic variants in chronic myelocytic leukemia (CML). Blood, 32:755-766, 1968.

113. Whang, J., Frei, E., Tjio, J.H., Carbone, P.P. and Brecher, G. The distribution of the Philadelphia chromosome in patients with chronic myelocytic leukemia. Blood, 22:664-673, 1963.

114. Wheldon, T.E. Mathematical models of oscillatory blood cell production. Math. Biosci., 24:289-305, 1975.

115. Wheldon, T.E., Kirk, J. and Finlay, H.M. Cyclical granulopoiesis in chronic granulocytic leukemia: A simulation study. Blood, 43:379-387, 1974.

116. Williams, D.M., Lynch, R.E. and Cartwright, G.E. Drug-induced aplastic anemia. Sem. Hemat., 10:195-223, 1973.

117. Wintrobe, M.M. Clinical Hematology, 7th ed., Philadelphia, Lea and Febiger, 1976.

118. Witts, L.J. Chronic granulocytic leukaemia: Comparison of radiotherapy and busulphan therapy. Br. Med. J., 1:201-208, 1968.