



## Cyclical Neutropenia and the Peripheral Control of White Blood Cell Production

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Cyclical neutropenia (CN) is an interesting dynamic hematological disease in which the neutrophils spontaneously oscillate from approximately normal levels to near zero with a period between 19 and 21 days. In the only known animal model for this disorder, the grey collie, the disease's single apparent difference from human CN is the smaller period of 11–15 days. CN can be treated using the cytokine G-CSF which decreases the period (to about 14 days in humans), increases the mean value, and elevates the amplitude of the oscillations. After reviewing the clinical and laboratory data on this disease, we examine the proposition that CN is due to a loss of stability in the peripheral negative feedback control of neutrophil production. This is accomplished by the development of a physiologically realist mathematical model for the system. We conclude that there is no consistent way in which such a destabilization can give rise to either the clinical or laboratory characteristics of CN. Rather it seems more likely that the oscillations of CN are generated within the pluripotential stem cell population.

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### 1. Introduction

Cyclical neutropenia (CN) is a relatively rare disorder, the hallmark of which is a periodic fall in the circulating neutrophil numbers from normal values to virtually zero in severe cases. This paper examines the potential role of instabilities in the peripheral control of neutrophil production (granulopoiesis) in generating the characteristic oscillations observed in the neutrophil counts of patients with CN. We also consider the grey collie—the naturally occurring laboratory model for this disease.

Following a review of normal granulopoiesis and granulopoiesis in CN (and previous models for CN) in Section 2, we present a model for the control of granulopoiesis in Section 3 including an estimation of the relevant parameters of the model for normals and in cyclical neutropenia, an analysis of the local stability properties of the model and numerical investigation of the full nonlinear model. The paper closes with a discussion in Section 4 of the

implications of this work for our understanding of the origin of CN.

### 2. Biological Background

#### 2.1. NORMAL GRANULOPOIESIS

Figure 1 is a cartoon representation of the organization of normal myelopoiesis that outlines the three major components of the myelopoietic system and the known or putative regulatory feedback loops. For more details, consult (Dexter & Spooner, 1987; Ogawa, 1993) and any recent hematology textbook, such as Beutler *et al.* (1995).

The control of granulopoiesis via the circulating neutrophil levels is obscure. One of the primary controlling agents is thought to be granulocyte colony stimulating factor (G-CSF). G-CSF is known to be absolutely essential for the growth of the granulocytic progenitor cells CFU-G *in vitro* (Williams *et al.*, 1990). CFU-G colony growth is a sigmoidally

increasing function of increasing G-CSF concentration (Hammond *et al.*, 1992; Avalos *et al.*, 1994).

The possible importance of G-CSF for *in vivo* control of granulopoiesis is shown in the work of Lieschke *et al.* (1994). In mice that lack G-CSF (due to an ablation of the G-CSF gene in embryonal stem cells) there is a pronounced neutropenia and reduction of the marrow granulocyte precursor cells by a factor of 50%. Exogenous G-CSF obliterates the neutropenia in one day and within 4 days restores the marrow composition to that of a normal wild type mouse. G-CSF also corrects neutropenia in humans (Bronchud *et al.*, 1987; Layton *et al.*, 1989; Leary *et al.*, 1992; Lord *et al.*, 1989; Lord, 1992; Ponchio *et al.*, 1995) and other mammals (Cohen *et al.*, 1987; Ikebuchi *et al.*, 1988; Lord *et al.*, 1991; Molineux *et al.*, 1990). In addition, there is a clear alteration of the neutrophil precursor maturation time characteristics under the action of G-CSF (Price *et al.*, 1996). These observations indicate that G-CSF most certainly plays some role in the normal maintenance of granulopoiesis.

Several studies have demonstrated an inverse relation between circulating neutrophil density and serum levels of G-CSF (Kearns *et al.*, 1993; Mempel *et al.*, 1991; Takatani *et al.*, 1996; Watari *et al.*, 1989).

Coupled with the *in vitro* colony growth characteristics cited above the observations on G-CSF knockout mice, this inverse relationship suggests that the nature of the neutrophil production regulation via G-CSF would be that of negative feedback. Thus, a fall in circulating neutrophil levels would lead to an increase in serum G-CSF levels and an eventual increase in marrow production of neutrophils. We address this control issue more quantitatively in Section 3.4.

## 2.2. CYCLICAL NEUTROPENIA

Our understanding of CN has been greatly aided by the discovery that the grey collie also suffers from the same disease. In the grey collie, this disease is apparently the same as in humans with the exception of the period which ranges from 11 to 15 days (Haurie *et al.*, 1998c), while in humans the period is typically reported to fall in the range of 19–21 days (Dale & Hammond, 1988) or 20–30 days (Haurie *et al.*, 1998b). Reviews of both human and canine cyclic neutropenia (Dale & Wolff, 1972; Dale & Hammond, 1988; Haurie *et al.*, 1998a; Jones & Lange, 1983; Lange & Jones, 1980; Lange, 1983; Page & Good, 1957; Quesenberry, 1983; Wright *et al.*, 1981) may be consulted for information not contained in the summary below.

In both human CN (Dale *et al.*, 1972a, b; Haurie *et al.*, 1998b; Hoffman *et al.*, 1974) and the grey collie (Guerry *et al.*, 1973; Haurie *et al.*, 1998c) there is not only a periodic fall in the circulating neutrophil levels, but also an oscillation of platelets, often the monocytes and eosinophils, and occasionally the reticulocytes. These oscillations all occur with the same period. In contrast to the neutrophils, the monocyte, eosinophil, and platelet oscillation levels range from normal to high levels, while the reticulocytes typically oscillate around normal values (Dale & Hammond, 1988).

The peripheral neutrophil kinetics indicate that the disappearance half time of circulating cells is normal (Dale *et al.*, 1972b). This implies that there is not a periodic modification of the peripheral loss rate but rather a periodic failure of marrow cell production.

In humans with CN, there is an orderly cell density wave that proceeds successively through the myeloblasts, promyelocytes, and myelocytes and then enters the post-mitotic maturation compartment (comprised of the metamyelocytes, the banded neutrophils, and the segmented, or polymorphonuclear, neutrophils) before being manifested in the circulation (Guerry *et al.*, 1973; Brandt *et al.*, 1975). Further studies have shown that this wave extends back into the committed stem cells CFU-G and

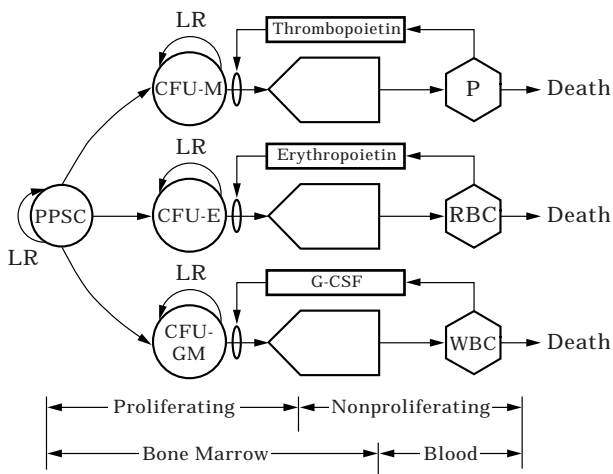


FIG. 1. The architecture of hematopoietic regulation. This figure gives a schematic representation of the architecture and control of platelet (P), red blood cell (RBC), and white blood cell (WBC, including neutrophils and monocytes) production. (Basophil and eosinophil lines not indicated.) Various presumptive control loops mediated by thrombopoietin (TPO), erythropoietin (EPO), and granulocyte colony stimulating factor (G-CSF) are indicated, as well as putative local regulatory (LR) loops within the various committed and pluripotential stem cell populations. CFU refers to the various colony forming units (M = megakaryocyte, E = erythroid, and GM = granulocyte/monocyte) which are the *in vitro* analogs of the *in vivo* committed stem cells (CSC). PPSC denotes the pluripotential myeloid stem cell population. Adapted from Mackey (1996).

TABLE 1  
*Distribution of maturation time parameters deduced from published data*

Condition	$\langle \tau \rangle$ (day)	$\sigma^2$ (day <sup>2</sup> )	$\tau_m$ (day)	Reference
Normal human	9.70	16.20	3.8	(Perry <i>et al.</i> , 1966)
CN human	7.57	12.01	1.2	(Guerry <i>et al.</i> , 1973)
30 $\mu$ g G-CSF human	6.27	4.60	2.4	(Price <i>et al.</i> , 1996)
300 $\mu$ g G-CSF human	4.86	2.30	2.0	(Price <i>et al.</i> , 1996)
Normal dog	3.68	0.198	3.0	(Deubelbeiss <i>et al.</i> , 1975)
Gray collie apogee	3.21	0.042	2.6	(Patt <i>et al.</i> , 1973)
Gray collie nadir	3.42	0.157	2.6	(Patt <i>et al.</i> , 1973)

See the text for details

CFU-M, and precedes the wave in the myeloblast compartment (Jacobsen & Broxmeyer, 1979). In the grey collie the same pattern exists as does waves of cell density in the CFU-G and CFU-E (Dunn *et al.*, 1977, 1978; Hammond & Dale, 1982; Jones & Jolly, 1982) and the BFU-E (burst forming units—erythroid which proceed the CFU-E) and CFU-GM (Hammond & Dale, 1982; Abkowitz *et al.*, 1988).

There are also changes in the distribution of transit times (cf. Section 3.1.2.) in CN. We discuss these in Section 3.1.3. and summarize them in Table 1. Briefly, in CN it is found that the neutrophil precursor mean maturation time is shortened, and the variance of the distribution is decreased relative to normal.

Cyclical neutropenia in the grey collie can be cured by lethal irradiation followed by transplantation of normal bone marrow (Dale & Graw, 1974; Jones *et al.*, 1975a). CN can be induced in normal collies by bone marrow transplant from a cyclical neutropenic littermate (Jones *et al.*, 1975a, b; Weiden *et al.*, 1974). The same is true in humans (Krance *et al.*, 1982). These studies suggest that the origin of the defect in CN is resident in one of the stem cell populations of the bone marrow.

In both the grey collie (Hammond *et al.*, 1990; Haurie *et al.*, 1997c; Lothrop *et al.*, 1988) and in humans with CN (Hammond *et al.*, 1989; Migliaccio *et al.*, 1990; Wright *et al.*, 1994) administration of G-CSF leads to an increase in the mean value of the peripheral neutrophil counts, an increase in the amplitude of the oscillations, and a decrease in the period of the oscillation. Exogenous G-CSF had no effect on the peripheral half-time for neutrophil disappearance, but there were significant changes in the distribution of marrow maturation times for neutrophil precursors in normal humans with a reduction in both the average maturation time and the variance of maturation times with G-CSF (Price *et al.*, 1996) (cf. Section 3.1.3.).

In the grey collie (Avalos *et al.*, 1994; Lothrop *et al.*, 1988) and in humans (Hammond *et al.*, 1992) the responsiveness of CD34<sup>+</sup> cells, which include

neutrophil precursor cells, to G-CSF is attenuated compared to normal dogs. Typical figures indicate that in CN the G-CSF concentration required to give half maximal colony growth ( $c_{50}$ ) is on the order of 7 to 9 times normal without any change in the stoichiometry. CD34<sup>+</sup> colony growth studies with GM-CSF show that in cells taken from cyclical neutropenic patients the  $c_{50}$  is increased by a factor of about 2.5 relative to normal marrow cells (Hammond *et al.*, 1992). In cells identified as CFU-GM from cyclical neutropenic patients, the  $c_{50}$  was elevated by a factor of 10 to 30 (Wright *et al.*, 1989).

### 2.3. HYPOTHESES FOR THE ORIGIN OF CYCLICAL NEUTROPENIA

Given the interesting dynamical presentation of CN in both its clinical and laboratory manifestations, it is not surprising that there have been a number of attempts to model this disorder mathematically. In this section we briefly review these attempts as they focus the work of this paper and simultaneously motivate the extensions that we have made.

The mathematical models that have been put forward for the origin of CN fall into two major categories. Reference to Fig. 1 will help place these in perspective. [See Dunn (1983); Fisher (1993) for other reviews].

The first group of these models takes a cue from the existence of oscillations in many of the peripheral cellular elements (neutrophils, platelets, and erythroid precursors, see Fig. 1) and postulates that the origin of CN is in the common pluripotential stem cell (PPSC) population feeding progeny into all of these differentiated cell lines. A loss of stability in the stem cell population is hypothesized to be independent of feedback from peripheral circulating cell types (see below) and would thus represent a relatively autonomous oscillation driving the three major lines of differentiated hematopoietic cells.

Mackey (1978) analysed a model for the dynamics of a stem cell population and concluded that one way the dynamic characteristics of cyclical neutropenia

could emerge from such a formulation was via an abnormally large cell death rate within the proliferating compartment. This hypothesis allowed the *quantitative* calculation of the period of the oscillation that would ensue when stability was lost. This hypothesis has been expanded elsewhere (Mackey, 1979; Mackey & Milton, 1990; Milton & Mackey, 1989) and allows a qualitative understanding of the observed laboratory and clinical effects of G-CSF and chemotherapy discussed above (Mackey, 1996). In spite of the resonance of this stem cell origin hypothesis in the clinical and experimental communities (Quesenberry, 1983; Ogawa, 1993) there has been little extension of this hypothesis in the modeling literature related to CN.

The second broad group of these models identifies the origin of CN with a loss of stability in the peripheral control loop, operating as a sensor between the number of mature neutrophils and the control of the production rate of neutrophil precursors within the bone marrow (cf. Fig. 1). This control has been uniformly assumed to be of a negative feedback type whereby an increase in the number of mature neutrophils leads to a decrease in the production rate of immature precursors. The other facet of this hypothesis is a significant delay due to the maturation times required between the signal to alter immature precursor production and the actual alteration of the mature population numbers. Typical examples of models of this type which have specifically considered CN are Kazarinoff & van den Driessche (1979); King-Smith & Morley (1970); MacDonald (1978); Morley *et al.* (1969); Morley & Stohlman (1970); Morley (1979); Reeve (1973); von Schulthess (1982); Shvitra *et al.* (1983), all of which have postulated an alteration in the feedback on immature precursor production from the mature cell population numbers.

As a subset of this second group of models invoking an instability in the peripheral control loop in the generation of CN, we would like to especially mention the work of Schmitz (1988). Elaborations of this have appeared in a series of papers over the past decade (Wichmann *et al.*, 1988; Schmitz *et al.*, 1990, 1993, 1994, 1995). These papers model a sequence of cell kinetic compartments in which peripheral control is mediated by circulating levels of G-CSF. The onset of CN (in humans and the grey collie) is attributed to a decreased variance in the density of the marrow maturation time distribution. Furthermore, it is postulated that the effects of G-CSF in modifying the period of the oscillations characteristic of CN

are due to a decrease in the minimal marrow transit time from 6.3 days to 1.5 days in humans, an increase in the number of mitoses in the myeloblast, promyelocyte, and myelocyte stages, and (to a minor extent) an increase in the variance of the density of the maturation time distribution.

A number of studies can be used to directly determine the distribution of maturation times in the neutrophil precursors, and modifications of this distribution in CN and after administration of G-CSF. Given the availability of these data and the quite specific nature of the Schmitz hypothesis, we were motivated to examine the notions concerning the destabilization of the peripheral feedback control of neutrophils with the involvement of the density of distributions of the maturation times. We have done this using a very general model (that captures the primary elements of previous models for peripheral neutrophil production, including the Schmitz model) in a form that allows us to specifically test elements of the hypothesis that destabilization of the peripheral control of neutrophil production is responsible for the dynamic aspects of CN.

### 3. Model for the Control of Granulopoiesis

#### 3.1. DEVELOPMENT

##### 3.1.1. Dynamical equation

We are now ready to translate the physiology of this system into a formal mathematical model based on our discussion of the control of granulopoiesis in Section 2. In the model development that follows reference to the lower part of Fig. 1, where the control of white blood cell production is outlined, will be helpful.

We let  $x(t)$  be the density of white blood cells in the circulation (units of cells/ $\mu$ l blood),  $\alpha$  be the random disappearance rate of circulating white blood cells ( $\text{days}^{-1}$ ), and  $\mathcal{M}_0$  be the production rate (cells/ $\mu$ l-day) of white blood cell precursors in the bone marrow.

The rate of change of the peripheral (circulating) white blood cell density is made up of a balance between the loss of white blood cells ( $-\alpha x$ ) and their production ( $\mathcal{M}_0(\tilde{x})$ ), or

$$\frac{dx}{dt} = -\alpha x + \mathcal{M}_0(\tilde{x}). \quad (1)$$

wherein  $\tilde{x}(t)$  is  $x(t - \tau)$  weighted by a distribution of

maturation delays.  $\tilde{x}(t)$  is given explicitly by

$$\begin{aligned}\tilde{x}(t) &= \int_{\tau_m}^{\infty} x(t-u)g(u) du \\ &\equiv \int_{-\infty}^{t-\tau_m} x(u)g(t-u) du.\end{aligned}\quad (2)$$

$\tau_m$  is the minimal maturation delay and  $g(\tau)$  is the density of the distribution of maturation delays as specified below in Section 3.1.2. Since  $g(\tau)$  is a density, it is normalized by definition:

$$\int_0^{\infty} g(u) du = 1.\quad (3)$$

To completely specify the semi-dynamical system described by eqns (1) and (2) we must additionally give an initial function

$$x(t') \equiv \varphi(t') \text{ for } t' \in (-\infty, 0).\quad (4)$$

### 3.1.2. Distribution of maturation times

A wide variety of analytic forms could be used for the density of the distribution of the maturation times in the bone marrow. We have chosen to use the density of the gamma distribution.

$$g(\tau) = \begin{cases} 0 & \tau \leq \tau_m \\ \frac{a^{m+1}}{\Gamma(m+1)} (\tau - \tau_m)^m e^{-a(\tau - \tau_m)} & \tau_m < \tau \end{cases}\quad (5)$$

with  $a, m \geq 0$ , as have Blythe *et al.* (1984); Cooke & Grossman (1982); Gatica & Waltman (1982, 1988) in a different context. This choice was predicated on two issues. First, we have found (see Section 3.1.3.) that we can achieve a good fit of the existing data on cellular maturation times using eqn (5). Second, the density of the gamma distribution has been used a number of times in the past (Kendall, 1948; Powell, 1955, 1958) to fit distributions of cell cycle times. When the parameter  $m$  in eqn (5) is a non-negative integer then the corresponding eqns (1) and (2) reduce to a system of linear ordinary differential equations coupled to a single nonlinear delayed equation with a discrete (not continuously distributed) delay (Fargue, 1973, 1974; MacDonald, 1989). This leads to analytic simplifications, though we do not use them here since we have typically found non-integer values for the parameter  $m$ . We did, however, use this reduction to test the accuracy of our numerical simulations of the full model (cf. Section 3.4.).

The parameters  $m$ ,  $a$ , and  $\tau_m$  in the density of the gamma distribution can be related to certain easily

determined statistical quantities. The average of the *unshifted* density is given by

$$\tau_2 = \int_{\tau_m}^{\infty} \tau g(\tau) d\tau = \frac{m+1}{a},\quad (6)$$

and thus the average maturation delay as calculated from eqn (5) is given by

$$\langle \tau \rangle = \tau_m + \tau_2 = \tau_m + \frac{m+1}{a}.\quad (7)$$

The variance (denoted by  $\sigma^2$ ) is given by

$$\sigma^2 = \frac{m+1}{a^2}.\quad (8)$$

Given the expressions (6), (7) and (8) in terms of the gamma distribution parameters  $m$  and  $a$ , we may easily solve for these parameters in terms of  $\tau_2$  and  $\sigma^2$  to give

$$a = \frac{\tau_2}{\sigma^2}\quad (9)$$

and

$$m+1 = \frac{\tau_2^2}{\sigma^2}.\quad (10)$$

Equations (9) and (10) will be used in Section 3.1.3.

### 3.1.3. Parameter estimation

Several studies have shown that labeled neutrophils disappear from the circulation with a half life  $t_{1/2}$  of about 7.6 hr in humans (Dancey *et al.*, 1976) and dogs (Deubelbeiss *et al.*, 1975) with a range of 7–10 hr. Furthermore, this disappearance rate is unaffected in human (Guerry *et al.*, 1973) and canine CN (Dale *et al.*, 1972b) and is not altered by the administration of exogenous G-CSF (Price *et al.*, 1996). Since the decay coefficient  $\alpha$  of eqn (1) is related to the  $t_{1/2}$  through the relation

$$\alpha = \frac{\ln 2}{t_{1/2}},\quad (11)$$

we have taken values of  $\alpha \in [1.664, 2.378]$  (days<sup>-1</sup>) in all of the numerical work reported here.

Distributions of maturation times were determined from published data on the emergence of the number of labeled circulating neutrophils following pulse labeling by tritiated thymidine. The published graphed data were scanned and the postscript file viewed with Ghostview. Ghostview gives coordinates for the position of the points which, using position of the axes, can be easily transformed to give the actual data points. The data

were adjusted for the random death occurring at a rate  $\alpha$  by using the method of (Dancey *et al.* (1976).

Assume that the neutrophils spend a period of time  $U$  in the bone marrow, and  $Y$  in the blood. Then the fraction,  $N(t)$ , of labeled cells in the blood at a time  $t$  is the probability that the time in the marrow is less than  $t$  and that the total time in the marrow and blood before death is greater than  $t$ . Let  $g(u)$  be the density of the distribution of the maturation times in the marrow, and remember that  $g(u)$  is the quantity that we wish to determine. Further note that, because of the experimentally observed random destruction of neutrophils in the circulation, if the rate of random destruction is  $\alpha$  then the density of the distribution of destruction rates is given by  $\alpha e^{-\alpha y}$ . With these observations, for  $N(t)$  we finally have

$$N(t) = \int_0^t \int_{t-u}^{\infty} \alpha e^{-\alpha y} g(u) dy du = \int_0^t e^{-\alpha(t-u)} g(u) du. \quad (12)$$

Thus,

$$e^{\alpha t} N(t) = \int_0^t e^{\alpha u} g(u) du, \quad (13)$$

and differentiating both sides with respect to  $t$  gives

$$\alpha e^{\alpha t} N(t) + e^{\alpha t} N'(t) = e^{\alpha t} g(t). \quad (14)$$

The final result for the density of marrow transit times is

$$g(t) = \alpha N(t) + N'(t). \quad (15)$$

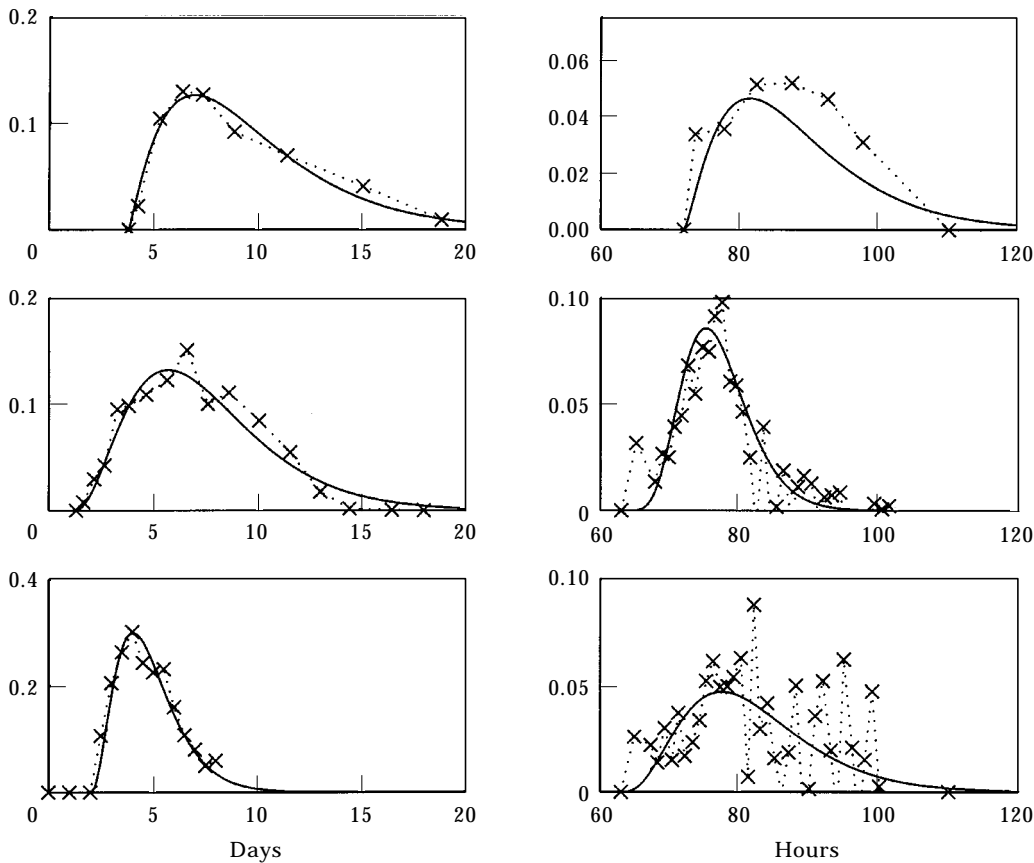


FIG. 2. Densities of distributions of maturation times and the least square fits to the data achieved using the density of the gamma distribution. The three left hand panels are for humans and show, from top to bottom, a normal human, data from a cyclical neutropenia patient, and a normal human receiving 300  $\mu\text{g}$  G-CSF. The three right hand panels are for dogs and correspond to (top to bottom) a normal dog, a grey collie at the apogee of the cycle and a grey collie at the nadir of the cycle. See Table 1 for the parameters used to fit the data and the references for the source of the data.

As we had discrete data points from the labeling data, we used the midpoint of two data points and the slope of the joining line in (15), and determined  $g(t)$  at the midpoint. The mean and variance were calculated from the new density, and the corresponding  $m$  and  $a$  determined from eqns (9) and (10) were used as the initial values in a nonlinear least squares fit to the data. The results of these determinations for a number of published data sets are summarized in Table 1. Figure 2 shows the raw data as well as the fits to the data using the density of the gamma distribution.

### 3.2. THE STEADY STATE AND STABILITY

#### 3.2.1. The steady state

The equilibrium solution for the functional differential equation (1)–(2) occurs when

$$\frac{dx}{dt} = 0 = -\alpha x + \mathcal{M}_0(\tilde{x}), \quad (16)$$

so the steady state  $x^*$  is defined implicitly by the solution of the equation

$$\alpha x^* = \mathcal{M}_0(x^*). \quad (17)$$

Given the presumptive monotone decreasing nature of the negative feedback production rate inferred from the biology, there can be but a single unique value for the steady state white blood cell density  $x^*$ . It is important to note that  $x^*$  is completely independent of the nature of the density  $g(\tau)$  of the distribution of the maturation times. However, the stability of  $x^*$  is dependent on  $g(\tau)$  as we show in the following section.

#### 3.2.2. Stability

One of the primary considerations of this paper has to do with the stability of the unique steady state, defined implicitly by eqn (17), and how that stability may be lost. In general, the question that one would always like to be able to examine is the global stability of a  $x^*$  to all perturbations away from  $x^*$ . However, there are no general global stability results for systems with dynamics described by eqns (1)–(2), and consequently the usual approach is to examine the stability of  $x^*$  in the face of very small deviations away from the steady state. This type of examination is called an analysis of the *local* stability of  $x^*$ . Though this analysis involves an approximation it is quite useful since the loss of local stability of a steady state implies the global loss of stability, and our goal here is to look for situations in which the steady state is globally unstable.

Throughout this analysis, an important parameter that will appear is the *slope* of the production function  $\mathcal{M}_0$  evaluated at the steady state, denoted by  $\mathcal{M}'_{0^*}$ . Because of our arguments at the end of Section 2.1. concerning the negative feedback nature of the peripheral control mechanisms acting on neutrophil production, we know that this slope must be non-positive (i.e. negative or zero).

To examine the local stability, we write out eqn (1) for small deviations of  $x$  from  $x^*$ . In the first (linear) approximation this gives

$$\frac{dx}{dt} \simeq -\alpha x + \mathcal{M}_{0^*} + (\tilde{x} - x^*)\mathcal{M}'_{0^*}, \quad (18)$$

wherein

$$\mathcal{M}_{0^*} \equiv \mathcal{M}_0(\tilde{x} = x^*) \quad (19)$$

and

$$\mathcal{M}'_{0^*} \equiv \left. \frac{d\mathcal{M}_0(\tilde{x})}{d\tilde{x}} \right|_{\tilde{x}=x^*}. \quad (20)$$

Utilizing eqn (17) and defining the deviation from equilibrium as  $z(t) = x(t) - x^*$ , we can rewrite eqn (18) in the form

$$\frac{dz}{dt} = -\alpha z + \mathcal{M}'_{0^*} \int_{-\infty}^{t-\tau_m} z(u)g(t-u) du. \quad (21)$$

To proceed, we make the *ansatz* that the deviation  $z$  from the steady state has the form  $z(t) \simeq \exp(\lambda t)$ , substitute this into eqn (21), carry out the indicated integrations and finally obtain

$$\lambda + \alpha = \mathcal{M}'_{0^*} \left( \frac{a}{\lambda + a} \right)^{m+1} e^{-\lambda\tau_m}. \quad (22)$$

Equation (22) for the eigenvalues  $\lambda$  may have a variety of solutions. If an eigenvalue  $\lambda$  is real, then a simple graphical argument shows that the eigenvalue will be negative and contained in the open interval  $(-\alpha, -a)$ .

Alternately, the eigenvalue solutions of (22) may be complex conjugate numbers, in which case the most interesting thing to know is when the real part of the eigenvalue is identically zero. This will define the boundary between a locally stable steady state  $\text{Re } \lambda < 0$  and a locally unstable steady state with  $\text{Re } \lambda > 0$ .

To investigate this possibility, we take  $\lambda = \mu + i\omega$  and substitute this into eqn (22) to give, with  $\mu = 0$ ,

$$i\omega + \alpha = \mathcal{M}'_{0^*} \left( \frac{a}{i\omega + a} \right)^{m+1} e^{-i\omega\tau_m}, \quad (23)$$

or rewriting

$$\left[ (i\omega + \alpha) \left( 1 + i \frac{\omega}{a} \right)^{m+1} \right] = \mathcal{M}'_{0^*} e^{-i\omega\tau_m} \quad (24)$$

This equation can be manipulated to give a set of parametric equations in  $\alpha$  and  $\mathcal{M}'_{0^*}$ . We start by setting

$$\tan \theta = \frac{\omega}{a}. \quad (25)$$

Using de Moivre's formula in eqn (24) gives

$$\begin{aligned} (\alpha + i\omega)(\cos[(m+1)\theta] + i \sin[(m+1)\theta]) \\ = \mathcal{M}'_{0^*} \cos^{m+1}\theta (\cos \omega\tau_m - i \sin \omega\tau_m) \end{aligned} \quad (26)$$

Equating the real and imaginary parts of eqn (26) gives the coupled equations

$$\alpha - \mathcal{M}'_{0^*} R \cos \omega\tau_m = \omega \tan[(m+1)\theta], \quad (27)$$

and

$$\alpha \tan[(m+1)\theta] + \mathcal{M}'_{0^*} R \sin \omega\tau_m = -\omega, \quad (28)$$

where

$$R = \frac{\cos^{m+1}\theta}{\cos[(m+1)\theta]}. \quad (29)$$

Equations (27) and (28) are easily solved for  $\alpha$  and  $\mathcal{M}'_{0^*}$  as parametric functions of  $\omega$  to give

$$\alpha(\omega) = -\frac{\omega}{\tan[\omega\tau_m + (m+1)\tan^{-1}(\omega/a)]} \quad (30)$$

and

$$\mathcal{M}'_{0^*}(\omega) = \frac{\omega}{\cos^{m+1}[\tan^{-1}(\omega/a)] \sin[\omega\tau_m + (m+1)\tan^{-1}(\omega/a)]} \quad (31)$$

respectively.

To show that the stability boundary defined implicitly by eqns (30) and (31) delimits a transition from a locally stable steady state to a locally unstable steady state as  $\mathcal{M}'_{0^*}$  decreases, we must show that the real part of the eigenvalue is negative on one side of the boundary and positive on the other. Thus, the real part of  $d\lambda/d\mathcal{M}'_{0^*}$ , or equivalently of  $(d\lambda/d\mathcal{M}'_{0^*})^{-1}$ , must be negative when  $\lambda = i\omega$ .

Implicit differentiation of eqn (22) yields

$$\begin{aligned} \left( \frac{d\lambda}{d\mathcal{M}'_{0^*}} \right)^{-1} &= \left( \frac{\lambda + a}{a} \right)^{m+1} e^{i\tau_m} \\ &+ \mathcal{M}'_{0^*} \frac{m+1}{\lambda + a} + \mathcal{M}'_{0^*} \tau_m, \end{aligned} \quad (32)$$

and the use of eqn (22) in (32) gives

$$\left( \frac{d\lambda}{d\mathcal{M}'_{0^*}} \right)^{-1} = \mathcal{M}'_{0^*} \left( \frac{1}{\lambda + \alpha} + \frac{m+1}{\lambda + a} + \tau_m \right). \quad (33)$$

Evaluating (33) at  $\lambda = i\omega$  and eliminating complex numbers in the denominators, we have

$$\begin{aligned} \left( \frac{d\lambda}{d\mathcal{M}'_{0^*}} \right)^{-1} \\ = \mathcal{M}'_{0^*} \left( \frac{\alpha - i\omega}{\alpha^2 + \omega^2} + \frac{(m+1)(a - i\omega)}{a^2 + \omega^2} + \tau_m \right) \end{aligned} \quad (34)$$

with

$$\begin{aligned} \text{Re} \left( \left( \frac{d\lambda}{d\mathcal{M}'_{0^*}} \right)^{-1} \right) \\ = \mathcal{M}'_{0^*} \left( \frac{\alpha}{\alpha^2 + \omega^2} + \frac{(m+1)a}{a^2 + \omega^2} + \tau_m \right). \end{aligned} \quad (35)$$

If  $\mathcal{M}'_{0^*}$  is negative (as in our case), then the right hand side of eqn (35) is negative indicating that for increases in  $\mathcal{M}'_{0^*}$  to more positive values at the boundary where  $\mu \equiv 0$ , the real part of the eigenvalue  $\lambda$  is crossing from positive to negative.

Thus, we conclude that the locus of points defined by eqns (30) and (31) define the location in  $(\alpha, \mathcal{M}'_{0^*})$  parameter space where a supercritical Hopf bifurcation takes place and a periodic solution of period

$$T_{\text{Hopf}} = \frac{2\pi}{\omega} \quad (36)$$

occurs.

### 3.3. IMPLICATIONS OF THE LOCAL STABILITY ANALYSIS

In Fig. 3 we have parametrically plotted  $\mathcal{M}'_{0^*}(\omega)$  vs.  $\alpha(\omega)$  ( $\omega$  is the parameter) [eqns (30) and (31)] to give the stability boundaries for a normal human and a human with CN using the data of Table 1. (Ignore the lines corresponding G-CSF for the time being). The two vertical dashed lines correspond to the normal range of  $\alpha$  values as discussed in Section 3.1.3., the lower dashed line is the stability boundary for the CN case, and the solid line is for the normal human. Regions above a given stability boundary in  $(\alpha, \mathcal{M}'_{0^*})$



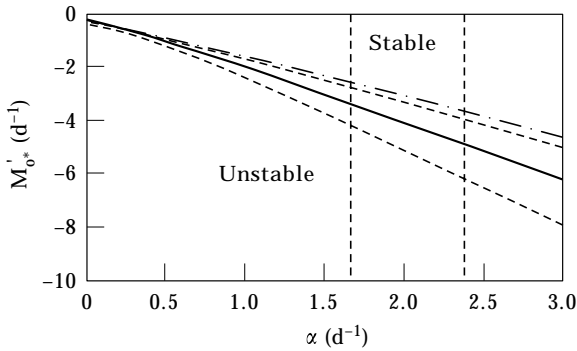


FIG. 3. A parametric plot of the regions of linear stability and instability based on data for normal humans (solid line) (Perry *et al.*, 1966), humans with CN (lower dashed line) (Guerry *et al.*, 1973), and normal humans administered G-CSF (upper dashed line is for 30  $\mu\text{g}$ , and the dash-dot line is for 300  $\mu\text{g}$ ) (Price *et al.*, 1996). In this and all subsequent stability diagrams, points  $(\alpha, \mathcal{M}'_{0^*})$  above a given stability line correspond to linear stability of the steady state and those below correspond to an unstable steady state. See the text for details.

parameter space correspond to a locally stable steady state neutrophil level, while regions below are unstable. For values of  $(\alpha, \mathcal{M}'_{0^*})$  exactly on a given line there is a bifurcation to a periodic solution with Hopf period  $T_{\text{Hopf}}$  as discussed above.

### 3.3.1. Implications for the origin of cyclical neutropenia

The first point to be noted is the following: if the model for granulopoiesis is stable for a normal human, then a simple alteration of the characteristics of the maturation time distribution to correspond to the value for cyclical neutropenia (Table 1) is incapable for singlehandedly inducing an instability. Furthermore, note that the unique steady state of the model as given implicitly by eqn (17) is *independent* of any alterations in the distribution of maturation times. However, the dynamically varying neutrophil levels in CN are often depressed relative to the normal state (Section 2.2.) thus implying that a simple alteration of the distribution of maturation times could not be the sole source of CN dynamics alone.

Examination of Fig. 3 shows that if the dynamic behaviour of CN is to be a result of an instability in this model then, in addition to the known alterations in the distribution of maturation times, there must be a concomitant decrease in  $\mathcal{M}'_{0^*}$  to more negative values such that  $(\alpha, \mathcal{M}'_{0^*})$  falls in the zone of parameter space where  $x^*$  is unstable. Since one of the hallmarks of CN is an oscillation about a reduced average neutrophil count, this decrease in  $\mathcal{M}'_{0^*}$  must also be accompanied by a decrease in  $\mathcal{M}'_{0^*}$  to account for the decrease in  $x^*$ . (Remember that  $\alpha$  is not altered

in CN, so an increase in  $\alpha$  cannot be the source of these depressed levels.)

Suppose for the sake of argument that in humans such a decrease in  $\mathcal{M}'_{0^*}$  has taken place—i.e. that  $\mathcal{M}'_{0^*}$  has become sufficiently negative for an unstable situation to occur. We can calculate exactly the period of the solution when the Hopf bifurcation to unstable behaviour occurs. In the case of the  $g$  parameters for the normal human  $T_{\text{Hopf}} \in [18.23, 17.79]$  days for  $\alpha \in [1.664, 2.378]$ . The corresponding range for the CN boundary is  $T_{\text{Hopf}} \in [14.18, 13.78]$  days. These values are significantly lower than the smallest observed periods in clinical CN as reviewed in Section 2.2. and as found in the analysis of Haurie *et al.* (1998b).

Turning to the case of canine CN, we have plotted stability boundaries for a normal dog and grey collies at the peak and nadir of their cycle in Fig. 4. The stability boundaries for all three situations (using the appropriate parameters from Table 1) fall virtually on top of one another. As with human cyclical neutropenia the local stability analysis suggests that, in contrast with the hypothesis of Schmitz *et al.* (1990), the origin of canine cyclical neutropenia is not a consequence of alterations in the distribution of marrow maturation times for neutrophil precursors alone. Rather, as in the human case, a shift in  $\mathcal{M}'_{0^*}$  to more negative values would be required to effect the requisite instability.

Assume for the grey collie that such a shift in  $\mathcal{M}'_{0^*}$  to values sufficiently negative to destabilize the system has taken place. What then are the predicted Hopf periods at the onset of the ensuing oscillation? Based on the data for normal dogs presented in Table 1, for  $\alpha \in [1.664, 2.378]$  the local stability analysis of Section 3.2.2. predicts that  $T_{\text{Hopf}} \in [8.46, 8.15]$  days. For the

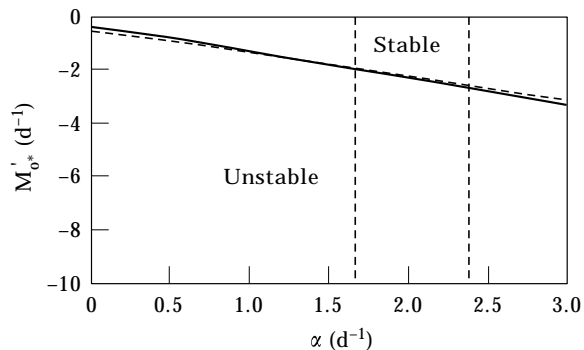


FIG. 4. A parametric plot of the regions of linear stability and instability based on data for normal dogs taken from Deubelbeiss *et al.* (1975) and from grey collies at the apogee and nadir of their oscillation as taken from Patt *et al.* (1973). Note that the three stability boundaries are virtually indistinguishable from one another.

grey collie maturation distribution data taken at the nadir of the cycle this range is reduced to  $T_{hopf} \in [7.95, 7.63]$  days, while the collie data from the apogee predicts  $T_{hopf} \in [7.35, 7.05]$  days. All of these estimates are below the reported ranges for the period of canine CN discussed in Section 2.2. and in Haurie *et al.* (1998c).

Thus, for both human and grey collie CN we conclude that there is no evidence from the linear stability analysis that the dynamics of CN are due to an instability in the peripheral control of granulopoiesis caused by a change in the distribution of cell maturation times.

### 3.3.2. Assessing the effects of G-CSF

The second point that we can address with the aid of the local stability analysis of Section 3.2.2. is the effect of G-CSF on the stability of the system in normal humans. In Fig. 3 we have plotted the stability boundaries for the data of Table 1 corresponding to the alterations in normal humans induced by 30 and 300  $\mu\text{g}$  G-CSF reported by Price *et al.* (1996). (Note that if the individuals in this study weighed 70 kg, then the dosage was either 0.43 or 4.3  $\mu\text{g}/\text{kg}\text{-day}$ , respectively.) It is clear from Fig. 3 that the region of parameter space in which the normal human control system is stable is actually *decreased* by the administration of G-CSF since the stability boundaries for both dosages of G-CSF lie above the stability boundary for a normal human. Unfortunately, we have been unable to locate any data for the effects of G-CSF on the density  $g$  of the distribution of maturation times in dogs, but based on the comparable data for humans we would not expect large quantitative differences.

If data were available for the effects of G-CSF on the density of the distribution of maturation times in humans with CN we could assess the potential role of G-CSF in altering the period as noted in the clinical literature. However, we must note that if the changes induced by G-CSF in CN are comparatively similar to those in normals, then it is unlikely that G-CSF could ever act to stabilize a peripheral instability in neutrophil numbers since its role seems to be a destabilizing one.

### 3.4. NUMERICAL BEHAVIOUR OF THE FULL MODEL

The linear stability analysis suggests that CN is not the result of a change in the distribution of maturation times. An examination of the full numerical behaviour of the complete nonlinear system once a control function  $\mathcal{M}$  is specified must be performed to determine if an alteration in the

feedback function can give the dynamics observed in CN. This section is devoted to the issue.

#### 3.4.1. A generic control function

In light of our discussion of the control of granulopoiesis in Section 2.1., though the *detailed* mechanisms whereby this control is exerted are unclear at this time it is equally clear that the net effect of the control elements regulating granulopoiesis are such that an elevation of peripheral neutrophil numbers eventually leads to a decrease in production and vice versa. Thus the production function  $\mathcal{M}_0$  can be assumed to be a monotone decreasing function of  $\bar{x}$ , and has a negative feedback character.

In this paper, for numerical computation purposes we assume that the production rate function  $\mathcal{M}_0$  is a Hill function of the form

$$\mathcal{M}_0(x) = \mathcal{M}_i A \frac{\theta^n}{\theta^n + x^n} \quad n > 0. \quad (37)$$

In eqn (37), the term represents  $\mathcal{M}_i$  the cellular input (in cells/ $\mu\text{l}\text{-day}$ ) of cells from the neutrophil stem cell precursors, while the Hill function portion of the feedback

$$A(x) = A_{max} \frac{\theta^n}{\theta^n + x^n} \quad (38)$$

represents the amplification factor assumed to operate within the precursor compartment. The maximal amplification is  $A_{max}$ .

To proceed with the numerical investigation of our system (1) with the (2), (5), and the nonlinearity (38) the parameters  $\mathcal{M}_i$ ,  $A_{max}$ ,  $\theta$  and  $n$  must be estimated. Given a normal value for the granulocyte turnover rate (GTR), and letting  $A(x^*)$  be the normal amplification (usually thought to be around 8) within the recognizable neutrophil precursors, we have

$$\mathcal{M}_i = \frac{\text{GTR}}{A(x^*)} \text{ cells/kg-day}. \quad (39)$$

It is easy to derive a relation between the normal neutrophil density  $x^*$ ,  $\theta$ ,  $n$ , and  $A_{max}$ . To do this, evaluate eqn (37) at the steady state, substitute (39), and define

$$\Gamma = \frac{A_{max}}{A(x^*)} \quad (40)$$

to give

$$\theta = \frac{x^*}{\sqrt[n]{\Gamma - 1}}. \quad (41)$$

With  $x^*$  and  $\Gamma$  known, (41) reduces to a relation between  $\theta$  and  $n$ . A second relation involving some of these parameters may be obtained by invoking the

TABLE 2

The primary data and the normal parameters derived from these for simulation purposes using the arguments outlined in the text

Quantity	Units	Humans	Dogs
GTR	cells/kg-day	$8.7 \times 10^8$	$1.65 \times 10^9$
$\alpha$	days <sup>-1</sup>	2.2 (1.7–2.4)	2.2 (1.7–2.4)
$x^*$	cells/ $\mu$ l	$4.5 \times 10^3$ (1.8–7.5)	$8.2 \times 10^3$ (6–12.5)
$A(x^*)$	—	8	8
$A_{max}$	—	128	128
$\Gamma$	—	16	16
$\mathcal{M}_i$	cells/kg-day	$1.1 \times 10^8$	$2.1 \times 10^8$
$\mathcal{M}'_{Hopf}$	days <sup>-1</sup>	-4.57	-2.47
$n_{max}$	—	2.22	1.19
$\theta(n_{max})$	cells/ $\mu$ l	$1.33 \times 10^3$	$8.4 \times 10^2$

requirement that the steady-state number of neutrophils,  $x^*$ , is stable in normal subjects. This in turn implies that  $\mathcal{M}'(x^*) \equiv \mathcal{M}'_0 > \mathcal{M}'_{Hopf}$  where  $\mathcal{M}'_{Hopf}$  is given by eqn (31). It is a straightforward calculation to show that

$$\mathcal{M}'(x^*) = -n \frac{\mathcal{M}(x^*)}{x^*} \left[ 1 - \frac{\mathcal{M}(x^*)}{\mathcal{M}_i A_{max}} \right] = -\frac{n}{\alpha} \cdot \frac{\Gamma}{\Gamma - 1}. \quad (42)$$

Requiring that the normal steady state be stable yields

$$-\frac{n}{\alpha} \cdot \frac{\Gamma}{\Gamma - 1} > \mathcal{M}'_{Hopf} \quad (43)$$

or

$$n < \frac{|\mathcal{M}'_{Hopf}|}{\alpha} \frac{\Gamma}{\Gamma - 1} \equiv n_{max}. \quad (44)$$

If CN is to arise because of an instability in the peripheral control loop, modeled here then all of the considerations of Section 3.3.1. become important. Specifically, it is clear that the parameters must change in such a way that the slope  $\mathcal{M}'(x^*_{CN})$  of the control function at a depressed and unstable steady state  $x^*_{CN}$  becomes sufficiently negative that it falls below the critical slope  $\mathcal{M}'_{Hopf}^{CN}$  at which the Hopf bifurcation takes place.

Three parameters in our feedback function must be estimated:  $\theta$ ,  $A_{max}$  and  $n$ .  $A(x^*)$ , the normal amplification, is usually thought to be around 8, and the maximum amplification is estimated to be as high as 64 (6 effective divisions) or 128 (7 divisions) under certain circumstances. Thus, the maximum values of the ratio  $\Gamma$  [eqn (40)] is between 8 and 16,  $\Gamma \in [8, 16]$ . This estimate is consistent with the work of Lord *et al.* (1991) on the effects of G-CSF in mice where a value of  $\Gamma \geq 14.5$  was found, and the work of Lord *et al.* (1989); Lord (1992) where a value of  $\Gamma \geq 9.4$  was found in humans treated with G-CSF. Here, we have

assumed a value of  $\Gamma = 16$ . This allows estimations of  $n_{max}$  and  $\theta$  for the normal state, noted in Table 2.

The experimental evidence cited earlier in our review of CN indicates that the responsiveness of neutrophil precursor cells to G-CSF is attenuated in CN compared to normal (Avalos *et al.*, 1994; Hammond *et al.*, 1992; Lothrop *et al.*, 1988) though the maximal value is unchanged. This suggests a shift in the feedback function resulting from a decrease in  $\theta$ . However, with the normal values given in Table 2, we found that a decrease of  $\theta$  alone is insufficient to decrease  $\mathcal{M}'$  below  $\mathcal{M}'_{Hopf}^{CN}$ . As a decrease in  $A_{max}$  increases the slope, we conclude that only an increase in  $n$  can destabilize the system.

### 3.4.2. Numerical methods

Equation (1) cannot be solved analytically. However, a numerical solution can be found to the integro-differential equation via initial value techniques used to solve ordinary differential equations, along with an integral solver. Other numerical work on similar problems has used the backward Euler method (Markowich & Renardy, 1983; Nevanlinna, 1978). We used the trapezoidal method to evaluate both the equation and the integral.

The accuracy of the program was first tested by a comparison with the predictions of a linear stability analysis. It was further checked by comparing the results of simulations of a gamma function of integer order with the results of simulations on xpp using the linear chain reduction (Fargue, 1973, 1974; MacDonald, 1989) with the same parameters. A copy of the program is available from the authors on request.

### 3.4.3. Results

In Fig. 5 we present the results of an extensive series of simulations for both humans and dogs. We ran the simulations for transient of 400 days in humans and 200 days in dogs with a constant initial function of one-half normal neutrophil values and then computed the mean value of the neutrophil numbers, the amplitude of the oscillation (maximum minus minimum), and the period of the oscillation for 100 days after the transient.  $A_{max}$  and  $\theta$  were systematically decreased from the values given in Table 2.  $n$  was studied in the range [3, 7.5] for humans and [1.25, 5.5] for dogs, which give unstable steady states.

As shown in Fig. 5, for small  $n$  the period was much smaller than is observed in CN. In humans, it was only for values of  $n \geq 5$  that we were able to obtain periodic neutrophil variation with a period approximating the lower range of the clinically observed period (19 days). However, as is clear from Fig. 6, having achieved a proper period, the simulations then

had completely inappropriate appearance, with maximum values substantially higher than found in CN (Dale & Wolff, 1972). Also, the mean values and amplitudes of the neutrophil oscillation are far in excess of typical CN data.

In summary, we were unable to produce numerical solution behaviour from the full nonlinear model that bore any resemblance to what has ever been reported in the clinical literature on CN. Furthermore, the values of the parameter  $n$  that were required to achieve this period exceed anything that would be expected in terms of the negative feedback characteristics between G-CSF and the response of primitive neutrophil progenitor cells (Avalos *et al.*, 1994; Hammond *et al.*, 1992; Lothrop *et al.*, 1988).

Without belaboring the point, we found the same qualitative failure of the model to produce behaviour similar to that seen in the grey collie. For completeness, we have also plotted the comparable mean, amplitude, and period data in Fig. 5.

These failures, which are consistent with the conclusions of Section 3.3., lead us to conclude finally

that given the current state of knowledge of cyclical neutropenia the origin of the dynamic nature of this disorder is not to be found in the peripheral control of neutrophil production. Further, we conclude that the response to treatment with G-CSF is not due only to the effects of G-CSF on the distribution of neutrophil precursor maturation times.

#### 4. Discussion and Conclusions

Our original motivation in carrying out the research reported here was to examine the hypothesis that CN was due to a loss of stability in the peripheral control of neutrophil production. Based on the considerations of Section 3.3. that are independent of the precise nature of the control function assumed, and the numerical computations of Section 3.4. we conclude that any alterations of parameters in this peripheral control system consistent with the extant laboratory and clinical data on CN are unable to reproduce either the characteristics of clinical CN or its laboratory counterpart in the grey collie. Further,

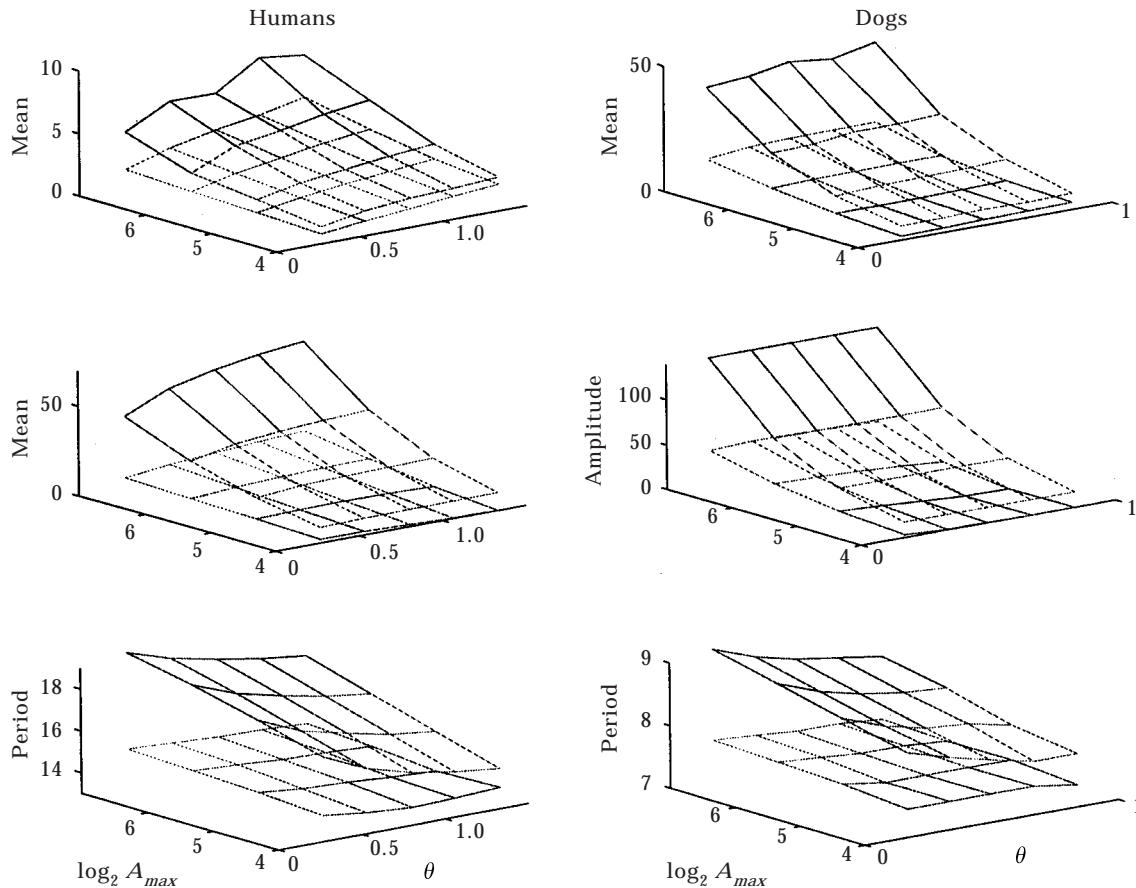


FIG. 5. Simulation results giving the numerically determined mean value ( $10^3$  cells/ $\mu$ l), amplitude ( $10^3$  cells/ $\mu$ l), and the period (in days) of neutrophil oscillations predicted by the model eqns (1), (2), (5) and the nonlinearity (38) with the parameters of Table 2. Plotted are  $n = 3$  (lower graphs) and  $n = 5.5$  for humans and  $n = 1.25$  (lower graphs) and  $n = 5.5$  for dogs. See the text for details.

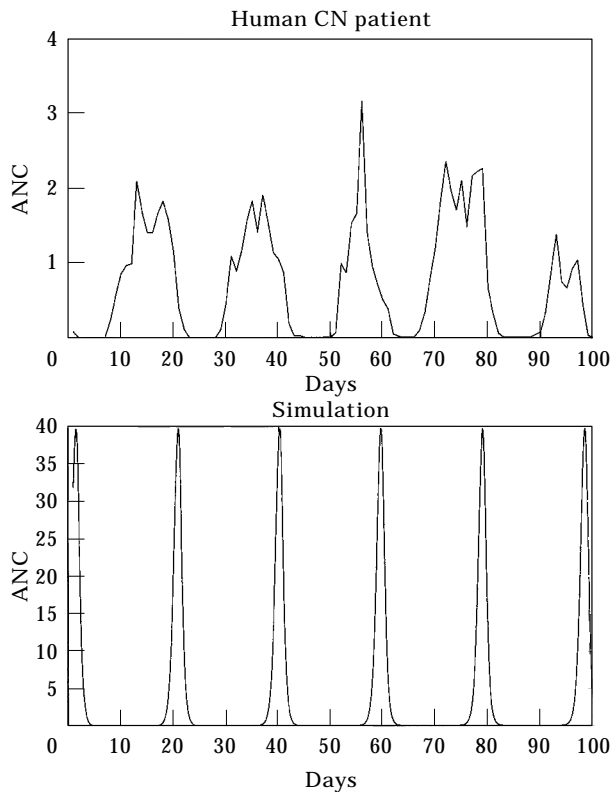


FIG. 6. Dynamics of the absolute neutrophil count (ANC,  $10^3/\mu\text{l}$ ) in a CN patient and in simulation results ( $n = 5.5$ ,  $\theta = 0.27$ ,  $A_{\max} = 128$ ). Note that in spite of the fact that the simulation period matches the one seen in CN, there is a huge discrepancy in the amplitudes, and that the simulation results predict much longer periods of severe neutropenia than observed.

we conclude that the dynamic effects of G-CSF treatment of CN are probably not primarily due to the alterations of the peripheral control dynamics.

Rather we tentatively conclude, as has Mackey (1996), that the dynamics of CN are due to a destabilization of the pluripotential stem cell population as originally proposed by Mackey (1978, 1979).

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