8. MATHEMATICAL MODELS OF HEMATOPOIETIC CELL REPLICATION AND CONTROL

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8.1 INTRODUCTION

The biological sciences offer an abundance of examples in which the dynamics are dependent on the past history of the system being studied. In the attempt to construct mathematical models for these processes, the dynamic equations are often framed either as functional equations or, more specifically, as differential delay equations. Often by studying the solution behavior of these model systems one can begin to understand the normal biological responses noted in the laboratory.

Even more interesting are those clinical situations in which the normal dynamics of a system are replaced, in disease situations, by dynamics with different characteristics. Sometimes these characteristics involve the destabilization of a steady state in favor of periodic or aperiodic behavior, or the replacement by a new periodic regime of the normal periodic one. Such diseases are called "periodic diseases" (Glass and Mackey 1988; Mackey and Glass 1977; Reimann 1963) and are quite interesting in that they can often give clues about the nature of the underlying disease expressed as a shift in parameters giving rise to a bifurcation.

Some of the most fascinating of these periodic diseases are the periodic diseases of the blood (periodic hematological diseases). It has long been suspected that periodic hematological diseases arise because of abnormalities in the feedback mechanisms which regulate blood-cell number (Dunn 1983; Kirk et al. 1968; Mackey 1978; Mackey 1979b; Mackey 1979a; Morley 1979; Wichmann and Loeffler 1988). 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 (Glass and Mackey 1979; Mackey and Glass 1977): (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 (Bai-Lin 1984; Degn et al. 1987; Glass and Mackey 1988). The clinical significance is that it may be possible to develop new diagnostic and therapeutic strategies based on manipulation of feedback (Glass and Mac-

key 1988; Mackey and Glass 1977; Mackey and an der Heiden 1982; Mackey and Milton 1987). In this chapter I examine some of these theoretical developments and discuss their clinical implications, using several different disease entities as case studies.

8.2 CONTROL OF BLOOD-CELL PRODUCTION

The organization of normal hematopoiesis (the production and maintenance of circulating blood cell numbers) is shown in Figure 8.1. It is generally believed that there exists a self-maintaining population of undifferentiated cells (pluripotential stem cells) capable of producing committed stem cells specialized for the red blood (erythroid), white blood (myeloid) or blood-clotting (thromboid) cell lines (Quesenberry 1990). The dynamics of pluripotential stem cells and the committed stem cells are regulated by two types of feedback mechanisms: (1) long-range humoral mechanisms (Cebon and Layton 1994; Sachs 1993; Sachs and Lotem 1994), e.g., renal erythropoietin for the erythrocytes, the colony-stimulating factors (G-colony-stimulating factor and GM-colony-stimulating factor) for the leukocytes (white blood cells), and thrombopoietin for the platelets; and (2) local environmental mechanisms, such as stem-cell factor (SCF) which are as yet poorly characterized (labeled as LR in Figure 8.1). An intrinsic property of these feedback mechanisms is the presence of time delays which arise because of finite nonzero cell-maturation times and cell-replication times. Thus many investigators have studied models of delayed feedback in order to investigate the periodic hematological diseases (Dunn 1983; Kazarinoff and van den Driessche 1979; Kirk et al. 1968; Glass and Mackey 1979; MacDonald 1978; Mackey and Glass 1977; Mackey 1978; Mackey 1979b; Mackey 1979a; Morley 1979; Nazarenko 1976; von Schulthess and Mazer 1982; Wheldon et al. 1974; Wheldon 1975).

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 hematopoiesis are the maturation times and the peripheral destruction rate(s).

8.3 RANDOM CELL LOSS FROM A COMPARTMENT

Let's consider the simplest possible situation, in which we have cells in a compartment that die randomly as time proceeds (Figure 8.2). We will let $X(t)$ be the number of cells (usually measured as a density, e.g., numbers

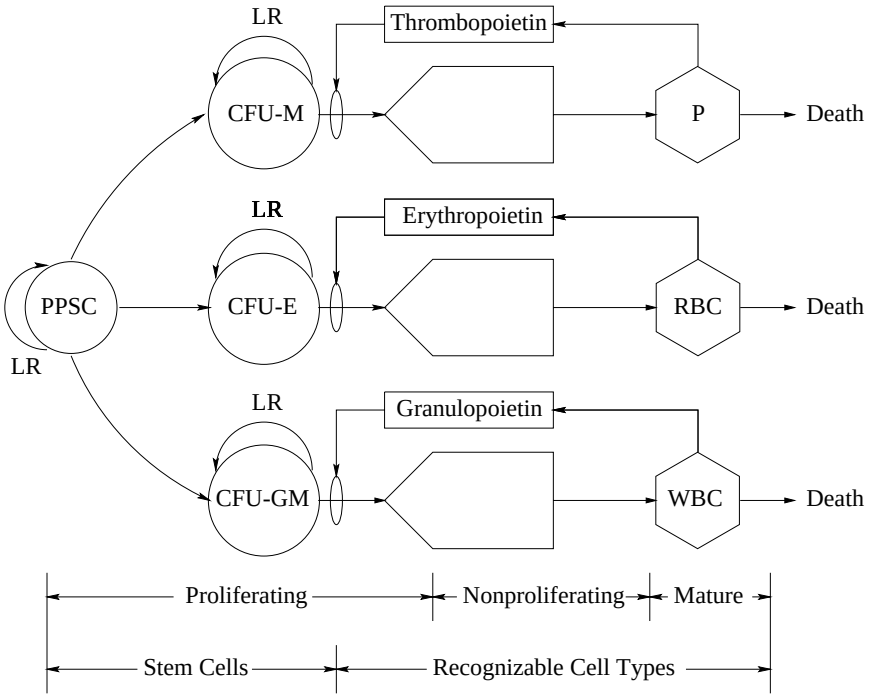

Figure 8.1. Hematopoietic regulation architecture. A schematic representation of the control of platelet (P), erythrocyte (RBC), and white blood cell (WBC) production (adapted from Quesenberry (1990)), showing loops mediated by the various poietins, as well as local regulatory (LR) loops within the various stem-cell compartments. CFU refers to the various colony-forming units (M = megakaryocytic, E = erythroid, GM = 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 (PPSCs).

per liter, numbers per unit body weight, etc.) at a given time t, and assume that cells are arriving in the compartment (for example, the peripheral circulation) with an input flux of $I(t)$ and are being lost at a random rate γ, so the efflux to death is $D(t) = \gamma X(t)$. In hematology, a typical example of a process whose dynamics are described by this situation is the kinetics of circulating white blood cells.

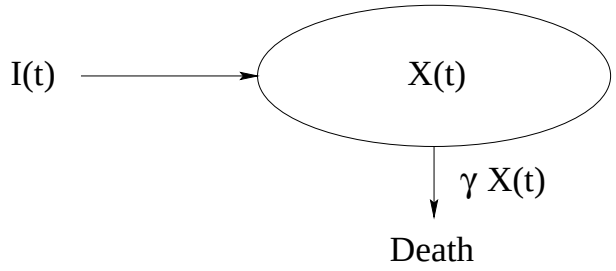

Figure 8.2. A schematic representation of our considerations of random cell loss.

Simple common sense concerning the conservation of cells tells us that we should have

$$\frac{dX}{dt} = I(t) - D(t) \tag{8.1}$$

$$= I(t) - \gamma X(t) \tag{8.2}$$

as the dynamical equation governing $X(t)$. This is a simple first order differential equation, and to solve it we have to specify an initial condition

$$X(t = t_0) = X_0. \tag{8.3}$$

The solution to (8.2) in conjunction with (8.3) is, of course, pretty easy to obtain. One could do it using Laplace transforms, or alternately by multiplying (8.2) by the integrating factor exp (γt) to obtain

$$e^{\gamma t}\left[\frac{dX(t)}{dt} + \gamma X\right] = \frac{d}{dt}\left(X(t)e^{\gamma t}\right) = I(t)e^{\gamma t} \tag{8.4}$$

Writing (8.4) in integral form and then integrating, using the initial condition, (8.3) and rearranging, we have

$$X(t) = X_0 e^{-\gamma(t-t_0)} + e^{-\gamma t}\int_{t_0}^{t} I(z)e^{\gamma z}\,dx. \tag{8.5}$$

If the input of cells I is a constant, then the solution (8.4) of the original problem (8.2)-(8.3) is even simpler and can be written as

$$X(t) = X_0 e^{-\gamma(t-t_0)} + \frac{I}{\gamma}\left[1 - e^{-\gamma(t-t_0)}\right]. \tag{8.6}$$

8.3.1 Example

To illustrate how hematologists can make use of such a formulation, consider the real situation in which a radioactive tracer is given in what is known as a *flash-labeling* mode, such that all of the circulating white blood cells pick up some of the label, but none of their precursors has any. Then, although unlabeled white blood cells will still be entering the circulation through the flux I, their absence of label will distinguish them from their labeled older sisters. If we denote the population of labeled white blood cells by X^*, then their dynamics are going to be given by

$$X^*(t) = X_0^* e^{-\gamma t}. \tag{8.7}$$

Now we can actually use (8.7) to determine the rate of peripheral cell death γ in the following straightforward way. If we let the fraction of labeled cells at a time t after the administration of the label be $F(t)$, then we can rewrite (8.7) in the more useful form

$$F(t) = \frac{X^*(t)}{X_0^*} = e^{-\gamma t}. \tag{8.8}$$

Equation (8.8) tells us that the fraction of labeled cells decreases exponentially with time, and if we can measure what F is at a given time, then we can find out what γ is. What is typically done is to find the time (denoted by $t_{1/2}$) at which the fraction F is $\frac{1}{2}$, i.e., $F(t_{1/2}) = \frac{1}{2}$. Using this notation in conjunction with (8.8), we have after a bit of algebra that

$$\gamma = \frac{\ln 2}{t_{1/2}}. \tag{8.9}$$

When this experiment is done with white blood cells from humans, the typical result is that the semilogarithmic plot of $F(t)$ versus time is a straight line, so it really does decay exponentially, and $t_{1/2}$ is approximately 6.9 hours, so that for humans

$$\gamma = \frac{\ln 2}{6.9}\ \mathrm{hr}^{-1} = 0.1\mathrm{hr}^{-1}. \tag{8.10}$$

8.4 DYNAMICS OF CELLS THAT AGE

As a next step in building our toolbox of techniques for dealing with cell-dynamics problems, let us consider a situation slightly more complicated that the random-loss one of the previous section.

Now, we consider the case in which cells are in a compartment and are lost by one of two means (Figure 8.3). First, as before, these cells are lost at a random rate (again denoted by γ), and second, they are lost because they just become too old. In addition, cells arrive into the population with a certain influx $I(t)$.

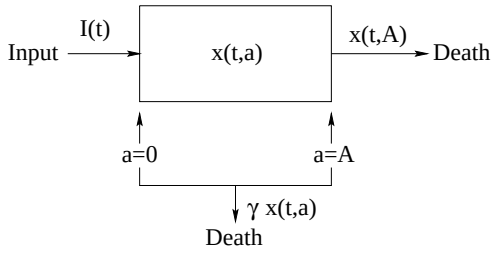

Figure 8.3. The situation in which we have cells living to a maximal age A, but also dying at a random rate γ.

This sounds somewhat like the situation facing humans or any other species — the birth rate is I, there are random deaths in the population as we age, and we eventually die when we get too old if something doesn't knock us off randomly. Clearly, there is a certain arbitrary nature to the distinction between death due to random events versus death due to senescence, but that won't bother us here.

We are going to let $x(t, a)$ be the number of cells at a given time t and age a. Further, we assume that cells can live to a maximum age A and that cells that immigrate with the influx $I(t)$ do so at an age $a = 0$. From a

dynamic point of view, the equation that governs the evolution of $x(t, a)$ is the first-order partial differential equation (or conservation equation)

$$\frac{\partial x(t,a)}{\partial t} + \frac{\partial x(t,a)}{\partial a} = -\gamma x(t,a). \tag{8.11}$$

To complete the specification of the problem, we will have to supplement (8.11) with the initial condition

$$x(t=0,a) = f(a) \tag{8.12}$$

as well as the boundary condition

$$x(t, a=0) = I(t). \tag{8.13}$$

How to deal with the system (8.11) through (8.13)? Well, the first thing to realize is that we can write the total number of cells of all ages between the minimum age $a = 0$ and the maximum age $a = A$ as

$$X(t) = \int_0^A x(t,a)\,da. \tag{8.14}$$

Thus, integrating (8.11) over the entire range of ages and using the definition (8.14), we have

$$\frac{dX(t)}{dt} + x(t,A) - x(t,0) = -\gamma X(t), \tag{8.15}$$

or, using the boundary condition (8.13), this becomes

$$\frac{dX(t)}{dt} = I(t) - \gamma X(t) - x(t,A). \tag{8.16}$$

Its easy to understand what each term in (8.16) means, for it just says that the total rate of change of all of the cells in the compartment is a balance between the input $I(t)$, the random loss $-\gamma X(t)$, and the loss to death of those individuals who made it to age A.

However, we can go even further by noting that, using the method of characteristics, the general solution of (8.11) is given by

$$x(t,a) = \begin{cases} x(0, a-t)e^{-\gamma t}, & 0 \le t < a, \\ x(t-a, 0)e^{-\gamma a}, & a \le t. \end{cases} \tag{8.17}$$

Further, making use of the initial condition (8.12) and the boundary condition (8.13), we can write the general solution (8.17) in the more explicit form

$$x(t,a) = \begin{cases} f(a-t)e^{-\gamma t}, & 0 \le t < a \\ I(t-a)e^{-\gamma a}, & a \le t. \end{cases} \tag{8.18}$$

Now (8.16) contains the term $x(t, A)$, and we clearly have, from (8.18), that

$$x(t, A) = \begin{cases} f(A-t)e^{-\gamma t}, & 0 \le t < A, \\ I(t-A)e^{-\gamma A}, & A \le t. \end{cases} \tag{8.19}$$

Thus we can finally write (8.16) in the final and useful form

$$\frac{dX(t)}{dt} = I(t) - \gamma X(t) - \begin{cases} f(A-t)e^{-\gamma t}, & 0 \le t < A, \\ I(t-A)e^{-\gamma A}, & A \le t. \end{cases} \tag{8.20}$$

8.5 PERIODIC AUTOIMMUNE HEMOLYTIC ANEMIA: CONTROL OF RED BLOOD CELL PRODUCTION AND DELAYED NEGATIVE FEEDBACK

Periodic autoimmune hemolytic anemia is a rare form of hemolytic anemia in humans (Gordon and Varadi 1962; Ranlov and Videbaek 1963), but it has been induced in rabbits (Figure 8.4) by using red blood cell autoantibodies (Orr et al. 1968). At certain levels of administration, these antibodies oscillate in red blood cell (erythrocyte) precursors (reticulocytes) with a period of 16 to 17 days. Rabbit autoimmune hemolytic anemia is one of the best understood periodic hematological diseases and arises from increases in the destruction rate of circulating erythrocytes.

The concept of delayed negative feedback can be introduced by considering the control of erythrocyte production as represented schematically in Figures 8.1 and 8.5.

To formulate this sequence of physiological processes in a mathematical model, we will make the following definitions (Mackey 1979b). Let:

$E(t)$ (cells/kg)	=	circulating density of red blood cell as a function of time
F (cells/kg-day)	=	cell influx from erythroid colony forming units, under erythropoietin control
τ (days)	=	time required to pass through recognizable precursors
γ (days^{-1})	=	loss rate of red blood cells in the circulation

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

$$\begin{aligned} \frac{dE(t)}{dt} &= \text{production} - \text{destruction} \\ &= F(E(t-\tau)) - \gamma E(t). \end{aligned} \tag{8.21}$$

It is important to remember that once a pluripotential stem cell is committed to the erythroid series, it undergoes a series of nuclear divisions and enters a

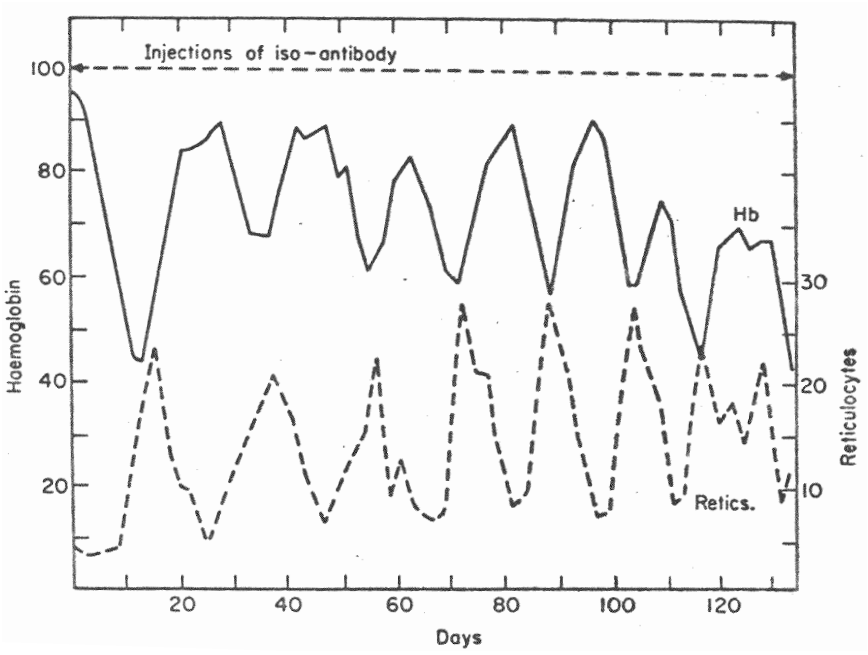

Figure 8.4. Laboratory-induced autoimmune hemolytic anemia. Oscillations in circulating hemoglobin and reticulocyte counts in a rabbit during constant application of red blood cell iso-antibody. (Redrawn from Kirk et al. (1968).)

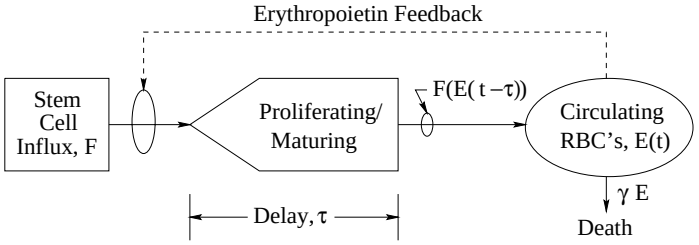

Figure 8.5. A fall in circulating erythrocyte numbers leads to a decrease in hemoglobin levels and thus in arterial oxygen tension. This decrease in turn triggers the production of renal erythropoietin, which increases the cellular production within the early committed erythrocyte series cells and thus the cellular efflux from the erythroid colony forming units into the identifiable proliferating and nonproliferating erythroid precursors, and ultimately augments circulating erythrocyte numbers (i.e., negative feedback) after a delay τ.

maturational phase for a period of time (τ approximately 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 red blood cell 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 $E(t-\tau)$, I will adopt here the usual convention of $E_\tau(t) = E(t-\tau)$, and I will not explicitly denote the time unless necessary. Thus, we can write our simple model (8.21) for red

blood cell dynamics in the alternate form

$$\frac{dE}{dt} = F(E_\tau) - \gamma E. \tag{8.22}$$

The next step in our model construction is to define some appropriate form for the production function F. *In vivo* measurements of erythrocyte production rates F in rats (Hodgson and Eskuche 1966) and other mammals including humans indicate that the feedback function saturates at low erythrocyte numbers and is a decreasing function of increasing red blood cell levels (i.e., negative feedback). A convenient function that captures this behavior, and which has sufficient flexibility to be able to fit the data, as well as easily handled analytic properties, is given by

$$F(E_\tau) = F_0 \frac{\theta^n}{E_\tau^n + \theta^n}, \tag{8.23}$$

where F_0 (units of cells/kg-day) is the maximal red blood cell production rate that the body can approach at very low circulating red blood cell numbers, n is a positive exponent, and θ (units of cells/kg) is a shape parameter. These three parameters have to be determined from experimental data related to red blood cell production rates.

Combining (8.22) and (8.23), we have the final form for our model of red blood cell control given in the form

$$\frac{dE}{dt} = F_0 \frac{\theta^n}{E_\tau^n + \theta^n} - \gamma E. \tag{8.24}$$

Equation (8.24) 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 DDEs 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

$$E(t') = \phi(t'), \qquad -\tau \le t' \le 0. \tag{8.25}$$

Usually here we will only consider initial functions that are constant, but it must be noted that differential delay equations (like their ordinary cousins) can display multistable behavior in which two or more coexisting locally stable solutions result, depending on the initial function (an der Heiden et al. 1981; an der Heiden and Mackey 1982; an der Heiden and Mackey 1987; Campbell et al. 1995; Foss et al. 1996; Mackey and an der Heiden 1984; Losson et al. 1993).

8.5.1 Oscillations in differential delay equations

Based on what you have seen so far, it might seem somewhat strange to claim that the apparently first-order differential delay equation (8.24) can have oscillatory solutions. It is well established that under appropriate circumstances, delayed negative-feedback mechanisms as in (8.24) can produce oscillations, and one of the reasons is that (8.24) isn't really a first-order equation

at all. Rather, it is an infinite-dimensional system; the reason is that the initial *function* is infinite dimensional in the sense that it takes an infinite number of points to specify it.

To illustrate the fact that differential delay equations can in fact oscillate, we continue our analysis of (8.24) by using an approximation system for which we can actually derive the analytic solution.

To do this, we imagine that in the nonlinear Hill function (8.23) we let $n \to \infty$, so the nonlinearity becomes progressively closer to the step-function nonlinearity illustrated in Figure 8.6. Under this circumstance, (8.24) becomes

$$\frac{dE(t)}{dt} = -\gamma E(t) + \begin{cases} F_0, & 0 \le E_\tau < \theta \\ 0, & \theta \le E_\tau. \end{cases} \tag{8.26}$$

In reality, the nonlinear differential delay equation (8.26) can be alternately viewed as a pair of ordinary differential delay equations, and which one we solve at any given time will depend on the value of the retarded variable E_τ with respect to the parameter θ. This method of solution, which we are going to carry out, is usually called the *method of steps*.

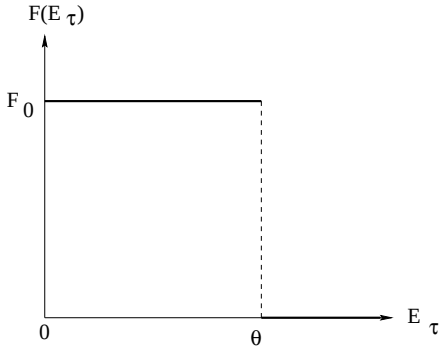

Figure 8.6. A piecewise constant nonlinearity approximating the negative-feedback function of (8.23) that is obtained in the limit as $n \to \infty$.

In preparation, we must first specify an initial function for (8.26) of the type in (8.25), and in point of fact it is almost immaterial what type of initial function we pick. Here, for concreteness, we will pick one that satisfies $\phi(t') > \theta$ for $-\tau \le t' \le 0$ and specify that $\phi(0) \equiv E_0$, a constant.

With this assumption (you could carry out the same analysis as here with a different initial function, and should do so to convince yourself that the ultimate conclusion we reach is independent of the initial function), we must first solve the equation

$$\frac{dE}{dt} = -\gamma E, \quad \theta < E_\tau, \quad E(t=0) \equiv E_0. \tag{8.27}$$

The solution is this equation is, of course, almost trivial to write down and is given by

$$E(t) = E_0 e^{-\gamma t} \tag{8.28}$$

and this solution will be valid until a time t_1 determined by the condition $\theta = E(t_1 - \tau)$ or, more specifically,

$$E(t_1 - \tau) = \theta \equiv E_0 e^{-\gamma(t_1 - \tau)} \tag{8.29}$$

from which we immediately deduce that

$$t_1 = \frac{1}{\gamma} \ln \left\{ \frac{E_0 e^{\gamma \tau}}{\theta} \right\} \tag{8.30}$$

Having this value of t_1, it is a simple matter to then show that the value of E at $t = t_1$ is given by

$$E(t = t_1) \equiv E_1 = \theta e^{-\gamma \tau}. \tag{8.31}$$

Thus, for the particular form of the initial condition that we have chosen we conclude that the solution is a decaying exponential given by (8.29), and that this solution is valid until a time t_1 given by (8.30), at which point the solution has the value E_1 given by (8.31).

Now to proceed for times longer than t_1 we must solve the other differential equation as given in (8.26), namely

$$\frac{dE}{dt} = -\gamma E + F_0, \quad E_\tau \leq \theta, \quad E(t_1) = E_1. \tag{8.32}$$

This is almost as easy as the first case, and the solution is given by the slightly more complicated relation

$$E(t) = E_1 e^{-\gamma(t - t_1)} + \frac{F_0}{\gamma} \left[1 - e^{-\gamma(t - t_1)} \right]. \tag{8.33}$$

According to our assumptions, the solution (8.33) will be valid until a time t_2 defined by $\theta = E(t_2 - \tau)$, or

$$E(t_2 - \tau) \equiv \theta = E_1 e^{-\gamma(t_2 - t_1)} + \frac{F_0}{\gamma} \left[1 - e^{-\gamma(t_2 - t_1)} \right], \tag{8.34}$$

from which we have, with a bit of algebra, that

$$t_2 = \frac{1}{\gamma} \ln \left\{ \left(\frac{E_0}{\theta} \right) \left[\frac{E_1 - (F_0/\gamma)}{\theta - (F_0/\gamma)} \right] e^{2\gamma \tau} \right\}. \tag{8.35}$$

We may then calculate the value of the solution at time t_2 [$E(t = t_2) \equiv E_2$] to obtain

$$E_2 = \frac{F_0}{\gamma} + \left(\theta - \frac{F_0}{\gamma} \right) e^{-\gamma \tau}. \tag{8.36}$$

Therefore, to summarize, for the period of time $t \in [t_1, t_2]$ the solution to (8.26) is an exponentially increasing function that terminates at a time t_2 given by (8.35) with a value of E_2 given in (8.36). These values then constitute the initial conditions for our determination of the third portion of the

solution and the method of steps is sufficiently clear that we need not write out things quite so explicitly.

In the computation of this third portion we must once again solve (8.27) subject to the end-point conditions just determined in the last computation. This then yields

$$E(t) = E_2 e^{-\gamma(t-t_2)}, \tag{8.37}$$

and from $\theta = E(t_3 - \tau)$ we find

$$t_3 = \frac{1}{\gamma} \ln \left\{ \left(\frac{E_0 E_2}{\theta^2} \right) \left[\frac{E_1 - (F_0/\gamma)}{\theta - (F_0/\gamma)} \right] e^{3\gamma\tau} \right\} \tag{8.38}$$

so $E(t_3) \equiv E_3$ is given by

$$E_3 = \theta e^{-\gamma\tau}. \tag{8.39}$$

Now comparison of (8.31) and (8.39) reveals that in point of fact $E_1 \equiv E_3$, and this in turn means that we have shown that (8.26) has a periodic solution! The argument that leads to this conclusion is as follows. Starting from two different initial values, namely E_0 and E_2 we have found that we arrive at precisely the same level $E_1 \equiv E_3$, and thus we know that proceeding further from E_3 at time t_3 will lead to a value of $E_4 \equiv E_2$ at a time $t_4 \equiv t_3 + (t_2 - t_1)$, and that this repeated cycling of the solution between a minimum value of E_1 and E_2 will continue indefinitely. Furthermore, since E_0 and E_2 both lead to the same minimum value of the exponentially decreasing portion of the solution, we could simply pick $E_0 \equiv E_2$ without any loss of generality, and thus t_3 takes the slightly simpler form

$$t_3 = \frac{1}{\gamma} \ln \left\{ \left(\frac{E_2^2}{\theta^2} \right) \left[\frac{E_1 - (F_0/\gamma)}{\theta - (F_0/\gamma)} \right] e^{3\gamma\tau} \right\}. \tag{8.40}$$

We can calculate the period of this periodic solution that we have derived by using $t_3 - t_1$, but this should just be equivalent to t_2 when we pick the special initial condition $E_0 \equiv E_2$. Denoting the period by T we obtain

$$T = \frac{1}{\gamma} \ln \left\{ \left(\frac{E_2}{\theta} \right) \left[\frac{E_1 - (F_0/\gamma)}{\theta - (F_0/\gamma)} \right] e^{2\gamma\tau} \right\}, \tag{8.41}$$

or after substituting the explicit value for E_2 from (8.36) we have

$$T = 2\tau + \frac{1}{\gamma} \ln \left\{ \left[\frac{F_0/(\gamma\theta)}{F_0/(\gamma\theta) - 1} - e^{-\gamma\tau} \right] \left[\frac{F_0}{(\gamma\theta)} - e^{-\gamma\tau} \right] \right\} \tag{8.42}$$

8.5.2 Steady states

A *steady state* (Appendix B) for the model (8.24) is defined by the requirement that the red blood cell number is not changing with time. This, in turn, can be translated to mean that

$$E(t) = E(t-\tau) = E_\tau(t) = \text{a constant, the steady state} = E^*, \tag{8.43}$$

and

$$\frac{dE}{dt} = 0 \qquad \text{so} \qquad F(E^*) = F_0 \, \frac{\theta^n}{E^* + \theta^n} = \gamma E^*. \tag{8.44}$$

Now generally we can't solve (8.44) to get an analytic form for E^*, but a simple graphical argument shows that only one value of E^* will satisfy (8.44). This value of the steady state occurs at the intersection of the graph of γE^* with the graph of $F(E^*)$, as indicated in Figure 8.7.

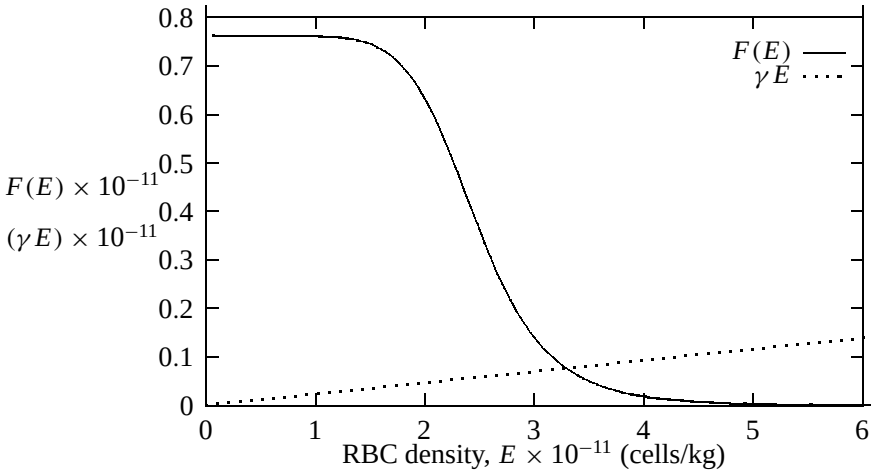

Figure 8.7. Determination of steady-state red blood cell numbers in rabbits. The unique steady state E_* of the model defined by (8.24) is determined by the intersection of the steady-state production curve $F(E)$ and the loss curve γE. Values of the parameters are selected to conform with the estimates of the normal parameter values as detailed in Section 8.5.4 below.

8.5.3 Stability of the steady state

Knowing the steady state of a model like (8.25), and how it depends on the various parameters of the problem, is certainly useful but is not of much help in understanding the dynamics of the oscillations seen in autoimmune hemolytic anemia as illustrated in Figure 8.4. 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 red blood cell 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

$$\lim_{t \to \infty} E(t) = E^*. \tag{8.45}$$

We would like to know what conditions on the parameters of our model (destruction rate, maximal production rate, etc.) are required to make sure that the

stability condition (8.45) holds, and even further we would like to know what happens if the stability condition (8.45) is violated.

Unfortunately, because of the nature of model (8.24) that describes this physiological process 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^*. Our assumption that the perturbation is small allows us to carry out a linear stability analysis of the steady state E^*, which we now proceed to do.

The nonlinearity of (8.24) comes from the term involving the red blood cell 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 F in the vicinity of E^*:

$$\begin{aligned} F(E_\tau) &\simeq F(E^*) + (E_\tau - E^*)F'(E_\tau = E^*) \\ &\quad + \frac{1}{2}(E_\tau - E^*)^2 F''(E_\tau = E^*) + \cdots \end{aligned} \tag{8.46}$$

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 (8.24) in the vicinity of E^* (with the notation that $F'(E^*) \equiv S$, for slope) as

$$\frac{dE}{dt} \simeq F(E^*) + (E_\tau - E^*)S - \gamma E. \tag{8.47}$$

Remember from (8.44) that the defining equation for the steady-state is $F(E^*) = \gamma E^*$. Using this in (8.47), we can rewrite it as

$$\frac{dE}{dt} \simeq (E_\tau - E^*)S - \gamma(E - E^*). \tag{8.48}$$

If we further set $z(t) = E(t) - E^*$, so z is the deviation of the red blood cell numbers from their steady-state value, then $z_\tau(t) = E_\tau(t) - E^*$ and $dE/dt = dz/dt$, so (8.48) can be rewritten as the linear differential delay equation

$$\frac{dz}{dt} = Sz_\tau - \gamma z. \tag{8.49}$$

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 to 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

$$z(t) \simeq e^{\lambda t} = e^{\mu t}\left\{\cos(\omega t) + i \sin(\omega t)\right\}. \tag{8.50}$$

If $\mu = \text{Re } \lambda < 0$, then the solution is a decaying oscillating function of time that approaches zero, so we have a stable situation. If $\mu = \text{Re } \lambda > 0$ 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 $\mu = \text{Re}\ \lambda = 0$, defines a *Hopf bifurcation*, which is characterized by an eigenvalue pair crossing from the left-hand to the right-hand complex plane (Appendix B).

After that wordy digression, let's assume that $z(t) \simeq e^{\lambda t}$ in (8.49). If we make this substitution, and carry out the algebra, then we are left with the equation

$$\lambda = Se^{-\lambda\tau} - \gamma \tag{8.51}$$

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

Under this assumption, $\lambda = i\omega$, and substituting this into (8.51) gives

$$i\omega = S[\cos\ (\omega\tau) - i\ \sin\ (\omega\tau)] - \gamma, \tag{8.52}$$

or after separating real and imaginary parts

$$\omega = -S \sin\ (\omega\tau) \tag{8.53}$$
$$\gamma = S \cos\ (\omega\tau) \tag{8.54}$$

If $|\gamma/S| < 1$ then (8.54) can be solved for $\omega\tau$ to give

$$\omega\tau = \cos^{-1}\left(\frac{\gamma}{S}\right). \tag{8.55}$$

Further, squaring and adding the two equations (8.53 and 8.54) gives

$$\omega = \sqrt{S^2 - \gamma^2}. \tag{8.56}$$

Combining (8.55) and (8.56), we find that the relation connecting τ, S, and γ that must be satisfied in order for the eigenvalues to have real part identical to zero is given by

$$\tau = \frac{\cos^{-1}(\gamma/S)}{\sqrt{S^2 - \gamma^2}}, \qquad |\gamma/S| < 1. \tag{8.57}$$

In general (Hayes 1950), the real parts of λ will be negative, and thus the linear equation (8.49) will have a locally stable steady state, if and only if $|\gamma/S| > 1$ *or* if $|\gamma/S| < 1$ *and*

$$\tau < \frac{\cos^{-1}(\gamma/S)}{\sqrt{S^2 - \gamma^2}}. \tag{8.58}$$

When the parameters satisfy (8.57), then we say that there has been a Hopf bifurcation. The period of the periodic solution that is guaranteed when (8.57) 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 (8.55) through (8.57)

$$T = \frac{2\pi\tau}{\cos^{-1}(\gamma/S)}. \tag{8.59}$$

Since the inverse cosine ranges from 0 to π, from (8.59) we know that at the Hopf bifurcation the period of the periodic solution must satisfy

$$2\tau \leq T. \tag{8.60}$$

In general, the period of an oscillation produced by a delayed negative-feedback mechanism is at least twice the delay (Hayes 1950; Mackey 1978). 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 (Mackey 1979b), i.e.

$$2\tau \leq T \leq 4\tau. \tag{8.61}$$

Since the maturational delay for erythrocyte production τ is approximately 6 days, we would expect to see oscillations in erythrocyte numbers with periods ranging from 12 to 24 days. This is in agreement with the observed periods of 16 to 17 days in rabbit autoimmune hemolytic anemia (Orr et al. 1968). What is surprising is the fact that these oscillations are so rarely observed. This paradox is illuminated in the following sections.

8.5.4 Parameter estimation

Having extracted about as much information as is possible from the linear analysis of our model for red blood cell production, we now need to turn to numerical simulations to see the full behavior. This, by necessity, requires that we have some estimation of the parameters in (8.24). Through a variety of data, we finally conclude that in the normal situation (i.e., not autoimmune hemolytic anemia):

$$\begin{aligned} \gamma &= 2.31 \times 10^{-2} \text{ day}^{-1}, \\ F_0 &= 7.62 \times 10^{10} \text{ cells/kg-day}, \\ n &= 7.6, \\ \theta &= 2.47 \times 10^{11} \text{ cells/kg}, \\ \tau &= 5.7 \text{ days}. \end{aligned}$$

These parameters correspond to a steady-state circulating red blood cell 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.

8.5.5 Explaining laboratory-induced autoimmune hemolytic anemia

The one fact we know about the induced autoimmune hemolytic anemia shown in Figure 8.4 is that the red blood cell destruction rate γ is increased

through the action of cell damage (lysis) by 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 $\gamma \simeq 5.12 \times 10^{-2}$ 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 (8.59). Our linear analysis tell us nothing about what happens in the full nonlinear equation (8.24) 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.

8.5.6 Numerical simulations

Numerical simulations of (8.24) with the parameters given above show that the linear analysis results quoted above give a very accurate picture of the full nonlinear behavior, including the values of γ at which stability of E^* is lost and regained, and the period of the solutions at these stability boundaries (Figure 8.8).

Figure 8.8 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 (Orr et al. 1968). A much more comprehensive model for the control of red blood cell production, and mathematically much more complicated, has properties (Bélair et al. 1995) similar to those of the simple model presented here. The observations in Figure 8.8 indicate that whether or not a proposed mechanism for periodic autoimmune hemolytic anemia 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 autoimmune hemolytic anemia.

It should be noted that the morphology of the oscillations shown in Figure 8.8 are quite simple; i.e., there is only one maximum per period. All studies to date of first-order delayed negative-feedback mechanisms have indicated that only oscillations with this simple morphology can be produced (an der Heiden and Mackey 1982; Glass and Mackey 1979; Longtin and Milton 1988; Mackey and Glass 1977), though second-order systems with delayed negative-feedback can have more complicated solution behavior as well as displaying multistability (an der Heiden et al. 1990; Campbell et al. 1995). More complex waveforms (i.e., more than one maximum per period) are, however, possible in first-order systems with multiple delayed negative-feedback loops (Glass et al. 1988) and with multiple time delays (Bélair and Mackey 1989).

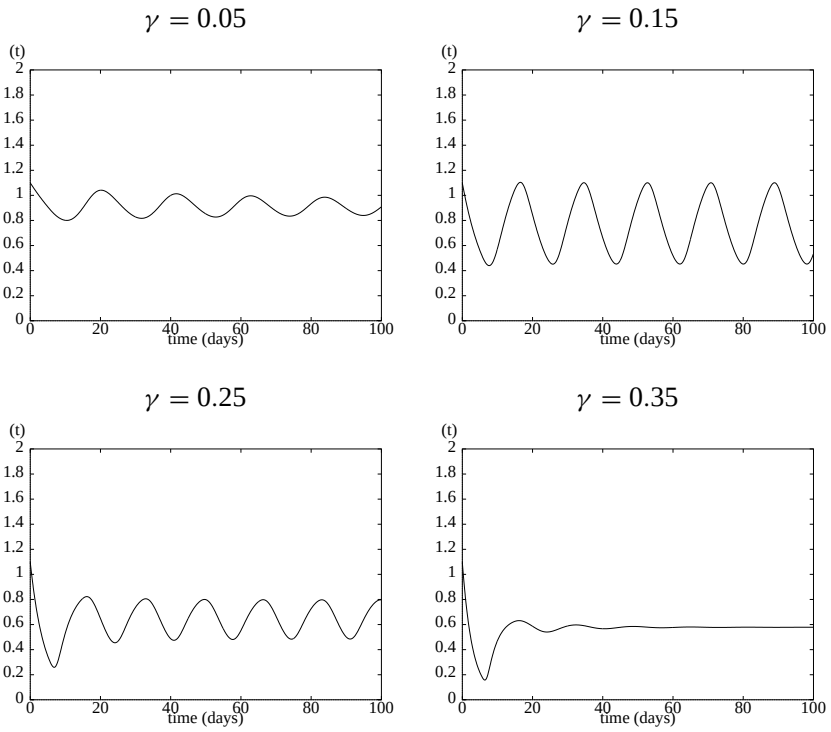

Figure 8.8. Computer simulations of the model (8.24) for erythrocyte production for four different peripheral destruction rates, γ, as indicated below each panel, all other parameters being held at the estimated normal values as given in the text. In every case, the plotted erythrocyte numbers are normalized to the normal steady state of $E^* = 3.3 \times 10^{11}$ cells/kg.

8.6 PERIODIC HEMATOPOIESIS AND PLURIPOTENTIAL STEM-CELL POPULATION STABILITY

The most common periodic hematological disease is periodic hematopoiesis. In humans, periodic hematopoiesis is a disease characterized by 17- to 28-day periodic oscillations in circulating white blood-cell numbers from approximately normal values to barely detectable numbers (Dale and Hammond 1988; Hoffman et al. 1974; Lange 1983; Wright et al. 1981). In addition to white blood cells, oscillations are seen for all the formed elements of blood with the same period. The oscillations for each of the blood-cell lines are out of phase, and the phase differences between the cell lines are consistent with the differences in the maturation times (Hoffman et al. 1974). These neutropenic (low circulating neutrophil numbers) episodes place the patients at increased risk for infective processes (e.g., abscesses, pneumonia, septicemia).

Experimental study of periodic hematopoiesis has been facilitated by the availability of suitable animal models. All gray collies have periodic hematopoiesis (Dale et al. 1972a; Dale et al. 1972b; Dale and Hammond 1988; Jones

et al. 1975; Jones and Lange 1983), and the only demonstrable quantitative difference between the human and canine form of periodic hematopoiesis is the period — in dogs the period ranges from about 10 to 17 days.

An abnormality in the regulation of the pluripotential stem cells in periodic hematopoiesis is suggested by the observation that the disorder can be transferred by bone-marrow transplantation (Jones et al. 1975; Krance et al. 1982; Quesenberry 1983). This is view is further supported by the observation (Abkowitz et al. 1988) that in the grey collie 12- to 13-day-period oscillations are found in marrow erythroid burst-forming units and that these are shifted in phase by two days from the oscillations in the erythroid colony-forming units. Furthermore, in the same study it was found that there were oscillations in the granulocyte-macrophage colony-forming cells and that these have phase differences of about five days preceding the neutrophils. These findings indicate that the defect in periodic hematopoiesis is resident in a cell population more primitive than these identifiable stem-cell populations.

Consequently most investigators have looked to abnormalities in the regulation of the pluripotential stem cells and delayed negative-feedback mechanisms as an explanation for periodic hematopoiesis (Dunn 1983; Mackey 1978; Mackey 1979a; Morley 1979; Wheldon et al. 1974; Wheldon 1975; Nazarenko 1976; MacDonald 1978; Kazarinoff and van den Driessche 1979; von Schulthess and Mazer 1982). A schematic representation of pluripotential stem cell population regulation is shown in Figure 8.9.

Interestingly in (Abkowitz et al. 1988) it was also found that the fraction of these progenitor cells in the DNA-synthesis phase is similar between the grey collie and normal collies, and the fraction does not show any sign of cyclic fluctuation.

A crucial clue to the potential origin of the defect in periodic hematopoiesis is the observation of the effect of continuous cyclophosphamide and busulfan administration in normal dogs (Morley and Stohlman 1970; Morley et al. 1970). Though in most animals these drugs led to a pancytopenia (generalized depression of the numbers of all circulating blood cells) whose severity was proportional to the drug dose, in some dogs low doses led to a mild pancytopenia, intermediate doses gave a periodic hematopoiesis-like behavior with a period between 11 and 17 days, and high drug levels led to either death or gross pancytopenia. When the periodic hematopoiesis-like behavior occurred, it was at circulating white blood cell levels of one-half to one-third normal. To this we must add the observation that patients undergoing hydroxurea therapy sometimes develop periodic hematopoiesis-like symptoms (Kennedy 1970), as do patients receiving cyclophosphamide (Dale et al. 1973).

Both cyclophosphamide and busulfan selectively kill cells within the DNA-synthetic phase of the cell cycle, and the fact that both drugs are capable of inducing periodic hematopoiesis-like behavior strongly suggests that the origin of periodic hematopoiesis as a disease is due to an abnormally large death rate (apoptosis) in the proliferative phase of the cell cycle of a population of pluripotential stem cells, which is at a level more primitive than the granulocyte-macrophage colony-forming cells and the marrow erythroid burst-forming units.

Here we interpret the effects of an increase in the rate of irreversible apoptotic loss from the proliferating phase of the pluripotential stem-cell population (γ in Figure 8.9) on blood-cell production (Mackey 1978).

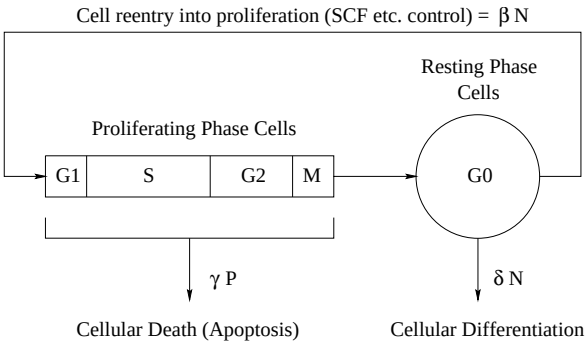

Figure 8.9. A schematic representation of the control of pluripotential stem-cell regeneration. Proliferating phase cells (P) include those cells in G_1, S (DNA synthesis), G_2, and M (mitosis) while, the resting-phase (N) cells are in the G_0 phase. Local regulatory influences are exerted via a cell-number dependent variation in the fraction of circulating cells. δ is the **normal** rate of differentiation into all of the committed stem-cell populations, while γ represents a loss of proliferating-phase cells due to apoptosis. See Mackey (1978; 1979a) for further details.

The dynamics of this pluripotential stem-cell population is governed (Mackey 1978; Mackey 1979a) by the pair of coupled differential delay equations (which can be derived using the techniques of (8.4)):

$$\frac{dP}{dt} = -\gamma P + \beta(N)N - e^{-\gamma\tau}\beta(N_\tau)N_\tau, \tag{8.62}$$

$$\frac{dN}{dt} = -[\beta(N) + \delta]N + 2e^{-\gamma\tau}\beta(N_\tau)N_\tau, \tag{8.63}$$

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

$$\beta(N) = \frac{\beta_0\theta^n}{\theta^n + N^n}. \tag{8.64}$$

An examination of (8.63) shows that this equation could be interpreted as describing the control of a population with a delayed mixed-feedback-type production term $[2e^{-\gamma\tau}\beta(N_\tau)N_\tau]$ and a destruction rate $[\beta(N) + \delta]$ that is a decreasing function of N.

For δ small enough relative to β_0, this model has two possible steady states. There is a steady state corresponding to no cells, $(P_1^*, N_1^*) = (0, 0)$, which is stable if it is the only steady state, and which becomes unstable whenever the second positive steady state (P_2^*, N_2^*) exists.

The stability of the nonzero steady state depends on the value of γ, and this is illustrated schematically in Figure 8.10. When $\gamma = 0$, this steady state cannot be destabilized to produce dynamics characteristic of periodic hematopoiesis. On the other hand, for $\gamma > 0$, increases in γ lead to a decrease in the pluripotential stem-cell numbers and a consequent decrease in the cellular efflux (given by δN) into the differentiated cell lines. This diminished efflux becomes unstable when a critical value of γ is reached, $\gamma = \gamma_1$, at which a supercritical Hopf bifurcation occurs (Appendix B). For all values of γ satisfying $\gamma_1 < \gamma < \gamma_2$, there is a periodic solution of (8.63) whose period is in

good agreement with that seen in periodic hematopoiesis. At $\gamma = \gamma_2$, a reverse bifurcation occurs and the greatly diminished pluripotential stem-cell numbers as well as cellular efflux again become stable.

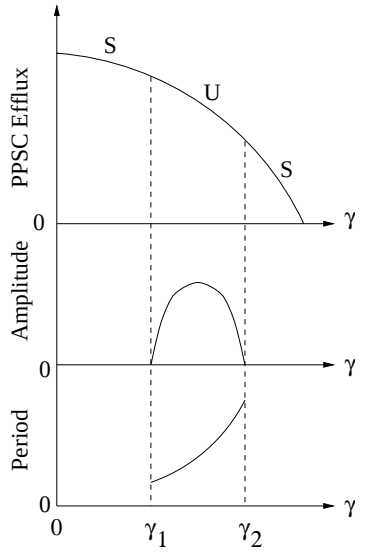

Figure 8.10. Schematic representation of the combined analytic and numerically determined stability properties of the pluripotential stem-cell model. See the text for details.

Separate estimations of the parameter sets for human and grey collie pluripotential stem cell populations give predictions of the period of the oscillation at the Hopf bifurcation that are consistent with those observed clinically and in the laboratory. These results are illustrated in Figures 8.11 and 8.12 for humans and grey collies, respectively.

Numerical simulations of (8.62) and (8.63) confirm the results of the local stability analyses displayed in Figures 8.11 and 8.12. 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 autoimmune hemolytic anemia 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 observed only for nonphysiological choices of the parameters. Thus, the observed irregularities in the fluctuations in blood-cell numbers in periodic hematopoiesis cannot be related to chaotic solutions of (8.63). These results suggest that periodic hematopoiesis is likely related to defects, possibly genetic, within the pluripotential stem-cell population that lead to an abnormal ($\gamma > 0$) apoptotic loss of cells from the proliferative phase of the cell cycle.

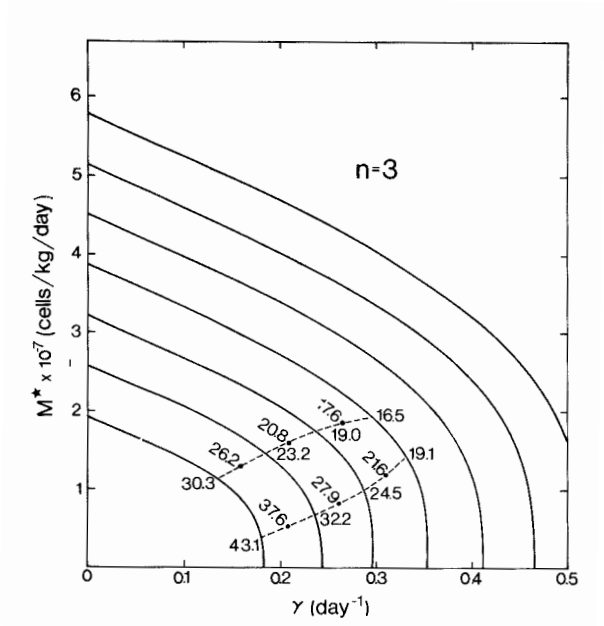

Figure 8.11. Variation of the total steady-state cellular-differentiation efflux ($M^* = \delta N$) as a function of the apoptotic death rate γ from the proliferating cell population in humans ($n = 3$). Parameters in the model were estimated assuming a proliferating fraction of 0.1 and an amplification of 16 in the recognizable erythroid, myeloid, and megakaryocytic precursors populations. See Mackey (1978; 1979a) for details. The pluripotential stem-cell parameters corresponding to each curve from the top down are: $(\delta, \beta_0, \tau, \theta \times 10^{-8}) = (0.09, 1.58, 1.23, 2.52)$, (0.08, 1.62, 1.39, 2.40), (0.07, 1.66, 1.59, 2.27), (0.06, 1.71, 1.85, 2.13), (0.05, 1.77, 2.22, 1.98), (0.04, 1.84, 2.78, 1.81), and (0.03, 1.91, 3.70, 1.62) in units (days^{-1}, days^{-1}, days, cells/kg). The dashed solid lines indicate the boundaries along which stability is lost in the linearized analysis, and the numbers indicate the predicted (Hopf) period (in days) of the oscillation at the Hopf bifurcation.

8.7 UNDERSTANDING LABORATORY-INDUCED CYCLICAL ERYTHROPOIESIS

To illustrate the usefulness of the model presented in Section 8.6 with a second 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 (Gibson et al. 1985; Gurney et al. 1981). W/W^v mice suffer from a defect in the pluripotential stem-cells, and in S1/S1^d mice the hematopoietic microenvironment is defective. Let us assume that the difference between W/W^v and S1/S1^d mice is related solely 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. The results of Section 8.6 predict that a higher γ would increase the likelihood that an oscillation in erythrocyte number occurs. Indeed, in contrast to W/W^v, approximately 40% of S1/S1^d mice have "spontaneous" oscillations in their hematocrit (Gibson et al. 1985). In both strains of mice, a single dose of ^{89}Sr is sufficient to increase γ into a

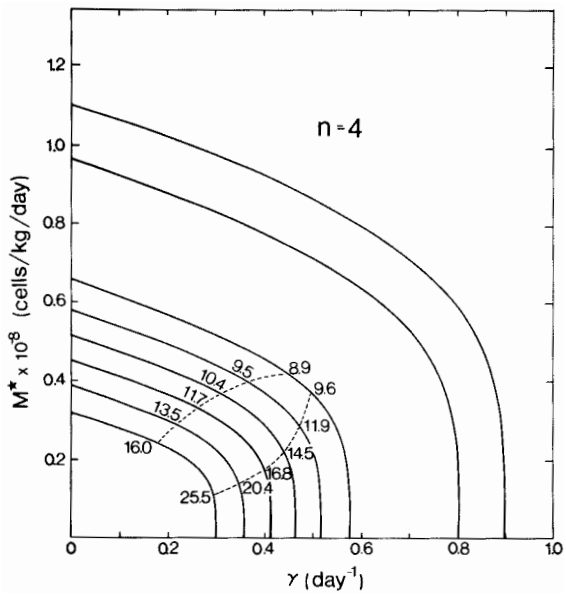

Figure 8.12. As in the previous figure, but all calculations done with parameters appropriate for dogs.

range associated with oscillations in erythrocyte number. Since the value of γ for the S1/S1^d mice is 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 equal doses of ^{89}Sr to both strains of mice, as shown in Figure 8.13. As predicted, experimentally the period of the oscillation is longer, the amplitude larger, and the mean hematocrit lower for S1/S1^d mice than for W/W^v mice.

8.8 UNDERSTANDING COLONY-STIMULATING-FACTOR EFFECTS IN PERIODIC HEMATOPOIESIS

Recent clinical and experimental work has focused on the modification of the symptoms of hematological disorders, including periodic hematopoiesis, by the use of various synthetically produced cytokines (Cebon and Layton 1994; Sachs 1993; Sachs and Lotem 1994), e.g., the recombinant colony stimulating factors rG-colony-stimulating factor and rGM-colony-stimulating factor, whose receptor biology is reviewed in Rapoport et al. (1992), and interleukin-3 (IL-3). These cytokines are now known to interfere with the process of apoptosis, or to lead to a decrease in γ within the context of the pluripotential stem-cell model of Section 8.6.

Human colony-stimulating factors increase both the numbers and proliferation rate of white blood cell precursors in a variety of situations (Bronchud et al. 1987; Lord et al. 1989; Lord et al. 1991). Furthermore, colony-stimulating factor in mice is able to stimulate replication in both stem cells and early erythroid cells (Metcalf et al. 1980).

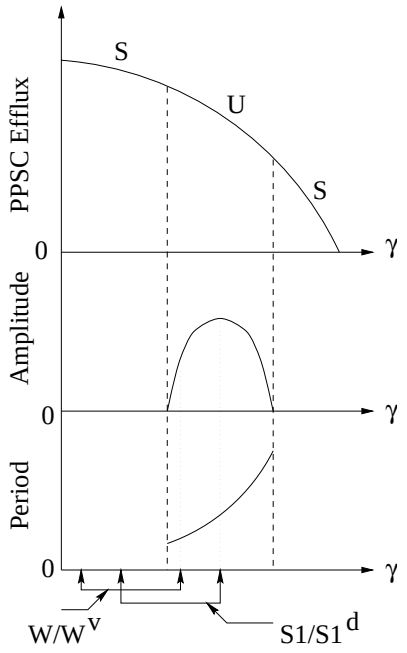

Figure 8.13. Schematic representation of the effects of administering the same dose of ^{51}Sr to W/W^v and S1/S1^d mice. See the text for details.

It is known that in aplastic anemia and periodic hematopoiesis there is an inverse relationship between plasma levels of G-colony stimulating factor and white blood cell numbers (Watari et al. 1989). Further it has been shown (Layton et al. 1989) that the $t_{1/2}$ of G-colony-stimulating factor in the circulation is short — of the order of 1.3 to 4.2 hours — so the dynamics of the destruction of G-colony-stimulating factor are unlikely to have a major role in the genesis of the dynamics of periodic hematopoiesis.

In the grey collie it has been shown that at relatively low doses of G-colony-stimulating factor the mean white blood-cell count is elevated (by 10 to 20 times), as is the amplitude of the oscillations (Hammond et al. 1990), while higher dosages (Hammond et al. 1990; Lothrop et al. 1988) lead to even higher mean white blood cell numbers but eliminate the cycling. Another interesting observation is that in the collie, G-colony-stimulating factor administration results in a decrease in the period of the peripheral oscillation. The elevation of the mean white blood cell levels and the amplitude of the oscillations, as well as an enhancement of the oscillations of platelets and reticulocytes, at low levels of G-colony-stimulating factor has also been reported in humans (Hammond et al. 1990; Migliaccio et al. 1990; Wright et al. 1994), and it has been also noted that the fall in period observed in the collie after G-colony stimulating factor administration occurs in humans with a fall in period from 21 to about 14 days. Finally it should be mentioned that treatment with G-colony stimulating factor in patients with agranulocytosis has also lead to a significant increase in the mean white blood cell counts and, in some patients, to the induction of white blood cell oscillations with periods ranging from 7 to 16 days.

Our major clue to the nature of the effects of G-colony stimulating fac-

tor comes from its prevention of apoptosis and from the work of Avalos et al. (1994) who have shown in dogs that there is no demonstrable alteration in the number, binding affinity, or size of the G-colony-stimulating-factor receptor on periodic-hematopoiesis dogs as compared to normal dogs. They thus conclude that periodic hematopoiesis "is caused by a defect in the G-colony-stimulating-factor signal-transduction pathway at a point distal to G-colony-stimulating-factor binding . . . ." The data of Avalos et al. (1994) can be used to estimate that

$$\gamma_{\max}^{PH} \simeq 7 \times \gamma_{\max}^{\text{norm}} \tag{8.65}$$

The results of Hammond et al. (1992) in humans are consistent with these results in dogs.

Less is known about the effect of GM-colony-stimulating factor, but it is known that administration of GM-colony-stimulating factor in humans gives an elevation of the mean white blood cell level but only by relatively modest amounts–1.5 to 3.9 times (Wright et al. 1994), but either dampens the oscillations of periodic hematopoiesis or eliminates them entirely. The same effect has been shown (Hammond et al. 1990) in the grey collie. It is unclear if the period of the peripheral cell oscillations has a concomitant decrease, as is found with G-colony-stimulating factor. The abnormal responsiveness of precursors to G-colony-stimulating factor in grey collies and humans with periodic hematopoiesis (Hammond et al. 1992; Avalos et al. 1994) is mirrored in the human response to GM-colony-stimulating factor (Hammond et al. 1992).

Thus, the available laboratory and clinical data on the effects of colony-stimulating factors in periodic hematopoiesis indicate that: (1) there is extensive intercommunication between all levels of stem cells; and (2) within the language of nonlinear dynamics, colony-stimulating factors may be used to titrate the dynamics of periodic hematopoiesis to the point of inducing a reverse Hopf bifurcation (disappearance of the oscillations). In the course of this "titration" there may also be a shift in the period.

The behavior in periodic hematopoiesis when colony-stimulating factor is administered is qualitatively consistent with the pluripotential stem-cell model discussed in Section 8.6, since it is known that colony-stimulating factor interferes with apoptosis and, thus, administration of colony-stimulating factor 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 hope this combined modeling and data-analysis project may give greater insight into the fundamental nature of the regulation of the mammalian cell cycle and, in the future, suggest more rational therapies for patients with periodic hematopoiesis.

8.9 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 to 4 times the delay but may be even longer. Thus it is not necessary to search for illusive and mystical entities (Beresford 1988), such as ultradian rhythms, to explain the periodicity of these disorders.

The observations in this chapter 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 behavior 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 hematological disorders.

As an extension to the concept of periodic diseases introduced by Reimann (1963) in 1963, the term "dynamical disease" has been introduced (Glass and Mackey 1979; Glass and Mackey 1988; Mackey and Glass 1977; Mackey and an der Heiden 1982; Mackey and Milton 1987). 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 readjust them to values associated with healthy behaviors. Developments in biotechnology and the analysis of physiological control mechanisms are occurring at so rapid a pace that the feasibility of such an approach may be just around the corner, as illustrated by the material described concerning the effects of the colony-stimulating factors in periodic hematopoiesis.

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