Cyclic Neutropenia (CN): A Clue to the Control of Granulopoiesis

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A simple quantitative feedback model of granulopoiesis is presented and discussed within the framework of existing data on granulopoiesis in both normals and patients with cyclic neutropenia (CN). The model assumes that the controlled compartment is the bone marrow pool of mature neutrophils (PMNs), which sends a negative feedback signal to the mitotic pool of early granulocyte precursors (i.e., CFU-C, myeloblasts, etc.) thus controlling the granulocyte production rate. Three parameters are found to play important roles in determining the response of the system to perturbations. These are: \( T_m \), the granulocyte maturation time; \( a \), a parameter reflecting the strength of the negative feedback exerted by mature PMNs on the granulocyte production rate; and \( b \), a parameter describing the leakiness of the bone marrow for PMN egress. It is shown that depending on the relative magnitudes of \( a \) and \( b \), the system will either respond to perturbations with a damped oscillation (\( a < b \): the normal state) or with a sustained oscillation (\( a > b \): the CN state). In both cases, the oscillation period is found to be approximately equal to \( 2T_m \).

Deductions of the values of \( a \), \( b \), and \( T_m \) from experimental data are consistent with the predictions of the model and show an increased value of \( a \) in CN relative to the normal state. This suggests an overly active feedback mechanism as the pathophysiologic basis of CN. In addition, the model can explain how various therapeutic agents correct CN and also provides insight into why other hematologic cell lines and CSA oscillate in CN.

Cyclic Neutropenia (CN) is a rare disease characterized by regular oscillations in the circulating neutrophil (PMN) count, and in many cases is associated with oscillations of other circulating blood cells. The oscillation period in man is typically 21 days, remains constant during many cycles, and seems not to vary substantially from patient to patient. CN is viewed by many researchers as a derangement of the feedback and control mechanisms regulating granulopoiesis, and for this reason has been actively investigated. These investigations have been greatly aided by the discovery of an animal model of this disease in the grey collie dog where all three cell lines exhibit cyclic oscillations but with a shorter period of 13 days.

In association with the experimental work on CN, several quantitative models of granulopoiesis have been proposed, most notably by King-Smith and Morley, Rubinow and Lebowitz, and Mackey. In the first two models, the basic assumption is that mature neutrophils can in some way modulate their own production. These authors further recognize that the maturation of a neutrophil from its precursors occurs over several days and that this maturation time creates a time delay in the feedback control of neutrophil production. From the theory of control systems it is known that such time delays can result in an oscillation of the controlled quantity. Within this framework CN can be viewed as the expression of such oscillations in the granulopoietic system. Using this concept, King-Smith and Morley assumed that the concentration of circulating PMNs represents the controlled quantity. In contrast, Rubinow and Lebowitz assumed that the population of bone marrow PMNs is the controlled variable. In the third model proposed by Mackey, both CN and aplastic anemia are explained in terms of the proliferative kinetics of the pluripotent stem cell pool. In this model, the mature neutrophils play no role in regulating granulocyte production.

While these models have been successful at simulating some aspects of normal and pathologic granulopoiesis, their considerable mathematical complexity has made it difficult to understand them in simple biologic and biochemical terms. Furthermore, the large number of free parameters contained in these models has impaired a direct comparison between theory and experimental data. At present, various aspects of these models remain unproved and have been questioned.

In this article we propose a new quantitative model for granulopoiesis that is mathematically much simpler than those given previously. The predictions of our model are explained in biologic terms, and these predictions are compared with the experimental data on normals and CN patients. Furthermore, we show that our model can explain how various therapeutic measures cure CN and also provides insight into the other oscillatory processes that are associated with CN. We believe that the usefulness of the present model is a result of its simplicity, its consistency with a wide variety of experimental data, and its ability to provide a conceptual framework within which one can attempt to understand and study the biochemical and

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cellular mechanisms responsible for the control of granulopoiesis.

**THE MODEL OF GRANULOPOIESIS**

The qualitative aspects of the model are represented in the schematic diagram of Fig. 1. The bone marrow contains a maturation sequence of granulocytes, including cells from the earliest progenitors (CFU-C) to the mature PMNs. It is generally accepted that cells up to the stage of the myelocyte can divide. Hence, the marrow compartment of the committed PMN precursors is divided into a mitotic and a postmitotic pool. The maturation time for a PMN will be the sum of the transit times through both pools. In man and dog, only the transit time through the postmitotic pool has been firmly established, with a value of about 6.6 days in man and 3.5 days in dogs. The transit time from early precursors to myelocyte (i.e., mitotic pool) is less well known but probably on the order of 3–4 days in man and dog. Using these data we estimate the total maturation time, denoted $T_m$, to be about 10–11 days in man and 6–7 days in dogs. Once mature, the bone marrow PMNs are released into the blood stream.

We assume in our model that there does exist a negative feedback loop by which mature PMNs can regulate the granulocyte production rate and that this feedback message originates from the bone marrow PMN compartment, as indicated in Fig. 1. In this way, our model is similar to that proposed by Rubinow and Lebowitz, but differs from the models of King-Smith and Morley and Mackey. We believe that the following observations support the assumption that the bone marrow (BM) PMNs constitute the regulated compartment. First, the possibility that local cell–cell interaction within the bone marrow can control granulopoiesis has been demonstrated by cell culture techniques. Second, there is biochemical evidence that colony-stimulating activity (CSA), the most likely candidate for the role of a granulopoietin, is produced by cells that reside within the bone marrow (lymphocytes, monocytes, and macrophages). The CSA production of these cells can therefore be under local control by neighboring mature BM-PMNs. Conversely, if the circulating PMNs were the major source of the negative feedback signal (as assumed by King-Smith and Morley), the following findings would be difficult to explain. First, in states of chronic neutropenia, CSA levels are not found to be elevated. Second, a peripheral neutrophilia would be expected to decrease CSA and thereby suppress PMN production, neither of which are found experimentally. If such events did occur, one could not easily rationalize the neutrophilic leukocytosis of acute infection in which an initial release of mature PMNs from the BM is followed by an increase in PMN production. Such behavior is, however, consistent with the present assumption of the BM-PMNs controlling the rate of granulopoiesis.

A second feature of the feedback loop concerns its point (or points) of action within the mitotic pool. As indicated in Fig. 1, the feedback signal can, in principle, affect all cells in the mitotic pool, but in view of the experimental evidence that CSA stimulates the proliferation of CFU-Cs, we believe that this may be an important location for the feedback signal to operate.

To quantitatively describe granulopoiesis in our compartment.
model we need to determine how the concentration of BM-PMNs, $C_{BM}(t)$, varies in time. This variation results from the difference between: (1) the rate at which BM-PMNs are being produced, $P(t)$, and (2) the rate at which the BM-PMNs egress into the blood stream, $E(t)$. A simple quantitative description of these two processes is now given.

**PMN Production $P(t)$**

Since a time, $T_M$, is required for an early precursor in the mitotic pool to mature into a PMN, it follows that the value of $P(t)$ can be equated* to the rate at which the precursors have proliferated at an earlier time, $t - T_M$.

According to the negative feedback concept proposed earlier, this proliferation rate will depend on the concentration, $C_{BM}$, at the time $t - T_M$. We shall assume that the proliferation rate has a maximum value of $P_{max}$ and decreases linearly with $C_{BM}$. It thus follows that the production rate, $P(t)$, is given by:

$$P(t) = P_{max} - aC_{BM}(t - T_M)$$  \hspace{1cm} (1)

where the parameter $a$ reflects the strength of the feedback inhibition exerted by the BM-PMNs. This linear dependence provides the simplest description of a negative feedback mechanism and is illustrated in Fig. 2.

**Bone Marrow Egress $E(t)$**

Depletion of the BM-PMN pool occurs as PMNs exit into the blood stream. For our purposes it is sufficient to assume that the marrow releases mature neutrophils by a random process at a rate that is proportional to the concentration in the marrow compartment, $C_{BM}$. $E(t)$ is thus given by:

$$E(t) = bC_{BM}(t)$$  \hspace{1cm} (2)

where $b$, the proportionality factor, reflects the "leakiness" of the BM compartment to the efflux of PMNs. This equation is also illustrated in Fig. 2. Presumably, the values of $b$ can be changed by signals from the periphery, as a mechanism for altering the blood PMN concentration.\(^{30-36}\) Such modulation of $b$, however, is not needed to explain the phenomenon of CN, and thus $b$ shall be taken as a constant in the following equations.

To analyze the fluctuations in the concentration of BM-PMNs, we equate the rate of change, $dC_{BM}(t)/dt$, with $P(t) - E(t)$. From equations 1 and 2 it follows that†:

$$\frac{dC_{BM}(t)}{dt} = P_{max} - aC_{BM}(t - T_M) - bC_{BM}(t)$$  \hspace{1cm} (3)

This equation contains the mathematical essentials of our granulopoiesis model and can be solved to relate the concentration $C_{BM}(t)$ to the parameters $P_{max}$, $a$, $b$, and $T_M$. A detailed analysis of the solution of equation 3 is given in Appendix 1. Here we shall summarize the qualitative features of this analysis making use of the graphical illustrations shown in Figs. 2 and 3. In addition, we will present some simple relationships between the parameters $T_M$, $a$, and $b$, which determine the important quantitative features of $C_{BM}(t)$.

First we analyze the steady-state operating point or "set point" of the system, which is derived from the condition that $P(t) - E(t) = 0$. From Fig. 2 this corresponds to the point of intersection of the production and egress curves, with the abscissa being identified as the set point BM-PMN concentration $C_{BM0}$, and the ordinate representing the granulocyte turnover rate $GTR_0$. In the normally functioning control system, the balance between production and egress will cause the system to operate at this point.

Next we analyze how $C_{BM}(t)$ varies with time if it initially differs from $C_{BM0}$. In general, $C_{BM}(t)$ will vary

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*Strictly speaking, $P(t)$ will equal the early precursor proliferation rate multiplied by a factor, representing the number of mitoses a granulocyte undergoes as it matures. This multiplicative factor is implicitly contained in the parameters $P_{max}$ (defined in the text below) and $a$ (also defined below) that appear in equation 1.

†It is interesting to note that equation 3 is similar in form to equation 2.4 in the model proposed by Mackey\(^4\) with his parameter $\lambda = 0$. Despite this mathematical similarity, the biologic concepts underlying the two models are entirely different and lead to different predictions concerning the origin and stability of PMN oscillations.
as a sinusoidal function whose amplitude either decreases or increases exponentially in time as illustrated in Fig. 3 A and B. In Fig. 3 A, \( C_{BM}(t) \) returns to the set point after an initial deviation, as would be expected in normal granulopoiesis, whereas in Fig. 3 B, \( C_{BM}(t) \) continues to oscillate around the set point as is seen in CN.‡

It is remarkable that a simple relationship between the parameters \( a \) and \( b \) determines whether the granulopoietic system behaves normally or as in the CN state. Specifically, we find that oscillations either decrease or increase depending on whether \( a \) is less than or greater than \( b \).

Normal State: Decaying oscillations \( a < b \) (4A)

CN State: Persistent oscillations \( a > b \) (4B)

A second simple relationship derived in Appendix 1 shows that the oscillation period \( T_{osc} \) in both the normal and CN state is approximately equal to twice the maturation time \( T_M \):

\[
T_{osc} \approx 2T_M \tag{5}
\]

Thus far, our analysis has dealt only with the behavior of BM-PMNs. In Appendix 2 we extend our model to describe the fluctuations in the concentration of PMNs in the blood compartment, \( C_B(t) \). This analysis shows that the fluctuations in \( C_B(t) \) but lag the latter by a short time (less than one-half day in the case of human CN). The oscillation period and stability criteria are identical to those derived for the BM compartment (equations 4 and 5). For this reason we will focus our attention primarily on the bone marrow, as the behavior of the blood neutrophil count will be analogous.

To see that the above mathematical results are intuitively reasonable, let us reexamine qualitatively the meaning of equation 3 and the important roles played by the parameters \( a, b, \) and \( T_M \). This equation shows in essence that two mechanisms attempt to restore \( C_{BM}(t) \) to the steady-state value. One mechanism, represented by the second term on the right-hand side in equation 3, is the feedback control of granulocyte production whose magnitude is governed by the parameter \( a \) (a measure of how sensitively the production rate is altered by changes in \( C_{BM} \)). The other mechanism, represented by the third term, is the leakage mechanism for PMN egress, whose rate, being proportional to \( C_{BM} \) (equation 2), will also return \( C_{BM} \) to its operating point. Were it not for the time delay \( T_M \), associated with the feedback term, both mechanisms would operate homeostatically and fluctuations would decay exponentially with a time constant given by \( (a + b)^{-1} \). However, the presence of the time delay, \( T_M \), renders the feedback mechanism unable to assess the adequacy of its efforts to “correct” the fluctuations in the BM-PMN compartment and results in a “hunting” behavior of the system. Thus, if the BM compartment is initially neutropenic, the system will increase granulocyte precursor formation with the result that an excess of mature PMNs appear at the time, \( T_M \).
later. At this point, the production rate becomes suppressed and a BM neutropenia will recur after a time $T_M$. Thus, we see clearly why the oscillation period for such neutropenic states should be approximately $2T_M$. The oscillations inherent in the production mechanism can, however, be overcome by the egress mechanism (which operates without a time delay), provided that it acts more strongly than the production term. For this to occur, the third term in equation 3 must be greater in magnitude than the second term. This implies that $b$ should be greater than $a$, as is rigorously deduced in Appendix 1.

**DISCUSSION**

In this section we shall use the available literature on granulopoiesis and CN to estimate numerical values for the parameters $T_M$, $a$, and $b$ corresponding to both normal and CN states, and compare these deductions with the predictions of equations 4 and 5. Furthermore, we will show how a variety of therapeutic interventions used to treat CN can be understood within the framework of our granulopoiesis model. A brief discussion of the oscillations in the other hematologic cell lines that are associated with CN will also be given. Finally, an interpretation of our results in biochemical and cellular terms is presented.

**The Maturation Time $T_M$**

We have previously estimated the values of $T_M$ for man and dog to be 10–11 days and 6–7 days, respectively. Assuming that these values are unchanged in CN, equation 5 of our model predicts the period of oscillation to approximately $\sqrt{2T_M}$, which gives values of 20–22 days in man and 12–14 days in dogs. These predictions are in excellent agreement with the experimental observations and constitute an important validation for the concept that the time delay associated with PMN maturation underlies the oscillatory process as noted in the work of King-Smith and Morley, and Rubinow and Lebowitz. The consistency of our predictions using estimates for $T_M$ that combine the transit times through the mitotic and postmitotic pools (Fig. 1) suggests that the major effect of the feedback signal regulating granulocyte production is felt early in the mitotic compartment, i.e., at the level of CFU-C. This view is supported by the observation that the cycling in CN involves waves of cellular proliferation that propagate from CFU-C to mature PMN in the time $T_M$. The fact that $T_M$ is about 3 days longer in man than dog, a result of the longer postmitotic transit time, explains the different oscillation periods in the two species according to our theory. Also, in the normal granulopoietic state, the response to perturbations appears to be as predicted in our model (Fig. 3A). For example, in mice and dogs a stress causing neutropenia results in a subsequent neutrophilia whose peak is reached after 5–10 days, followed by a few decaying oscillatory cycles in the granulocyte concentrations, suggesting that $a < b$ in these normal animals.

**The “Feedback Response” Parameter $a$ and the “Marrow Release” Parameter $b$**

According to our model, the relative magnitude of the feedback response parameter $a$ and the marrow release parameter $b$ determine whether the PMN fluctuations will decay as a damped oscillation ($a < b$) or persist in an oscillatory fashion ($a > b$). We shall now estimate these parameters in both normals and CN patients so as to see whether the inequalities predicted by our theory are satisfied.

The parameter $b$. The value of the parameter $b$ in normal man may be estimated from the relationship $GTR_b = bC_{BM}$, which is represented by the point of intersection in Fig. 2. Using the literature values for $GTR_b$ of $0.85 \times 10^9$ cells/kg/day and $C_{BM}$ of $2.2 \times 10^9$ cells/kg, we deduce a value of $b = 0.40$ day$^{-1}$. Whether $b$ is different for CN patients than for normals is not known, as there is insufficient data to determine its value in CN patients. In view of this we will choose $b$ to be the same for both groups. We believe this assumption to be reasonable as long as a CN patient is neither chronically infected nor subject to other factors that alter PMN egress from bone marrow (through presumed changes in $b$), such as leukocyte-inducing factor, endotoxin, etiocholanolone, and prednisone. Furthermore, we recall that our model assumes $b$ to remain constant throughout the cycle in CN. While it is possible that $b$ may increase at the nadir of a CN cycle (as assumed by King-Smith and Morley), such variation is not required to explain the phenomenon of CN and for reasons of simplicity is not considered in the present model.

The parameter $a$. The parameter $a$ measures the strength of coupling between an excess or deficit in the BM-PMN pool and the granulocyte production rate (see Fig. 2). Its magnitude is more difficult to derive from existing data than the magnitude of $b$. Nevertheless, there is sufficient information available from...
estimated production rates

Fig. 4. Number of bone marrow myelocytes on day t + 4 of the cycle versus the number of mature bone marrow PMN's on day t of the cycle. Data points for CN patient (●) and for a non-cycling normal (○) are taken from the study of Meuret and Fliedner.43 Both the myelocyte and PMN counts have been normalized by the erythroid count. These data provide estimates for the dependence of production rate and egress rate on C as discussed in the text. Also shown is the estimated egress curve (---) for a CN patient treated with prednisone as derived from data by Wright et al.

quantitative bone marrow analyses43 to permit estimates of the ratio a/b in CN and normals. To do this we have inferred the dependence of the granulocyte production rate on CBM using data on the periodic fluctuations of BM myelocytes and PMNs in a patient with CN and the steady-state values defined in normals. We have assumed that the myelocyte count found on a given day of the cycle is proportional to the granulocyte precursor production rate 4 days earlier. Thus a plot of the BM myelocyte count at time t + 4 days versus the mature PMN count (PMNs plus late bands) at time t yields the estimated P versus CBM data shown in Fig. 4. (The bone marrow erythroid count, which remained constant during the cycle in these studies, was used as a normalizing factor for both myelocyte and mature PMN counts in the data analysis.) Note that the solid points corresponding to the CN patient fall approximately on a straight line, the slopes of which should be proportional to a. In the same way we have used the BM myelocyte count measured in normals to estimate the steady-state production rate and have plotted this versus the corresponding BM-PMN count43 as the open circle in Fig. 4. (The bone marrow erythroid count, which remained constant during the cycle in these studies, was used as a normalizing factor for both myelocyte and mature PMN counts in the data analysis.) The latter finding is consistent with experimental data of bone marrow neutrophil cellularity,6,7,43 which fluctuates between zero and normocellular during the cycle. Also shown in Fig. 4 is the dotted line representing the bone marrow egress curve for a CN patient successfully treated with prednisone.7 The significance of this line will be discussed below.

Using the ratios deduced above and the previous estimate of b = 0.40 day−1, we find \( a_{CN} = 0.70 \pm 0.15 \) day−1 and \( a_{normal} = 0.25 \pm 0.05 \) day−1. In this way we have used the available literature data to make estimates of the three major parameters of our model for normals. The ratio a/b is found by constructing an estimate of the bone marrow egress curve (as in Fig. 2) by connecting the origin to the open circle in Fig. 4.† This line will have a slope proportional to b, with the same proportionality factor that relates the slope of the production line to a. The ratio between the slopes of the production and egress curves is thus equal to the ratio a/b, which we find to be 0.6 ± 0.2 in normals and 1.7 ± 0.4 in the patient with CN. These ratios are consistent with the inequalities predicted in our theory (equations 4A and 4B) and constitute a second validation of our model. Figure 4 shows that the operating point of the system (intersection of the production and egress curves) is characterized by a decreased GTRO and a decreased CBM, in the CN patient compared to normals. The latter finding is consistent with experimental data of bone marrow neutrophil cellularity,6,7,43 which fluctuates between zero and normocellular during the cycle. Also shown in Fig. 4 is the dotted line representing the bone marrow egress curve for a CN patient successfully treated with prednisone.7 The significance of this line will be discussed below.

†The use of the same egress curve for CN and normals is based on our previous assumption that b does not differ in the two states.
(a, b, and $T_m$) for both normals and CN patients and find that it is sufficient to account for the principal features of the CN state (i.e., the persistence of oscillations, the oscillation period, and the granulocyte cellularity) solely through an increase in the parameter $a$.

Additional experimental evidence that $a$ is larger in CN than normals comes from the BM tissue culture studies of Dresch et al.\textsuperscript{44} In these studies the incorporation of $^3$H-TdR into normal BM cells was used as a marker for BM cell production to study the suppressive effects of mature PMNs on the production rate. It was found that mature PMNs from a CN patient suppressed $^3$H-TdR incorporation 2–4 times more than normal mature PMNs when incubated at the same concentration with the BM cells, suggesting that the inhibitory feedback signal coming from the CN-PMNs is stronger than that from normal PMNs.

**Correction of CN by Various Therapeutic Measures**

It has been found that agents like endotoxin,\textsuperscript{49,50} prednisone,\textsuperscript{7} and lithium\textsuperscript{51} can eliminate the oscillatory phenomena in CN. In the context of our model such agents must either: increase the parameter $b$, decrease the parameter $a$, or affect both parameters, such that $a$ becomes less than $b$ as in the normal state. In the case of prednisone, Wright et al.\textsuperscript{7} have performed bone marrow studies on a successfully treated patient that allow one to assess which of these mechanisms is relevant. In the steady state, which resulted after prednisone treatment, the ratio of myelocytes to mature BM-PMNs is found to equal 0.7 ± 0.1, which is nearly twice the ratio 0.4 ± 0.04 found in normals (as deduced from the data of Meuret and Fliedner\textsuperscript{43} and Dancey et al.\textsuperscript{21}). From the discussion of Fig. 4, these ratios are proportional to the slope of the bone marrow egress curves (i.e., the parameter $b$). Using the value of $b$ in normals of 0.40 day$^{-1}$, we estimate the value of $b_p$ (the prednisone-treated state) to be 0.7/0.4 × 0.4 = 0.70 (±0.1 day$^{-1}$). The value of $b_p$ has now increased so that it approximately equals the value of $a_{CN}$ deduced previously (0.70 ± 0.15 day$^{-1}$). Thus, the system is on the borderline of stability and the PMN oscillations should slowly decay in time (see equation 4). In fact, using the results of Appendix 1, an analysis of the decay of the neutrophil oscillations observed in this patient confirms that the system is only marginally stable. Within the uncertainties of our estimates, the increase in $b$ appears sufficient to account for the correction of CN by prednisone in this patient. It is conceivable that the failure of prednisone to uniformly “cure” CN patients\textsuperscript{51} results from its inability to increase the value of $b$ to be greater or equal than $a$.

Returning to Fig. 4, we see that the larger value of $b_p$ shifts the bone marrow egress curve counterclockwise, resulting in a new point of intersection with the production rate curve. This counterclockwise rotation is reflected in the observed “left shift” of the BM myeloid series (i.e., an increase in the ratio of myelocytes to mature PMNs). The new point of steady-state operation is characterized by a concentration of BM-PMNs that is roughly half the normal value, which is in good agreement with the BM-PMN reserve measurements by Wright et al.\textsuperscript{7} using an etiocholalone injection. The present model thus explains a number of the central features of Prednisone therapy in terms of an increase in the “leakiness” of the bone marrow for PMN egress as reflected by the parameter $b$.

Lithium has been used to correct CN in dogs.\textsuperscript{51} There the BM data show no left shift in the treated state relative to normal dogs.\textsuperscript{20} This suggests that lithium does not change the value of $b$ and therefore may operate by decreasing the value of $a$. There is, in fact, experimental evidence that lithium could affect $a$ in this way through a possible modulation of CSA-producing cells.\textsuperscript{47}

**Other Oscillatory Phenomena in CN**

Monocytes, reticulocytes, and platelets have also been found to fluctuate in CN with the same period, but different phases relative to the PMNs.\textsuperscript{6,7,10} The same is true for both urinary and serum CSA.\textsuperscript{52} The fact that all oscillations occur with the same period intrinsic to the granulocyte control system suggests that the other cell lines oscillate as a result of a secondary coupling to the PMN oscillations. In the case of monocytes, the circulating count reaches its maximum prior to the blood PMN count by about 6–8 days in man\textsuperscript{6,44,51,56} and 3–4 days in grey collies.\textsuperscript{10} This finding is consistent with the view that monocytes proliferate simultaneously with the granulocytes as they derive from the same stem cell (CFU-C), but that they appear earlier in the blood than the PMNs because of their shorter BM transit time of about 2.5 days.\textsuperscript{18,55} The observation that CSA levels in blood and urine oscillate in phase with the monocyte levels may reflect the actual production of CSA by these cells\textsuperscript{23,58} or be due to peripheral spillage of CSA that originated in the BM to stimulate granulocyte (and monocyte) production. In the latter case, the short maturation time of the monocyte would imply a close correlation between monocyte production (measured as CSA) and the circulating monocyte count.

Although platelet and reticulocyte oscillations are universally seen in the grey collie,\textsuperscript{10} some humans appear to exhibit only fluctuations in their granulo-
cyte-macrophage (GM) precursor series\textsuperscript{43,44} or in the GM and erythroid series.\textsuperscript{56} Where platelet and reticulocyte oscillations are seen, their peaks occur about 0.25–0.5 cycles\textsuperscript{7,10} and 0.35–0.6 cycles\textsuperscript{6,7,10,39,59,60} after the granulocyte peak. If these oscillations are coupled to the PMN oscillations through simultaneous proliferative bursts in all three hematologic cell lines as suggested by various authors,\textsuperscript{5,6} then the shorter BM transit times of the platelet and reticulocyte precursors (5–7 days in man\textsuperscript{61–63}) would imply that they would appear 0.1–0.3 of a CN cycle before the granulocyte, but after the monocyte. This does not appear to be consistent with the data. However, if there is some form of competition for pluripotent stem cells between the three cell lines,\textsuperscript{10,29,50} then one would expect the proliferative rates of erythroid and thrombocytic cells to be low when the stimulus for GM proliferation is high, and vice versa. This “competition coupling” mechanism would predict the platelets and reticulocytes to appear 0.25–0.4 cycles after the PMNs, which appears to be consistent with the data.

**Biochemical and Cellular Aspects of CN**

From the theory and discussion given in this article, the phenomenon of CN appears to represent a derangement in which the feedback inhibition of granulocyte production by mature PMNs is overactive and in which other cell lines are secondarily affected. It is believed that the basis of this feedback inhibition is due to an interaction between mature BM-PMNs and the sources or stem cell receptors of CSA.\textsuperscript{23} If this interaction is the result of a product secreted by the PMN, as suggested by Broxmeyer and others,\textsuperscript{43–48} then the biochemical defect in CN implied by our model could result from: (1) an increased secretion rate of the product; (2) a qualitative alteration in the product (increasing its potency); (3) an alteration in those cells responding to the product, or (4) an alteration in the sensitivity of the stem cells to CSA. Of these possibilities the first two would be consistent with the tissue culture studies of Dresch et al.\textsuperscript{44}

The important observations that multiple cell lines oscillate in CN and that bone marrow transplantation can both induce\textsuperscript{64} and abolish\textsuperscript{65} these oscillations in grey collies has lead to the concept that CN is a “stem cell defect.”\textsuperscript{64–66} To reconcile this view with our previous conclusions, we note that any of the possible causes for the overactive feedback loop could result from an abnormality initially present in the genome of the stem cells and hence the BM-PMN precursors, as in the grey collie, or be an acquired abnormality, as seems to be the case at least in some humans.\textsuperscript{6} Thus, our model is also consistent with the view that CN can be considered a stem cell defect. However, as discussed earlier, the defect causing the overactive feedback loop could reside in the stem cell, CSA-producing cells, or in the mature PMN.

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oscillation frequency $v$ only on the parameters $a$, $b$, and $c$, giving the coordinates of the "set point" in Fig. 2.

\[
\frac{d\text{CBM}(t)}{dt} = -a\text{CBM}(t) - b\text{CBM}(t) - c\text{BM}(t)
\]  

(A-1)

where $\text{CBM}(t)$ is the concentration of mature BM-PMNs at the time $t$, $P_{\text{max}}$ is the maximum production rate of mature BM-PMNs, $T_M$ is the maturation time, $a$ is a measure of the strength of the feedback signal affecting production (see equation 1) and $b$ is a measure of the leakiness of the BM for PMN egress (see equation 2).

**Steady State**

In the steady state, granulocyte production and egress are equal and can be identified with the granulocyte turnover rate $G_{\text{TR}}$, $d\text{CBM}(t)/dt = 0$ for all times and $\text{CBM}(t) = \text{CBM}(t - T_M) = \text{CBM}_0$, where $\text{CBM}_0$ is the set point value of BM-PMN concentration. These conditions and definitions imply (see Fig. 2):

\[
P_{\text{max}} = a\text{CBM}_0 - b\text{CBM}_0 - G_{\text{TR}}
\]  

(A-2)

From equation A-2 we can relate $G_{\text{TR}}$ and $\text{CBM}_0$ to the independent parameters $P_{\text{max}}$, $a$, and $b$:

\[
\text{CBM}_0 = \frac{P_{\text{max}}}{a + b}
\]  

(A-3)

\[
G_{\text{TR}} = \frac{b}{a + b} P_{\text{max}}
\]

(A-3)

giving the coordinates of the "set point" in Fig. 2.

**Perturbations From the Steady State**

To analyze the response of our granulopoietic model to perturbations, we rewrite equation A-1 in terms of the variable $\delta\text{Cbm}(t) = \text{CBM}(t) - \text{CBM}_0$. It follows that $d\delta\text{Cbm}(t)/dt$ is given by:

\[
\frac{d\delta\text{Cbm}(t)}{dt} = -a\delta\text{Cbm}(t) - b\delta\text{Cbm}(t) - c\text{BM}(t)
\]  

(A-4)

which shows that the time dependence of the perturbation depends only on the parameters $a$, $b$, and $T_M$. Equation A-4 can be solved using the "trial solution" method employed by Benedek and Villars in analyzing a different feedback problem. The interested reader should refer to them for further details.) Using this trial solution method we assume that $\delta\text{Cbm}(t)$ has the following form:

\[
\delta\text{Cbm}(t) = \delta\text{Cbm}(0)e^{-\gamma t}\cos vt
\]  

(A-5)

where $\delta\text{Cbm}(0)$ is the initial perturbation imposed on the system, and $\gamma$ and $v$ characterize the response of the system in terms of the oscillation frequency $v$ and the damping constant $\gamma$ (which can be positive or negative, see Fig. 3).

We wish to determine how the parameters $a$, $b$, and $T_M$ are related to $a$, $b$, and $T_M$. By substituting equation A-5 into equation A-4 and requiring that the coefficients of the terms in $\cos vt$ and $\sin vt$ correspond to each other on both sides of the resulting equation, we obtain two transcendental equations for $\gamma$ and $v$:

\[
\gamma = a e^{i\gamma} \cos \gamma T_M + b
\]  

\[
v = a e^{i\gamma} \sin \gamma T_M
\]  

(A-6)

In order to solve these equations for $\gamma$ and $v$, it is advantageous to change the variables of the problem in the following way: $X = a' T_M$, $Y = \gamma T_M$, $a_1 = a T_M$, and $a_2 = b T_M$. Equation A-6 then becomes:

\[
X = a_1 e^{\gamma} \sin X
\]  

\[
Y = a_2 e^{\gamma} \cos X + a_2
\]  

(A-7A)

(A-7B)

We must now solve these equations to obtain $X$ and $Y$ as functions of $a_1$ and $a_2$. To solve for $X$ it is useful to make the additional change -- $Y' = Y - a_2$ and $a' = a e^{\gamma}$, from which equations A-7 become:

\[
X = a' e^{\gamma} \sin X
\]  

\[
Y' = a'e^{\gamma} \cos X
\]  

(A-8)

Equation A-8 are identical in form to the ones solved in ref. 16. From that analysis it can be shown that for each value of $a'$ there will be an infinite number of solutions $(X, Y)$ that satisfy equation A-8, each giving rise to a particular oscillation frequency $v$, determined by the value of $X$. However, the solution having the lowest frequency (smallest $X$) will be the one which determines the overall stability of the oscillatory response (i.e., whether the oscillations decay or grow) and thus plays the dominant role in the behavior of the system. It can be shown that in the limit of large $a'$ (i.e., $v$) this "low frequency solution" has an $X$ value that asymptotically approaches $\pi$ from below. Thus, under these conditions (which are attained if $a_1 \geq 4$ and $a_2 \geq 4$), the oscillation frequency $v$ will approximately equal $\pi/2 T_M$, which implies that the oscillation period $T_M$ is given by:

\[
T_M \approx 2 T_M
\]  

(A-9)

given in equation 5 of the text.

Having solved equation A-8 for $X$ (i.e., the low frequency solution), this result may be substituted into equation A-7B to permit the deduction of $Y$ using an iterative numerical method. However, one can easily derive the criterion by which $Y$ (i.e., $\gamma$) is either positive or negative, and thus see whether the oscillations decay ($Y > 0$) or increase ($Y < 0$). For this purpose we use the result that $X = \pi$ and therefore $\cos X = -1$. Furthermore, as we are analyzing the case where $Y$ is close to zero we can set $\gamma = 1$. Substituting these results into equation A-7B, it follows that $Y \approx a_1 + a_2$. Hence, we see that $Y$ will be positive for $a_1 > a_2$ and negative for $a_1 < a_2$. Alternatively, this implies that it is only the relative magnitudes of $a$ and $b$ that determine whether an initial perturbation will result in damped or persistent oscillations:

Damped oscillations: $b > a$

Persistent oscillations: $a > b$

(A-10)

as previously given in equation 4).

The exact deduction of $Y$ for arbitrary values of $a_1$ and $a_2$ requires the full analysis of equation A-7B and the results of such an analysis are shown in Fig. 5, where curves of constant $Y$ are plotted as functions of $a_1$ and $a_2$. This exact treatment again illustrates the simple criterion concerning the sign of $Y$ given above.

---

\*Most generally, $\delta\text{Cbm}(t)$ will be a sum of terms, each having the form given by equation A-5. We restrict our attention however to the lowest order term (i.e., lowest frequency $v$), as that provides the dominant behavior of the system.

\*In this limit the next higher frequency solution has $X \approx 2 \pi$ and thus corresponds to an oscillation period of approximately $T_M$. A periodogram analysis of actual CN data indicates the presence of this second harmonic.

\*It can be shown from the results of ref. 16 that for the higher frequency solutions of equation A-8, the values of $Y'$ (and hence $Y$) will be greater than the values corresponding to the low frequency solution $(X = \pi)$ given in Fig. 5. As a result, these high frequency solutions do not determine the stability of the system.
It is straightforward to develop a differential equation for the concentration of blood PMNs, \( C_8(t) \). The rate of change \( dC_8(t)/dt \) is equal to the rate at which BM-PMNs enter the blood minus the rate at which circulating PMNs leave the blood:

\[
\frac{dC_8(t)}{dt} = b C_{BM}(t) - \frac{ln2}{TB} C_8(t)
\]  

where the first term on the right side is identical to the rate at which BM-PMNs egress from the bone marrow, and the second term expresses the random exit of circulating PMNs in terms of their half-life in the circulation, \( TB \).

**Steady State**

In the steady state, \( dC_8(t)/dt = 0 \) and equation A-11 implies that the set point concentration of circulating PMNs, \( C \), will be given by:

\[
C_8 = C_{BM0} e^{-\frac{ln2}{TB}}
\]  

where \( C_{BM0} \) is the steady-state concentration in the BM given earlier by equation A-3. It is interesting to point out that \( ln2/b \) is actually the half-life of mature PMNs in the bone marrow (denoted \( TB_{M,B} \)), and thus, equation A-12 in essence shows that the ratio \( C/C_0 \) is identical to the ratio \( T_{B,M,B}/TB_{M,B} \).

The value of \( TB \) is generally believed to be 0.3 days,\(^6\) whereas \( T_{B,M,B} \) as estimated from our deduction of \( b = 0.4 \) day equals 1.7 days. Thus, \( C/C_0 \) equals 0.11 (i.e., about 1/6), consistent with the deduction of others.\(^{20} \)

**Perturbations From the Steady State**

As discussed in Appendix 1, a perturbation in \( C_{BM} \) results in either a damped or persistent oscillation in \( C_{BM}(t) \). This oscillation will influence \( C_8(t) \) through the first term in equation A-11. By substituting the previous equation for \( C_{BM}(t) \) (equation A-5) into equation A-11, and solving the resulting linear differential equation, one finds that the solution \( C_8(t) \) will be given by:

\[
C_8(t) = C_{1,0} + b C_{0}(e^{-\gamma t} \cos(\nu t - \theta))
\]  

where \( \gamma \) and \( \nu \) are the same parameters appearing in equation A-5 and where \( \delta C_8(t) \) and \( \theta \) are new quantities. It is straightforward to show that these new quantities are related to \( \delta C_{BM}(o) \), \( \gamma \), \( \nu \), and \( TB_{M,B} \) by the following relations:

\[
\delta C_{8}(o) = \frac{\delta C_{BM}(o) b T_{B,M,B}/ln2}{[1 - \gamma B_{M,B}/ln2]^2 + (\nu T_{B,M,B}/ln2)^2]^{1/2}}
\]  

and

\[
\theta = \tan^{-1} \left( \frac{\gamma T_{B,M,B}/ln2}{1 - \gamma T_{B,M,B}/ln2} \right)
\]

From these results we see that \( C_{8}(t) \) simply follows the fluctuations in \( C_{BM}(t) \) with a phase angle \( \theta \) given by equation A-15. The criteria for decaying or sustained oscillation remain identical to those discussed previously. In the case of human CN, our deductions of \( a, b, \) and \( TB \), imply that the phase angle is quite small (≈6°), and thus the peripheral PMN count will essentially reflect the oscillations of the mature PMNs in the BM compartment.
Cyclic neutropenia (CN): a clue to the control of granulopoiesis

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