

**FEEDBACK, DELAYS AND THE ORIGIN  
OF BLOOD CELL DYNAMICS**

By

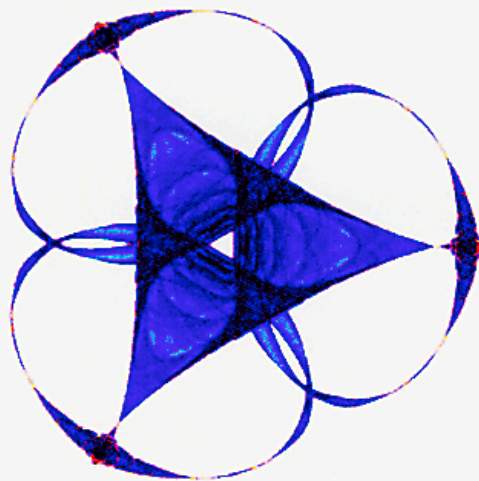
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## Feedback, Delays and the Origin of Blood Cell Dynamics

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## ABSTRACT

This paper reviews a variety of models for the regulation of hematopoiesis that are most naturally framed as nonlinear differential delay equations, and which are often obtained from partial differential equations. We review the development of these models, and point out a number of interesting mathematical questions raised by the biology, including the effects of multiple delays, distributions of delays, state dependent delays, and nonconstant initial functions. The range of insight given by the modelling to experimentally and clinically observed dynamics is discussed.



## INTRODUCTION

The dynamics of many biological variables,  $x(t)$ , can be modeled by the differential equation<sup>1-2</sup>

$$\begin{aligned}\frac{dx}{dt} &= \text{Production rate} - \text{Destruction rate} \\ &= P-D\end{aligned}\tag{1}$$

Typically  $P$  and/or  $D$  are highly nonlinear functions of the state variable at some time,  $\tau$  in the past,  $x(t - \tau)$ , as well as depending on the current value of  $x(t)$ , i.e.

$$\frac{dx}{dt} = P(x(t), x(t - \tau), \mu_1, \mu_2, \dots) - D(x(t), x(t - \tau), \mu'_1, \mu'_2, \dots)\tag{2}$$

where the  $\mu_i, \mu'_i, i = 1, 2, \dots$  are control parameters which, in comparison to the state variable, either do not change with time, or change so slowly that they can be regarded as constant by the investigator. In order to solve the differential delay equation (2) it is necessary to specify an initial function on the interval  $[-\tau, 0]$ . In this sense differential delay equations like eq. (2) are infinite dimensional systems. Differential delay equations have a rich literature<sup>3-4</sup>.

Equation (2) is to be contrasted with models typically encountered in the physical sciences where the dynamics are often described by differential equations in which the right hand side is an instantaneous function of  $x(t)$ . In biology, the time delays are often inherent properties of the control mechanisms and arise, for example, because of non-zero conduction times in the nervous system, the cell cycle time or maturation time in replicating cellular populations, delays due to transcription or translation in the genome and circulatory times in the cardiovascular system<sup>5-8</sup>.

Differential delay equations have been used to model the control of respiration<sup>5-6</sup>, the pupil light reflex<sup>9-12</sup>, blood cell dynamics<sup>13-25</sup>, simple neural networks<sup>26-28</sup>, population



growth<sup>29</sup>, protein synthesis<sup>30-31</sup>, optical<sup>32-33</sup> and acousto-optical<sup>34</sup> bistability and the dynamics of economic commodity markets<sup>35-36</sup>. Here we focus on applications of Eq. (2) for modelling the control of blood cell numbers with references to other physiological contexts. Mathematical investigations of the control of blood cell dynamics continue to provide important insights into the properties of the hematological regulatory systems. In addition, we draw attention to experimental and clinical observations which suggest the importance of extending these studies to situations which, to our knowledge, have not been explored mathematically.

## CONTROL OF HEMATOPOIESIS

The formation of red blood cells (erythrocytes), white blood cells (granulocytes) and platelets (thrombocytes) primarily occurs in the bone marrow (see references 37-39 for a general discussion). A simplified schematic representation of normal hematopoiesis is shown in Figure 1. As depicted there, a self-maintaining pluripotential stem cell population (PPSC) is thought to exist which is capable of producing committed stem cells (CSC) specialized for the erythroid, granuloid and thromboid cell lines. The influx of cells from the PPSC to the CSC lines is regulated in two ways: 1) long range humoral mechanisms (labeled LR in Figure 1); and 2) local environmental mechanisms which are as yet poorly understood

The long range humoral substances regulating the erythroid (erythropoietin) and thromboid (thrombopoietin) cell lines are primarily produced by the kidney, but extra-renal sites such as liver may also be of importance. A variety of substances possessing granulopoietic activity have been isolated from T lymphocytes, monocytes, endothelial cells and fibroblasts. An intrinsic property of these feedback mechanisms is the presence of time delays which range from seconds (e.g. circulatory time) to days (e.g. maturational times).

Under normal circumstances marked fluctuations in blood cell number do not seem to be present. In humans abnormal oscillations in blood cell number occur in a group of disorders collectively referred to as periodic hematological diseases (for a recent review see reference 40). The periodic hematological diseases include periodic hematopoiesis (also referred to as cyclic neutropenia)<sup>41-42</sup>, cyclic thrombocytopenia<sup>43</sup>, cyclic eosinophilic myositis and hyper-immunoglobulin E syndrome<sup>44</sup> and the periodic variants of chronic myelogenous leukemia<sup>45-46</sup> and autoimmune hemolytic anemia<sup>47,48</sup>. In addition, oscillations in granulocyte number have been observed following the administration of chemotherapeutic agents to patients with chronic myelogenous leukemia<sup>49</sup>.

Experimental study of the periodic hematological diseases has sometimes been facilitated by the availability of suitable animal models. For example, all gray collies have periodic hematopoiesis<sup>50-51</sup>, and periodic erythropoiesis can be induced in mice<sup>52-53</sup> by the administration of a single dose of the marrow-seeking radioisotope <sup>89</sup>Sr and in rabbits<sup>54</sup> by the administration of red blood cell auto-antibodies.

In principle, there are three types of mechanisms which can account for oscillations in peripheral blood cell number.

1. Oscillations can occur because of instabilities in the long range feedback mechanisms.
2. Oscillations in peripheral blood cell number may simply be a reflection of oscillations in PPSC number due to abnormalities in PPSC regulatory mechanisms.
3. Oscillations in blood cell number may arise because of interactions between two or more cell lines.

In the following, we survey the work that has been done in each of these areas, as well as offering some possible unexplored, or incompletely explored, extensions.

## LONG RANGE HUMORAL MECHANISMS

### a). Delayed negative feedback: Single delay

The control of erythrocyte production can be characterized as a delayed negative feedback mechanism<sup>37</sup> (Figure 2a). Hypoxia (due, for example, to a fall in erythrocyte number) induces the release the hormone erythropoietin from the kidney. Erythropoietin, in turn, increases the production rate within the early committed erythroid series cells, ultimately augmenting circulating erythrocyte numbers (i.e. negative feedback). There is a significant delay between the receipt of this erythropoietin signal and a consequent change in the influx of circulating erythrocytes. This is because once a cell from the PPSC is committed to the erythroid series it takes an average of  $\tau_m \sim 5.7$  days for it to complete a series of nuclear divisions and undergo maturation before being released into the circulation.

Oscillations in erythrocyte number,  $E$ , are rarely observed in humans<sup>47-48</sup>, but can be induced in rabbits by using a red blood cell auto-antibody to increase the peripheral erythrocyte destruction rate<sup>54</sup>. In terms of eq. (2), a possible model for induced rabbit auto-immune hemolytic anemia (AIHA) is

$$\frac{dE}{dt} = f(E_{\tau_m}) - \gamma E, \quad (3)$$

where

$$f(E_{\tau_m}) = \frac{\beta_o \theta^n}{\theta^n + E_{\tau_m}^n}, \quad (4)$$

$\gamma > 0$  is the random rate of erythrocyte destruction, and  $n, \beta_o$ , and  $\theta$  are constants (i.e. control parameters) characterizing the erythrocyte production function<sup>14</sup>. The notation  $E_{\tau_m}$  denotes  $E$  at a time  $\tau_m$  in the past, i.e.  $E_{\tau_m} = E(t - \tau_m)$ . A monotone decreasing  $f(E_{\tau_m})$  is consistent with *in vivo* measurements of erythrocyte production rates in rats<sup>55</sup>. Increasing the parameter  $n$  increases the maximal slope of the feedback function  $f$ , the



parameter  $\theta$  controls the location of the point of maximum slope, while  $\beta_0$  controls the maximal erythroid production rate. Increases in  $n$  are equivalent to increasing the gain in the feedback loop<sup>10-11</sup>.

The properties of eq. (3) have been extensively studied using analytical and numerical methods<sup>5-6,56</sup>. Two types of stable solutions are known to exist: 1) a locally stable equilibrium point,  $E^* > 0$ , defined by  $dE/dt = 0$ , and given implicitly by

$$\gamma E^* = \frac{\beta_0 \theta^n}{\theta^n + (E^*)^n}; \quad (5)$$

and 2) a stable limit cycle oscillation of simple type, i.e. one maximum per period<sup>56</sup>. A supercritical Hopf bifurcation between these two types of solutions occurs<sup>11</sup> when the control parameters  $n$ ,  $\gamma$ , and  $\tau_m$  satisfy

$$\omega \tau_m = \cos^{-1} \left( \frac{\gamma}{f'(E^*)} \right), \quad (6a)$$

where

$$\omega^2 = [f'(E^*)]^2 - \gamma^2 \quad (6b)$$

and  $f'(E^*) = (\partial f / \partial E_{\tau_m})_{E_{\tau_m} = E^*}$ . The period,  $T_H = 2\pi/\omega$ , of the oscillatory solution at the Hopf bifurcation<sup>57</sup> is easily shown to be bounded by  $2\tau_m \leq T_H \leq 4\tau_m$ . For  $\tau_m \sim 6$  days, these bounds predict oscillations with periods between 12-24 days, but use of the strict relation  $T_H = 2\pi/\omega$  with estimates of the other parameters<sup>14</sup> sharpens the estimated period to about 21 days which is in good agreement with the observed period of 16-17 days in induced AIHA.

Because of the nonlinear nature of the problem, numerical calculations must be used to explore the global behavior of the solutions of eq. (3) as the control parameters are increased beyond the Hopf bifurcation. To obtain insight into the nature of the dynamics

of AIHA, changes in  $\gamma$  are important since red cell auto-antibody causes random erythrocyte lysis. As  $\gamma$  is increased, there is a progressive fall in the steady state number of circulating erythrocytes as would be expected. However, the other feature that appears as  $\gamma$  is increased is related to a loss of the stability of this steady state for some biologically important ranges of the parameters  $\beta_0, n$ , and  $\tau_m$ . In these ranges, for small values of  $\gamma$ , the steady state is locally stable. At a critical value of  $\gamma = \gamma_{crit,1}$  which may be calculated exactly from eqs. (6a,b), there is a supercritical Hopf bifurcation and the steady state  $E^*$  is locally unstable. This instability persists for all  $\gamma_{crit,1} < \gamma < \gamma_{crit,2}$ . Finally, at  $\gamma = \gamma_{crit,2}$  there is a reverse Hopf bifurcation, and the (depressed) steady state  $E^*$  is once again stable for  $\gamma > \gamma_{crit,2}$  (Figure 2b). This sequence explains the observation in rabbit AIHA that reticulocyte levels may be either depressed at constant levels or oscillate around depressed levels depending on the severity of the hemolytic anemia<sup>54</sup>.

Once the supercritical Hopf bifurcation has occurred and the positive steady state  $E^*$  is unstable ( $\gamma_{crit,1} < \gamma < \gamma_{crit,2}$ , it is found numerically that increasing the control parameters  $n$  and  $\beta_0$  controlling the gain changes the shape of the oscillation with little change in frequency<sup>10-11</sup>. The more complex waveforms typically associated with the occurrence of higher-order bifurcations<sup>58</sup>, such as period-doubling bifurcations or bifurcations from a limit cycle to a two torus, are not seen and the results of Kaplan and Yorke<sup>56</sup> make it likely that no other bifurcations exist.

In the special case of piecewise constant negative feedback, when  $f(E_{\tau_m})$  is replaced by  $F(E_{\tau_m})$  given by

$$F(E_{\tau_m}) = \begin{cases} \alpha & \text{if } E_{\tau_m} < \theta \\ 0 & \text{if } E_{\tau_m} > \theta, \end{cases} \quad (7)$$

and  $\alpha, \theta$  are positive constants, it has been possible to prove that only one type of stable oscillatory solution exists<sup>1</sup>.

These results indicate that this model accurately replicates the essential elements of the dynamic behaviour of induced AIHA. However, the oscillations in erythrocyte number observed in AIHA show variations in both waveform morphology and inter-peak interval. Recently the possibility that noise-like fluctuations of these types may be of deterministic origin, i.e. 'chaotic', has attracted a great deal of attention<sup>5-7,59-61</sup>. Thus, these modeling observations further indicate that chaotic dynamics can not be produced by nonlinear negative feedback mechanisms of the type described by eq. (3) in the absence of stochastic inputs.

#### **b). Mixed feedback: Single delay**

The type of feedback which arises in the regulation of neutrophil numbers differs from that involved in the regulation of erythrocyte numbers. At sufficiently high neutrophil numbers, the neutrophil production rate decreases as the number of neutrophils increases (i.e. negative feedback). However, at low neutrophil numbers the production rate falls to zero. Thus in the range of low neutrophil numbers, the production rate increases as the neutrophil number increases (i.e. positive feedback). The resulting feedback function has the 'humped' shape shown in Figure 2a. This type of feedback has been referred to as 'mixed' feedback<sup>1-2</sup>. Though the feedback function for neutrophil regulation has a different functional characteristic (mixed) than does the one for erythrocytes (negative), they are similar in that the feedback in both is delayed. For the neutrophils, there is once again a significant delay of  $\tau_m \simeq 5-7$  days between when the feedback signal is sensed by the primitive neutrophil precursors and when the effect of this signal is seen in the periphery. Finally, from studies utilizing labeled neutrophils, it is known that these cells have an exponential disappearance from the circulation, consistent with random destruction at a constant rate.



Oscillations in neutrophil number occur in a type of human leukemia referred to as periodic chronic myelogenous leukemia (CML). A simple model for the regulation of neutrophil number,  $N$ , which incorporates the delayed mixed feedback is<sup>5-6</sup>

$$\frac{dN}{dt} = g(N_{\tau_m}) - \gamma N \quad (8)$$

where  $\gamma$  is again the random destruction rate and the delayed production function is

$$g(N_{\tau_m}) = \frac{\beta_0 N_{\tau_m} \theta^n}{\theta^n + N_{\tau_m}^n}. \quad (9)$$

In contrast to the erythroid control system, this model predicts that there are potentially two steady state levels of neutrophil numbers:  $N_1^* = 0$ , and a second positive steady state  $N_2^*$  given explicitly by

$$N_2^* = \theta \left[ \frac{\beta_0 - \gamma}{\gamma} \right]^{1/n}$$

which exists whenever  $\beta_0 > \gamma$ . As for the model of erythroid production, it is possible to analyze the local stability of both of these steady states, and the results of this analysis indicate that whenever  $N_2^* > 0$  exists, the steady state corresponding to no neutrophils,  $N_1^* = 0$ , is unstable. For fixed values of the random peripheral neutrophil destruction rate  $\gamma$ , increases in either  $\beta_0$  and/or  $n$ , which both increase the gain of the system at the steady state, and in  $\tau_m$  will eventually lead to a loss of stability of  $N_2^*$  through a supercritical Hopf bifurcation and the onset of limit cycle behaviour. The criteria for this bifurcation are given by eqs. (6a,b) with  $g$  replacing  $f$ .

The behaviour of eq. (8) has been studied numerically<sup>5-6</sup>, and these studies indicate that the dynamics of eqs. (8) and (9) is much richer than for the simple negative feedback model of erythrocyte production. Increases in  $\tau_m$  are of particular interest since a prolongation of the neutrophil maturation time is inferred in patients with CML<sup>62</sup>. As  $\tau_m$  is increased an initially stable equilibrium becomes unstable and stable periodic solutions

appear. Further increases in  $\tau_m$  lead to a sequence of period-doubling bifurcations which ultimately culminates in an apparently chaotic or aperiodic regime (an example of which is shown in Figure 2c). In the aperiodic regime, the choice of the initial function determines the evolution of the dynamics. In addition, for some choices of initial functions stable, but complex periodic oscillations may also be observed. Precisely the same mathematical observations have been made<sup>63</sup> using a different analytic mixed feedback form for the function  $g$ .

Figure 2c compares a computer simulation of eq. (8), when the maturation time is increased from a normal value of  $\sim 5-7$  days to 20 days, to the changes in neutrophil number measured for a CML patient. Not only does the simulation correctly predict the overall period of the observed oscillations in neutrophil number, but it also duplicates the irregular fluctuations. Thus in the case of mixed feedback it is not easy to distinguish fluctuations which arise from stochastic inputs (“noise”) from the inherent dynamics of the control mechanism. To our knowledge, this simple model for the production of neutrophils was the first association of “intrinsic” chaos in a continuous time deterministic equation with a pathological process.

As in the case of negative feedback, it has been possible to obtain greater analytical insight into the properties of mixed feedback by considering the special case of piecewise constant mixed feedback<sup>1</sup>. In particular,  $g(N_{\tau_m})$  in eq. (8) is replaced by  $G(N_{\tau_m})$  where

$$G(N_{\tau_m}) = \begin{cases} 0 & \text{if } N_{\tau_m} \leq \theta_1 \\ \alpha & \text{if } \theta_1 < N_{\tau_m} < \theta_2 \\ \delta & \text{if } \theta_2 \leq N_{\tau_m}, \end{cases} \quad (10)$$

and  $\alpha > \delta > 0$ . In this case it has been proved<sup>1</sup> that for constant initial functions there exist stable equilibria, stable and unstable limit cycles, Li and Yorke<sup>64</sup> type chaos, and mixing and exact motions for various parameter values.

### c). Multiple delays

In the models for erythrocyte and neutrophil regulation, the destruction rates only accounted for cell loss due to random processes in the circulation such as immune-mediated cytolysis and loss of cells in hemostatic-thrombotic events. For neutrophils this is an accurate representation of the underlying physiology, but erythrocytes also age and are removed from circulation as a consequence of senescence. Laboratory studies indicate that in humans, the lifespan of erythrocytes is on the order of 120 days<sup>37</sup>. Figure 3a illustrates a possible model for erythrocyte production which incorporates loss due to senescence. If we denote the erythrocyte senescence time by  $\tau_s$ , then the dynamics will be more accurately described by

$$\frac{dE}{dt} = f(E_{\tau_m}) - \gamma E - f(E_{\tau_m + \tau_s})e^{-\gamma\tau_s} \quad (11)$$

In contrast to eq. (3), eq. (11) contains two different time delays,  $\tau_m$  and  $\tau_s$ . Numerical simulations of eq. (11) indicate that for  $\tau_s = 120$  days, the dynamics do not differ significantly from those produced by eq. (3) (M.C. Mackey, unpublished data). This is not surprising since for large  $\tau_s$ ,  $\gamma\tau_s \gg 1$  and  $e^{-\gamma\tau_s} \sim 0$ , so eq. (11) is approximated by eq. (3).

However, in other situations multiple delays may have profound effects on the predicted dynamics. One example comes from a model for the production of platelets, whose dynamics are of interest because of the as yet unexplained condition of cyclical thrombocytopenia<sup>43</sup>. In this disease, platelet levels oscillate from high to low levels with a period of approximately 28 days. If the platelet count at the nadir of the oscillation is sufficiently low, the patient is at risk for a stroke, and indeed this is the cause of death for many of these patients. Initial reports of this condition were in women, and it was thought that the oscillation might be driven by the menstrual cycle. However, there are



other clinical studies of the same disease in men thus ruling out this explanation for all cases.

Platelets are produced from megakaryocytes by a fragmentation processes<sup>65</sup>. Normally, the total time between the appearance of a recognizable megakaryocyte and when it starts to produce platelets is  $\tau_m \sim 9$  days<sup>65</sup>. Once released into the circulation, the platelet lifetime is  $\tau_s \sim 10$  days<sup>37</sup>. Thus two delays arise: one associated with the platelet lifetime ( $\tau_s$ ) and another with the megakaryocyte maturation time ( $\tau_m$ ). In contrast to the situation for erythrocyte production, these two delays are of comparable magnitude.

A simple yet realistic model for platelet production is shown schematically in Figure 3b and can be expressed mathematically<sup>16</sup> as

$$\frac{dP}{dt} = -\gamma P + e^{-\delta\tau_m} [\beta(P_{\tau_m}) - \beta(P_{\tau_s+\tau_m})e^{-\gamma\tau_s}] \quad (12)$$

where the feedback function  $\beta$  is of the mixed form like that described by eq.(9),  $\gamma$  is the random rate of destruction of platelets in the circulation, and  $\delta$  is the random rate of megakaryocyte destruction.

As in the model for neutrophil dynamics of the previous section, this model has two steady state values of platelet levels, one zero ( $P_1^* = 0$ ) which always exists, and a second positive one ( $P_2^* > 0$ ) that exists for all values of  $\gamma$  satisfying  $0 < \gamma < \gamma_{crit}$ . However, the existence of the two time delays controlling the dynamics makes the stability analysis at these steady states much more complicated than the analysis when only one delay is present<sup>66</sup>. One of the surprising predictions<sup>66</sup> of the model for platelet regulation is that the nonzero platelet steady state is *unstable* for low platelet destruction rates  $\gamma$ , and that increases in  $\gamma$ , in addition to decreasing the unstable steady state platelet levels, will ultimately result in a reverse supercritical Hopf bifurcation so that the platelet numbers are eventually stabilized by increasing platelet destruction rates. Precisely the

same behaviour is found following increases in  $\delta$ . These results suggest that a possible, but certainly non-intuitive, treatment for the potentially life-threatening condition of cyclical thrombocytopenia might be to increase platelet and/or megakaryocyte destruction rates in a carefully controlled fashion.

#### d). Distributed delays

In writing eqs. (3), (8), (11) and (12) we have assumed that the maturation time is the same for all cells. In general, however, this is not the case and there will be a distribution of maturation times. Similar problems arise in the modeling of recurrent inhibitory pathways in the nervous system, where there is not a single delay but rather a distribution of delays due to a distribution of fibre diameters and conduction path lengths, and a consequent distribution of conduction times in the feedback pathways.

Considerations like these lead to models framed in terms of integro-differential equations. For example, eq. (3) becomes

$$\frac{dE}{dt} = f(y(t)) - \gamma E \quad (13a)$$

where

$$y(t) = \int_{-\infty}^{t-\tau_{min}} \phi(t-u)E(u) du, \quad (13b)$$

the kernel  $\phi$  gives the distribution of maturation times and  $\tau_{min} \geq 0$  is the minimum maturation time. Clearly if  $\phi$  is a dirac delta function,  $\phi(t-u) = \delta(t-u-\tau_m)$  with  $\tau_m \geq \tau_{min}$ , then we just have  $y(t) = E(t-\tau_m)$  and the original model of eq. (3) is recovered.

It has been known for some time<sup>8,67-68</sup> that if a smooth kernel corresponding to a gamma distribution of maturation times is chosen,

$$\phi(q) = \frac{a^{m+1}}{m!} q^m e^{-aq}, \quad a, m \geq 0,$$

with  $m$  an integer, then the system (13) can be rewritten as a system of  $m$  linear differential equations coupled to one nonlinear differential delay equation. In the case that  $\tau_{min} = 0$ , then the system just reduces to a system of ordinary differential equations. Furthermore, in this latter case it is easy to calculate that the average delay corresponding to the gamma distribution kernel is simply  $\tau_{av} = (m + 1)/a$  and thus taking the double limit  $m, a \rightarrow \infty$  with  $\tau_{av}$  held constant we have

$$\lim_{\substack{m, a \rightarrow \infty \\ \tau_{av} \text{ const}}} \phi(q) \simeq \delta(t - \tau_{av}),$$

so  $y(t) \simeq E(t - \tau_{av})$ . The dynamics of systems with no minimum delay ( $\tau_{min} = 0$ ) and a continuous gamma distribution of delays as the parameter  $m$  is increased have been studied<sup>69</sup>. Mackey (unpublished) has compared the numerical behaviour of differential delay equations with delta function delays (both negative and mixed feedback) with that of the same equations but with a gamma distribution of delays and  $\tau_{av} = \tau_{min}$ , and found that there seemed to be no new qualitative behaviors introduced by the distribution of delays. The system (13) with very general kernels  $\phi$  may be approximated by systems of ordinary differential equations<sup>70</sup>.

### e). State-dependent delays

To now we have assumed that the time delays are constant. However, in the case of platelet production there is clear evidence that the megakaryocyte age at which platelets are produced is, in fact, not constant but is a function of platelet number<sup>71</sup>. Megakaryocytes mature by undergoing repeated nuclear divisions without cytokinesis to the point of disintegration in three principal ploidy classes<sup>65</sup>:  $8n$ ,  $16n$ , and  $32n$ . Since more nuclear divisions are required to produce a  $32n$  versus a  $8n$  megakaryocyte, ploidy is a convenient marker of megakaryocyte age. In the normal situation the majority of circulating platelets are produced by  $16n$  megakaryocytes. In response to a fall in platelet number<sup>71</sup>, there



is a poorly characterized increase in the velocity of megakaryocyte maturation leading to an eventual decrease in  $\tau_m$ . In addition  $\tau_m$  may be altered under pathological conditions because the different ploidy classes respond differently to stimulation and suppression of thrombopoiesis<sup>72</sup>, i.e. differing proportions of circulating platelets may be derived from the various ploidy classes in these situations.

In view of these observations, eq. (12) should be modified to read

$$\frac{dP}{dt} = -\gamma P + e^{-\delta\tau_m(P)}[\beta(P_{\tau_m(P)}) - \beta(P_{\tau_s+\tau_m(P)})e^{-\gamma\tau_s}] \quad (14)$$

where the dependence of  $\tau_m$  on platelet number is explicitly recognized<sup>73</sup>. Now, the platelet dynamics are described by a nonlinear differential delay equation with two delays, one of which is constant ( $\tau_s$ ) and the second of which is a monotone increasing function of the state variable,  $\tau_m(P) \geq 0$ .

Though little is presently known about the dynamics of the solutions of problems with state dependent delays<sup>74-75</sup>, a local stability analysis of the positive steady state of eq. (14) indicates that when the megakaryocyte destruction rate is positive,  $\delta > 0$ , the inclusion of the state dependent delay  $\tau_m(P)$  is equivalent to an augmentation of the random platelet destruction rate  $\gamma$ . Thus, if the positive steady state  $P_2^*$  of the original platelet model given by eq. (12) was unstable, the effect of the state dependent delay would be to move the steady state closer to the stability boundary. This stabilizing effect of the state dependent delay is a consequence of the fact that the delay is an increasing function of the circulating platelet numbers.

State-dependent delays also arise in models of economic commodity dynamics and of recurrent inhibition in the nervous system. In the economic context, the state dependent delays arise because producers are able to store commodities for a period of time that is dependent on market price<sup>35</sup>. In this situation, it would be expected that the delay would

be a decreasing function of the state variable, market price. In the recurrent inhibitory models, the average value of the distribution of delays mentioned in the previous section is expected to be a decreasing function of the state variable which is the difference between the excitatory and inhibitory potentials in a population of post synaptic cells. In both of these models, based on a local stability analysis the existence of the state dependent delay is a destabilizing factor.

## REGULATION OF PLURIPOTENTIAL STEM CELL PRODUCTION

The most common periodic hematological disease in humans is periodic hematopoiesis (PH)<sup>41-42</sup>. PH is characterized by 17-28 day periodic oscillations in all the formed elements of the blood and is currently thought to be due to a defect in the regulation of the pluripotential stem cells (PPSC)<sup>76-77</sup>. Although control mechanisms within the PPSC populations are not well understood, there is evidence that short range interactions are more important than long range circulating regulators for limiting stem cell numbers.

A schematic representation of a possible model for the production of PPSC is shown in Figure 4. Stem cells are classified as being in either a proliferative (cycling) phase (C) or resting phase (R). Cells travel through proliferation to undergo mitosis after a fixed time ( $\tau$ ). Proliferating cells entering R may exit randomly to either re-enter proliferation at a rate  $\beta$  (known to be a monotone decreasing function of the number of resting phase cells) or to be irreversibly lost via differentiation into the hematopoietic cell lines at an approximately constant rate  $\delta$ . In addition proliferating cells may be lost from any phase of the cell cycle at a rate  $\gamma$ .

The dynamics of this PPSC population is governed by the pair of coupled differential delay equations<sup>13,15</sup>

$$\frac{dC}{dt} = -\gamma C + \beta(R)R - e^{-\gamma\tau} \beta(R_\tau)R_\tau \quad (15a)$$

$$\frac{dR}{dt} = -(\beta(R) + \delta)R + 2e^{-\gamma\tau} \beta(R_\tau)R_\tau \quad (15b)$$

where  $\tau$  is the time required for a cell to traverse the proliferative phase, and the resting to proliferative phase feedback rate is taken to be

$$\beta(R) = \frac{\beta_0 \theta^n}{\theta^n + R^n}.$$

An examination of eq. (15b) shows that this equation could be interpreted as describing the control of a population with a delayed mixed feedback type production term [ $2e^{-\gamma\tau} \beta(R_\tau)R_\tau$ ] and a destruction rate [ $\beta(R) + \delta$ ] that is a decreasing function of  $R$ .

Once again, this model has two possible steady states. There is a steady state corresponding to no cells,  $(C_1^*, R_1^*) = (0, 0)$  which is stable if it is the only steady state, and which becomes unstable whenever the second positive steady state  $(C_2^*, R_2^*)$  exists. The stability of the non-zero steady state depends on the value of  $\gamma$ . When  $\gamma = 0$  (assumed to characterize the normal situation), this steady state cannot be destabilized to produce dynamics characteristic of PH. On the other hand for  $\gamma > 0$ , increases in  $\gamma$  lead to a decrease in the PPSC numbers and a consequent decrease in the cellular efflux (given by  $\delta R$ ) into the differentiated cell lines. This diminished efflux becomes unstable when a critical value of  $\gamma$  is reached,  $\gamma = \gamma_{crit,1}$ , at which a supercritical Hopf bifurcation occurs. For all values of  $\gamma$  satisfying  $\gamma_{crit,1} < \gamma < \gamma_{crit,2}$ , there is a periodic solution of eq. (15) whose period is in good agreement with that seen in PH. At  $\gamma = \gamma_{crit,2}$ , a reverse bifurcation occurs and the greatly diminished PPSC numbers as well as cellular efflux again becomes stable. These results suggest that PH is likely related to defects, possibly genetic, within the PPSC population that lead to an abnormal ( $\gamma > 0$ ) losses of cells from the proliferative phase of the cell cycle. Indeed oscillations can be induced in the peripheral reticulocyte number of mice<sup>52-53</sup> using <sup>89</sup>Sr to increase  $\gamma$ .



Numerical simulations of eqs. (15) bear out the results of the above local stability analyses. When all the parameters in the model are set to the values estimated from laboratory and clinical data, no other types of bifurcations are found. Although these simulations also indicate the existence of multiple bifurcations and chaotic behaviors, these more complex dynamics are only observed for non-physiological choices of the parameters (M. C. Mackey, unpublished). Thus the observed irregularities in the fluctuations in blood cell numbers in PH cannot be related to chaotic solutions of eq. (15).

### CHAOS IN MATURING CELL LINES

The model for the regulation of the PPSC population given by eqs. (15) was derived by balancing cellular fluxes in each of the cell compartments. The fact that cells are both maturing (or aging) and proliferating simultaneously suggests that a model for PPSC production might be better expressed in terms of partial differential equations rather than differential delay equations. However for biologically appropriate boundary conditions it has been previously shown<sup>15</sup> that the description of PPSC production in terms of partial differential equations reduces to the differential delay equations given by eqs. (15). Since complex and chaotic dynamics are observed for eqs. (15), this observation suggests that similar dynamics might also be observed in partial differential equations. This is indeed the case. Here we illustrate this point by considering a model for a population of cells that are proliferating and maturing in a continuous manner. A similar model for a single cellular population has appeared previously<sup>78</sup>.

Characterize each cell in the population by two internal variables:  $a$ , the age of the cell in the cell cycle, and  $m$ , the maturation level of the cell. For concreteness and convenience, one could think of erythroid cells and take the intracellular hemoglobin content as an index of maturation. At birth, cells have age  $a = 0$  and their age increases with a velocity  $V_a$

until cell division occurs at age  $a = a_D$ . In terms of maturation, cells are assumed to first become identifiable members of the population under consideration at a maturation level  $m = m_0$ . These cells mature at a velocity  $V_m$  until they reach the maturation level  $m = m_1$  of a totally mature cell. During this entire process of maturation, the cells proliferate and they may, in addition, also die at a constant random rate  $\gamma$ . It is important to emphasize that this process explicitly allows cellular movement through the cell cycle to proceed hand in hand with cellular maturation. This hypothesis is sufficient to explain existing hematopoietic cell kinetic data<sup>79</sup>.

Denote the number of cells of age  $a$  and maturation level  $m$  at time  $t$  by  $n(t, m, a)$ . By our description of the assumed progression of cells through the age-maturation space,  $n(t, m, a)$  must satisfy a continuity equation of the form

$$\frac{\partial n}{\partial t} + \frac{\partial(V_m n)}{\partial m} + \frac{\partial(V_a n)}{\partial a} = -\gamma n(t, m, a) \quad (18)$$

along with the mitotic flux condition

$$V_a(t, m, 0)n(t, m, 0) = 2V_a(t, m, a_D)n(t, m, a_D). \quad (19)$$

At any given time  $t$  and maturation level  $m$  the total number of cells of all ages is simply

$$N(t, m) = \int_0^{a_D} n(t, m, a) da. \quad (20)$$

If the velocity of maturation,  $V_m$ , is independent of cellular position within the cell cycle, then eq. (18) may be integrated over cellular age  $a$  and the result combined with (19) and (20) to yield a corresponding continuity equation for  $N$ :

$$\frac{\partial N}{\partial t} + V_m \frac{\partial N}{\partial m} = \left[ \beta(t, m, N) - \frac{\partial V_m}{\partial m} - \gamma \right] N. \quad (21)$$

In (21), the *relative proliferation rate*  $\beta$  is defined by

$$\beta(t, m, N) = \frac{V_a(t, m, a_D)n(t, m, a_D)}{N(t, m)}, \quad (22)$$

and plays a role analogous to the function  $\beta$  in the PPSC model of the previous section.

We assume that the maturation velocity  $V_m$ , as a function of maturation level  $m$ , is given by

$$V_m(t, m) = r(m - m_0), \quad (23)$$

where  $m_0 \leq m \leq m_1$  and  $r > 0$  is constant. Equations (21) through (23), in addition to the initial condition

$$N(0, m) = \tilde{v}(m), \quad (24)$$

complete the specification of the model.

One of the parameters is superfluous and may be eliminated by a judicious choice of variables. To this end we first define a dimensionless maturation variable  $x$  by

$$x = \frac{m - m_0}{m_1 - m_0},$$

so  $0 \leq x \leq 1$ . With this change, equations (21) through (23) may be transformed and combined to yield

$$\frac{\partial N}{\partial t} + rx \frac{\partial N}{\partial x} = [\beta(t, x, N) - r - \gamma] N. \quad (25)$$

with the associated initial condition

$$N(0, x) = v(x). \quad (26)$$

Partial differential equations of the form<sup>80</sup>

$$\frac{\partial u}{\partial t} + c(x) \frac{\partial u}{\partial x} = f(x, u) \quad (27)$$

with the initial condition  $u(0, x) = v(x)$  have a unique solution only if  $c(0) = 0$ . However, this requirement for the uniqueness of the solution has some other surprising consequences.



Namely, under certain mild regularity conditions on the function  $f(x, u)$  one of two behaviours may occur. If the initial function  $v(x)$  satisfies  $v(0) > 0$ , then there is always a *stable* unique solution  $u(t, x)$ . However, this stability is lost if the initial function is such that  $v(0) = 0$ , and the solutions are chaotic in a function space.

Our cell proliferation and maturation model is described by an equation (25) exactly of the type of eq. (27), and it is rather easy to illustrate the nature of the unstable solutions that Lasota's results<sup>80</sup> guarantee. To this end, we pick a relative proliferation rate given by  $\beta = \beta_0 - N$ , where  $\beta_0 > 0$  is constant. Then, eq. (25) takes the form

$$\frac{\partial N}{\partial t} + rx \frac{\partial N}{\partial x} = [\beta_0 - r - \gamma - N] N. \quad (28)$$

With the initial condition (26), it is straightforward to show that the solution of eq. (28) is given by

$$N(t, x) = \alpha \frac{v(xe^{-rt})e^{\alpha t}}{1 - v(xe^{-rt})[1 - e^{\alpha t}]}, \quad (29)$$

where we have set  $\alpha = \beta_0 - r - \gamma$ . Clearly, if the solutions  $N(t, x)$  are to be biologically meaningful, then we must have  $\alpha > 0$  and under this condition it is an immediate consequence that  $\lim_{t \rightarrow \infty} N(t, x) = \alpha$  whenever  $v(0) > 0$ , thus illustrating the stability result of Lasota<sup>80</sup>.

If, however,  $v(0) = 0$  then a much different situation occurs. Let  $\nu$  denote the exponential rate at which  $v(xe^{-rt})$  approaches 0. Then it is clear that

$$\lim_{t \rightarrow \infty} N(t, x) = \begin{cases} 0 & 0 < \alpha < \nu \\ \alpha \frac{v(x)}{1 + v(x)} & \nu = \alpha \\ \alpha & \nu < \alpha, \end{cases} \quad (30)$$

which shows the nature of the instability and the sensitive dependence of the eventual behaviour of  $N(t, x)$  on the initial function  $v(x)$ .

The situation of  $v(0) = 0$  is quite likely in hematopoietic systems since, for example, it is known from *in vitro* observations that if cell numbers within the PPSC fall to low values

then differentiation into the neutrophilic and erythroid cell lines ceases. When this occurs, the result would be precisely  $v(0) = 0$  and the instability predicted by the mathematical results of Lasota<sup>80</sup> and demonstrated by equation (30) could easily be observed if there were fluctuations in  $\alpha$  sufficiently large to cause switching between various dynamic behaviours. These fluctuations could arise, for example, because of fluctuations in the rate  $r$  of cell maturation or in the rate  $\gamma$  of cell death within the simultaneously proliferating and maturing cells. The consequent switching in dynamic behaviour would be mirrored in the output flux of cells from this compartment, proportional to  $N(t, 1)$ , and eventually in the numbers of circulating mature cells.

## INTERACTIONS BETWEEN DEVELOPING CELL LINES

Interactions between different committed stem cell compartments occur<sup>81</sup>. For example, *in vivo* experimental maneuvers expected to increase (decrease) the erythropoietin drive to the CSC-E, there is an accompanying decrease (increase) in the number of circulating neutrophils as well as their primitive precursors. Thus changes in the proliferative activity in one population gives rise to changes in the proliferative activity in another. Here we examine whether certain periodic hematological diseases arise from abnormalities in the interactions between different stem cell compartments.

This question has recently been examined<sup>82</sup> in the context of  $n$  coupled first-order partial differential equations of the form of eq. (21). The coupling of proliferative activity between different committed stem cell compartments is mediated by cell number<sup>81</sup>. This coupling will be reflected through the dependence of the relative proliferative rate,  $\beta$ , on the numbers,  $N_i$  of the various stem cells. For  $N_i$  stem cell populations coupled in this way

$$\frac{\partial N_i}{\partial t} + V_m \frac{\partial N_i}{\partial m} = \left[ \beta(t, m, N_1, \dots, N_i) - \frac{\partial V_m}{\partial m} \right] N_i \quad (31)$$

In the special case that

$$\beta(t, m, N_1, \dots, N_i) = \frac{\theta_i^{n_i}(m)}{\theta_i^{n_i} + (\sum_j B_{ij}(m)N_j)^{n_i}} \quad (32)$$

it has been possible to show using local stability results (K. Loskot, unpublished) and numerical simulations, that oscillatory dynamics do not occur. This observation suggests that periodic hematological diseases likely have their origin in other destabilizing mechanisms.

## CONCLUDING REMARKS

Here we have discussed the origins of periodic hematological diseases from the context of the properties of nonlinear delay and partial differential equations. These disorders likely arise as a result of destabilization of feedback control mechanisms. In the case of diseases such as AIHA and CML this destabilization may occur in the long range humoral feedback control mechanisms, whereas for diseases such as PH it probably occurs within the short range feedback mechanisms in the PPSC compartment. In contrast, it is unlikely that oscillations in blood cell number arise because of abnormalities in the interactions between different stem cell compartments.

Under certain conditions, nonlinear delay and partial differential equations can produce (in addition to regular oscillations) complex, aperiodic, irregular fluctuations in blood cell numbers. These “chaotic” dynamics are so complex that in some cases they could be misinterpreted as, or mistaken for, noise and/or experimental error. This observation has caused many investigators to search for a deterministic origin for all of the complex, noisy fluctuations seen in the real world<sup>83–84</sup>. However attractive these claims may be, obtaining solid evidence to support them has been problematic. The strongest case can be made in those experimental situations in which it is possible to observe theoretically predicted dynamics (periodic as well as chaotic) for corresponding parameter values<sup>7,85–86</sup>. The study of hematological control mechanisms is well suited for this approach. In this



case the nature of the control mechanisms are well understood experimentally, a variety of qualitatively different dynamics are observed in both clinical and laboratory settings, and the values of the relevant control parameters can either be measured directly, or readily estimated.

In all the models discussed in this review, oscillations in blood cell number which resemble those observed in human disease and laboratory animals are produced. The fact that these dynamics are observed in the model for the measured ranges of the control parameters indicates that these models provide, at the very least, plausible explanations for the observed dynamics. However, these models do not, in general, readily account for the observed irregular fluctuations in blood cell number. Delayed negative feedback mechanisms (eq. 3) are incapable of generating chaotic dynamics and in the case of the PPSC model (eqs. 15) complex dynamics are not observed for physiologically relevant values of the control parameters. Only in the case of the mixed feedback model for CML (eq. 8) is a deterministic origin of irregular blood cell fluctuations plausible. These observations provide little evidence to support the possibility of a deterministic origin to noise-like fluctuations in blood cell number.

An alternate point of view is that complex, noisy dynamics observed in the real world reflect an interplay between deterministic and stochastic processes. Indeed in order to compare the predictions of any model to experimental and/or clinical data it would seem to be prudent to explore the predictions of the model in the face of noisy perturbations (additive and/or multiplicative). The study of the stochastic nonlinear delay and partial differential equations that arise in the description of physiological phenomena has received little attention.

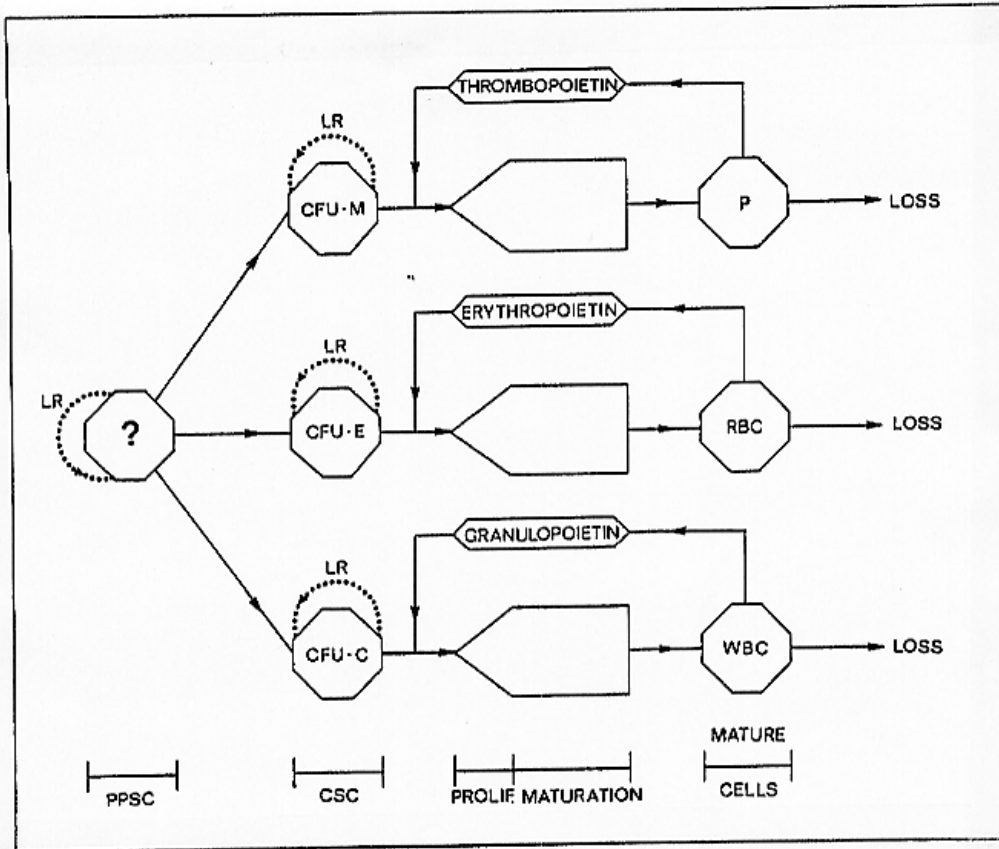
From a clinical point of view, the potentially most important observation gained from

modelling studies is the realization that qualitative changes can occur in blood cell dynamics as quantitative changes are made in control parameters. This suggests the possibility of treating certain periodic hematological diseases by careful manipulation of control parameters<sup>5-7,87-88</sup>. Given the rapid advances in understanding the molecular biology of hematological control mechanisms and the development of molecular probes, such an approach may soon be feasible. It can be anticipated that modelling studies, such as those reviewed here, will play an important role in implementing these treatment strategies.

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## FIGURE LEGENDS



**Figure 1.**

**Hematopoietic Regulation Architecture.** A schematic representation of the control of platelet (P), erythrocyte (RBC), and white blood cell (WBC) production<sup>40</sup>. The peripheral control loops are mediated by the various poietins, and there are in addition local regulatory (LR) loops within the various stem cell compartments. CFU stands for the various colony forming units (M = megakaryocytic, E = erythroid, C = granulocyte/macrophage) which are thought to be the *in vitro* analogs of the *in vivo* committed stem cell (CSC) populations, all of which arise from the pluripotent stem cells (PPSC).



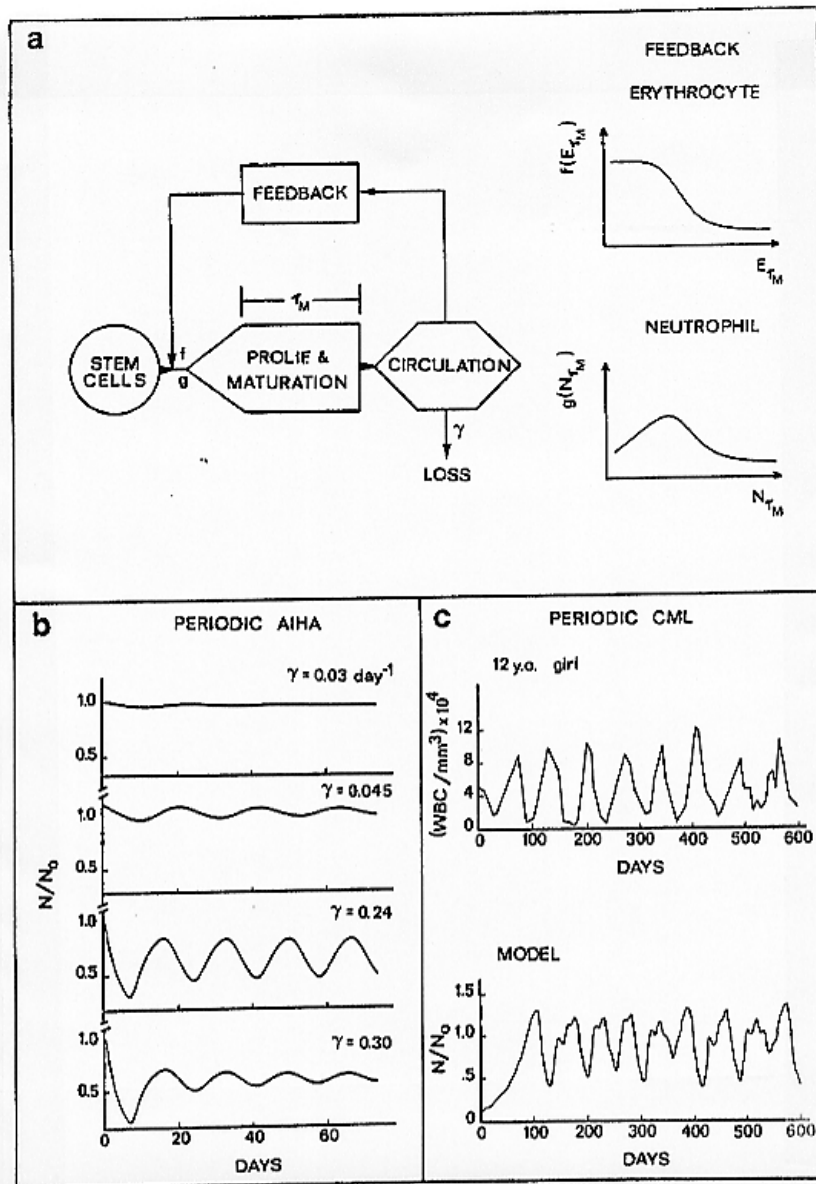
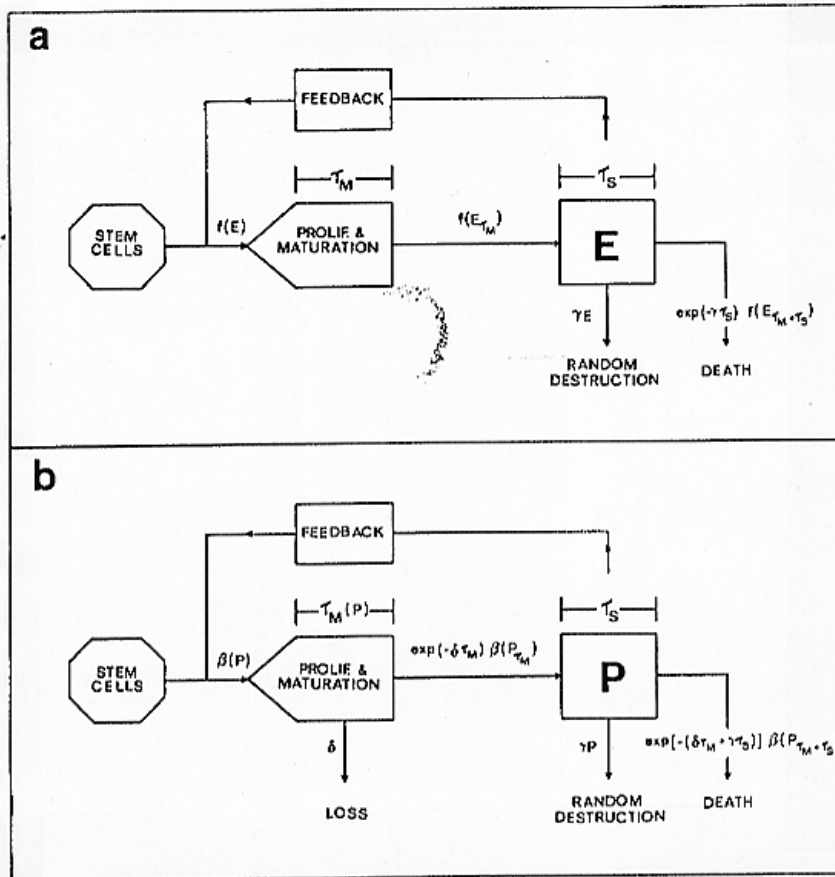


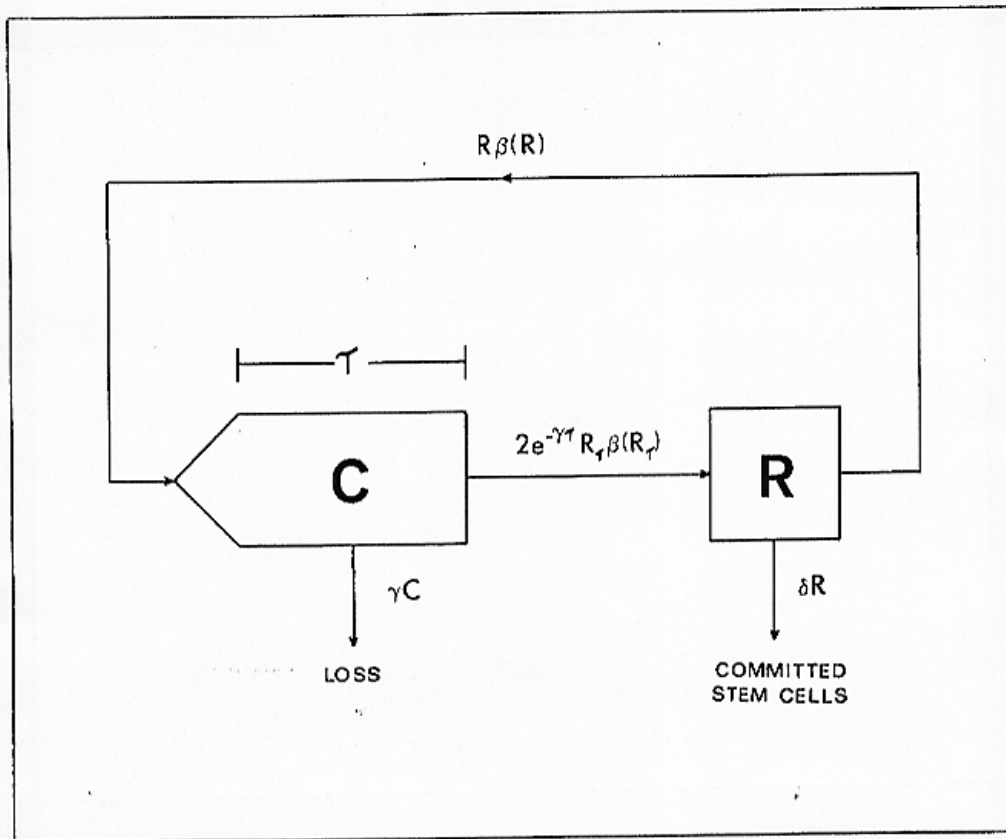
Figure 2.

**Peripheral Hematopoietic Regulation.** a). The generic peripheral regulatory system of Figure 1, with the qualitative dependence of the feedback mechanisms (right) on circulating cell numbers for erythrocyte (top) and neutrophil (bottom) production.  $\gamma$  is the rate of random loss of cells from the circulation. b). Computer simulations of the model for erythrocyte production, defined by eqs. (3,4), for four different peripheral destruction rates,  $\gamma$ . The predicted reticulocyte numbers have been plotted relative to their normal steady state values. See the text for further discussion. c). A comparison between the temporal evolution of WBC numbers in a patient with periodic CML (from ref. 45) and the predictions of the model defined by eqs. (8,9) when the neutrophil production delay is abnormally long<sup>5,6</sup>. See text for further discussion.



**Figure 3.**

**Peripheral Regulation—Variation on a Theme.** a). An elaboration of the erythroid peripheral control, taking into account the senescence time  $\tau_s$  of circulating erythrocytes which leads to a two delay problem as in eqn. (11). b). The peripheral control of platelet production, including the variable maturation time  $\tau_m(P)$  within the megakaryocyte compartment and the senescence time  $\tau_s$  of circulating platelets, as described by eq. (12).



**Figure 4.**

**Central Hematopoietic Regulation.** A schematic representation of the control of PPSC regeneration. Proliferating phase (C) cells include those cells in  $G_1$ , S (DNA synthesis),  $G_2$ , and M (mitosis) while the resting phase (R) cells are in the  $G_0$  phase. Local regulatory influences are exerted via a cell number dependent variation  $[R\beta(R)]$  in the flux of cells reentering proliferation.  $\delta$  is the normal rate of differentiation into all of the CSC populations, while  $\gamma$  represents an abnormal loss of proliferating phase cells. See the text for further details.



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