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Synchrony of bone marrow proliferation and maturation as the origin of cyclic haemopoiesis

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Abstract. Cyclic haemopoiesis in Grey Collie dogs is characterized by stable oscillations in all haemopoietic lineages. It is proposed that in these animals, in contrast to normal animals, the maturation process of haemopoietic (in particular granuloid) cells from the primitive progenitors to the functional cells is characterized by an abnormally strong synchrony. It is conjectured that the marrow maturation time has a very small variance compared with non-cyclic normal dogs. With a mathematical model of haemopoiesis it is shown that small fluctuations are amplified via regular feedback processes such that stable granuloid oscillations are established. Erythroid oscillations are induced indirectly by granuloid feedback to the stem cell pool. The model calculations further show that the synchrony hypothesis of bone marrow maturation can quantitatively explain the following experimental results: (1) the maintenance of stable cycles of granuloid and erythroid bone marrow and blood cells with a period of approximately 14 d; (2) the disappearance of granuloid and erythroid cycles during the administration of the colony stimulating factor rhG-CSF; (3) the reappearance of oscillations when the administration of CSF is discontinued; (4) the cessation of cycles during endotoxin application; and (5) the persistence of cycles during erythroid manipulations (bleeding anaemia, hypoxia, hypertransfusion). We therefore conclude that cyclic haemopoiesis is not caused by a defect in the regulatory control system but by an unusual maturation process.

Cyclic haemopoiesis (CH) in dogs was first described by Lund, Padgett & Ott in 1967 and is the only known naturally occurring animal model of CH. The phenomenon is often compared with a rare human haematological disorder called cyclic neutropenia. CH in Grey Collie dogs is characterized by periodic fluctuations of haemopoietic blood cells (reticulocytes, neutrophils, lymphocytes and thrombocytes) with a stable period of 11–14 d (Lund *et al.*, 1967; Dale,

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Alling, & Wolff, 1972; Lange & Jones, 1980; Jones & Lange, 1983). Fluctuations in the peripheral cell counts are preceded by oscillations in the percentages of the (morphologically identifiable) granuloid and erythroid bone marrow cells (Patt, Lund & Maloney, 1973) and by the committed progenitor cells of the erythropoietic burst forming unit—BFU-E, the erythropoietic colony forming unit—CFU-E and the granulocyte–macrophage colony forming unit—GM-CFU (Dunn *et al.*, 1977, 1982; Abkowitz, Holly & Hammond, 1988). The concentrations of the growth factors erythrocyte stimulating factor (ESF), erythrocyte stimulating activity (ESA), and granulocyte–macrophage colony stimulating factor (GM-CSF) and activity (GM-CSA) also exhibit stable oscillations (Adamson, Dale & Elin, 1974; Yang *et al.*, 1974; Jones *et al.*, 1975; Lange & Jones, 1976; Dunn *et al.*, 1977).

Bone marrow transplantation experiments show that dogs with CH can be cured by transplantation of bone marrow from normal dogs (Dale & Graw, 1974). On the other hand, normal dogs acquire CH after bone marrow transplantation from Grey Collie donors (Weiden *et al.*, 1974). Bleeding anaemia and hypoxia do not alter the characteristic cycles (Adamson *et al.*, 1974; Lange & Jones, 1976). In contrast, chronic application of the colony stimulating factor (CSF) rhG-CSF (Lothorp *et al.*, 1988), endotoxin (Maloney, Lund & Patt, 1974) or lithium (Hammond & Dale, 1980) is reported to eliminate the granuloid oscillations. Hypertransfusion of washed homologous red cells suppresses the reticulocyte count to nearly zero but leaves the neutrophil oscillations unaffected (Adamson *et al.*, 1974). Maloney *et al.* (1974) were the first to point out that a high dose of endotoxin eliminates the cycles of neutrophils and reticulocytes. Their observations were confirmed by other authors (Hammond, Price & Dale, 1978; Hammond, Engelking & Dale, 1979; Hammond, Adamson & Dale, 1982).

There is at present no conclusive explanation for CH. Most hypotheses focus on defects at the level of the pluripotent stem cells. Patt *et al.* (1973) conjectured that the phenomenon reflects a defect in competition of the differentiated cells (erythroid *v.* granuloid) for a limited pool of pluripotent stem cells. This feedback mechanism was assumed to generate an alternating differentiation trigger to the stem cells, leading to an oscillating input into erythro- and granulopoiesis. In a similar way, Dale *et al.* (1972) postulated a defect in haemopoietic regulation at the stem cell level with a complete suppression for nearly one day. Abkowitz *et al.* (1988) conjectured that cycling is due to a commitment of cells more primitive than BFU-E and CFU-GM at discrete intervals. A lack of pluripotent stem cells or a defect of their function was proposed by Quesenberry (1983). A temporary stop in maturation of the differentiating cells in the bone marrow was discussed by Lund *et al.* (1967). Dunn, Lange & Jones showed that the conditioned media from the bone marrow of dogs with CH inhibits and stimulates the replication of murine pluripotent stem cells (Lange & Jones, 1980; Dunn *et al.* 1982). A similar effect of adherent bone marrow cells on CFU-GM was observed (Jones & Jolly, 1982). These authors conjectured that CH occurs due to a defect in the factors controlling the proliferation of the stem cells.

The interpretation of the experimental results with respect to a possible pathological mechanism encounters two serious problems. On the one hand, it is currently not possible to measure the pluripotent stem cells in dogs, thus, making conclusions about defects at this level at least debatable. On the other hand, it is difficult to distinguish between cause and effect in CH because a start of oscillations at any level in this feedback-controlled system will induce cycling at many other levels as a natural consequence. Any explanation therefore has to be based on an understanding of the dynamic properties of the system and the control processes involved. We report the results obtained by applying a comprehensive mathematical model of haemopoietic regulation and maturation to study CH from a theoretical point of view, and propose a new explanation for the phenomenon.

MATERIALS AND METHODS

Model structure

The mathematical model used to describe normal haemopoiesis in dogs is schematically illustrated in Fig. 1. The structure and the mathematical formulae used have been described in detail elsewhere (Wichmann & Loeffler, 1985; Loeffler *et al.*, 1989). The basic assumptions of the model are briefly summarized: it is a compartmental model in which the different maturation and differentiation stages (compartments) are connected by cell fluxes. All granuloid and erythroid cells originate from the pluripotent stem cells (CFU-S-analogue). Differentiating pluripotent stem cells develop into committed stem cells (BFU-E, CFU-E, CFU-GM) which give rise to the morphologically identifiable precursors. These cells mature and finally form the functional blood cells. Three types of interrelated negative feedbacks are assumed: I for the autoregulation of pluripotent stem cells, II for the influence of progenitor and precursor cells on the stem cells and III for the feedback of mature cells on the immature cells within the bone marrow (Wichmann, Loeffler & Schmitz, 1988). Each model compartment is characterized by the transit time, the number of mitoses and the fraction of actively proliferating cells (Table 1). The model parameters are either taken directly from the literature or are fixed in the simulation of various experiments (described in Wichmann & Loeffler, 1985; Wichmann *et al.*, 1988; Loeffler *et al.*, 1989). The change of cell numbers of any compartment with time is described by ordinary differential equations.

Regulation of stem cells

Two properties of the pluripotent stem cells are regulated via feedbacks I and II: the fraction of cells actively cycling and the self-renewal. The first parameter determines the turnover rate of stem cells and thus the rate of cell production. The latter parameter determines whether the number of stem cells increases or decreases. A reduction in stem cell number increases self-renewal and cycling; a lack of differentiated cells also stimulates stem cell cycling but induces a decrease in the self-renewal probability (increase in differentiation probability).

Regulation of committed cells

The committed cells are predominantly regulated with respect to the number of cell divisions that take place (feedback III). The model assumptions on erythroid regulation have been described elsewhere (Loeffler *et al.*, 1989). For granulopoiesis the model presented here is an extension of the model proposed by Wichmann, Loeffler & Schmitz (Schmitz, 1988). The model equations are given in Appendix I. The level of the feedback hormone, CSF, depends on the number of mature granuloid cells and influences the amplification (number of cell divisions) of the early progenitors in the granuloid lineage.

Variance of the bone marrow transit time

Transit times quantify the duration of stay of cells in a compartment. Table 1 gives the average values used. The variance of transit time depends to a large degree on the age structure of the respective compartments. The effect of the variance of transit time on a whole haemopoietic lineage is schematically shown in Fig. 2. On the left, a sequence of three distinct cohorts of cells enter granulopoiesis. A small variance implies that the cohorts stay distinct and unmixed during differentiation and maturation. The cohorts leave the marrow in the same order as they entered. In this case the cells matured in synchrony. In contrast, a large variance of transit times leads to a mixture of the cohorts on their way through the bone marrow. Thus, a considerable number of cells leave the bone marrow either earlier or later than expected by the average transit time. Although the sequential order of the cohorts is conserved, they are largely

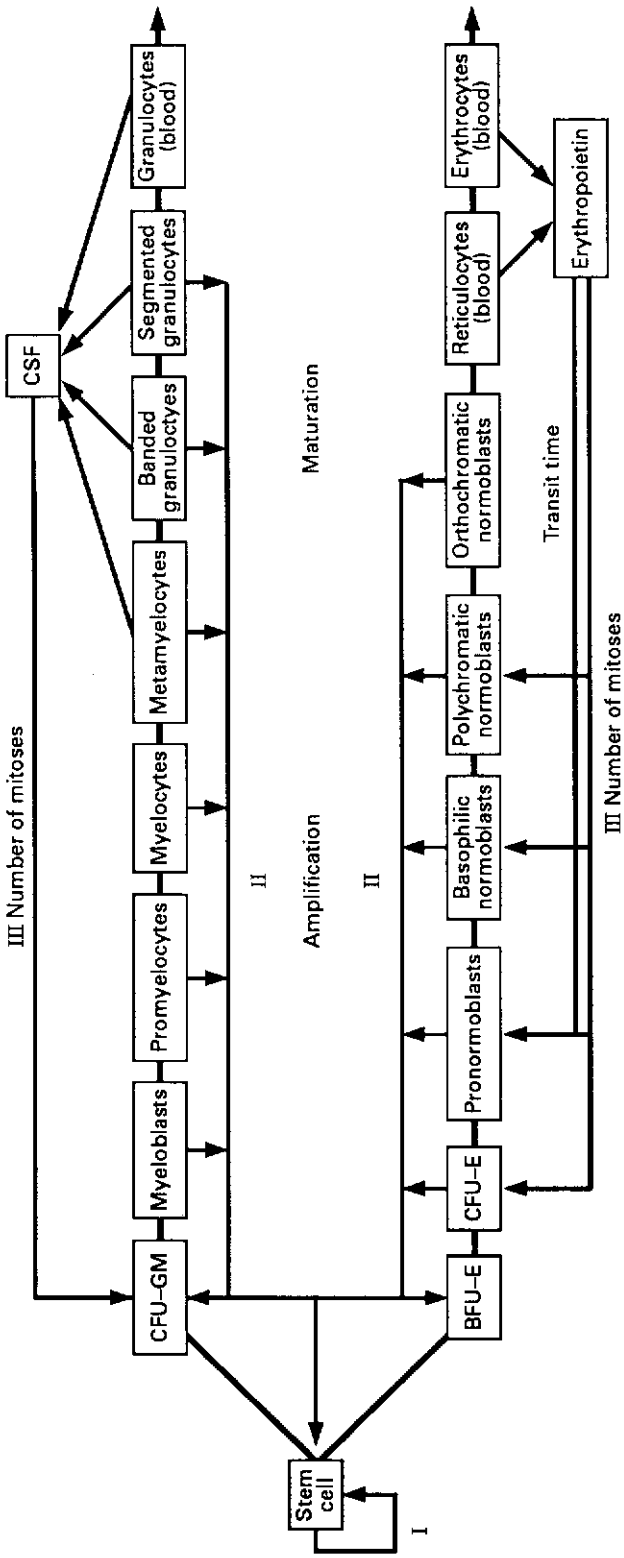

Fig. 1. Mathematical model to describe normal haemopoiesis in dogs. This is a compartmental model in which the different maturation and differentiation stages are connected by cell fluxes. Originating from the pluripotent stem cells, all cells of granulo- and erythropoiesis are described. Thrombopoiesis is neglected. Haemopoiesis is regulated by three types of feedbacks: I for the autoregulation of stem cells, II for the influence from the progenitors and precursors on the stem cells and III for the feedback of the mature cells on the immature bone marrow cells. Each compartment is characterized by the average transit time, the number of mitoses and the fraction of actively proliferating cells and the values of these parameters are given in Table 1. In feedback II, mainly the maturing precursors are responsible for regulation. (CSF = colony stimulating factor.)

Table 1. Model parameters of canine haemopoiesis: where available, results for proliferative fraction, transit time and number of mitoses are shown as normal/minimum/maximum values

					Normoblasts			
	CFU-S	BFU-E	CFU-E	CFU-GM	Pro-/basophilic	Polychromatic	Orthochromatic	Myeloblasts
Proliferative fraction	0·15/0·01/1	0·33/0·3/1	1	0·33/0·3/1	1	1	—	1
Transit time (h)	8	40	40	42	16/8/16	24/10/24	32/13/32	48
No. of mitoses	*	5	5/0/6·5	6/2/10	2/0/2·33	2/0/2·33	0	1
No. of sub-compartments (normal/cyclic)	1/1	1/10	1/10	1/10	1/10	1/10	1/10	1/10

				Granulocytes		Blood		
	Promyelocytes	Myelocytes	Meta-myelocytes	Banded	Segmented	Reticulocytes	Erythrocytes	Neutrophils
Proliferative fraction	1	1	—	—	—	—	—	—
Transit time (h)	30	34	29	36	11	32/32/51	77·7 days	10
No. of mitoses	1	1	0	0	0	0	0	0
No. of sub-compartments (normal/cyclic)	1/10	1/10	1/10	1/10	1/10	1	1	1

* Self-renewal probability (norm/min/max) = 0·5/0·4/0·6.

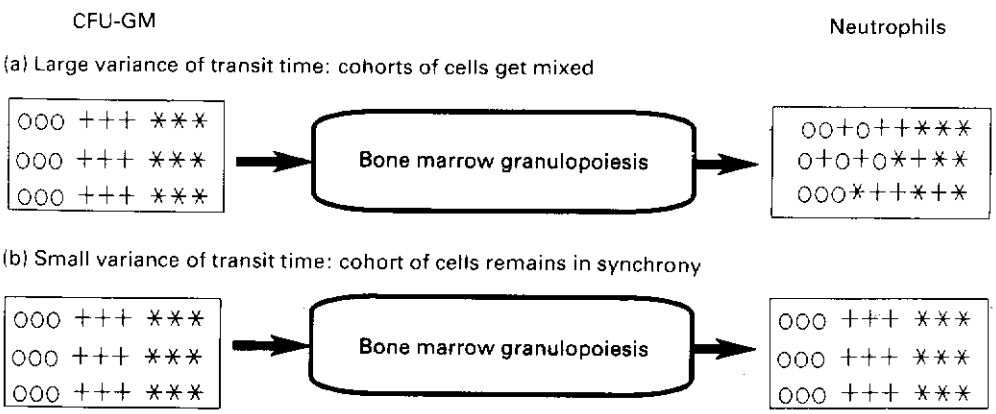

Fig. 2. The variance of the granuloid marrow transit times is illustrated: imagine three cohorts of cells in a certain order in time in the CFU-GM compartment. (a) A large variance of transit time implies that for a cohort CFU-GM the time it takes to pass through the bone marrow deviates around the mean within a wide range. This results in changes of the exact order to cells throughout their differentiation. The cells are mixed when they leave the bone marrow. (b) In the case of a small variance of transit time the deviation around the mean is small. In the extreme, the variance is zero and all cells leave the bone marrow after exactly the same time. This results in the conservation of the primary order. Model analysis shows that a large variance is realized in normal haemopoiesis and a small variance is the origin of cyclic haemopoiesis.

mixed. Technically, in the model the variance of the transit time of a compartment can be reduced by introducing a series of sub-compartments all described by similar ordinary differential equations. This mathematical subdivision of each compartment does not change any of the model parameters, such as the number of mitoses, the proliferative fraction or the total compartment transit time. What it does change is the age structure of each compartment. In the standard model of normal haemopoiesis the age structure of each compartment is characterized by an exponential distribution of the transit time. After subdivision into n sub-compartments, the distribution of transit times is gamma-distributed. In this case the coefficient of variation is known to be proportional to $1/\sqrt{n}$ (Takahashi, 1966).

Model of CH

In the standard model of normal canine granulopoiesis seven bone marrow compartments, representing the biological cell stages, are assumed ($n = 7$). In the model of CH each granuloid bone marrow compartment is divided into 10 sub-compartments, thus yielding a synchronization of cells. The same is assumed for erythropoiesis. With these assumptions the model shows an oscillating normal steady state. Small deviations develop into stable oscillations in the sense of a limit cycle.

Model simulations of experimental situations

Hypertransfusion is simulated in the following way (Wulff *et al.*, 1989):

1. The initial values of the reticulocytes and erythrocytes are increased according to the transfused red cell volume (up to 5·3 times the normal red cell volume).
2. The average transit time of the transfused erythrocytes is assumed to be 2/3 of the normal value.
3. The plasma volume is kept constant.

Based on the evaluation of an experimental data collection including chronic bacterial infections, chronic application of endotoxin is simulated as follows (Kreutzfeldt, 1983):

1. The number of mitoses in the CFU-GM is maximum (10 instead of 6).
2. The average transit time of the blood neutrophils is prolonged to 180% of normal (18 h instead of 10 h).

Chronic application of CSF is simulated similarly:

1. The number of mitoses of the CFU-GM is kept on its maximum level.
2. The blood neutrophil transit time is prolonged to 300% of normal.

Experimental data

The experimental data presented in this paper which are used for comparison with the model results are either taken from the literature or are provided by two of us (R.D.L. & I.B.J.). The morphologically identifiable bone marrow cells are determined from marrow aspirates which are smeared and stained with Wright-Giemsa. Bone marrow was obtained from four CH dogs and four normal dogs on Monday, Wednesday and Friday throughout a 14-day cycle and on particular days of other cycles. Collections were made from the ribs and the long bones while the dogs were heavily sedated with xylazine and oxymorphone. The collections from the CH dogs were all related to cycle days as designated by the onset of neutropenia. They were counted as total differentials on 1–7 determinations for each of the cycle days. Cycle day 1 in both experiment and model curves is identified as the day the blood neutrophils fall for the first time below 1600 cells/mm^3.

RESULTS

Model of CH

Table 2 shows the relationship of the coefficient of variation of the total granuloid marrow transit time to the amplitude of the blood neutrophils within the model when the number of sub-compartments are varied. Oscillations are induced by a perturbation of the system from its steady state. The kind of perturbation we used is a single initial value reduction of the pluripotent stem cells. Decreasing coefficients of the variance of transit time lead to increasing amplitudes and to less efficient dampening. Knowing the experimental amplitudes, the coefficient of variation is set to be 0·12. This leads to stable oscillations. Fig. 3 shows that this

Table 2. Coefficient of variation of the total granuloid marrow transit time (230 h)

No. of sub-compartments	Coefficient of variation	Amplitude of neutrophils*
7	0·39	0
14	0·27	2·1
21	0·22	4
35	0·17	7·9
70	0·12	13

* In units of the normal neutrophil number.

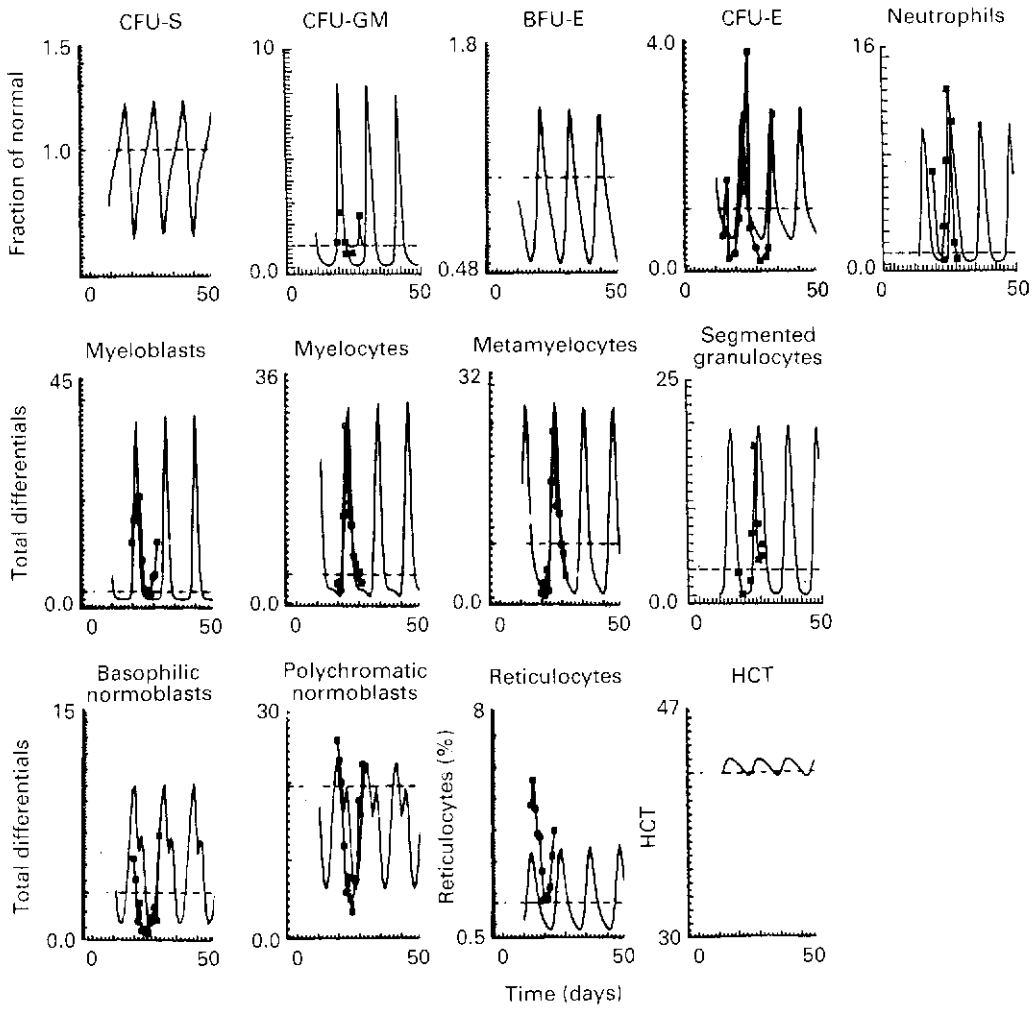

Fig. 3. Comparison of experimental (-■-■-) and model (——) data. The model data is generated with the standard model, assuming a small variance of transit times. Both the experimental and theoretical data are expressed in the same units. The number of morphologically identifiable bone marrow cells are given as total differentials. The results for CFU-S, CFU-GM, BFU-E, CFU-E and blood neutrophils are normalized to their values in dogs with normal haemopoiesis (– – –) and expressed as fractions of normal values. The experimental CFU-GM, CFU-E and % of reticulocytes are from Dunn *et al.*, 1977, 1978); all other data are from two of us (J. B. J., & R. D. L.).

coefficient of variation does not only induce the correct amplitudes but also results in a good quantitative fit of the theoretical and experimental data.

In the model, the period of oscillations (14 d) is within the range reported experimentally (11–14 d). The model stem cells are decreased for a slightly longer period than they are increased (8 d *v.* 6 d). Although the average stem cell number is slightly decreased, an increased cell flux is maintained (1·66 instead of 1). BFU-E and CFU-E are both suppressed for nearly twice as long as they are stimulated, a property which is confirmed by the experimental data for the CFU-E.

The phase relationship between CFU-E and reticulocyte counts is also reproduced within the model, since the maximum of CFU-E occurs during the time of the reticulocyte minimum.

While the theoretical curves for CFU-GM show some quantitative differences to the experiments, the fit between the theoretical and experimental results is satisfactory for the morphologically identifiable bone marrow cells.

The blood neutrophils oscillate in the model, as in the data, with an increased average value. The model average value is increased three-fold above normal.

Mechanism of CH in the model

In the model, CH results from the tight synchrony of the cells on their maturation path through the bone marrow. The synchrony, described by a small variance of the bone marrow transit time, leads to an instability of the feedback which is necessary to produce the cycles. Starting from a steady state value, a single, small perturbation of the normal cell numbers leads, via granuloid feedback, to an amplification of the perturbation and finally to stable cycles with a period of 14 d. Via feedback II, the oscillating number of granulocytic precursors leads to changes in the self-renewal probability and the cycling fraction of the pluripotent stem cells and, thus, induces secondary oscillations in this cell stage also. The oscillations of stem cells subsequently induce oscillations also in the erythroid cell lineage.

Alternative model scenarios tested

Table 3 lists alternative model scenarios tested within the standard model of normal haemopoiesis (seven compartments, no sub-compartments) to determine whether they would generate CH. In the first scenario different patterns of oscillating cell fluxes are used as input into the CFU-GM model compartment. None of the investigated input patterns, which included very sharp and extreme peaks, could generate the required neutrophil oscillations because the peaks were rapidly dampened during maturation. In the second scenario the effects of extremely oscillating CSF levels were simulated. Again, these oscillations did not induce large enough oscillations in granulopoiesis. Although the oscillating CSF did induce oscillations in the CFU-GM compartment, these oscillations were dampened during their further maturation through the bone marrow. In the third scenario the parameters regulating the number of pluripotent stem cells (namely feedback II) were modified. It is only possible to produce cycles by changing the sign of the feedback from negative to positive (Wichmann & Loeffler, 1982). However, due to the strong dampening of the system, even this fundamental inversion of the

Table 3. Alternative model scenarios tested: when these scenarios were simulated within the standard model of normal haemopoiesis, only minor oscillations of the numbers of bone marrow and blood cells could be generated within the standard model of normal haemopoiesis (parameter in Table 1). These small and weak oscillations are not comparable to the experimentally observed values

1.	Oscillating influx into the CFU-GM
2.	Oscillating levels of the feedback hormone CSF
3.	Variation of the feedback on the proliferative fraction a_s and self-renewal probability p of the pluripotent stem cells
4.	Oscillating influx into erythropoiesis

normal regulatory characteristics could not quantitatively reproduce the experimental data. In the fourth scenario an oscillating influx of cells into erythropoiesis was simulated. As for the previous scenarios, this simulation did not generate the experimentally observed oscillations.

CH under erythroid manipulations

Transfusion of washed homologous red cells decreased the production of reticulocytes while the neutrophil oscillations were maintained (Fig. 4). The model curves for the simulation of hypertransfusion exhibit the same patterns as the data.

Mechanism of the model behaviour

The large number of red cells suppresses the erythroid stimulation and, thus, almost no cell divisions occur within the CFU-E and erythroblast compartments. The production of reticulocytes drops to nearly zero. The oscillations reappear as soon as the suppression of erythropoiesis disappears. Cycles in granulopoiesis continue because of the decisive feedback from the mature granuloid cells to the committed granuloid stem cells and the synchrony of maturation is not affected.

The model simulation of anaemia (not presented here) does not change the cyclic behaviour of the system either—as found experimentally by Adamson *et al.* (1974).

CH under granuloid stimulation

During continued administration of endotoxin the cycles of neutrophils and reticulocytes are eliminated. After cessation of endotoxin treatment the cycles reappear. Fig. 5 shows the

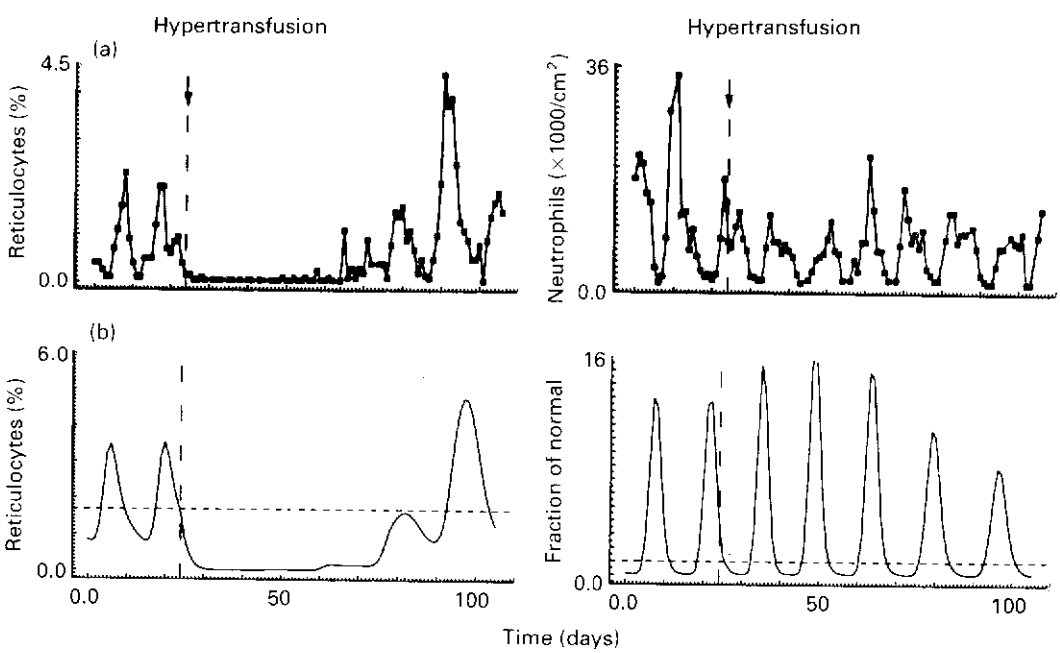

Fig. 4. Comparison of experimental (–■–■–) and (b) model (——) data before and after hypertransfusion of washed homologous red cells on day 24 in dogs with cyclic haemopoiesis. Normal values are shown (– –). Experimental data from Adamson *et al.* (1974). After red cell transfusion the cycles remain but cannot be observed in erythropoiesis since the number of reticulocytes is reduced to zero.

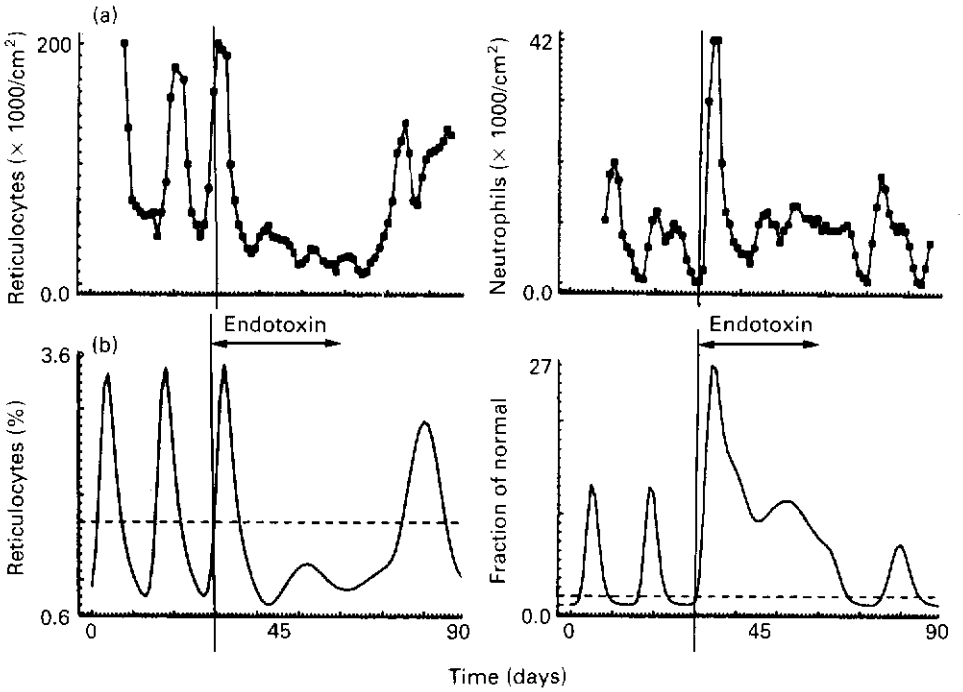

Fig. 5. Comparison of experimental (-■-■-) and (b) model (——) data before, during and after daily application of endotoxin from day 30 to day 60 in Grey Collie dogs. Normal values are shown (– – –). Experimental data from Maloney *et al.* (1974); daily dose of endotoxin 18·8 g kg^{-1} body weight. During the application of a sufficiently high dose of endotoxin both the neutrophil and reticuloycte oscillations are eliminated.

corresponding model simulations together with the experimental observations. Data and model curve are consistent.

The continued administration of rhG-CSF has similar effects on the cycles (Lothorp, 1988). The corresponding model curve and the experimental data are shown in Fig. 6.

Mechanism of the model behaviour during G-CSF and endotoxin

Endotoxin, as well as G-CSF, leads to a strong and continuous stimulation of the granuloid system. In the simulations the CSF concentration is kept at a high constant level. A constant high level of cell divisions is stimulated in granulopoiesis over the total period of the drug administration (different values for endotoxin and G-CSF). The cycles disappear because feedback III is interrupted and the cell numbers of granulopoiesis stabilize on an elevated level. Indirectly, via feedback II, this influences stem cells and erythropoiesis. The oscillations of the stem cell number disappear but their total number is predicted to increase. Furthermore, the elevated number of granuloid precursors leads to a suppression of the active fraction of stem cells and, thus, to a reduced turnover. Therefore, the flux of cells into erythropoiesis is reduced and the number of reticulocytes falls below normal. As soon as the administration of the drugs ceases, the cycles re-establish.

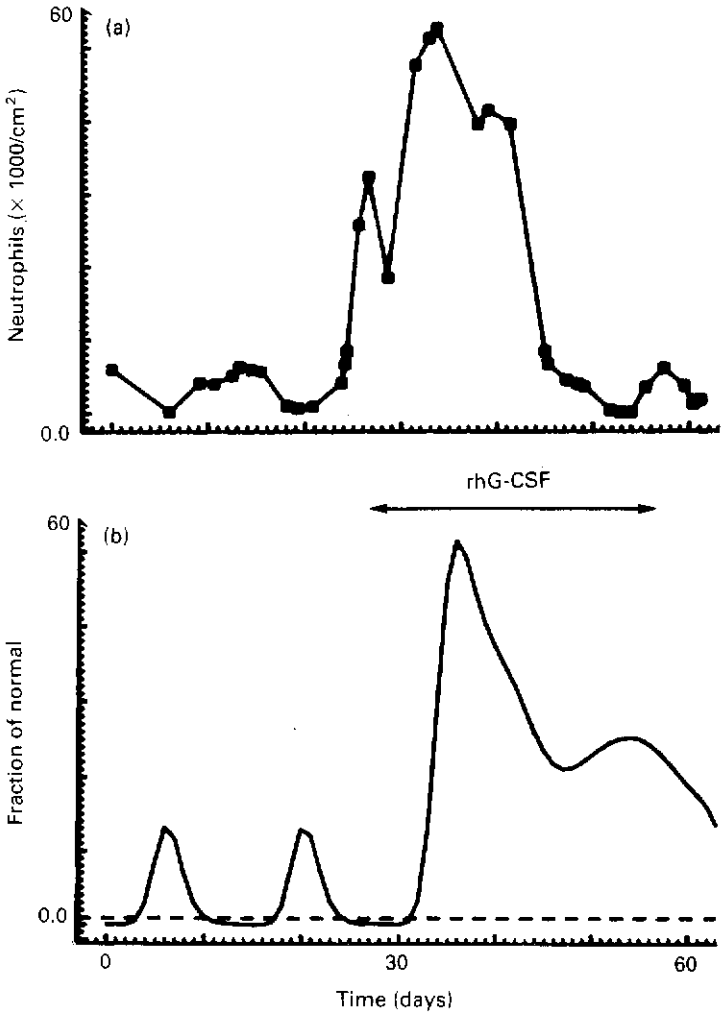

Fig. 6. Comparison of (a) experimental (-■-■-) and (b) model (——) data before and during daily rhG-CSF application from day 26 to 56. Experimental data from Lothorp *et al.* (1989). Daily application of 10 g kg^{-1} body weight subcutaneously in Grey Collie dogs. The administration of rhG-CSF leads to a steep increase of blood neutrophils and the temporary elimination of cycles.

DISCUSSION

Several authors have tried to elucidate the mathematical conditions for the origin of CH, but did not necessarily aim to quantitatively reproduce the experimental data (Morley, King-Smith & Stohlman, 1970; Wheldon, Kirk & Finley, 1974; Macdonald, 1978, 1981; Mackey, 1978; Schulthess & Maser, 1982). Wheldon *et al.* (1974) presented a qualitative analysis of a four-compartment model for granulopoiesis which includes two time-delayed feedbacks. They showed that small perturbations lead to dampened oscillations. The period of the oscillations

mainly depended on the time delay and the bone marrow transit time. The model parameters were not adjusted to the experiments. Mackey (1978) investigated CH with a stem cell model which includes a time delay for the proliferating cells. He explained CH on the basis of a defect in the stem cell area. In his model the onset of cycles required the presence of a continuous cell loss of proliferative stem cells. No detailed comparison with experimental data was performed. Schulthess & Maser examined the relationship of the bone marrow transit time to the period of oscillations in granulopoiesis (Schulthess & Maser, 1982). They found the oscillation period to be approximately twice the bone marrow transit time and a fixed time delay in the response of feedback signals. Morley & King-Smith examined the interaction of a destabilizing feedback and a stabilizing feedback acting simultaneously (Morley *et al.* (1970)). The two feedbacks act in a three-compartment model. The sensitivity of the system, e.g. the reactivity to small perturbations, depends on the relative strength of both feedbacks. CH in their model occurred if the relative strength of both feedbacks was varied in favour of the destabilizing feedback. The normal steady state is said to be the best mixture of sensitivity and stability. No detailed comparison with experimental data was performed. In a preliminary study neglecting the pluripotent stem cell compartment, three of us described the pattern of influx into erythro- and granulopoiesis which is necessary to reproduce the experimental data within a model with a small variance of transit times (Schmitz, Loeffler & Wichmann, 1986). A mathematical model of neutrophil production in man was presented by Rubinow & Lebowitz (1975). It is a five-compartment model which neglects the pluripotent stem cells and erythropoiesis. A model of canine granulopoiesis was developed by Steinbach (Steinbach *et al.*, 1980). It includes seven cell compartments and one hormone compartment. It describes the possibility of changing the age structure of the compartments by introducing sub-compartments. The system is stable due to the feedbacks to the mitotic cells. Only experimental CFU-C and blood neutrophils were compared with the model.

All of these former models focus on different aspects of CH and do not give a comprehensive reproduction of the experimental data. None of the models describes all the cell stages of erythro- and granulopoiesis together with the pluripotent stem cells. In that sense they are not extensive enough, neglecting the complex interactions between stem cells, erythropoiesis and granulopoiesis which are considered here in greater detail. Therefore it is debatable whether the results of the former models still hold in more realistic models. It should be stressed that the novelty of the present model is its comprehensiveness. Every experimentally measurable cell stage of erythro- and granulopoiesis is described. The model is challenged in the simulation of various experiments. It should be made clear that the former models are not necessarily exclusive. Some models investigated the impact of exact time delays on the stability of the systems. The Morley & King-Smith model (Morley, King-Smith & Stohlman, 1970) as well as the Schulthess & Maser model (1982) were based on equations with exact time delay. They showed that a time delay may be a necessary condition for destabilizing the model systems. They failed to show that this condition alone is sufficient to generate CH in a comprehensive model of haemopoiesis.

The model presented here was not developed solely to describe CH. It is based on, and is an extension of, the former model of Wichmann & Loeffler. No basic assumption of their model had to be abandoned to cope with the reality of CH. The model presented here quantitatively describes granulo- and erythropoiesis together with the pluripotent stem cells in dogs with normal haemopoiesis and those with CH and also reproduces the results of various experiments. Before generating the model hypothesis, different scenarios for the generation of CH were systematically investigated within the standard model of normal haemopoiesis (Table 3). These simulations show that in (the model of) normal haemopoiesis oscillation of the feedback

hormones or the stem cells alone cannot generate the characteristic cycles. Any oscillation in the model of normal haemopoiesis is strongly dampened. This is due to the large variance necessarily assumed for the normal marrow transit time. In accordance with these results, a small variance of transit times (synchrony) is simulated in the model. In conjunction with the normal granuloid feedback III, this synchrony destabilizes the system and is responsible for the generation and the maintenance of the cycles. Without a feedback III (CSF regulation), the synchrony alone would not be sufficient to generate the cycles. Thus, the primary defect of CH appears not to be a pathological cycling of the feedback hormone CSF, or impaired pluripotent stem cells or CFU-GM, although their cycling follows as a consequence. Neither is it the time delay of the feedbacks. A time delay in the granulopoietic feedback III alone, with the normal variance of the bone marrow transit time of granulopoiesis, does not generate the experimentally observed oscillations. The primary defect of CH is the small variance of the transit times.

The period of the oscillations in the model is found to be 14 d, which is within the experimentally observed period of 12–14 d. The cycle period of individual dogs is relatively stable, varying only by one day if counted over several cycles. The model period is roughly twice the time it takes for cells to mature from the middle of the CFU-GM compartment to the middle of the banded granulocytes. This relation has already been described by Schulthess in relation to human neutropenia (Schulthess & Maser, 1982; Deubelbeiss *et al.*, 1975; Cronkite, 1979).

Besides synchrony and normal CSF-regulation, no further assumption is necessary to allow a comprehensive description of the various phenomena in CH under different experimental conditions. Clearly, additional effects cannot be excluded. Thus, the present hypothesis is proposed as the minimum and most simple explanation for CH.

From the theoretical results it is predicted that even under chronic application of endotoxin and under chronic application of rhG-CSF, the synchrony of granuloid cells should still be observable in an appropriately designed experiment. Since the synchrony of granuloid bone marrow cells is not affected, the chronic application of endotoxin or rhG-CSF does not cure the underlying defect of CH. Apparently there is only one way to stop the oscillations fundamentally and that is bone marrow transplantation (Dale *et al.*, 1974).

The transplantation experiments, the pattern of heredity (Lund, Padgett & Gorham, 1970) and the synchrony hypothesis together suggest that the CH defect is genetically fixed in the pluripotent stem cells but becomes apparent and effective only in maturing cell stages. The phenomenon should be of particular interest for molecular biologists studying the steps involved in haemopoietic maturation. These cyclic dogs should be excellent subjects in which to follow the genetic changes involved in maturation and differentiation, since the changes occur in many cells simultaneously.

The constancy of cycles in individual dogs (there are 11-d as well as 14-d dogs) could suggest a clonal origin. It is possible that cyclic dogs exhibit only one, or very few, clones of haemopoiesis. If this is true, the cascade of genetic switches involved in haemopoietic maturation would be strongly preprogrammed already at the stem cell level. It is tempting to speculate whether transfusion of a 14-d cycle marrow into an 11-d cycle animal or *vice versa* can abrogate the cycles. We think that this is very possible.

In contrast to CH, a large variance may be an essential constitutive element of normal haemopoiesis. In general, no oscillations are observed in normal animals after acute irradiation, hypoxia and bleeding anaemia (Lund *et al.*, 1967; Lange *et al.*, 1976; Dunn *et al.*, 1982; Gerhartz, Nothdurft & Fliedner, 1982). This stability of normal haemopoiesis is not compatible with a small variance of transit times. If one assumes the existence of feedbacks which act as amplifiers, dampening properties must exist to prevent the system from being destabilized. A large variance of the transit times may therefore be an important property to stabilize normal

haemopoiesis. Normal haemopoiesis may be polyclonal (in the above sense) and therefore exhibit more variation which stabilizes control.

The model hypothesis presented here has still to be proven experimentally. For this purpose it will be necessary to set up experiments in which the variance of the bone marrow transit time is measured for normal dogs and for dogs with CH in comparable circumstances.

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APPENDIX I

Model equations for granulopoiesis

For the committed stem cells CFU-GM it holds that:

$$dY_{CG}(t)/dt = A_G \cdot Z_{CG} \cdot Y_S^{OUT}(t) - Y_{CG}(t) \cdot a_{CG}/T_{CG}$$

with: $Y_i(t)$ = number of cells in the compartment i at the time t;

Z_{CG} = Amplification factor = $2^{(\text{number of mitoses})}$;

Y_j^{OUT} = cell flux out of the compartment j;
a_{CG} = proliferative fraction of the CFU-GM;
T_k = transit time in the compartment k (if all cells proliferate);
A_G = fraction of differentiating pluripotent stem cells entering granulopoiesis.

For the proliferative compartments G1–G3 it follows:

$$d\,Y_{Gi}\,(t)/dt = Z_{Gi} \cdot Y_{Gi-1}^{OUT}(t) - Y_{Gi}(t)/T_i$$

with $i = (1,2,3)$ and $Gi{-}1 = G_{CG}$.

For the non-proliferative compartments it is:

$$d\,Y_{Gj}(t)/dt = Y_{Gj-1}^{OUT}(t) - Y_{Gj}(t)/T_{Gj}$$

with j = (4,5,6)

For the number of blood neutrophils:

$$d\,Y_N(t)/dt = Y_{G6}^{OUT}(t) - Y_N(t)/T_N$$

For the hormone compartment:

$$d\,Y_{CSF}(t)/dt = Y_{CSFPROD}(t) - Y_{CSF}(t)/T_{CSF}$$

with the production of CSF:

$$Y_{CSFPROD}(t) = A - B \cdot \exp(-C \cdot G(t))$$

with G = the normalized sum of all granuloid cells;
$A = CSF_{min}$
$B = CSG_{min} - CSF_{max}$;
$C = \ln[(CSF_{min} - CSF_{max})/(CSF_{min} - CSF_{norm})]$.

For the amplification factor Z_{CG} which is CSF dependent it holds that:

$$Z_{CG} = D - E \cdot \exp(-F \cdot CSF(t)/CSF_{norm})$$

with: $D = Z_{CG}^{max}$
$E = Z_{CG}^{max} - Z_{CG}^{min}$;
$F = \ln[(Z_{CG}^{max} - Z_{CG}^{min})/(Z_{CG}^{max} - Z_{CG}^{norm})]$.

The general form of the differential equations for any compartment Y is:

$$d\,Y/dt = Y_{-1} - k \cdot Y$$

with Y_{-1} input from the foregoing compartment.

Assuming the compartment Y is subdivided into n sub-compartments Y_i it holds that:

$$d\,Y_i/dt = Y_{i-1} - k_i \cdot Y_i.$$

In any sub-compartment, the transit time is exponentially distributed with the average transit time $1/k_i$ and a variance $1/k_i^2$. The average transit time of the compartment Y is:

$$T = \sum_{i=1}^{n} 1/k_i.$$

For the variance of the transit time it holds that:

$$v^2 = \sum_{i=1}^{n} 1/k_i^2.$$

The coefficient of variation is v/T.
If all the sub-compartments are equal, it is:

$$T = n/k_i; \qquad v^2 = n/k_i^2 \qquad \text{and} \qquad v/T = 1/\sqrt{n}.$$