Variable Maturation Velocity and Parameter Sensitivity in a Model of Haematopoiesis

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Abstract

We analyse an age-structured model for haematopoiesis, describing the development of specialized cells in the blood from undifferentiated stem cells and including the controlling effects of hormones. Variation in the length of time for maturing of precursor cells in this model has a stabilising influence. When the maturing process does not vary, then the age-structured model reduces to a delay differential equation. Depending on the death process considered, either a differential equation with two time delays or a differential equation with a state-dependent delay is obtained. Each of these is analysed in turn, for its linear stability. A sensitivity analysis of the parameters in this model shows which biochemical processes in the negative feedback most strongly affect the solutions.

Keywords: Age-structured models; haematopoiesis; stability; bifurcations; oscillations.

1 Introduction

A number of haematological dysregulations are characterised by oscillations in one or more of the circulating haematopoietic cell lines. Examples of these periodic haematological disorders include cyclical neutropenia (Dale, Alling and Wolff 1972, Dale and Hammond 1988, Haurie, Dale and Mackey 1998, Jones and Lange 1983, Lange 1983, Schmitz, Franke, Wichmann and Diehl 1995, von Schulthess and Mazer 1982, Wright, Dale, Fauci and Wolff 1981, Wright, Kenney, Oette, LaRussa, Boxer and Malech 1994), chronic myelogenous leukemia (Chikkappa, Borner, Burlington, Chanana, Cronkite, Ohl, Pavelec and Robertson 1976, Delobel, Charbord, Passa and Bernard 1973, Fortin and Mackey 1999, Gatti, Robinson, Deinare, Nesbit, McCullough, Ballow and Good 1973, Kennedy 1970, Mastrangelo, Stabile, Parenti and Cimatti 1976, Mastrangelo, Stabile, Parenti and Segni 1974, Rodriguez and Lutcher 1976, Shadduck, Winkelstein and Nunna 1972, Wheldon, Kirk and Finlay 1974), periodic auto-immune haemolytic anemia (Gordon and Varadi 1962, Orr, Kirk, Gray and Anderson 1968, Ranlov and Videbaek 1963), polycythemia vera (Morley 1969), and cyclical thrombocytopenia (Bernard and Caen 1962, Brey, Garner and Wells 1969, Caen, Meshaka, Larrieu and Bernard 1964, Cohen and Cooney 1974, Demmer 1920, Engstrom, Lundquist and Soderstrom 1966, Goldschmidt and Fono 1972, Lewis 1974, Skoog, Lawrence and Adams 1957, Swinburne and Mackey 1999, Wasastjerna 1967, Wilkinson and Firkin 1966). These diseases are believed to result from abnormalities in the regulatory control processes, but details for the breakdown of the controls are lacking. Haurie *et al.* 1998 have recently reviewed the basic regulation of haematopoiesis and some of the mathematical models used to study those haematological disorders. In a series of articles (Bélair, Mackey and Mahaffy 1995, Mahaffy, Bélair and Mackey 1998, Mahaffy, Polk and Roeder 1999), the authors and co-workers describe the process of erythropoiesis with its physiological control by erythropoietin and develop age-structured models for normal human subjects and rabbits with an induced auto-immune haemolytic anemia.

The process of haematopoiesis begins with undifferentiated stem cells, which have the potential to be highly proliferative. Several growth factors act to differentiate these stem cells into the various cell lineages, which eventually proliferate into large colonies of specialised cells, such as erythrocytes, granulocytes, monocytes, or platelets. These cell lines are regulated by several lineage-specific hormones, which promote cellular production or decrease preprogrammed cell death (apoptosis). In addition, the hormones appear to accelerate the maturing process. Since many hormones and growth

factors are involved in the regulatory process, there are many ways in which this system may be affected by disease.

In this study, we examine age-structured models for haematopoiesis and identify the sensitivity of the model to variations in the parameters. An analysis of the parameters in the mathematical models can determine which ones have the greatest effect on stability of the model and may give insight into which biological controls are most important in haematopoiesis. The next section contains a brief background of haematopoiesis and presents the mathematical models. In Section 3, an analysis of the effect of a variable velocity of maturation for the age-structured model is performed, showing that this process stabilises the model. Numerical studies are presented to quantify this effect using parameter values for both normal and diseased states. An additional study shows the difference between the age-structured model of Bélair *et al.* 1995, where the mature cells have a specified lifespan, and the model of Mahaffy *et al.* 1998, which has a fixed flux of mature cells dying to allow for variable lifespans of these cells. In the next section, we examine how the other parameters of the mathematical model affect the stability of the model. Finally, we relate the biological controls to the sensitivity analysis in a discussion section.

2 Age-structured Models for Haemopoiesis

Specialised cells in the blood provide a range of essential functions for the body. Erythrocytes carry O_2 to the tissues, neutrophils attack disease agents and remove dying and defective cells from the body, and platelets provide the biochemical factors necessary for clotting. All of these cell types are derived from undifferentiated stem cells, primarily in the bone marrow. The course of developing from stem cells to mature specialised cells in the blood during haematopoiesis follows similar pathways.

Haematopoiesis begins with either the recruitment of undifferentiated stem cells or a self-sustaining population of committed cells (burst forming units, BFU, or colony forming units, CFU), which are difficult to distinguish from the stem cells. A collection of cells begins a rapid proliferative stage to amplify the population of a particular cell-type, under the influence of the appropriate hormones, here denoted $H(t)$: Primarily, erythropoietin (EPO) for erythrocytes, thrombopoietin (TPO) for platelets, or granulocyte stimulating factor (G-CSF) for neutrophils. It is believed that many precursor cells, noted $p(t, \mu)$ at time t and maturity level μ, enter this proliferative stage, but then are destroyed by apoptosis from insufficient hormonal con-

centrations to continue development. After a few days the proliferative stage ends, and the cells enter a new phase where they become more specialised without cellular division. In erythropoiesis, the cells become reticulocytes, which accumulate haemoglobin while most other cellular components, including the nucleus, degenerate. These cells actually shrink in size and eventually leave the bone marrow to become mature erythrocytes circulating in the blood. Granulopoiesis undergoes a similar specialisation to the neutrophil, but maintains its nucleus and has a very different role in the body. In fact, the macrophages are among the neutrophils and play a key role in destroying the aged erythrocytes.

The mature cells, denoted $m(t,\nu)$ at age t and maturity level ν, enter the blood stream, then act to perform their specialised functions. For example, the erythrocytes circulate in the blood for about 120 days (in humans) carrying O_2 before they are actively destroyed by macrophages. Neutrophils rapidly leave the bloodstream and enter the lymphatic system for 4 to 5 days as they patrol for harmful agents, including aged erythrocytes. Neutrophils themselves are destroyed during defensive action of the immune system, or by senescence. Based on the concentration of these mature cells, specific receptors release hormones to control the particular haematopoietic system. For example, the partial pressure of O_2 is sensed by renal cells, which release EPO using a negative feedback to control erythropoiesis. These hormones also affect haematopoiesis by varying the rate at which precursor cells mature, accelerating the process at higher hormone concentrations.

The age-structured model, developed in Mahaffy *et al.* 1998, is given by a system of partial differential equations connected by its boundary conditions and a differential equation that describes the controlling hormone. The velocity of maturing for the precursor and mature populations are given by $V(H)$ and W, respectively, since the maturing velocity of the mature cells is taken to be constant. We assume that the net birth rate for the precursor cells is $\beta(\mu,H)$, and the death rate of mature cells is $\gamma(\nu)$. The partial differential equations are given by:

$$\frac{\partial p}{\partial t} + V(H)\frac{\partial p}{\partial \mu} = V(H)\beta(\mu,H)p, \quad t>0, \quad 0<\mu<\mu_F, \tag{2.1}$$

$$\frac{\partial m}{\partial t} + W\frac{\partial m}{\partial \nu} = -W\gamma(\nu)m, \qquad t>0, \quad 0<\nu<\nu_F, \tag{2.2}$$

where μ and ν represent the respective levels of maturity in precursor and mature cells, and μ_F and ν_F represent the respective maximal maturity levels that each cell class can reach. Two different destruction mechanisms are postulated for the mature cells: either a fixed number of cells are destroyed

in a given time or all cells die at the same age. They lead to different systems of delay equation models, both of which are described below.

Let $S_0(H)$ represent the number of cells recruited into the proliferating precursor population. The boundary conditions for the model are given by:

$$V(H)p(t,0) = S_0(H), \tag{2.3}$$

$$V(H)p(t,\mu_F) = Wm(t,0), \tag{2.4}$$

$$(W - \dot{\nu}_F(t))m(t,\nu_F(t)) = Q, \tag{2.5}$$

where Q represents a fixed removal rate of mature cells. Boundary condition (2.4) assumes that a fixed level of maturity in the precursor cells automatically promotes them to new mature cells. The last boundary condition (2.5) is a moving boundary condition, which indicates that a fixed number of mature cells are destroyed in any given time step (Mahaffy et al. 1998).

The hormone level H is governed by a differential equation with a negative feedback. Let $M(t)$, the total population of mature cells, be given by:

$$M(t) = \int_0^{\nu_F(t)} m(t,\nu)d\nu, \tag{2.6}$$

then the differential equation for H is:

$$\frac{dH}{dt} = f(M) - kH, \tag{2.7}$$

where k is the decay constant for the hormone and $f(M)$ is a monotone decreasing function of M, since there is a negative feedback by the total circulating mature population on the release of the hormone, H. We shall consider the following form of f:

$$f(M) = \frac{a}{1 + KM^r}, \tag{2.8}$$

which is a Hill function that often occurs in enzyme kinetic problems.

Mahaffy *et al.* 1998 made several simplifying assumptions and applied the method of characteristics to the age-structured model above to reduce the mathematical model to a system of delay differential equations. The most important assumption needed in the reduction of the age-structured model to a system of delay differential equations is that both velocities of aging are constant. For simplicity, they are both normalised to the value one, *i.e.,* $V(H) = 1$ and $W = 1$. The second assumption is that the precursor cells grow exponentially for a given period of time μ_1, then stop dividing, so

$$\beta(\mu, H) = \begin{cases} \beta, & \mu < \mu_1, \\ 0, & \mu \geq \mu_1, \end{cases} \tag{2.9}$$

for some constant growth rate β. Finally, they assumed that the decay rate $\gamma(\nu)$ is a constant γ. With these assumptions and letting $T = \mu_F$, the model finally becomes the following system of delay differential equations with a fixed delay T and a state dependent delay occurring in the equation governing the age at which mature cells die:

$$\begin{aligned} \frac{dM(t)}{dt} &= e^{\beta\mu_1} S_0(H(t-T)) - \gamma M(t) - Q, \\ \frac{dH(t)}{dt} &= f(M(t)) - kH(t), \\ \frac{d\nu_F(t)}{dt} &= 1 - \frac{Qe^{\gamma\nu_F(t)}}{e^{\beta\mu_1} S_0(H(t-T-\nu_F(t))}. \end{aligned} \tag{2.10}$$

Note that the $\dot{\nu}_F(t)$ equation is uncoupled from the other two equations and that the equation for $\dot{M}(t)$ has only the single time delay T, simplifying the stability analysis of the system.

As mentioned above, this last model assumes that mature cells are removed at a constant rate. The alternate model, which assumes that mature cells have a fixed, specific lifespan ν_F, has been derived in Bélair *et al.* 1995. This second model would be more appropriate for neutrophils, for example, if the mature cells had a preprogrammed cell death, controlled by apoptosis. Our study below shows the difference in the characteristic equations of these models and how this may explain the different numerical simulations. This model also assumed constant velocity of maturation; for simplicity of comparison, we assume $V(H) \equiv 1$, so that the age-structured model reduces to a system of delay differential equations. From Bélair *et al.* 1995, the model becomes

$$\begin{aligned} \frac{dM(t)}{dt} &= e^{\beta\mu_1}\left[S_0(H(t-T_1)) - e^{-\gamma T_2} S_0(H(t-T_1-T_2))\right] \\ &\qquad -\gamma M(t), \\ \frac{dH(t)}{dt} &= f(M(t)) - kH(t), \end{aligned} \tag{2.11}$$

where $T_1 = \mu_F$ and $T_2 = \nu_F$.

3 Analysis of the Age-structured Model with Variable Velocity

Mahaffy *et al.* 1999 observed in their numerical simulations of the age-structured model with constant flux boundary conditions presented in

the previous section that allowing $V(H)$ to be an increasing function of H increased the stability of the equilibrium. Biologically, this suggests that by allowing the precursor cells to mature at different rates depending on the concentration of the hormone, a haematopoietic cell-line is better able to maintain homeostasis. Although it cannot be reduced to a system of delay differential equations, the age-structured model including the variable velocity, $V(H)$, can be analysed for its linear stability.

3.1 Linearisation

The linear analysis begins by finding the unique equilibrium solution of (2.1)–(2.7). We retain the assumptions on W, β, and γ from the previous section, but now we allow $V(H)$ to vary, and be a function of the hormone level H. By removing the time dependence of p and m, Eqns. (2.1) and (2.2) are easily solved with the boundary conditions (2.3) and (2.4), then inserted into (2.6) to give:

$$\overline{M} = \frac{S_0(\overline{H})e^{\beta\mu_1}}{\gamma}(1 - e^{-\gamma\overline{\nu}_F}). \tag{3.1}$$

The boundary condition (2.5) yields an equation for $\overline{\nu}_F$,

$$e^{\beta\mu_1} S_0(\overline{H}) e^{-\gamma\overline{\nu}_F} = Q.$$

From (2.7), an equilibrium equation relating the hormone level to the total size of the mature population is obtained as

$$f(\overline{M}) = k\overline{H}.$$

From the assumed forms, essentially the monotonicity of $S_0(H)$ and $f(M)$, the three equations above produce a unique equilibrium $\overline{H}$, $\overline{M}$, and $\overline{\nu}_F$.

We next linearise the age-structured model about its equilibrium. As usual, we seek solutions of the form:

$$\begin{pmatrix} M(t) - \overline{M} \\ H(t) - \overline{H} \\ \nu_F(t) - \overline{\nu}_F \end{pmatrix} = \begin{pmatrix} A \\ B \\ C \end{pmatrix} e^{\lambda t} \tag{3.2}$$

to find the characteristic equation. As in Mahaffy *et al.* 1998, the method of characteristics is used, where only the long time solutions are considered. Eqn. (2.6) and the solution of (2.2) along characteristics give

$$M(t) \quad = \quad \int_0^{\nu_F(t)} m(t - \nu, 0) e^{-\gamma\nu} d\nu,$$

$$
\begin{aligned}
&= \int_0^{\nu_F(t)} V(H(t-\nu))p(t-\nu,\mu_F)e^{-\gamma\nu}d\nu, \\
&= e^{\beta\mu_1}\int_0^{\nu_F(t)} V(H(t-\nu))\frac{S_0(H(t-\nu-\tau))}{V(H(t-\nu-\tau))}e^{-\gamma\nu}d\nu, \qquad (3.3)
\end{aligned}
$$

where

$$
p(t-\nu,\mu_F) = \frac{S_0(H(t-\nu-\tau))}{V(H(t-\nu-\tau))}e^{\beta\mu_1},
$$

is obtained by solving (2.1) along its characteristics, and τ satisfies

$$
\mu_F = \int_{t-\tau}^{t} V(H(r))dr.
$$

The integrand in (3.3) is linearised about $\overline{H}$ by a Taylor expansion to first order to give

$$
M(t) \simeq e^{\beta\mu_1}\int_0^{\nu_F(t)} \left[S_0(\overline{H}) + V_1(H(t-\nu)-\overline{H}) + V_2(H(t-\nu-\tau)-\overline{H})\right] e^{-\gamma\nu}d\nu,
$$

where

$$
(3.4)\quad V_1 \equiv \frac{V'(\overline{H})S_0(\overline{H})}{V(\overline{H})} \qquad \text{and} \qquad V_2 \equiv \frac{V(\overline{H})S_0'(\overline{H}) - V'(\overline{H})S_0(\overline{H})}{V(\overline{H})}.
$$

From (3.2), we have $H(z) - \overline{H} = Be^{\lambda z}$, which allows the evaluation of the integral in the expression for $M(t)$ above. It follows that

$$
\begin{aligned}
M(t) &\simeq e^{\beta\mu_1}\int_0^{\nu_F(t)} \left[S_0(\overline{H}) + Be^{\lambda(t-\nu)}(V_1 + V_2e^{-\lambda\tau})\right] e^{-\gamma\nu}d\nu \\
&\simeq e^{\beta\mu_1}\left[S_0(\overline{H})\left(\frac{1-e^{-\gamma\nu_F(t)}}{\gamma}\right)\right. \qquad (3.5) \\
&\qquad \left. + Be^{\lambda t}(V_1 + V_2e^{-\lambda\tau})\left(\frac{1-e^{-(\lambda+\gamma)\nu_F(t)}}{\lambda+\gamma}\right)\right],
\end{aligned}
$$

which is still nonlinear in $\nu_F(t)$.

Substituting the expression for the equilibrium $\overline{M}$ given by Eq. (3.1) and linearising $\nu_F(t)$ using

$$
e^{-\gamma\nu_F(t)} \simeq e^{-\gamma\overline{\nu}_F}(1-\gamma(\nu_F(t)-\overline{\nu}_F)),
$$

Eq. (3.5) now becomes

$$(3.6)\qquad M(t) \simeq \overline{M} + e^{\beta\mu_1} S_0(\overline{H}) e^{-\gamma\overline{\nu}_F}(\nu_F(t) - \overline{\nu}_F) + Be^{\beta\mu_1} e^{\lambda t}(V_1 + V_2 e^{-\lambda\tau}) \left(\frac{1 - e^{-(\lambda+\gamma)\nu_F(t)}}{\lambda+\gamma}\right).$$

We note that the last term in the expression above has both τ and $\nu_F(t)$ in the exponentials and τ has a linear expansion in H. However, since the entire last term is already multiplied by B (making it a first order expression), we only need, for the linear expansion we are seeking, zeroeth order expansions for τ and $\nu_F(t)$, which are μ_F and $\overline{\nu}_F$, respectively. From (3.2), we substitute $M(t) - \overline{M} = Ae^{\lambda t}$ and $\nu_F(t) - \overline{\nu}_F = Ce^{\lambda t}$ in Eq. (3.7), then divide by $e^{\lambda t}$ to obtain:

$$(3.7)\quad A = Ce^{\beta\mu_1} S_0(\overline{H}) e^{-\gamma\overline{\nu}_F} + Be^{\beta\mu_1}(V_1 + V_2 e^{-\lambda\mu_F}) \left(\frac{1 - e^{-(\lambda+\gamma)\overline{\nu}_F}}{\lambda+\gamma}\right).$$

The linearisation of (2.7) with the substitutions from (3.2) yield:

$$(3.8)\qquad \lambda B e^{\lambda t} = f'(\overline{M}) A e^{\lambda t} - Bke^{\lambda t}$$

Finally, the boundary condition (2.5) gives the differential equation:

$$(3.9)\qquad \dot{\nu}_F = 1 - \frac{Q}{m(t, \nu_F(t))},$$

where

$$\begin{aligned} m(t, \nu_F(t)) &= V(H(t - \nu_F(t)))p(t - \nu_F(t), \mu_F) e^{-\gamma\nu_F(t)} \\ &= \frac{V(H(t - \nu_F(t)))S_0(H(t - \nu_F(t) - \mu_F))}{V(H(t - \nu_F(t) - \mu_F))} e^{\beta\mu_1} e^{-\gamma\nu_F(t)}. \end{aligned}$$

Using $Q = e^{\beta\mu_1} e^{-\gamma\overline{\nu}_F} S_0(\overline{H})/V(\overline{H})$, we can now linearise Eq. (3.9) to

$$\begin{aligned} \dot{\nu}_F \simeq -\gamma(\nu_F(t) - \overline{\nu}_F) &- \left(\frac{V'(\overline{H})}{V(\overline{H})}\right)(H(t - \overline{\nu}_F) - \overline{H}) \\ &+ \left(\frac{S_0'(\overline{H})}{S_0(\overline{H})} - \frac{V'(\overline{H})}{V(\overline{H})}\right)(H(t - \overline{\nu}_F - \mu_F) - \overline{H}). \end{aligned}$$

By using (3.2) in the this last equation and dividing by $e^{\lambda t}$, we obtain

$$(3.10)\quad C\lambda = -C\gamma - B\left[\left(\frac{V_1}{S_0(\overline{H})}\right)e^{-\lambda\overline{\nu}_F} + \left(\frac{V_2}{S_0(\overline{H})}\right)e^{-\lambda(\overline{\nu}_F + \mu_F)}\right].$$

The eigenvalue problem can now be stated by combining (3.7), (3.8), and (3.10). From the coefficients of A, B, and C, we have the following matrix equation

$$\begin{pmatrix} 1 & -e^{\beta\mu_1}(V_1+V_2e^{-\lambda\mu_F})\frac{(1-e^{-(\lambda+\gamma)\overline{\nu}_F})}{\lambda+\gamma} & -e^{\beta\mu_1}S_0(\overline{H})e^{-\gamma\overline{\nu}_F} \\ -f'(\overline{M}) & \lambda+k & 0 \\ 0 & -\frac{e^{-\lambda\overline{\nu}_F}}{S_0(\overline{H})}(V_1+V_2e^{-\lambda\mu_F}) & \lambda+\gamma \end{pmatrix} \begin{pmatrix} A \\ B \\ C \end{pmatrix} = \mathbf{0}. \tag{3.11}$$

The determinant of this 3×3 matrix can be expanded by the first column to obtain the characteristic equation,

$$(\lambda+\gamma)[(\lambda+\gamma)(\lambda+k) - e^{\beta\mu_1}f'(\overline{M})(V_1+V_2e^{-\lambda\mu_F})] = 0. \tag{3.12}$$

Notice that $V_1+V_2e^{-\lambda\mu_F}$ can be written, using Eqs. (3.4), as

$$\frac{V'(\overline{H})S_0(\overline{H})}{V(\overline{H})} + \left(\frac{V(\overline{H})S_0'(\overline{H}) - V'(\overline{H})S_0(\overline{H})}{V(\overline{H})}\right)e^{-\lambda\mu_F},$$

showing the role played by the velocity $V(H)$ in the characteristic equation.

In the case of constant velocity V, where $V'(\overline{H})=0$, the simplified characteristic equation can be directly computed from a linearisation of system (2.10). Indeed, since $S_0(H)$ is monotonically increasing and $f(M)$ is a negative feedback function, there is a unique equilibrium $(\overline{M},\overline{H},\overline{\nu}_F)$ for (2.10). The simplified model given by (2.10) can thus be directly linearised about its equilibrium,

$$\begin{aligned} \dot{M}(t) &= e^{\beta\mu_1}S_0'(\overline{H})H(t-T) - \gamma M(t), \\ \dot{H}(t) &= f'(\overline{M})M(t) - kH(t), \\ \dot{\nu}_F(t) &= \frac{1}{\overline{H}}H(t-T-\overline{\nu}_F) - \gamma\nu_F(t). \end{aligned} \tag{3.13}$$

The characteristic equation for the eigenvalues corresponding to (3.13) was shown in Mahaffy *et al.* 1998 to be

$$(\lambda+\gamma)\left[(\lambda+\gamma)(\lambda+k) - e^{\beta\mu_1}S_0'(\overline{H})f'(\overline{M})e^{-\lambda T}\right] = 0, \tag{3.14}$$

which is equivalent to $V'(\overline{H})=0$ in (3.12).

A similar analyis can be performed for the age-structured model with a fixed lifespan, ν_F described in Section 2 and in Bélair *et al.* 1995. This model

replaces $\nu_F(t)$, with $\nu_F = T_2$, a constant, which eliminates the boundary condition (2.5) and changes the upper limit of integration in (2.6) from $\nu_F(t)$ to T_2. With these changes, and assuming $\mu_F = T_1$, then a very similar linearisation to the one above yields the characteristic equation

$$(\lambda+\gamma)(\lambda+k) - e^{\beta\mu_1} f'(\overline{M})(V_1 + V_2 e^{-\lambda T_1})(1 - e^{-(\lambda+\gamma)T_2}) = 0. \tag{3.15}$$

(Note that the equation corresponding to the matrix equation (3.11) in this model can be obtained by removing the third row and third column.) If the factor $(\lambda+\gamma)$ is divided from (3.12), then (3.15) is very similar, except that it has the additional factor $\left(1 - e^{-(\lambda+\gamma)T_2}\right)$ included. The inclusion of the second delay significantly increases the complexity of analysing the characteristic equation, as has been seen in other works (Bélair and Campbell 1994, Bélair et al. 1995, MacDonald 1989, Mahaffy, Zak and Joiner 1995).

Bélair *et al.* 1995 examined the corresponding two delay model for the fixed lifespan model and no variable velocity of maturation. The linearisation of (2.11) about its equilibrium is given by

$$\begin{aligned} \frac{dM(t)}{dt} &= e^{\beta\mu_1}\left[S_0'(\overline{H})H(t-T_1) - e^{-\gamma T_2} S_0'(\overline{H})H(t-T_1-T_2)\right] \\ &\qquad -\gamma M(t), \\ \frac{dH(t)}{dt} &= f'(\overline{M})M(t) - kH(t). \end{aligned} \tag{3.16}$$

The characteristic equation, obtained as the determinant of a 2×2 matrix, is

$$(\lambda+\gamma)(\lambda+k) - e^{\beta\mu_1} S_0'(\overline{H}) f'(\overline{M}) \left(e^{-\lambda T_1} - e^{-\gamma T_2} e^{-\lambda(T_1+T_2)}\right) = 0. \tag{3.17}$$

This equation agrees with (3.15) where $V_1 = 0$ and $V_2 = S_0'(\overline{H})$, which is the case for $V'(\overline{H}) = 0$.

3.2 Stability Analysis

Our study concentrates on the effects of variable velocity in the model with constant flux boundary conditions for mature cells. This more general case, where the haematopoietic model has $V(H)$ as a non-decreasing, non-negative function based on physiological assumptions, uses the characteristic equation given by (3.12). Since $\gamma > 0$, the factor $\lambda+\gamma$ cannot contribute to the destabilisation of the equilibrium. Thus, we can need only consider

$$(\lambda+\gamma)(\lambda+k) + \alpha_1 + (\alpha_2 - \alpha_1)e^{-\lambda T} = 0, \tag{3.18}$$

where α_1 and α_2 are non-negative constants and we have written T for μ_F. (Note that when V is not constant, then $\alpha_1 > 0$.) This equation has been studied extensively, by numerous investigators (Bhatt and Hsu 1966, Boe and Chang 1989, Boe and Chang 1991, Boese 1989, Boese and van den Driessche 1994, Cooke and Grossman 1982, Heiden 1979, MacDonald 1989, Stépán 1989). Although complicated dynamical behaviour is possible in nonlinear second order delay differential equations (Campbell, Bélair, Ohira and Milton 1995), the values of the parameters in Eq. (3.18) are such that only the mildest instability can occur. Since the constants γ, k, α_1 and α_2 are all positive, a Poincaré-Bendixson (Mallet-Paret and Sell 1996) result holds, and we only have, in the words of Campbell *et al.* 1995, "less interesting dynamics." However, we show that as α_1 increases, the region of stability also increases.

A complete stability diagram of (3.18) can be analytically determined, as in (Campbell et al. 1995, Cooke and Grossman 1982). Of the many possible relations between the different parameters (α_1, α_2, k and γ), we only consider those that occur in the parameter estimation below. In particular, we assume that γ is non-negative; also, illustrative values of the other parameters are $\alpha_1 = 0.221$, $\alpha_2 = 3.362$ and $k = 6.65$.

First, it is clearly impossible for 0 to be a root of Eq. (3.18). Thus, the null solution of the latter equation can only lose stability by a pair of complex eigenvalues acquiring positive real part. If we let $\lambda = i\omega$ in Eq. (3.18), then separating real and imaginary parts of the resulting equation yields the system

$$k\gamma + \alpha_1 - \omega^2 + (\alpha_2 - \alpha_1)\cos\omega T = 0 \quad , \tag{3.19}$$

$$\omega(k+\gamma) - (\alpha_2 - \alpha_1)\sin\omega T = 0 \quad , \tag{3.20}$$

which can be written as

$$\cos\omega T = \frac{\omega^2 - (k\gamma + \alpha_1)}{\alpha_2 - \alpha_1} \quad , \tag{3.21}$$

$$\sin\omega T = \frac{\omega(k+\gamma)}{\alpha_2 - \alpha_1} \quad . \tag{3.22}$$

Squaring both sides of both equations in the latter system and using the fundamental trigonometric identity, we are lead to the equation

$$\omega^4 + (k^2 + \gamma^2 - 2\alpha_1)\omega^2 + (k\gamma + \alpha_1)^2 - (\alpha_2 - \alpha_1)^2 = 0 \quad . \tag{3.23}$$

This is a quadratic equation in ω^2, whence we extract

$$\omega^2 = \frac{1}{2}\left[2\alpha_1 - (k^2 + \gamma^2) \pm \sqrt{(k^2 + \gamma^2 - 2\alpha_1)^2 - 4((k\gamma + \alpha_1)^2 - (\alpha_2 - \alpha_1)^2)}\right] \quad .$$

In the case $2\alpha_1 - k^2 < \gamma^2$, which, as we shall shortly see holds in our experimental conditions, only the positive sign of the latter equality yields a real root. Furthermore, the expression under the square root is positive only when γ lies in the interval $\left(0, \dfrac{\alpha_2 - 2\alpha_1}{k}\right)$, provided that $2\alpha_1 < \alpha_2$. Therefore, we are led to the single value of ω given by

$$\omega = \sqrt{\alpha_1 - \frac{k^2+\gamma^2}{2} + \frac{\sqrt{(k^2+\gamma^2-2\alpha_1)^2 - 4((k\gamma+\alpha_1)^2 - (\alpha_2-\alpha_1)^2)}}{2}}$$

when $0 \leq \gamma < \frac{\alpha_2 - 2\alpha_1}{k}$. Note that stability of the equilibrium is assured for all values of T when $\gamma > \frac{\alpha_2 - 2\alpha_1}{k}$ (Cooke and Grossman 1982).

Once the value of ω has been so determined, we can substitute it back into either one of Eqs. (3.21) or (3.22), or, equivalently, into

$$T = \frac{1}{\omega} \arctan\left[\frac{\omega(k+\gamma)}{\omega^2 - (k\gamma + \alpha_1)}\right] \quad . \tag{3.24}$$

It remains to determine the appropriate branches of the inverse tangent function.

From Eq. (3.22), $\sin \omega T > 0$ whenever $\alpha_2 > \alpha_1$, so that ωT must lie in some interval $(2j\pi, (2j+1)\pi)$, where j is an integer. Furthermore, $\cos \omega T$ must have the same sign as $\omega^2 - (k\gamma + \alpha_1)$, which is readily seen from above to be the sign of

$$4k\gamma^3 + 4(\alpha_1 - k^2)\gamma^2 + 4k(k^2 + 2\alpha - 1)\gamma - 4[(\alpha_1 - \alpha_2)^2 - \alpha_1 k^2] \; .$$

This cubic polynomial in γ is monotone increasing for the values of the parameters k, α_1 and α_2 considered below. It takes a negative value when $\gamma = 0$ and, as γ increases, quickly becomes positive, well before γ takes the value $\frac{\alpha_2 - 2\alpha_1}{k}$. The argument ωT must thus move, as ω so increases, from a value in an interval $((2j + \frac{1}{2})\pi, (2j+1)\pi)$ to a value in an interval $(2j\pi, (2j + \frac{1}{2})\pi)$, where j is an integer. Of course, the limit of the stability region corresponds to the smallest (but positive) value of T determined from (3.24).

The stability diagram is illustrated in Fig. 3.1. We display the boundary of the stability region for both cases of constant and variable velocities. It is worth noticing that a velocity with positive slope, corresponding to $\alpha_1 > 0$, can only stabilise the system, when compared to the solution where $V(H) \equiv 1$, corresponding to $\alpha_1 = 0$. Hence, the region of stability obtained with positive values of α_1 is larger than the one obtained when α_1 is null.

The age-structured model with a fixed lifespan, ν_F, and variable velocity of maturation of the precursor cells has a more complicated characteristic equation because of the two delays in this problem. If we define $T_1 = T$, $\nu_F = T_2$, and $\beta_1 = e^{-\gamma T_2} < 1$, then using the same α_1 and α_2 as in (3.18), we find the characteristic equation for this model has the form

$$\begin{aligned} &(\lambda+\gamma)(\lambda+k) + \alpha_1 + (\alpha_2 - \alpha_1)e^{-\lambda T} \\ &-\beta_1 \left(\alpha_1 + (\alpha_2 - \alpha_1)e^{-\lambda T}\right) e^{-\lambda T_2} = 0. \end{aligned} \tag{3.25}$$

This characteristic equation is similar to (3.18), but has the complicating term at the end, resulting from the second delay, T_2. If β_1 is sufficiently small, then a perturbation analysis using the argument principle shows that the stabilising nature of α_1 seen for (3.18) carries through for this characteristic equation. However, the more general case with larger β_1 is significantly more complex, and it is possible that for some parameter values, α_1 increasing could be destabilising. A geometric examination of (3.25) using the argument principle does suggest that for most parameter values, increasing α_1 results in increasing stability of the system. These more involved changes in the stability structure are unlikely to occur in the parameter ranges of physiological interest (not unlike the codimension two bifurcation points of Figure 6 of (Bélair et al. 1995), which do not present a biological interest) .

4 Parameter Sensitivity for the Models

First, we perform a numerical investigation of solutions beyond the Hopf bifurcation lines determined in the last section, using parameter values from two sets of clinical studies. The first of these is based on data from Orr *et al.* 1968. They induced an auto-immune haemolytic anemic in a group of rabbits by regular injections of iso-antibodies for their erythrocytes. Mahaffy *et al.* (1998,1999) used the age-structured and delay differential equation model of Sec. 2, (2.1)–(2.7) and (2.10), respectively, to simulate the data for one of the rabbits. We begin our investigation by using the variable velocity,

$$V(H) = \frac{\kappa_1 H}{\kappa_2 + H},$$

where $\kappa_1 = 1.5$ and $\kappa_2 = 5$. These parameters assume that the maximum increase in the velocity of maturation is 50% above normal and that the normal level of H is 10. In the anemic state, we assume that the equilibrium satisfies $\overline{M} = 2.63$ (75% of the normal based on the observations of Orr *et*

al. 1968, $\overline{H} = 71.1$, and $\overline{\nu}_F = 50$. For the partial differential equation part of the model, the parameters are taken as $\beta = 2.773$, $\mu_1 = 3$, $\mu_F = 5.9$, and $\gamma = 0.073$. Assuming $S_0(H)$ is linear, we compute $S_0'(\overline{H}) = 0.00277$ and $Q = 0.00512$. In Eq.(2.8), we take $a = 15,600$, $K = 0.0382$, $r = 6.96$, and also the decay rate of the hormone as $k = 6.65$.

We illustrate in Fig. 4.1 a simulation overlaying the data with the above parameters. Details on the numerical procedure are found in Mahaffy *et al.* 1999. This produced a periodic solution with a period of 15.8 days and EPO concentrations fluctuating with an amplitude of 157 mU/ml between peak and trough. The figure also includes the results of the simulation when $V(H) \equiv 1$. As predicted by the analysis of the last section, this simulation is further away from the stable regime, with amplitudes of the erythrocyte counts and EPO concentrations 4.40 and 2.68 times larger, respectively. The period of the oscillation in this case is 1.63 times larger than in the case of the variable velocity.

The local analysis, however, does not predict the dramatic differences between the two simulations seen in Fig. 4.1. Analysis of the characteristic equation (3.12) for the simulations give a pair of leading eigenvalues $\lambda = 0.044 \pm 0.33i$ for the variable velocity case and $\lambda = 0.060 \pm 0.32i$ when the velocity is constant, $V(H) \equiv 1$. We see a moderate difference in the real part, which should govern the amplitude. However, there is almost no difference in the imaginary part, which should affect the period, yet the two simulations display substantially different periods for the oscillations. This difference confirms that if the rate of maturation for the precursor cells varies with the hormone concentration, then the age-structured model becomes more stable. In other words, hormone levels and mature cell populations oscillate less, or approach the equilibrium values quicker, when variability of the rate of maturation of the precursor cells is allowed.

The second numerical study compares the mathematical model with a fixed lifespan for erythrocytes (2.11) to the model with constant flux for the death of erythrocytes (2.10). (For this comparison, we let $V(H) \equiv 1$.) We use the parameters from Mahaffy *et al.* 1998, namely $\mu_F = 4.1$ and $\gamma = 0.065$. These changes result in $S_0'(\overline{H}) = 0.0025$ and $Q = 0.0069$. For (2.11), we take $\nu_F = 50$. The numerical simulations overlaying the data from Orr *et al.* 1968 are shown in Fig. 4.2. A comparison of the characteristic equations gives the pair of leading eigenvalues as $\lambda = 0.0171 \pm 0.4155i$ for (3.12) and $\lambda = 0.0173 \pm 0.4186i$ for (3.17). The leading eigenvalues are almost identical in their real part and differ by 1% in their imaginary parts. In the simulation, the period of the solution of (2.10) is 15.9 days, and the concentration of EPO fluctuated between 20.2 and 154.5 mU/ml. For the

solution of (2.11), the period is 15.2 days with EPO concentration bounded between 25.2 and 140.3 mU/ml.

The simulation in Fig. 4.2 illustrates how a fixed boundary condition for death of mature cells can stabilise the system. The period of oscillation is primarily determined by the delay T in system (2.10) or the delay T_1 in system (2.11). It is roughly four times this delay. The sum of the delays $T_1 + T_2$ in system (2.11) is about 3.5 times the period. Significantly, this means that the destruction rate is half a period out of phase, so its effect is diminished over the constant rate Q in (2.10), leading to enhanced stability. When the simulations are performed with $\overline{\nu}_F = 58$, both models have a period of 15.8 days, but in this case the amplitude of oscillation for the EPO concentration in (2.11) is about 1.1 times the amplitude observed for (2.10). Thus, the two-dela. model (2.11) is very similar to the constant flux model (2.10), but there are complicated variations caused by the complexity of a two-delay model.

We now turn to an examination of how systematic changes in the parameters affect the model. Already we have seen that flexibility in the rate of maturation of the precursor cells significantly increases the stability of the haematopoietic models. Our sensitivity study examines five of the parameters in the haematopoietic model for a normal human subject, and an experimental rabbit with an auto-immune haemolytic anemia. By knowing which parameters are most sensitive to perturbations and understanding their role in the control of the haematopoietic system, we can determine which elements of the model are most active in diseased states. Ultimately, this could suggest where therapeutic efforts would be most effective in treatment of a disease.

We begin with a study of an experimental rabbit with an auto-immune haemolytic anemia. Again to simplify our analysis, we let $V(H) \equiv 1$ so that the delay differential equation (2.10) and its corresponding characteristic equation (3.14) are used for the study. For comparison purposes, we chose to maintain the equilibrium solution at $(\overline{M}, \overline{H}, \overline{\nu}_F) = (2.63, 71.1, 50)$. The parameters of interest in the equation for $\dot{M}$ are the time delay, T, for the maturation time of the precursor cells and the random destruction of mature cells, γ. The unknown constant $S_0'(\overline{H})$ is adjusted appropriately to maintain the same equilibrium value. In the equation for $\dot{H}$, the parameters K and r in the Hill function $f(M)$ for production of H are treated separately, and the random destruction rate k is the final parameter considered. To maintain the same equilibrium values, the parameter a is adjusted.

We systematically considered variations of 10% in the values of the parameters given in Mahaffy *et al.* 1998 for (2.10). The results and the set

% CHANGE				
Parameter	10% decrease		10% increase	
	Period	Amplitude	Period	Amplitude
γ	-1.9	-54	+1.2	+35
T	-14	-54	+11	+38
K	0	-1.8	0	+1.5
r	-2.6	stable	+280	+50
k	< 1	+2	< 1	-1.5

Table 4.1: The table entries show the percent change in the period and amplitude of oscillation for the concentration of EPO as the parameters are varied in the model Eq.(2.10).

parameter values are displayed in Table 4.1. The parameters that are varied in the differential equation for the mature population are γ and T. We discuss the influence of each of the parameters in turn.

A 10% decrease in γ caused a 10% decrease in the real part of the eigenvalue and negligible change in the imaginary part. The resultant solution had only a 2% decrease in its period, while the amplitude of oscillation for the EPO concentration decreased by more than 50%. Similarly, a 10% increase in γ resulted in small increases in the period, but a 35% increase in the amplitude of oscillation for EPO.

Much stronger changes were seen when T was the parameter being varied. A 10% decrease in T caused a 14% decrease in the period with the oscillation having about a fifth of the amplitude, when compared to the original simulation. Equally significant increases were observed when T is increased by 10%.

In the second equation for (2.10), when either k or K were varied by 10%, the effect on either the period or the amplitude of oscillation was negligible. For the parameter r, a 10% decrease resulted in the real part of the eigenvalue becoming negative. Hence, (2.10) becomes stable. A 10% increase in r causes the largest change in amplitude, which increased by 50% though the period only increased about 4%. The variation of r had the strongest effect of any parameter of the system.

For the normal human subject, a change of 10% in any of the parameters listed above was unable to destabilise the model. Thus, the normal state of this model appears to be quite insensitive to perturbation, so far as stability is determined. The largest shifts in the eigenvalues were seen when the

parameters T and r were modified, which agrees with the results for the anemic rabbit. A 10% decrease in either of these parameters actually shifted the leading eigenvalue to the real axis, though it remained negative.

5 Discussion

We have presented a generalisation for the analysis of age-structured models for haematopoiesis developed earlier (Bélair et al. 1995, Mahaffy et al. 1998, Mahaffy et al. 1999). Using the method of characteristics this age-structured model can be reduced to a system of threshold-type delay differential equations. In the case detailed here, namely when a variable velocity of maturation of precursor cells is introduced in the age-structured model, the linear analysis must be performed on the threshold-type differential equations. This is analytically more intricate than the usual delay-differential equation (Bélair et al. 1995, Mahaffy et al. 1998). We have nevertheless been able to establish that any physiologically based function representing the variable velocity of maturation will have a stabilising influence.

We also performed a sensitivity analysis of the many parameters in the model to determine which ones had the greatest effects on the stability of the system. For this analysis, data from a normal human male and an experimental rabbit with an auto-immune haemolytic anemia were used. The parameters were chosen to maintain the same equilibria as studied in Mahaffy *et al.* 1998, which allowed five parameters to vary. By allowing a change of 10% in either direction for the parameters, we determined an ordering of significance in the modeling parameters. The greatest effect was attributed to changes in r, the Hill coefficient: this parameter reflects the degree of cooperativity in the O_2 sensors in the kidneys to the release of EPO, or, possibly, the degree of cooperativity for the binding of EPO to the precursor cells. An increase in r denotes an increase in the gain of the negative feedback function, and decreases stability of the equilibrium in the model. The next most significant parameter is the time delay for maturation, T. As T increases, the equilibrium point becomes less stable and the period of oscillation increases. The random destruction rate of mature cells, γ, has an effect on the amplitude of the oscillation which is similar to that of the parameter T, but not nearly such an effect on the period of the oscillation.

Our parameter sensitivity study confirms previous work on delay differential equations, showing that the gain in the negative feedback function and the delays are the most destabilising elements. A contrario, the variable ve-

locity of maturation for our age-structured model has been shown to have a very significant stabilising effect. This stabilising influence is taking place in addition to the equally stabilising influence of a variable lifespan in the sub-population of mature cells, when cells are removed according to a constant flux boundary condition. In both instances, it seems that organisms have developed mechanisms to increase stability of equilibria in haematopoietic systems. This may be yet another example of redundant control evolved to compensate for the inherent instabilities caused by time delays, helping to maintain homeostasis of this physiological system.

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FIGURE CAPTIONS

Figure 3.1
Stability diagram for eq. (3.14) in the case of constant maturation velocity (lower curve, $\alpha_1 = 0$) and positive velocity (upper curve, $\alpha_1 > 0$). The bullet labelled 'Model' corresponds to the parameter values derived in section 4 for the anemic rabbit, with simulations displayed in Figure 4.1.

Figure 4.1
Top: Data from Orr *et al.* 1968 on the circulating erythrocytes of a rabbit suffering from auto-immune haemolytic anemia (dashed-dotted line) superimposed on the simulation of eqs. (2.1)-(2.8) with variable velocity $V(H)$ and parameter values established at the beginning of section 4 (full line) and the corresponding model with constant velocity, $V(H) = 1$ (dashed line). Notice the increase in amplitude when the velocity is constant, and the much better fit to the data obtained by allowing the velocity to vary.
Bottom: Hormone levels corresponding to the simulations of the top figure. Variable velocity (dashed-dotted line) and constant velocity (dashed line). Notice the effect of the variable velocity on both amplitude and period of the oscillations, as discussed in section 4.

Figure 4.2
The model (2.10) with moving boundary conditions, $\mu_F = 4.1$ and $\gamma = 0.065$, (dashed line) is fitted to, and compared with, the data of Orr *et al.* 1968 which is given by the dashed-dotted line. The model (2.11) with $\nu_F = 50$ and other parameters matching ones for (2.10) is shown with a solid line.

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