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Reduced variance of bone-marrow transit time of granulopoiesis—a possible pathomechanism of human cyclic neutropenia

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Abstract. Human cyclic neutropenia (CN) is a haematological disorder characterized by oscillations in the numbers of neutrophilic granulocytes and other blood cells with a stable period of approximately 21 days. In most cases the neutrophils oscillate well below normal values such that these patients are chronically neutropenic. A comprehensive concept of the origin of CN is proposed. It assumes an abnormally small variance of the transit time of bone marrow cells (compared to normal human granulopoiesis) for the origin of the characteristic cycles. Furthermore, a reduced responsiveness of the immature granulopoietic bone marrow cells to the mitotic feedback stimuli is assumed to account for the subnormal neutrophil peaks. Together with feedback control provided in a simulation model of normal human granulopoiesis these two abnormalities can explain experimental and clinical cell kinetic data for bone marrow and blood in CN.

Human cyclic neutropenia (CN) is a rare, benign haematological disorder characterized by oscillations in the number of blood neutrophils with a stable period of 21 days. In most cases the neutrophils oscillate well below normal values such that these patients are chronically neutropenic. Severe neutropenic phases (neutrophil counts < 250 cells/μl) last 4–10 days. During these phases patients frequently develop malaise, upper respiratory tract infections, stomatitis, fever, cervical lymphadenopathy, anorexia, headache and myalgia. With increasing levels of blood neutrophils the infections and accompanying symptoms normally vanish (Wright *et al.* 1981, Lange 1983, Dale & Hammond 1988). Other blood cells also cycle in CN (Guerry *et al.* 1973, Wright *et al.* 1981, Lange 1983, Dale & Hammond 1988). The cycling of reticulocytes and monocytes is usually easily detectable whereas the detection of the cycles of the lymphocytes, eosinophils and platelets often requires long serial blood counts.

Bone marrow examinations show that the cycling of the peripheral blood neutrophils is preceeded by oscillations of the granulopoietic bone marrow differentials (Moore *et al.* 1974, Brandt *et al.* 1975, Greenberg *et al.* 1976, Dresch *et al.* 1977, Verma *et al.* 1982). During the beginning of severe neutropenia a wave of granulopoietic precursors (predominantly

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promyelocytes and myelocytes) can be detected which is followed by an increase of the metamyelocytes and band neutrophils. Just at the end of the neutropenic phase, bone marrow examinations show a decrease of early granulopoietic cells and a high proportion of mature neutrophils (Wright *et al.* 1981). G-CSF levels are also reported to cycle within CN. Tsunogake *et al.* (1991) reported that the serum G-CSF level was slightly elevated during the neutropenic nadir. Similar G-CSF patterns were found by Watari *et al.* (1989), Misago *et al.* (1991) and Yujiri *et al.* (1992).

More recent papers describe an abnormal response of early granulopoietic bone marrow cells to human granulopoietic growth factors. Hammond *et al.* (1992) show that *in vitro* colony assays of bone marrow mononuclear cells from patients with CN required higher concentrations of added G-CSF and GM-CSF to achieve half-maximal growth. Similarly, Tsunogake *et al.* (1991) report an abnormal response of CFU-G to G-CSF in patients with CN. Wright *et al.* (1989) found that half maximal-growth of CFU-GM colonies from patients with a childhood-onset CN required up to ten times higher rhGM-CSF concentrations than CFU-GM colonies from normal volunteers. This abnormality of the GM-CSF responsive growth of myeloid progenitors was reported to be independent throughout the cycle (neutrophil nadir or recovery phase).

The pathomechanistic interpretation of experimental and clinical data of CN is complicated by the fact that in a dynamical feedback system like hematopoiesis it is difficult to distinguish between cause and effect. Oscillations occurring on one level of hematopoiesis may induce cycling at other levels via feedbacks. Thus, any explanation requires understanding of the dynamical properties of the whole system. In such circumstances mathematical modelling can be an appropriate method. Therefore we applied an established simulation model of normal human hematopoiesis to analyze available cell kinetic CN data.

In contrast to former models we assume that the pathomechanism of CN is given by a significantly reduced variance of the bone-marrow transit time in which CN differs from normal granulopoiesis. The simulations show that this hypothesis, combined with the detected reduced mitotic responsiveness of granulopoietic precursors, is sufficient to explain experimental and clinical cell kinetic data in CN.

MATERIALS AND METHODS

Clinical data

Clinical data were taken from the literature (Guerry *et al.* 1973, Dresch *et al.* 1977, Jacobsen & Broxmeyer 1979, Wright *et al.* 1981, Inoue *et al.* 1984, Ohta *et al.* 1987, Watari *et al.* 1989, Wright *et al.* 1989, Migliaccio *et al.* 1990, Misago *et al.* 1991, Tsunogake *et al.* 1991, Hammond *et al.* 1992, Yujiri *et al.* 1992). All data sets on bone marrow cells and characteristic blood neutrophil counts are compared with the corresponding model curves.

Mathematical model of normal hematopoiesis

Structure

The mathematical model used to describe normal human hematopoiesis is schematically shown in Figure 1. Each biological cell stage is represented by a model compartment characterized by transit time, number of mitoses and the fraction of actively proliferating cells. Model parameters are given in Table 1. For normal granulopoiesis they are taken directly from the literature or are derived from published experimental data (Donohue *et al.* 1958, Cronkite *et al.* 1960, Killmann *et al.* 1962, Cartwright, Athens & Wintrobe 1964, Cronkite & Fliedner 1964, Cronkite *et al.* 1965, Cronkite & Vincent 1969). Our estimation of the somewhat controversial values for CFU-GM is

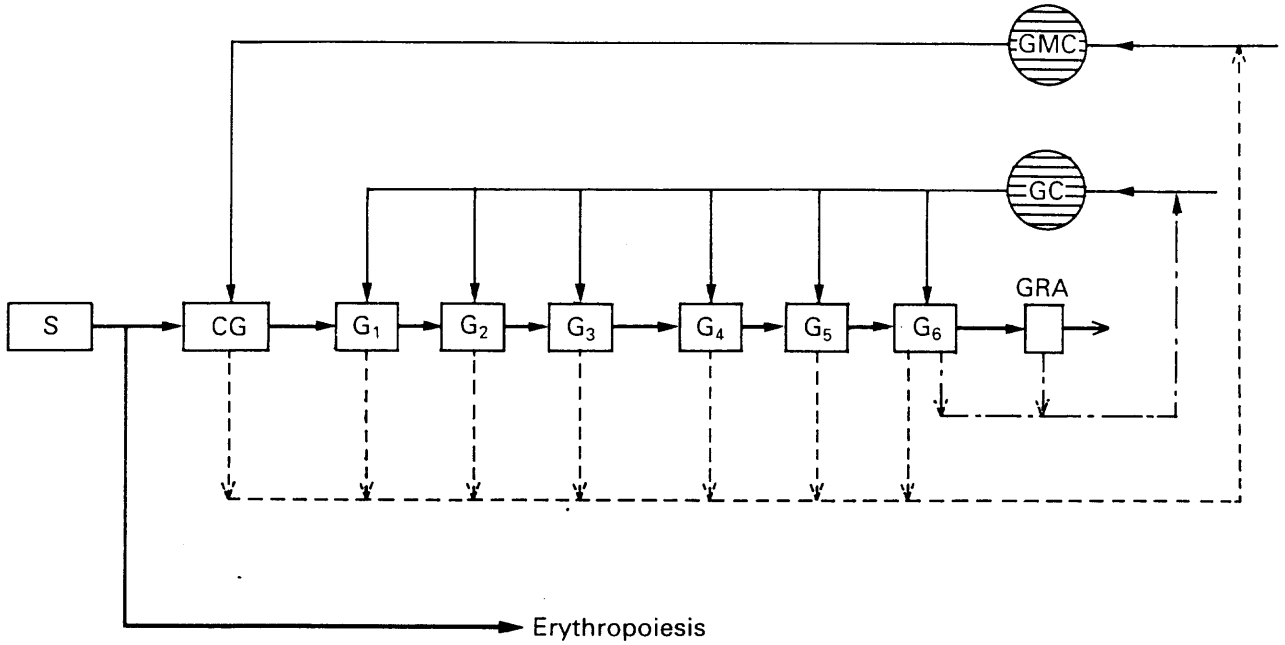

Figure 1. Scheme of granulopoiesis in the model of normal human hematopoiesis. The efflux from the compartment of the pluripotent stem cells CFU-S (S) differentiates towards granulopoiesis and erythropoiesis (thick lines). The granulopoietic lineage is represented by the committed cells CFU-GM (CG) and by the compartments of the proliferating granulopoietic precursors myeloblasts (G_1), promyelocytes (G_2), myelocytes (G_3) and the postmitotic maturing stages of metamyelocytes (G_4), band (G_5) and segment forms (G_6). Mature granulocytes leave the bone marrow and enter the neutrophil compartment (GRA) in the peripheral blood. The mitotic activity of the CG is regulated (thin line) by the model hormone GMC which itself is controlled by the total granulopoietic bone-marrow cell count (CG and G_1–G_6, dotted lines). Cell divisions of the precursors G_1–G_3 and early release from maturing cell stages G_4–G_6 are controlled by another feedback loop via the model hormone GC (thin line). The production of GC depends on the number of the peripheral cells GRA and a fraction of the most mature G_6 cells (broken lines).

Table 1. Basic model parameters of normal human granulopoiesis

Cell stage		Average transit time (h)	Mitoses
Bone marrow			
CG	CFU-GM	112	6
G_1	Myeloblasts	22	1
G_2	Promyelocytes	24	1
G_3	Myelocytes	102	2
G_4	Metamyelocytes	30	0
G_5	Band forms	50	0
G_6	Segment forms	72	0
Blood			
GRA	Circulatory pool of granulocytes	5	0

based on colony-growth data reported for CFU-C by Pike & Robinson (1970). From the 8.2, 9, 10.2, 10.3 mitoses resulting from their colony size on days 8, 11, 13, 18, respectively, we conclude six mitoses for CFU-GM (CG) taking into account (the well established) four mitoses in the subsequent stages of myeloblasts through myelocytes (G_1–G_3). Moreover, if the G_1–G_3 parameters listed in Table 1 are correct, two inequalities for transit times can be derived from the

observation of eight mitoses on day 8: $T_{CG}+T_{G_1}+T_{G_2}<8$ days and $T_{CG}+T_{G_1}+T_{G_2}+T_{G_3}/2>8$ days, hence $95\text{ h}<T_{CG}<146\text{ h}$. We chose $T_{CG}=112$ h to get the best fit of the neutrophil data. The change in the number of cells with time in each compartment is described by a differential equation, thus yielding a system of coupled nonlinear ordinary differential equations. Erythropoiesis and granulopoiesis originate from the pluripotent stem cells (S). Passing through several cell stages the cells give rise to the morphologically identifiable bone marrow cells which finally form the functional blood cells.

Regulation

The self-renewal probability and proliferative fraction of the pluripotent stem cells obey feedback loops not shown in Figure 1. Committed cells and early bone marrow cells of granulopoiesis are regulated by two feedback loops as follows (Figure 1): The number of all granulopoietic bone marrow cells controls the growth of the CFU-GM (CG) via the hypothetical model hormone GMC. Reduced numbers of granulopoietic bone marrow cells induce additional mitoses in the mitotic pedigree at the CG stage. The number of amplifying mitoses at the proliferating granulopoietic precursor cell stages, myeloblasts through myelocytes (G_1–G_3), is regulated by the number of granulopoietic blood cells via the second hypothetical model hormone GC. This hormone also shortens the transit time of the postmitotic stages, metamyelocyte through segment forms (G_4–G_6), inducing an early release into the circulation (GRA). It is expected that the model hormones GMC and GC reflect, in some aspects, the role of the growth factors GM-CSF and G-CSF, respectively, in human granulopoiesis. Details of the model and its biomathematical realization are described elsewhere (Wichmann & Loeffler 1985, Wichmann, Loeffler & Schmitz 1988, Loeffler *et al.* 1989b).

Assumed defects in cyclic neutropenia

Reduced variance of the granulopoietic bone-marrow transit time

To simulate a reduced variance in the maturation process of granulopoiesis, a series of subcompartments is introduced into each compartment (Takahashi 1966). This leads to a well-peaked distribution of the total granulopoietic transit time (Figure 2a) but does not change any of the model parameters such as the average transit time, number of mitoses or fraction of actively cycling cells. Details are described elsewhere (Schmitz *et al.* 1990).

Reduced mitotic response of the granulopoietic bone marrow cells

A reduced responsiveness of the committed stem cells (CG) and proliferating precursor cells (G_1–G_3) to the feedback hormones GMC and GC is realized within the model by a shift of the dose-response curve (Figure 2b, c).

RESULTS

Effect of oscillatory influx into granulopoiesis

Assuming the large variance of the total granulopoietic bone-marrow transit time (59 d^2) required to model normal hematopoiesis, an oscillating cell flux originating from the pluripotent stem cells cannot explain CN (Figure 3a). Two different oscillating influxes into the CFU-GM (CG) are simulated in the model of normal granulopoiesis. The initially pronounced oscillations are dampened progressively on their way through the bone marrow and have nearly disappeared when they reach the pool of blood neutrophils. Although box influxes considerably differ in their amplitudes the resulting neutrophil curves are very similar. Obviously, the small fluctuations of the model neutrophils are inconsistent with the clinically observed characteristic oscillations in CN.

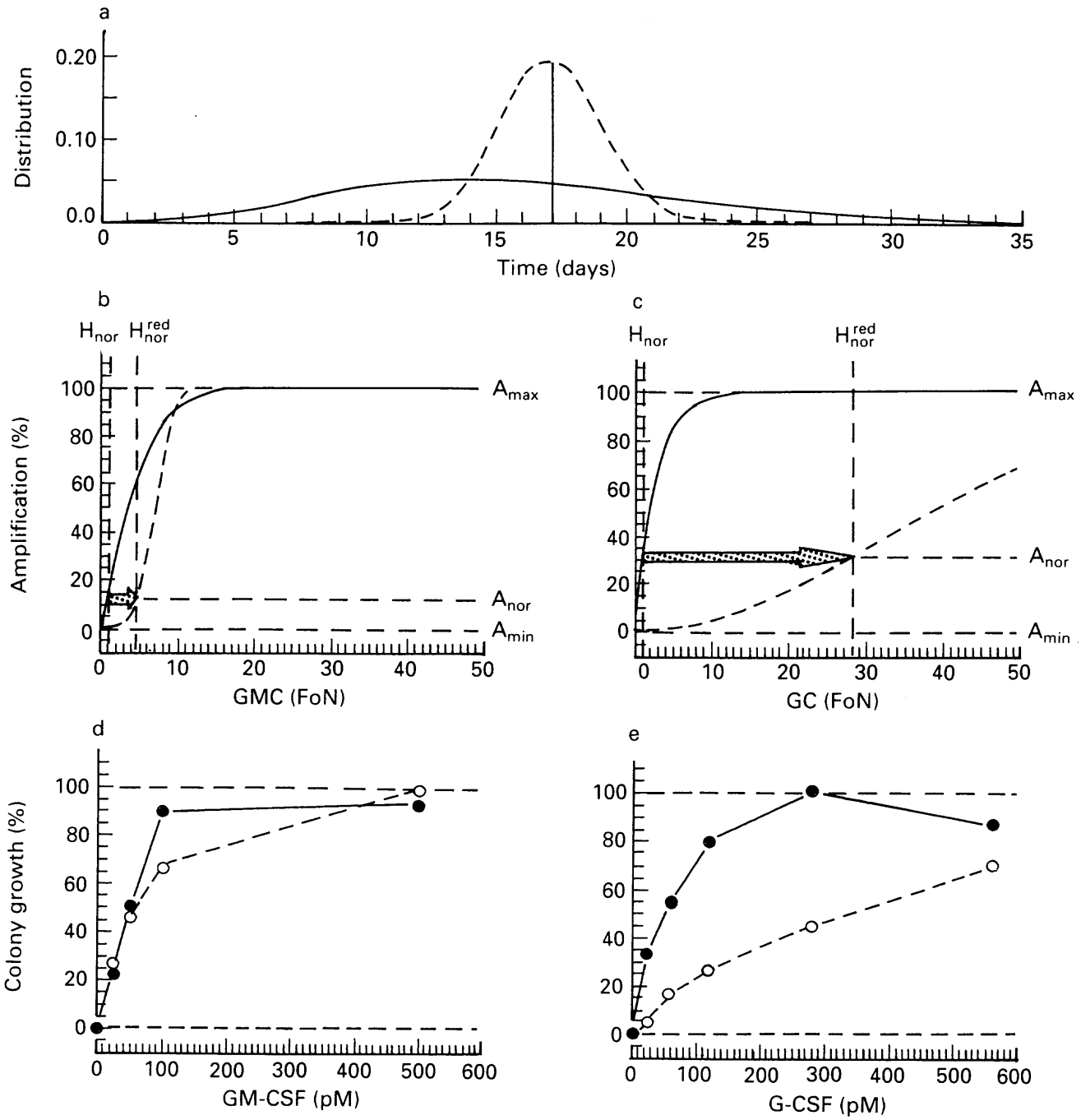

Figure 2. a, b, c Assumed defects and **d, e** *in vitro* data in CN. Reduced variance of the granulopoietic bone-marrow transit time, reduced responsiveness of granulocyte-committed progenitor cells to the model hormones GMC and GC in the model of CN, and to growth factors GM-CSF and G-CSF in patients with CN. Curves for normal and CN cells are indicated by full and broken thick lines, respectively. **a** distribution of the transit time from earliest progenitors CFU-GM (CG) to entrance into the blood (GRA), **b** model amplification of CFU-GM (CG) as a function of GMC, **c** model amplification of myeloblasts through myelocytes (G_1–G_3) as a function of GC, **d** experimental colony growth of nonadherent bone marrow mononuclear cells (BMMCs) stimulated by GM-CSF, **e** experimental colony growth of nonadherent BMMCs stimulated by G-CSF. The transit time distributions are calculated by Γ-functions for variances $\sigma^2 = 59$ d^2 (normal) and $\sigma^2 = 4$ d^2 (CN), the average transit time is $\langle T \rangle = 17$ d. The model amplification $A = 2^n$ of n mitoses is plotted in percent of the maximum value, the model hormone H (GMC or GC) is given in fraction of the normal level (FoN). The experimental growth of day-21 colony formation is plotted in percent of maximal growth as a function of growth factor concentration in picomolar ranges (pM). Reduction can be quantified by the shift of the response curve (indicated by arrow) which is defined by the hormone ratio H_{nor}^{red}/H_{nor}. The hormones for normal amplification A_{nor} on the normal and reduced dose-response curve are denoted by H_{nor} and H_{nor}^{red} respectively. Similar to GM-CSF and G-CSF the response reduction to the model hormone is smaller for GMC (shift by a factor of 5) than for GC (shift by a factor of 28). Experimental data are taken from Hammond *et al.* (1992).

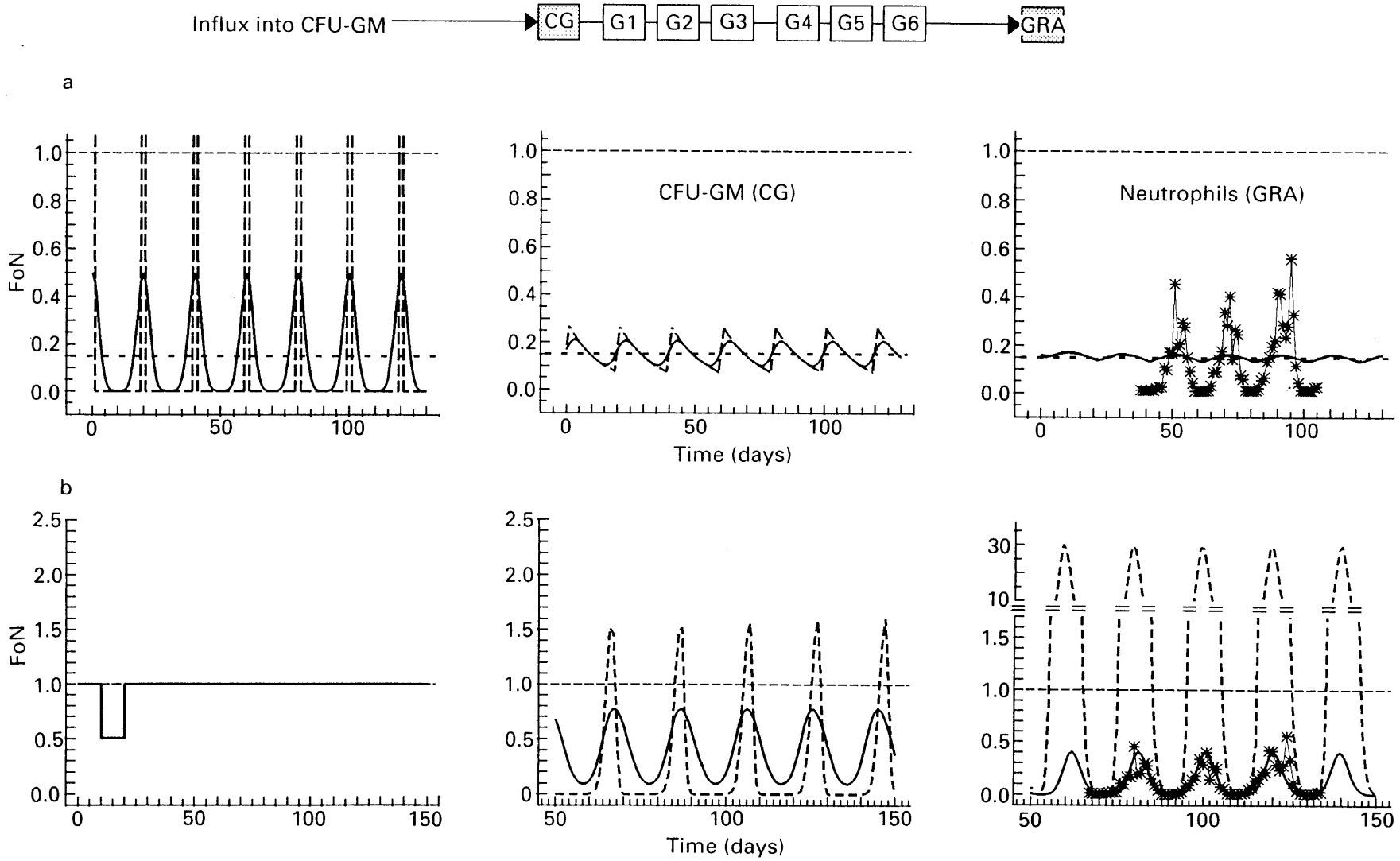

Figure 3. a Effect of an oscillating influx into granulopoiesis. Granulopoiesis with a large variance of the transit time dampens oscillations progressively in the different cell stages: Even pronounced oscillating influxes into CFU-GM (CG) result in only weak fluctuations in the peripheral neutrophil compartment GRA which cannot explain the clinically observed cycles of the neutrophil count. Model curves (thick lines) and data * of Wright *et al.* (1981) are plotted in fractions of normal values (FoN). Both influx curves have the same mean value 0.15 (broken straight line). They differ only by the amplitudes of 0.5 (full line) and 5 (broken line). **b** Effect of a small variance of transit time and reduced feedback response. Granulopoiesis with a small variance of transit time amplifies a perturbation (short decrease) of the steady state influx into CFU-GM via feedback to stable oscillations. However, average and peak values of the neutrophils (GRA) are much larger (broken line) than observed in CN. Combination of the small-variance effect and the reduced responsiveness to GMC and GC reproduces the data * of Wright *et al.* (1981) (full line). Model curves (thick lines) and data are plotted in fractions of normal values (FoN).

Effect of small variance of transit time and reduced mitotic feedback response

The effect of introducing a small variance of the total granulopoietic transit time (4 d^2) is shown in Figure 3b (broken curve). After a slight perturbation of a constant influx into granulopoiesis the system destabilizes, resulting finally in stable oscillations. The neutrophils show marked oscillations with a period of 20 days. However, whereas the predicted length of the cycle fits well to the clinically observed period of 21 days the maximum cell counts are too large in the model. Additionally an assumed reduced mitotic responsiveness of the early granulopoietic bone marrow cells to mitotic feedback stimuli reduces the peak values. If the amplification of the CFU-GM (CG) as a function of the hormone GMC and the amplification of the myeloblasts through myelocytes (G_1–G_3) as a function of GC are reduced (as shown in Figure 2b, c) the predicted maximum neutrophil counts become subnormal (Figure 3b, full curve).

Model of CN

The combination of a small variance of granulopoietic bone marrow transit time with a reduced mitotic responsiveness of the granulopoietic bone marrow cells in the complete model of hematopoiesis (regulated pluripotent stem cells and erythropoiesis included) yields simulation results which are in good agreement with the clinically observed blood neutrophil counts and all other granulopoietic bone marrow cell stages measured (Figure 4). The model qualitatively and quantitatively reproduces the granulopoietic clinical data for bone marrow and blood in CN. The curve of the model hormone GC is in good accordance with the time courses experimentally determined for G-CSF serum levels.

Reduced mitotic responsiveness

Figure 2 shows the dose-response curves of the feedback hormones GMC (Figure 2b) and GC (Figure 2c) used in the model of CN to reproduce the clinical data of Figure 4 in relation to the dose-response curves used in the model of normal hematopoiesis. These model dose-response curves are compared with experimental *in vitro* data of Hammond *et al.* (1992). In Figures 2d and 2e the percentages of maximum growth of colony formation of nonadherent bone marrow mononuclear cells from normal volunteers and from patients with CN are plotted in response to the growth factors GM-CSF and G-CSF. Similar to the experimental results the magnitude of the response reduction used in the model is less pronounced from the more primitive cells (GM-CSF responsive cells) and more pronounced for the more mature cells (G-CSF responsive cells).

DISCUSSION

Cyclic neutropenia is not only a human hematologic disorder, it is also known in grey collie dogs (Lange & Jones 1980). Extensive experimental effort was performed to elucidate the underlying defect. In humans and in dogs it was shown that the disease can be transferred by bone marrow transplantation (Dale & Graw 1974, Krance *et al.* 1982). Due to these transplantation experiments most hypotheses for the explanation of CN focus on the pluripotent stem cells. From the canine studies it was conjectured that CN reflects a defect in competition of the erythroid and granuloid differentiated cells for a limited pool of stem cells thus leading to an oscillating input into both lineages (Patt, Lund & Maloney 1973). Dale, Alling and Wolff (1972) discussed a defect in stem cell regulation completely suppresing the stem cells for one day. Similar mechanisms were proposed by Quesenberry (1983) and Abkowitz, Holly and Hammond (1988). A temporary maturation step of differentiating cells in the bone marrow was presumed by Lund, Padgett and Ott (1967). Other authors conjectured defective factors controlling proliferation of stem cells (Lange & Jones 1980, Dunn *et al.* 1982, Jones & Jolly 1982).

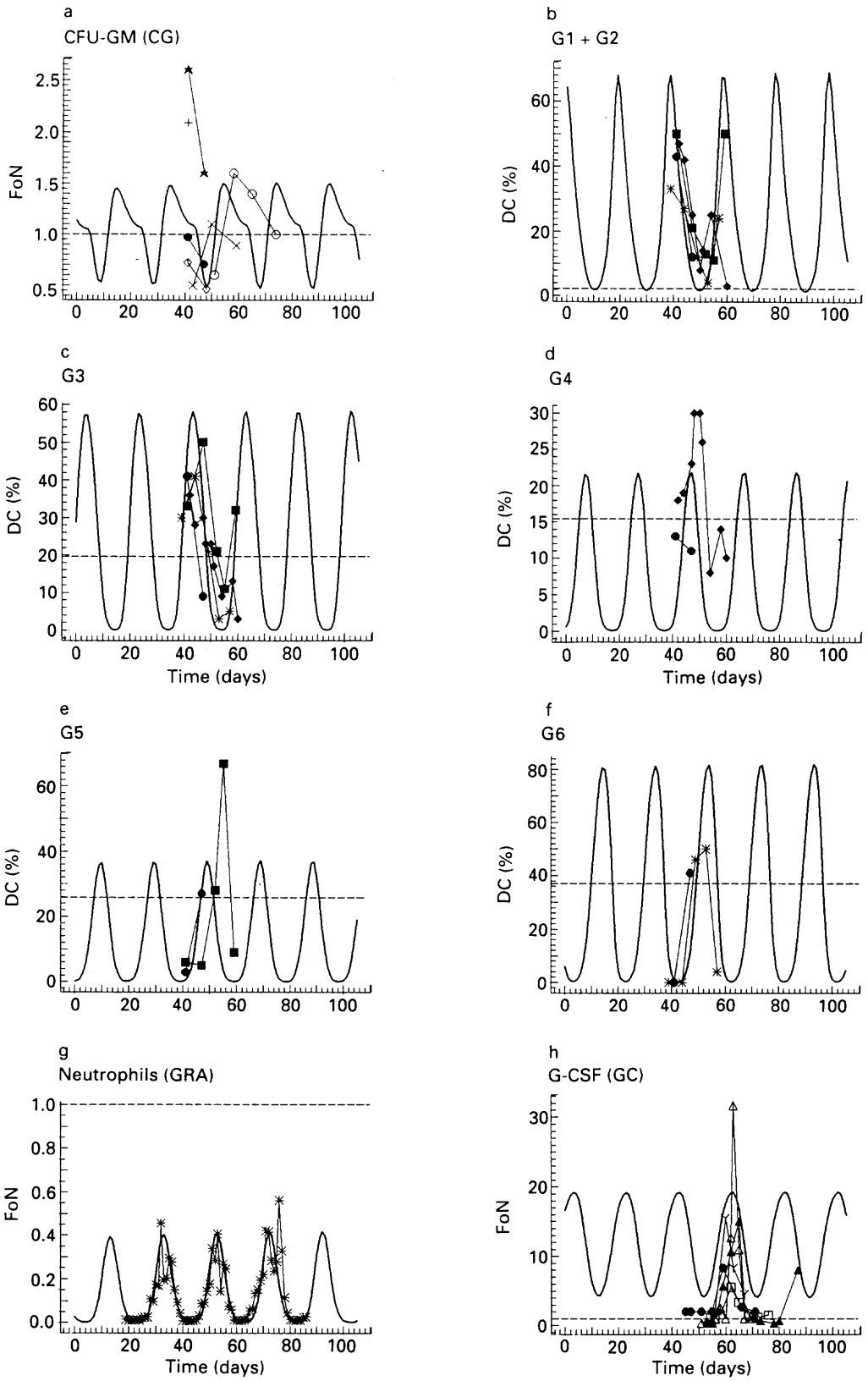

Figure 4. Model simulation curves for cyclic neutropenia compared with cell kinetic clinical data. **a** CFU-GM (CG), **b** differential count (pooled) of myeloblasts and promyelocytes, **c** differential count of myelocytes, **d** differential count of metamyelocytes, **e** differential count of band forms, **f** differential count of segment forms, **g** blood neutrophils (GRA), **h** G-CSF (GC). In **a, g** and **h** the model curves (thick lines) are plotted in fractions of normal values (FoN), **b–f** show the differential count (DC) in percent of all granulopoietic precursors G_1–G_6, normal values are indicated by broken straight lines. Clinical data are taken from Guerry *et al.* (1973) ■; Dresch *et al.* (1977) ◆; Jacobsen *et al.* (1979) ○; Wright *et al.* (1981) *, Inoue *et al.* (1984) X; Ohta *et al.* (1987) ◇; Watari *et al.* (1989) □; Wright *et al.* (1989) ★; Migliaccio *et al.* (1990) +; Misago *et al.* (1991) △, ▲; Tsunogake *et al.* (1991) ●; Yujiri *et al.* (1992) Y.

Stimulated by the discussion of clinicians and experimentalists periodic hematopoiesis also received attention from theoreticians. Mackey (1978) developed a model for the pluripotent stem cell population and found conditions for oscillating growth by irreversible loss of proliferative stem cells. Other investigators recognized the importance of a delayed feedback control involving peripheral cells for the understanding of CN. King-Smith and Morley (1979) proposed a model with two feedback loops. The granulocyte production rate is regulated by the blood granulocyte concentration with a delay representing the time for proliferation, maturation and storage in the bone marrow. A second short-range feedback from blood granulocytes to bone marrow granulocytes influences the storage time. Experimental evidence for this additional regulation is provided by the early release of bone marrow granulocytes into the blood observed in neutropenic stress situations. In normal granulopoiesis the second feedback dampens oscillations resulting from the time-delayed first feedback. Impaired granulopoiesis with a depressed production rate shortens permanently the storage time and inactivates the short-range loop. Without dampening, stable large-amplitude oscillations can arise. Wheldon, Kirk and Finlay (1974) used a similar time-delayed model to describe cyclic granulopoiesis in chronic granulocyte leukemia. Schulthess and Mazer (1982) introduced a model of CN which is also based on a time-delayed production rate of bone marrow granulocytes. In contrast to King-Smith and Morley, they assumed that the controlled quantity is given by the bone-marrow granulocyte concentration itself, and that dampening is provided by the undelayed granulocytic egress from bone marrow into the circulation. Oscillatory or normal behaviour depends on the relative strength of the two mechanisms.

Most of the proposed models assume that cyclic and normal granulopoiesis are regulated by a time-delayed feedback. They show that the origin of cycling is due to this time delay. In normal granulopoiesis the resulting oscillatory tendency is compensated by dampening mechanisms. Depressed granulopoiesis diminishes the efficacy of these mechanisms and the oscillations become visible. There are experiments supporting this concept. Normal dogs were treated with cyclophosphamide, and in some animals oscillating neutrophil counts were seen in the blood (Morley & Stohlman 1970). Similar observations are reported for the red cell lineage (Wheldon *et al.* 1974).

On the other hand, there is also evidence for depressed granulopoiesis without regular cycling. In general, no continuous oscillatons are observed in normal humans e.g. after extended irradiation, chemotherapy or drug-induced neutropenia, hypoxia or bleeding anemia. This stability of normal and depressed granulopoiesis suggests a somewhat altered concept. Normal granulopoiesis is regulated by relatively undelayed feedbacks. Some degree of delay, however, may exist by first-in first-out like maturing processes through the cell stages G_4–G_6, indicated by labeling experiments of Fliedner *et al.* (1964). They found a minimum emergence time of 2–3 days for labelled granulocytes into the peripheral blood. The normal distribution of transit time sketched in Figure 2a (variance $\sigma^2 = 59$ d^2) is consistent with these observations. Strongly time-delayed feedback regulation of granulopoiesis is assumed only for CN. Time delay is provided by an essentially narrowed distribution curve ($\sigma^2 = 4$ d^2). Thus the pathomechanism of CN is given by a significantly reduced variance of the bone-marrow transit time compared to normal granulopoiesis.

Based on this concept the result of this simulation study shows that CN can be understood by two defects of normal granulopoiesis. First, a small variance of the granulopoietic bone-marrow transit time distribution, and second, a reduced responsiveness of the early granulopoietic bone marrow cells to mitotic stimuli exerted by feedback hormones. Beside these two defects no further assumption is necessary to allow a comprehensive and quantitatively correct description of CN. The model used to analyze the data is not particularly designed to describe CN. It is developed

from a model of normal hematopoiesis utilized in earlier descriptions and explanation of various hematologic questions (Wichmann & Loeffler 1985, Wichmann *et al.* 1988, Loeffler *et al.* 1989a, b, Schmitz *et al.* 1990, Scheding *et al.* 1992, Schmitz *et al.* 1993). Including the whole granulopoietic system cell kinetic clinical data of any cell stage are reproduced.

According to the proposed concept that normal granulopoiesis is a system with a considerable dampening capacity, cycling stem cells cannot explain the periodic pattern of CN. Figure 3 demonstrates that neither the rate nor the shape of influx into the CFU-GM compartment is crucial to a cyclic outcome. The oscillatory behaviour of the system is rather determined by the extent of time delay effective in its feedback regulation. Figure 3a shows that any initially pronounced oscillating influx into CFU-GM is progressively dampened and dispersed during its development through the bone marrow by a large variance. Only moderate oscillations of the blood neutrophils remain. In the same way, oscillating feedback hormones lead only to minor peripheral oscillations (data not shown). On the other hand, small variance of the granulopoietic bone-marrow transit time reduces the dampening property (Figure 3b, broken curve). In that case cycling can occur without any driving oscillator. Even a slight random perturbation of a constant steady-state influx into CFU-GM is sufficient to induce the transition to a state with stable oscillations. Whereas the period of the oscillations (dependent on transit times) fits well to the clinical data, the maximum neutrophil counts predicted are much too large. The inconsistency can be removed by the additional assumption of reduced mitotic responsiveness for the early granulopoietic bone marrow cells (Figure 3b, unbroken curve). Abnormally reduced responsiveness of granulocyte-committed progenitor cells of CN patients to GM-CSF and G-CSF was more recently described by several authors (Wright *et al.* 1989, Tsunogake *et al.* 1991, Hammond *et al.* 1992). In Figure 2 the model amplification dose-response curves are compared with experimentally derived *in vitro* data of colony growth (Hammond *et al.* 1992). Although the model curves cannot be identified with the experimental curves they may describe the same effect. We conjecture that GC may be identified with G-CSF. In contrast to GC, only slight response reduction is necessary for GMC to reproduce GM-CSF data in CN. This tendency can also be noticed in the *in vitro* data neglecting other discrepancies, especially at low concentrations of GMC.

Figure 4 shows that the model of normal granulopoiesis modified by the two proposed defects of CN predicts bone marrow and blood data (phases in subsequent cell stages included) quantitatively correct. Only one characteristic data set of the numerous but similar blood neutrophil counts is shown. In accordance with the clinical data the period is predicted to be 20 days and the duration of the severe neutropenic phase is reproduced (Figure 4g). The period of the oscillations depends mainly on the transit times. The model value chosen for the CFU-GM (112 h) is within the estimated range from 95 h to 146 h (see Materials and Methods). Other values, somewhat higher or lower, yield comparable results. Seveal authors (Watari *et al.* 1989, Misago *et al.* 1991, Tsunogake *et al.* 1991, Yujiri *et al.* 1992) reported serum G-CSF levels peaking during the severe neutropenic phase. This observation can be understood if G-CSF is identified with the model hormone GC depending on peripheral granulopoietic cell numbers (Figure 4h). Despite the very low blood neutrophil nadirs the numbers of CFU-GM are not comparably reduced in CN. All CFU-GM nadirs are reported not to oscillate below 50% of normal (Jacobsen & Broxmeyer 1979, Inoue *et al.* 1984, Ohta *et al.* 1987, Wright *et al.* 1989, Tsunogake *et al.* 1991). The model curve agrees with this experimental finding if CFU-GM (CG) growth is regulated by a separate loop and a somewhat reduced mitotic responsiveness to the hormone GCM (Figure 4a). In the present model small variance of transit time is not assumed for erythropoiesis. Due to the cycling of granulopoiesis, dampened oscillations of the red cells are induced by the common feedback loops controlling self-renewal probability and proliferative

fractions of stem cells and early progenitors CFU-GM and BFU-E. Although oscillating numbers of reticulocytes are obtained, fluctuations in the erythrocyte compartment are offset by the considerable transit time of 128 days (curves not shown), as observed in patients (Guerry *et al.* 1973).

Adapting a very similar mathematical model to normal canine hematopoiesis we previously showed that a small variance of bone marrow transit time quantitatively explains cyclic hematopoiesis in grey collie dogs as well (Schmitz *et al.* 1990). However, different to human CN, the maximum neutrophil blood counts in the animals are well above normal. In agreement with this experimental fact, it was not necessary to assume reduced mitotic responsiveness in order to explain the cyclic hematopoiesis in grey collie dogs. It would be interesting to support this assumption experimentally.

Whereas there is experimental evidence for the defect of reduced mitotic responsiveness, variance in transit time and molecular-biological mechanisms responsible for its reduction in CN remain to be determined. Since CN can be transferred by bone marrow transplantation it may be a cellular defect rather than a defect of the hematological microenvironment. The measured constancy of the cycle period over years could suggest a clonal origin. However, it seems obscure whether hemopoietic maturation in cells follows a precise internal clock, or whether cooperative phenomena play a role. Further research is required to reveal the genetic nature of the defect in humans and dogs.

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