PERIODIC HAEMATOLOGICAL DISEASES

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I. INTRODUCTION

Nearly 2,400 years ago Hippocrates associated disease with a change in the regularity of a physiological process. Present day clinical medicine often focuses on diseases in which these changes occur on time scales ranging from milliseconds to hours, for example, the generation of cardiac and respiratory arrhythmias, tremors and seizures. More puzzling have been those diseases, collectively referred to as the "periodic diseases", in which symptoms recur at seven-day intervals, or multiples thereof (1). The most common of these disorders are the periodic haematological diseases, i.e. cyclic neutropenia (also known as periodic haematopoiesis) (2, 3), cyclic thrombocytopenia (4), cyclic eosinophilic myositis and hyper-immunolobulin E syndrome (5), and the periodic variants of chronic myelogenous leukemia (6, 7) and autoimmune hemolytic anemia (8, 9).

It has long been suspected that periodic haematological diseases arise because of abnormalities in the feedback mechanisms which regulate blood cell number (10-16). Indeed this observation has provided a major impetus for mathematicians to determine the conditions for oscillation onset in these mechanisms. There have been two surprising predictions of these studies (17, 18): 1) qualitative changes can occur in blood cell dynamics as quantitative changes are made in feedback control; and 2) under appropriate conditions, these feedback mechanisms can produce aperiodic, irregular fluctuations ('chaotic' in the current vernacular) which could easily be mistaken for noise and/or experimental error (19-21). The clinical significance is that it may be possible to develop new diagnostic and therapeutic strategies based on manipulation of feedback (17, 18, 21-23). In these notes I examine some of these theoretical developments and discuss their clinical implications using three different disease entities as case studies.

II. CONTROL OF BLOOD CELL PRODUCTION

The organization of normal haematopoiesis is shown in Figure 1. It is generally believed that there exists a self-maintaining pluripotential stem cell population (PPSC) capable of producing committed stem cells (CSC) specialized for the erythroid, myeloid or thromboid cell lines (24). The influx of cells from the PPSC to the CSC lines is regulated by two types of feedback mechanisms: 1) long range humoral mechanisms, e.g. renal erythropoietin for the erythrocytes, and the colony stimulating factors for the white blood cells; and 2) local environmental mechanisms which are as yet poorly characterized (labeled as LR in Figure 1). An intrinsic property of these feedback mechanisms is the presence of time delays which arise because of finite cell maturation times and cell replication times. Thus most investigators have studied models of delayed feedback in order to investigate the periodic haematological diseases (10-15, 17, 18, 25-30).

In order to appreciate how oscillations develop in blood cell number and their properties such as period and morphology, three steps are necessary: 1) development of a simple, but physiologically realistic, model for the relevant control mechanism; 2) investigation of the properties of the model typically by the use of stability analysis and computer simulations; and 3) comparison of the model's predictions to experimental and/or clinical observations. Current analytic and numerical work determine the time-dependent changes in blood cell number as certain quantities, referred to as control parameters, are varied. Control parameters are those quantities which in comparison to blood cell number either do not change with time, or change very little and hence are regarded by the investigator to be constant. Examples of control parameters in the regulation of haematopoiesis are the maturation times and the

peripheral destruction rate(s). It must be emphasized that quantitative predictions are usually unique to the individual model. Here we discuss only those qualitative properties of delayed feedback mechanisms which appear to be more generally applicable.

Figure 1. Hematopoietic Regulation Architecture. A schematic representation of the control of platelet (P), erythrocyte (RBC), and white blood cell (WBC) production, adapted from (24). There are peripheral control loops mediated by the various poietins, as well as local regulatory (LR) loops within the various stem cell compartments. CFU refers to the various colony forming units (M = megakaryocytic, E = erythroid, C = granulocyte/macrophage) which are the *in vitro* analogs of the *in vivo* committed stem cell (CSC) populations, all of which arise from the pluripotential stem cells (PPSC).

III. PERIODIC AUTO IMMUNE HEMOLYTIC ANEMIA (AIHA) CONTROL OF RED BLOOD CELL PRODUCTION AND DELAYED NEGATIVE FEEDBACK

Periodic AIHA is a rare form of hemolytic anemia in humans (8, 9), but it has been induced in rabbits (cf. Figure 2) by using red blood cell auto-antibodies (32). Rabbit AIHA is one of the best understood periodic haematological diseases and arises from increases in the destruction rate of circulating erythrocytes.

Figure 2. Laboratory Induced AIHA. Oscillations in circulating hemoglobin and reticulocyte counts in a rabbit during constant applications of RBC iso-antibody, taken from (10).

The concept of delayed negative feedback can be introduced by considering the control of erythrocyte production as represented schematically in Figure 1. A fall in circulating erythrocyte numbers leads to a decrease in hemoglobin levels and thus in arterial oxygen tension. This decrease in turn triggers

the production of renal erythropoietin (EPO) which increases the cellular production rate within the early committed erythrocyte series cells, and thus the cellular efflux from the CFU-E into the identifiable proliferating and nonproliferating erythroid precursors, and ultimately augments circulating erythrocyte numbers (i.e. negative feedback).

To formulate this sequence of physiological processes in a mathematical model, we will make the following definitions (12). Let:

$$E(t)(\text{cells/kg}) = \text{circulating density of RBC as a function of time}$$
$$\beta(\text{cells/kg-day}) = \text{cell influx from CFU-C, under EPO control}$$
$$\tau(\text{days}) = \text{time required to pass through recognizable precursors}$$
$$\gamma(\text{days}^{-1}) = \text{loss rate of RBC's in the circulation}$$

Using this notation, we can write a balance equation that states that the rate of change of erythrocyte numbers is a balance between their production and their destruction:

$$\begin{aligned}\frac{dE(t)}{dt} &= \text{production} - \text{destruction} \\ &= \beta(E(t-\tau)) - \gamma E(t). \end{aligned} \tag{1}$$

It is important to remember that once a cell from the PPSC is committed to the erythroid series, it undergoes a series of nuclear divisions and enters a maturational phase for a period of time ($\tau \sim 5.7$ days) before release into circulation. The argument in the production function is $E(t-\tau)$, and not $E(t)$, because a change in the peripheral RBC numbers can only augment or decrease the influx into the circulation after a period of time τ has elapsed. Thus, changes that occur at time t were actually initiated at a time $t-\tau$ in the past. To avoid the cumbersome notation $E(t-\tau)$, in these notes I will adopt the usual convention of $E_\tau(t) = E(t-\tau)$, and also not explicitly denote the time unless necessary. Thus, we can write our simple model (1) for RBC dynamics in the alternate form

$$\frac{dE}{dt} = \beta(E_\tau) - \gamma E. \tag{2}$$

The next step in our model construction is to define some appropriate form for the production function β. *In vivo* measurements of erythrocyte production rates β in rats (31) and other mammals including humans indicate that the feedback function saturates at low erythrocyte numbers, and is a decreasing function of increasing RBC levels (i.e. negative feedback). A convenient function that captures this behaviour, and which has sufficient flexibility to be able to fit the data, as well as easily handled analytic properties, is given by

$$\beta(E_\tau) = \beta_0 \frac{\theta^n}{E_\tau^n + \theta^n}, \tag{3}$$

where β_0 (units of cells/kg-day) is the maximal RBC production rate that the body can approach at very low circulating RBC numbers, n is a positive exponent, and θ (units of cells/kg). These three parameters have to be determined from experimental data related to RBC production rates.

Combining equations (2) and (3) we have the final form for our model of RBC control given in the form

$$\frac{dE}{dt} = \beta_0 \frac{\theta^n}{E_\tau^n + \theta^n} - \gamma E. \tag{4}$$

Equation (4) is a differential delay equation (DDE). In contrast to ordinary differential equations, for which we need only to specify an initial condition as a particular point in the phase space for a given time, for DDE's we have to specify an initial condition in the form of a function defined for a period of time equal to the duration of the time delay. Thus we will select

$$E(t') = \phi(t'), \qquad -\tau \le t' \le 0. \tag{5}$$

Usually we will only consider initial functions that are constant in these notes.

It is well established that under appropriate circumstances, delayed negative feedback mechanisms as in (4) can produce oscillations. To illustrate this point we continue our analysis.

Steady States. A *steady state* for the model (4) is defined by the requirement that the RBC number is not changing with time. This, in turn, can be translated to mean that

$$E(t) = E(t-\tau) = E_\tau(t) = \text{a constant, the steady state} = E^*,$$

and

$$\frac{dE}{dt} = 0 \qquad \text{so} \qquad \beta(E^*) = \beta_0 \frac{\theta^n}{E^* + \theta^n} = \gamma E^*. \tag{6}$$

Now generally we can't solve (6) to get an analytic form for E^*, but a simple graphical argument shows that there is only value of E^* that will satisfy (6). This value of the steady state occurs at the intersection of the graph of γE^* with the graph of $\beta(E^*)$, as indicated by the circle.

Stability of the Steady State. Knowing the steady state of a model like (5), and how it depends on the various parameters of the problem, is certainly useful, but not of much help in understanding the dynamics of the oscillations seen in AIHA as illustrated in Figure 2. To further our investigation, we must examine the *stability* of the steady state E^* that we determined above.

What does stability mean? In words, it just means that if the body had a steady state value E^* of RBC numbers and we perturbed this number (by, for example, blood donation or transfusion) to a value E less than or greater than E^*, then over time we would find that

$$\lim_{t\to\infty} E(t) = E^*. \tag{7}$$

We would like to know what the conditions are on the parameters of our model (destruction rate, maximal production rate, etc) that are required to make sure that the stability condition (7) holds, and even further we would like to know what happens of the stability condition (7) is violated.

Unfortunately, because the production of RBC's is a nonlinear process, and the corresponding model (4) that describes this physiological process is also nonlinear, we cannot answer these questions in total generality. Rather, we must be content with understanding what happens when we make a **small** perturbation of E away from E^*. The fact that we are assuming that the perturbation is small allows us to carry out what is known as a linear stability analysis of the steady state E^*, which we now proceed to do.

The nonlinearity of (4) comes from the term involving the RBC production function, which is highly nonlinear. What we want to do is replace this nonlinear term by a linear function in the vicinity of the steady state E^*. This involves writing out the expansion of β in the vicinity of E^*:

$$\beta(E_\tau) \simeq \beta(E^*) + (E_\tau - E^*)\beta'(E_\tau = E^*) + \frac{1}{2}(E_\tau - E^*)^2\beta''(E_\tau = E^*) + \cdots \tag{7}$$

remembering that we are assuming that $E_\tau - E^*$ is pretty small so $(E_\tau - E^*)^2$ is even smaller and therefore negligible, and finally writing the approximate version of (4) in the vicinity of E^* as

$$\frac{dE}{dt} \simeq \beta(E^*) + (E_\tau - E^*)\beta'(E_\tau = E^*) - \gamma E. \tag{8}$$

Remember from equation (6) that the defining equation for the steady state is $\beta(E^*) = \gamma E^*$. Using this in equation (8), we can rewrite it as

$$\frac{dE}{dt} \simeq (E_\tau - E^*)\beta'(E_\tau = E^*) - \gamma(E - E^*). \tag{9}$$

If we further set $z(t) = E(t) - E^*$, so z is the deviation of the RBC numbers from their steady state value, then $z_\tau(t) = E_\tau(t) - E^*$ and $dE/dt = dz/dt$ so (9) can be rewritten as the linear differential delay equation

$$\frac{dz}{dt} = \beta'(E^*)z_\tau - \gamma z. \tag{10}$$

Now linear equations, with or without delays, are a lot easier to work with than nonlinear ones. The usual procedure, loosely speaking, to find a solution is to assume that the solution has the form $z(t) \simeq e^{\lambda t}$,

and find out the requirements on the parameters of the equation such that there is an eigenvalue λ allowing z to be written in this form. Usually the eigenvalue λ is a complex number, $\lambda = \mu + i\omega$ so the solution can actually be written in the alternative form

$$z(t) \simeq e^{\lambda t} = e^{\mu t}\left\{\cos(\omega t) + i\sin(\omega t)\right\}. \tag{11}$$

If $\mu = \mathrm{Re}\lambda < 0$, then the solution is a decaying oscillating function of time that approaches zero, so we have a stable situation. If $\mu = \mathrm{Re}\lambda > 0$ on the other hand, then the solution diverges to infinity in an oscillatory fashion and the solution is unstable. The boundary between these two situations, where $\mu = \mathrm{Re}\lambda = 0$, defines a *Hopf bifurcation* in which an eigenvalue crosses from the left hand to the right hand complex plane.

After that wordy digression, lets assume that $z(t) \simeq e^{\lambda t}$ in equation (10). If we make this substitution, and carry out the algebra, then we are left with the equation

$$\lambda = \beta'(E^*)e^{-\lambda\tau} - \gamma \tag{12}$$

that λ must satisfy. In general, determining the criteria such that $\mathrm{Re}\lambda = \mu < 0$ involves a lot of messy algebra. However, determining the relation between the parameters such that $\mathrm{Re}\lambda = \mu = 0$ is a lot easier, and so lets look at it.

Under this assumption, $\lambda = i\omega$ and substituting this into equation (12) gives

$$i\omega = \beta'(E^*)[\cos(\omega\tau) - i\sin(\omega\tau)] - \gamma,$$

or after separating real and imaginary parts

$$\omega = -\beta'(E^*)\sin(\omega\tau) \tag{13a}$$

$$\gamma = \beta'(E^*)\cos(\omega\tau) \tag{13b}$$

Equation (13b) can be solved for $\omega\tau$ to give

$$\omega\tau = \cos^{-1}\left(\frac{\gamma}{\beta'(E^*)}\right). \tag{14}$$

Further, squaring and adding the two equations (13) gives

$$\omega = \sqrt{\beta'(E^*)^2 - \gamma^2}. \tag{15}$$

Combining equations (14) and (15), we find that the relation connecting τ, $\beta'(E^*)$, and γ that must be satisfied in order for the eigenvalues to have real part identically to zero is given by

$$\tau = \frac{\cos^{-1}\left(\dfrac{\gamma}{\beta'(E^*)}\right)}{\sqrt{\beta'(E^*)^2 - \gamma^2}}. \tag{16}$$

In general (33), after the messy algebra mentioned above, the real parts of λ will be negative, and thus the linear equation (10) will have a locally stable steady state, if and only if the parameters satisfy the inequality

$$\tau < \frac{\cos^{-1}\left(\dfrac{\gamma}{\beta'(E^*)}\right)}{\sqrt{\beta'(E^*)^2 - \gamma^2}}. \tag{17}$$

When the parameters satisfy (16), then we say that there has been a Hopf bifurcation. The period of the periodic solution that is guaranteed when (16) is satisfied can be easily derived by noting that

$\omega = 2\pi f = 2\pi/T$, where f and T are the frequency and period of the solution respectively, and thus from (14) through (16)

$$T = \frac{2\pi\tau}{\cos^{-1}\left(\frac{\gamma}{\beta'(E^*)}\right)}. \tag{18}$$

Since the inverse cosine ranges from 0 to π, from (18) we know that at the Hopf bifurcation the period of the periodic solution satisfies

$$2\tau \leq T. \tag{19}$$

In general, the period of an oscillation produced by a delayed negative feedback mechanism is at least twice the delay (11, 33). Moreover, for our model of erythrocyte production it can be shown that the period of the oscillation should be no greater than four times the delay (12), i.e.

$$2\tau \leq T \leq 4\tau. \tag{20}$$

Since the maturational delay for erythrocyte production is $\tau \sim 6$ days, we would expect to see oscillations in erythrocyte numbers with periods ranging from 12-24 days. This is in excellent agreement with the observed periods of 16-17 days in rabbit AIHA (32). What is surprising is the fact that these oscillations are so rarely observed. This paradox is illuminated in the following sections.

Parameter Estimation. Having extracted about as much information as is possible from the linear analysis of our model for RBC production, we now need to turn to numerical simulations to see the full (nonlinear) behavior. This, by necessity, requires that we have some estimation of the parameters in equation (4). Through a variety of data, we finally conclude that in the normal situation (i.e., not AIHA):

$$\begin{aligned}
\gamma &= 2.31 \times 10^{-2}\text{day}^{-1} \\
\beta_0 &= 7.62 \times 10^{10}\text{cells/kg-day} \\
n &= 7.6 \\
\theta &= 2.47 \times 10^{11}\text{cells/kg} \\
\tau &= 5.7\text{days}
\end{aligned}$$

These parameters correspond to a steady state circulating RBC mass of $E^* = 3.3 \times 10^{11}$cells/kg, and from the linear analysis of the previous section it is predicted that this steady state is stable.

Explaining Laboratory Induced AIHA. The one fact that we know about the induced AIHA shown in Figure 2 is that the RBC destruction rate γ is increased through the cell lysis action of the injected iso-antibody. The linear analysis presented above predicts that the steady state E^* will be stable in the face of increased γ until $\gamma \simeq 5.12 \times 10^{-2}\text{day}^{-1}$, and when it becomes unstable at this point there will be an oscillation about the steady state with a period of $T \simeq 20.6$ days as given by equation (18). Our linear analysis tell us nothing about what happens in the full nonlinear equation (4) after this stability is lost, but it does predict that when γ is further elevated to $\gamma \simeq 2.70 \times 10^{-1}$ then another (reverse) Hopf bifurcation should occur and that the period of the periodic solution will be $T \simeq 16.6$ days. Further increases in γ such that $\gamma > 2.70 \times 10^{-1}$ are predicted to result in a stabilization of E^* about a low value.

Numerical Simulations. Numerical simulations of equation (4) with the parameters given above show that the linear analysis results quoted above give a very accurate picture of the full nonlinear behaviour, including the values of γ at which stability of E^* is lost and regained, and the period of the solutions at these stability boundaries (cf. Figure 3).

Figure 3 shows a computer simulation of the model as a function of the peripheral destruction rate (γ). As can be seen, when γ is low, as normally occurs, oscillations in erythrocyte numbers do not occur. As γ increases, regular oscillations occur whose period increases as γ increases. However, for high γ, no oscillation occurs. Interestingly, depending on the severity of the hemolytic anemia induced in the rabbit model, reticulocyte levels were observed to be either depressed at constant levels or to oscillate (32). A much more comprehensive model for the control of RBC production, and mathematically much

more complicated, shows properties (49) that are virtually identical with those of the simple model presented here. The observations in Figure 3 indicate that whether or not a proposed mechanism for periodic AIHA produces an oscillation critically depends on whether the value of the control parameter, i.e. the peripheral destruction rate (γ), lies in some crucial range. This may explain why oscillations in erythrocyte number are so rarely seen in patients with AIHA.

Figure 3. Computer simulations of the model (4) for erythrocyte production for four different peripheral destruction rates, γ. The predicted reticulocyte numbers have been plotted relative to normal values, E_0.

It should be noted that the morphology of the oscillations shown in Figure 3 are quite simple, i.e. there is only one maxima per period. All studies to date of delayed negative feedback mechanisms have indicated that only oscillations with this simple morphology can be produced (17, 18, 34, 35). More complex waveforms (i.e. more than one maximum per period) are, however, possible with multiple delayed negative feedback loops (36) and with multiple time delays (37).

IV. PERIODIC CHRONIC MYELOGENOUS LEUKEMIA CONTROL OF WHITE BLOOD CELL PRODUCTION AND DELAYED MIXED FEEDBACK

In the previous section we only considered delayed negative feedback mechanisms. However, this is not the only type of feedback which can occur. A more complicated type of feedback arises for the control of circulating neutrophil numbers. Over a wide range of circulating neutrophil levels, the neutrophil production rate decreases as the number of neutrophils increases (i.e. negative feedback). However, due to a variety of factors, it is expected that at very low neutrophil numbers the production rate falls to zero. Thus in the range of low neutrophil numbers, the production rate must increase as neutrophil number increases (i.e. positive feedback). This type of feedback is a mixture of positive and negative feedback, i.e. 'mixed' feedback (34-35, 45).

In order to contrast the dynamics that arise in delayed negative and mixed feedback mechanisms we will consider the disorder periodic chronic myelogenous leukemia (CML). Periodic CML is a variant of CML in which peripheral neutrophil numbers oscillate around elevated levels with a period of 30-70 days even in the absence of clinical interventions (6-7). In most patients the oscillations in neutrophil numbers range from approximately normal values to levels that are approximately 10 times normal. Although occasionally parallel oscillations in thrombocytes and reticulocytes have been reported (7), here we consider only those patients with oscillations in neutrophil levels. This disorder may be more common than is generally appreciated since it is known that oscillations can occur in the number of S-phase CFU-C in CML patients in the absence of peripheral leukocyte oscillations (46).

Figure 4 shows the serial white cell counts (6) in a 12 year-old girl with periodic CML. There are oscillations in neutrophil number with a period of $\sim$ 60-70 days. On closer inspection it can be seen that the number of days between successive maximum numbers of neutrophils is not constant, but varies by a few days. Moreover the morphology of each waveform differs slightly and there are shoulders on some of

them. The standard explanation for the origin of these departures from a regular periodic oscillation is that they reflect sampling errors and/or the influence of uncontrollable "random" fluctuations in blood cell number. Here we discuss the possibility that these irregularities are intrinsic properties of the underlying control mechanism.

Figure 4. (a) WBC numbers as a function of time in a patient with CML. (b) Simulated solution of the model given by equation (21) assuming all parameters are normal except for an elevated maturation/proliferation delay, which was taken to be 20 days.

A simple model for the regulation of neutrophil number, N, which incorporates the delayed mixed feedback is really virtually identical with the model of equation (4) for RBC production:

$$\frac{dN}{dt} = g(N_\tau) - \gamma N \tag{21}$$

where γ is again the random destruction rate and the delayed mixed feedback production function operating through the peripheral colony stimulating factor is

$$g(N_\tau) = \frac{\beta_0 N_\tau \theta^n}{\theta^n + N_\tau^n}. \tag{22}$$

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

$$N_2^* = \theta \left[\frac{\beta_0 - \gamma}{\gamma} \right]^{1/n} \tag{23}$$

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 γ, increases in either β_0 and/or n, which both increase the gain of the system at the steady state, and in τ 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 equation (14) with $g'(N^*)$ replacing $\beta'(N^*)$.

The behaviour of equation (21) has been studied numerically (17,18,22), and these studies indicate that the dynamics of equations (21) and (22) is much richer than for the simple negative feedback model of erythrocyte production. Increases in τ are of particular interest since a prolongation of the neutrophil maturation time is inferred in patients with CML (47). As τ is increased an initially stable equilibrium becomes unstable and stable periodic solutions appear. Further increases in τ 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 4b). 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 using different analytic mixed feedback forms for the function g.

Figure 4b shows a computer simulation when the maturation time τ is increased from the normal value of $\sim 5-6$ days to 20 days. Not only does the simulation correctly predict the overall period of the observed oscillations in the CML patient, it also has irregular fluctuations. This is in contrast to the general conception that random fluctuations are caused by random inputs. Here the model predicts that levels of circulating neutrophils are random simply as a consequence of their own deterministic evolution.

The complex, aperiodic fluctuations produced by deterministic mechanisms are currently referred to as chaos (19). The realization that physiological control mechanisms can generate exceedingly complex oscillations, such as chaos, is currently a subject of great interest (17-21). It is quite possible that both interesting and relevant dynamical changes are often observed, but their significance is wrongly ascribed to environmental noise and/or experimental error. Careful attention to these dynamical behaviors may eventually provide important insights into the properties of the underlying control mechanisms.

V. PERIODIC HEMATOPOIESIS (PH) AND PPSC STABILITY

The most common periodic haematological disease is PH. In humans, PH is a disease characterized by 17-28 day periodic oscillations in circulating neutrophil numbers from approximately normal values to barely detectable numbers (2-3, 54, 60). In addition to neutrophils, oscillations are seen for all the formed elements of blood with the same period. The oscillations for each blood cell lines are out of phase and the phase differences between the cell lines are consistent with the differences in the maturation times. Neutropenic episodes place the patients at increased risk for infective processes (e.g. abscesses, pneumonia, septicemia) and up to 20 % of patients have died during these episodes.

An abnormality in the regulation of the PPSC in PH is suggested by the observation that the disorder can be transferred by bone marrow transplantation (38, 39, 67). Consequently most investigators have looked to abnormalities in the regulation of the PPSC and delayed negative feedback mechanisms as an explanation for PH (11, 13-15, 25-30, 55, 56). Recent evidence has suggested that the abnormality in feedback may be related to defective lymphocyte production of granulocyte-macrophage colony-stimulating factor (GM-CSF) in response to a monocyte-derived recruiting activity (40, 41).

A schematic representation of PPSC regulation is shown in Figure 5. Experimental study of periodic haematological disorders has been facilitated by the availability of suitable animal models. All gray

Figure 5. A schematic representation of the control of PPSC regeneration. Proliferating phase cells (C) 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 in the fraction of circulating cells. δ is the **normal** rate of differentiation into all of the CSC populations, while γ represents a loss of proliferating phase cells due to apoptosis. See (11, 13) for further details.

collies have PH (38, 42, 54, 59) and periodic erythropoiesis can be induced in mice by the administration of a single dose of the marrow-seeking radioisotope ^{89}Sr (42, 43). Here we interpret the effects of an increase in the rate of irreversible apoptotic loss from the proliferating phase of the PPSC (γ in Figure 5) on blood cell production (17).

The dynamics of this PPSC population is governed (13,15) by the pair of coupled differential delay equations

$$\frac{dC}{dt} = -\gamma C + \beta(R)R - e^{-\gamma\tau}\beta(R_\tau)R_\tau \tag{24a}$$

$$\frac{dR}{dt} = -(\beta(R) + \delta)R + 2e^{-\gamma\tau}\beta(R_\tau)R_\tau \tag{24b}$$

where τ is the time required for a cell to traverse the proliferative phase, and the resting to proliferative phase feedback rate β is taken to be

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

An examination of Equation (24b) 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.

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 γ. When $\gamma = 0$, this steady state cannot be destabilized to produce dynamics characteristic of PH. On the other hand for $\gamma > 0$, increases in γ lead to a decrease in the PPSC numbers and a consequent decrease in the cellular efflux (given by δR) into the differentiated cell lines. This diminished efflux becomes unstable when a critical value of γ is reached, $\gamma = \gamma_{crit,1}$, at which a supercritical Hopf bifurcation occurs. For all values of γ satisfying $\gamma_{crit,1} < \gamma < \gamma_{crit,2}$, there is a periodic solution of equation (24) 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. Separate estimations of the parameter sets for human and grey collie PPSC populations give predictions of the period of the oscillation at the Hopf bifurcation that are consistent with those observed clinically and in the laboratory.

Numerical simulations of equations (24) bear out the results of the above local stability analyses. As expected an increase in γ is accompanied by a decrease in the average number of circulating cells. For certain values of γ an oscillation appears. Over the range of γ in which an oscillation occurs, the period increases as γ increases. However, the amplitude of the oscillation first increases and then decreases. (Similar observations hold for the model of AIHA as the control parameter γ is increased.) 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. Thus the observed irregularities in the fluctuations in blood cell numbers in PH cannot be related to chaotic solutions of equation (24). 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.

VI. UNDERSTANDING LABORATORY INDUCED CYCLICAL ERYTHROPOIESIS

To illustrate the usefulness of the preceding model with a second concrete example, we will compare these predictions to experimental observations obtained for ^{89}Sr-induced cyclic erythropoiesis in two congenitally anemic strains of mice, W/W^v and S1/S1^d (Figure 3c)(43, 44). W/W^v mice suffer from a defect in the PPSC and in S1/S1^d mice the hematopoietic micro-environment is defective. Let us assume that the difference between W/W^v and S1/S1^d mice is solely related to differences in γ. The observation that S1/S1^d mice are more refractory to erythropoietin than W/W^v suggests that γ is higher in S1/S1^d. From Figure 3a we see that a higher γ would increase the likelihood that an oscillation in erythrocyte number occurs. Indeed, in contrast to W/W^v, $\sim$ 40 % of S1/S1^d mice have "spontaneous" oscillations in their hematocrit (44). In both strains of mice, a single dose of ^{89}Sr is sufficient to increase γ into a range associated with oscillations in erythrocyte number. Since the value of γ for S1/S1^d was greater than that for W/W^v prior to ^{89}Sr, it is reasonable to expect that it will also be higher following administration of ^{89}Sr. Thus as predicted from Figure 3b, the period of the oscillation is longer, the amplitude is larger and the mean hematocrit is lower for S1/S1^d mice.

VII. UNDERSTANDING CSF EFFECTS IN PH

Recent clinical and experimental work has focused on the modification of the symptoms of periodic hematopoiesis by recombinant colony stimulating factors, whose receptor biology is reviewed in (68).

Human colony stimulating factors increase both the numbers and proliferation rate of neutrophil precursors in a variety of situations (53, 61, 62). Furthermore, colony stimulating factor in mice is able to stimulate replication in both stem cells and early erythroid cells (65). In human periodic hematopoiesis, it is found (57, 64) that the administration of low levels of colony stimulating factor has several effects: (1) It elevates the mean level around which the peripheral neutrophil counts oscillate; (2) It decreases the period from 21 days to about 14 days; and (3) It increases the amplitude of the oscillations. In canine periodic hematopoiesis, use of colony stimulating factor had the same qualitative effects as in human periodic hematopoiesis, with the added finding that: (4) The effects were dose dependent, and high doses of colony stimulating factor are able to obliterate the cycling characteristic of canine periodic hematopoiesis (61, 66). There is circumstantial evidence that the same cessation of cycling may occur in humans.

Thus, the available laboratory and clinical data on the effects of colony stimulating factor in periodic hematopoiesis indicate that: (1) There is extensive inter-communication between all levels of stem cells; and (2) Within the language of nonlinear dynamics, colony stimulating factor may be used to titrate the dynamics of periodic hematopoiesis to the point of inducing a reverse Hopf bifurcation (disappearance of the oscillations).

The behaviour in periodic hematopoiesis when colony stimulating factor is administered is qualitatively consistent with the PPSC model discussed in Section V since it it known that CSF interferes with apoptosis and thus administration of CSF is equivalent to a decrease in the apoptotic death rate γ. This is a current active area of research in conjunction with Profs. David Dale and William Hammond of the University of Washington (Seattle). We expect that this combined modeling and data analysis project will give greater insight into the fundamental nature of the regulation of the mammalian cell cycle and may, in the future, suggest more rational therapies for patients with periodic hematopoiesis.

VIII. CONCLUDING REMARKS

Delayed feedback mechanisms are important for regulating blood cell numbers. Under certain conditions, delayed feedback mechanisms can produce oscillations whose period typically ranges from 2-4 times the delay, but which may be even longer. The observation that periodic haematological diseases have periods which are multiples of seven may simply be a consequence of the combination of delayed feedback mechanisms with maturation times which are of the order of 5-7 days. Thus it is not necessary to search for illusive and mystical entities (48), such as ultradian rhythms, to explain the periodicity of these disorders.

The observations in these notes emphasize that an intact control mechanism for the regulation of blood cell numbers is capable of producing behaviors ranging from no oscillation to periodic oscillations to more complex irregular fluctuations, i.e. chaos. The type of behaviour produced depends on the nature of the feedback, i.e. negative or mixed, and on the value of certain underlying control parameters, e.g. peripheral destruction rates or maturation times. Pathological alterations in these parameters can lead to periodic haematological disorders.

As an extension to the concept of periodic diseases introduced by Riemann (1) in 1963, the term 'dynamical disease' has been introduced (17, 18, 21-23). A dynamical disease is defined as a disease that occurs in an intact physiological control system operating in a range of control parameters that leads to abnormal dynamics. Clearly the hope is that it may eventually be possible to identify these altered parameters and then re-adjust them to values associated with healthy behaviors. Developments in biotechnology and the analysis of physiological control mechanisms are occurring at such a rapid pace that the feasibility of such an approach may be just around the corner.

Often overlooked in the advance of medical science is the essential role that must be assumed by the practicing physician. The identification of a dynamical disease as well as its response to clinical maneuvers can only be assessed at the bedside. Indeed it is possible that both interesting and relevant dynamical changes have been observed, but not published because their theoretical significance is not fully appreciated by the clinician. In the words of the French neurologist, J. M. Charcot: " Disease is very old and nothing about it has changed. It is we who change as we learn to recognize what was formerly imperceptible".

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References.

1. H. A. Riemann (1963) *Periodic Diseases.* Philadelphia: F.A. Davis Co.
2. D. G. Wright *et al.* (1981) *Medicine (Baltimore)*, **60**, 1.
3. R. D. Lange (1983) *Experimental Hematology*, **11**, 435.
4. T. Cohen and D. P. Cooney (1974) *Scandinavian Journal of Haematology*, **12**, 9.
5. W. A. Symmans *et al.* (1986) *Annals of Internal Medicine*, **104**, 26.
6. R. A. Gatti *et al.* (1973) *Blood*, **41**, 771.
7. G. Chikkappa *et al.* (1976) *Blood*, **47**, 1023.
8. P. Ranlov and A. Videbaek (1963) *Acta Medica Scandinavica*, **174**, 583.
9. R. R. Gordon and S. Varadi (1962) *Lancet*, **i**, 296.
10. J. Kirk *et al.* (1968) *British Journal of Haematology*, **15**, 35.
11. M. C. Mackey (1978) *Blood*, **51**, 941.
12. M. C. Mackey (1979) *Bulletin of Mathematical Biology*, **41**, 829.
13. M. C. Mackey (1979) In *Biophysical and Biochemical Information Transfer in Recognition.* (Eds J. Vassilova-Popova and E.V. Jensen) p373. New York: Academic Press.
14. A. Morley (1979) *Blood Cells*, **5**, 283.
15. C. D. R. Dunn (1983) *Experimental Hematology*, **11**, 779.
16. H.-E. Wichmann and M. Loeffler (1988) *Mathematical Modeling of Cell Proliferation: Stem Cell Regulation in Hemopoiesis* p 55. Boca Raton: CRC Press.
17. M. C. Mackey and L. Glass (1977) *Science*, **197**, 615.
18. L. Glass and M. C. Mackey (1979) *Annals of the New York Academy of Sciences*, **316**, 214.
19. H. Bai-Lin (1984) *Chaos*, Singapore: World Scientific.
20. H. Degn *et al.* (1987) *Chaos in Biological Systems*, New York: Plenum Press.
21. L. Glass and M. C. Mackey (1988) *From Clocks to Chaos: The Rhythms of Life.* Princeton: Princeton University Press.
22. M. C. Mackey and U. an der Heiden (1982) *Funktionelle Biologie & Medizin*, **1**, 156.
23. M. C. Mackey and J. G. Milton (1987) *Annals of the New York Academy of Sciences*, **504**, 16.
24. P. Quesenberry and L. Levitt (1979) *New England Journal of Medicine*, **301**, 755, 819.
25. T. E. Wheldon *et al.* (1974) *Blood*, **43**, 379.
26. T. E. Wheldon (1975) *Mathematical Biosciences*, **24**, 289.
27. V. G. Nazarenko (1976) *Biofizika*, **21**, 352.
28. N. MacDonald (1978) In *Biomathematics and Cell Kinetics.* (Eds. A.-J. Valleron and P.D.M. Macdonald) p 287. New York: Elsevier.
29. N. D. Kazarinoff and P. van den Driessche (1979) *Science*, **203**, 1348.
30. N. D. von Schulthess and N. A. Mazer (1982) *Blood*, **59**, 27.
31. G. Hodgson and I. Eskuche (1966) *Archives of Biological and Medical Experiments*, **3**, 85.
32. J. S. Orr *et al.* (1968) *British Journal of Haematology*, **15**, 23.
33. N. D. Hayes (1950) *Journal of the London Mathematical Society*, **25**, 226.
34. U. an der Heiden and M. C. Mackey (1982) *Journal of Mathematical Biology*, **16**, 75.
35. A. Longtin and J. G. Milton (1988) *Mathematical Biosciences*, **90**, 183.
36. L. Glass *et al.* (1988) *Mathematical Biosciences*, **90**, 111.
37. J. Bélair and M. C. Mackey (1989) *Journal of Dynamics and Differential Equations*, **1**, 299.
38. J. B. Jones *et al.* (1975) *British Journal of Haematology*, **30**, 215.
39. R. A. Krance *et al.* (1982) *Blood*, **60**, 1253.
40. T. P. Loughran and W. P. Hammond (1986) *Journal of Experimental Medicine*, **164**, 2089.
41. W. P. Hammond *et al.* (1988) *Experimental Hematology*, **16**, 389.
42. D. C. Dale *et al.* (1972) *Journal of Clinical Investigation*, **51**, 2190.
43. C. W. Gurney *et al.* (1981) *Experimental Hematology*, **9**, 118.
44. C. M. Gibson *et al.* (1984) *Experimental Hematology*, **12**, 343.
45. N. MacDonald (1978) *Time Lags in Biological Models.* New York: Springer-Verlag.

46. T. Olofsson and I. Olsson (1976) *Blood*, **48**, 351.
47. P. C. Vincent (1974) In *Leukemia* (Eds F. Gunz and A.G. Baikie) p 189, New York: Grune and Stratton.
48. C. H. Beresford (1988) *Journal of the Royal College of Physicians of London*, **22**, 94.
49. J. Bélair *et al.* (1995) *Mathematical Biosciences*, in press.
50. J. G. Milton and M. C. Mackey (1989) *Journal of the Royal College of Physicians of London*, **23**, 236.
51. M. C. Mackey and J. G. Milton (1990) *Comments in Theoretical Biology*, **1**, 299.
52. J.L. Abkowitz *et al. Experimental Hematology* (1988) **16**, 941.
53. M.H. Bronchud *et al. British Journal of Cancer* (1987) **56**, 809.
54. D.C. Dale & W.P. Hammond. *Blood Reviews* (1988) **2**, 178.
55. T.M. Dexter & E. Spooncer. *Annual Reviews of Cell Biology* (1987) **3**, 423.
56. M.Y. Gordon. *Blood Reviews* (1993) **7**, 190.
57. W.P. Hammond *et al. New England Journal of Medicine* (1989) **320**, 1306.
58. W.P. Hammond *et al. Blood* (1990) **76**, 523.
59. J.B. Jones & R.D. Lange. *Immunology and Hematology Research Monographs* (1983) **1**, 33.
60. R.D. Lange. *Experimental Hematology* (1993) **11**, 435.
61. B.I. Lord *et al. Proceedings of the National Academy of Sciences, USA* (1989) **86**, 9499.
62. B.I. Lord *et al. Blood* (1991) **77**, 2154.
63. C.D. Lothrop *et al. Blood* (1988) **72**, 1324.
64. A.R. Migliaccio *et al. Blood* (1990) **75**, 1951.
65. D. Metcalf *et al. Blood* (1980) **55**, 138.
66. A. Morley & F. Stohlman. *New England Journal of Medicine* (1970) **282**, 643.
67. P.J. Quesenberry. *Immunology and Hematology Research Monographs* (1983) **1**, 2.
68. A.P. Rapoport *et al. Blood Reviews* (1992) **6**, 43.