Blood Cells (1988) 14:411–429

Blood Cells
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# A Concept of Hemopoietic Regulation and Its Biomathematical Realization

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ABSTRACT. **Although the amount of experimental data on the behavior of the hemopoietic system after various perturbations is considerable, a conclusive understanding of hemopoietic regulation is still absent. In the last years, we have examined murine erythropoiesis, thrombopoiesis, granulopoiesis, and stem cell hemopoiesis by means of mathematical modeling in order to identify some of the underlying principles. Our results can be summarized in four hypotheses. 1) The regulation of hemopoiesis is governed by three interrelated control loops: autoregulation of stem cells, feedback from progenitors and precursors to the stem cells, and feedback from mature cells to progenitor and precursor cells. 2) The feedback from mature cells to the progenitor and precursor cells predominantly varies the number of cell divisions taking place during hemopoietic maturation. 3) Two distinct properties of the stem cells are regulated: their cyclic activity and their self-renewal. Both are under the control of stem cell autoregulation and the feedback from progenitors and precursors. 4) A large variance in the maturation time from the stem cells to the mature cells stabilizes the hemopoietic control. The mathematical formulation of these assumptions allows us to understand a broad range of experimental observations including recovery from stem cell damage, hypoproliferative and hyperproliferative situations, and interactions between different cell lines.**

KEY WORDS: **Hemopoiesis – Erythropoiesis – Granulopoiesis – Regulation – Mathematical Model**

## INTRODUCTION

The first mathematical model of hemopoietic regulation had been proposed by Laszlo Lajtha and colleagues in 1962 to describe stem cell proliferation and differentiation [1]. The authors subdivided the stem cell pool into resting $G_0$ cells ("available stock") and cells in active cycle ("triggered stock").

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The stem cells differentiated from the resting compartment where the differentiation rate was regulated by a hormone. That article has stimulated many biologists and biomathematicians and since then, numerous concepts have been cast into the frame of mathematical models (see review [2]).

In the last few years, our group, as did others, attempted to identify some of the underlying principles of hemopoietic control [3–7]. Based on this experience, the constituents of our concept are summarized in four hypotheses we consider essential to a comprehensive understanding of hemopoietic regulation. After discussing each of them, their consequences are illustrated by comparing experimental data with model simulations.

Some of the assumptions involved in the hypotheses have been formulated by other authors. Because these publications have been reviewed in detail elsewhere [2, 3] it may be legitimate to concentrate in this presentation on one concept rather than discussing many.

## FOUR HYPOTHESES

### *Hypothesis I. The Regulation of Hemopoiesis Is Governed by Three Interrelated Control Loops: Autoregulation of Stem Cells, Feedback From Progenitors and Precursors to the Stem Cells, and Feedback From Mature Cells to Progenitors and Precursors*

Figure 1 demonstrates a simplified schematic representation of this hypothesis. It is generally accepted that the stem cells participate in the control of their own population. There appears to exist a self-control that stabilizes the population size at a certain "normal" level and counteracts any depletion or elevation. Such a process would explain the recovery of the stem cell number after severe depletion due to irradiation or cytotoxic drugs and it would explain hemopoietic reconstitution after marrow transplantation of only a few stem cells. Beyond this, a self-control of this type appears to prevent an inadequate expansion of the stem cell population. Any increase above normal appears to be only temporary. Stem cell numbers can adopt subnormal or supranormal values, but as long as regulation is intact, the number returns to normal after a perturbation has disappeared.

The feedback from mature cells back to their ancestors in the bone marrow (and spleen) was identified very early and is, for example, reflected by an increase of erythropoietic progenitors (CFU-E) and precursors (erythroblasts) after bleeding or during hypoxia. The decline of bone marrow erythroblasts after red cell transfusion is a consequence of the same control process. The trigger of control can be an associated parameter (such as the hematocrit or the tissue oxygen pressure) rather than the mature cells themselves. Similarily, one finds increases in megakaryocyte number and volume in case of thrombocytopenia. Some "growth factors" involved in the feedback loops from blood to bone marrow have been identified (EPO in erythropoiesis, various CSFs in granulopoiesis, thrombopoietin activity in thrombopoiesis). It is becoming increasingly clear, however, that there

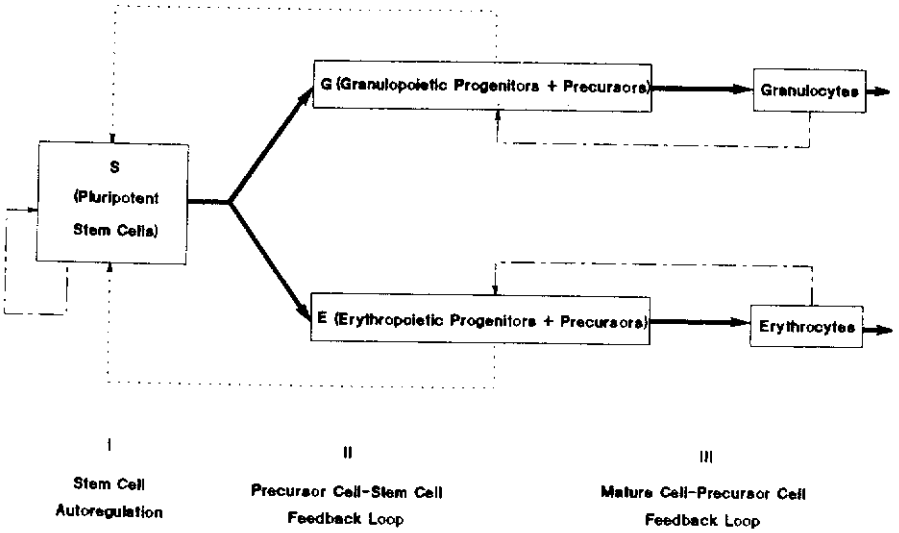


Fig. 1. General concept of hemopoietic regulation. Three types of interrelated feed back loops are identified: one for the autoregulation of stem cells, another for the feedback from the progenitor and precursor cells to stem cells, and a third for a feedback from the mature cells to the immature marrow progenitors and precursors.

exists a network of regulatory cells, e.g., fibroblasts, monocytes and macrophages, lymphocytes – and other factors – interleukins, interferons, tumor necrosis factor – that interact in a complicated way to make the bone marrow produce the right type and number of functional cells.

In the early era of stem cell research, one thought that stem cells were also under some control related to the mature cell stages. Later it became evident that EPO and CSF do not act on pluripotent stem cells but on committed cell stages (CFU-E, CFU-GM). Consequently, a gap in our knowledge became apparent. How could changes in stem cell number or their cyclic activity, which were obviously associated with changes in the demand of the mature cell stages, be explained if no direct message from these mature cells to the stem cells could be found? We therefore investigated whether feedback from progenitors and precursors to stem cells could be a possibility [3, 6]. Instead of being regulated by parameters associated with the mature cells, stem cell behavior could be under some control of the progenitor (BFU-E, CFU-E, CFU-GM) or precursor cells (myeloblasts, myelocytes, neutrophils, normoblasts) that are, in turn, regulated by the mature cells. If the contribution to control is approximately the same from each individual cell within each lineage, the precursors will be more important in such a feedback than will the progenitor cells because they are more numerous.

The basic idea of hypothesis I is that hemopoietic regulation is based on the interaction of several (at least three) control processes. The advantage would be that each of the control levels can already cope with a broad range of requirements, thereby protecting some parts of the hemopoietic

system, whereas others may react in response to demands. Weakly coupled feedback loops in this sense could have major relevance for the stability and flexibility of the overall system.

In the subsequent two hypotheses we will focus on the presumptive nature of these control processes.

*Hypothesis II. The Feedback From Mature Cells to the Progenitor and Precursor Cells Predominantly Varies the Number of Cell Divisions Taking Place During Hemopoietic Maturation*

The implications of this hypothesis can best be illustrated in erythropoiesis. Although the number of cell divisions in the erythropoietic pathway is not precisely known, it may be in the order of 12–17. Thus, from each immature cell entering this pathway, tens of thousands of mature descendents will originate. Omission or addition of one cell division will halve or double the overall cell production of mature blood cells.

We believe that the regulation of the number of cell divisions in CFU-E and proliferative erythroblasts is the major control point of the feedback from the mature cells. The number of mitoses should depend in a dose-response fashion on a parameter associated with mature cells as for example, on the hematocrit (see Fig. 2, bottom). The two curves represent the basic model features for mice (dashed) and rats (full). Any reduction in hematocrit will lead to an increase in the number of cell divisions; any increase of the hematocrit above the normal value will lead to a reduced number of cell divisions. However, there are limitations. Under extreme conditions, not more than three to four divisions can be added, and up to six mitoses can be skipped.

Addition (omission) of cell divisions is not associated with a prolongation (shortage) of the marrow maturation time. Rather, the opposite is the case (see Fig. 2, top). A reduced hematocrit leads to a shortage of the average marrow transit time. Thus, more cell divisions take place in a shorter period. Because there are minima for the cell cycle time and the maturation time, the number of cell divisions cannot increase beyond a certain maximum. Conversely, for suppressed erythropoiesis, cell maturation can go on at a somewhat slower pace, but does not cease completely.

It should be pointed out, however, that control of the marrow transit time alone would not be a mechanism to satisfy the demand for more cells over a longer period. Any acceleration in maturation that is not accompanied by more cell divisions would only result in a temporary elevation of the blood cell counts. Therefore, the control of the cell division rate appears as the superior control mechanism. This concept implies the prediction that cell division rate and cell maturation are only weakly correlated processes in amplifying precursor cell stages. Biologically, this means that cells entering such a cell stage (e.g., CFU-E) may undergo just a few additional cell divisions in vivo before finally maturing to the next stage (e.g., proerythroblast).

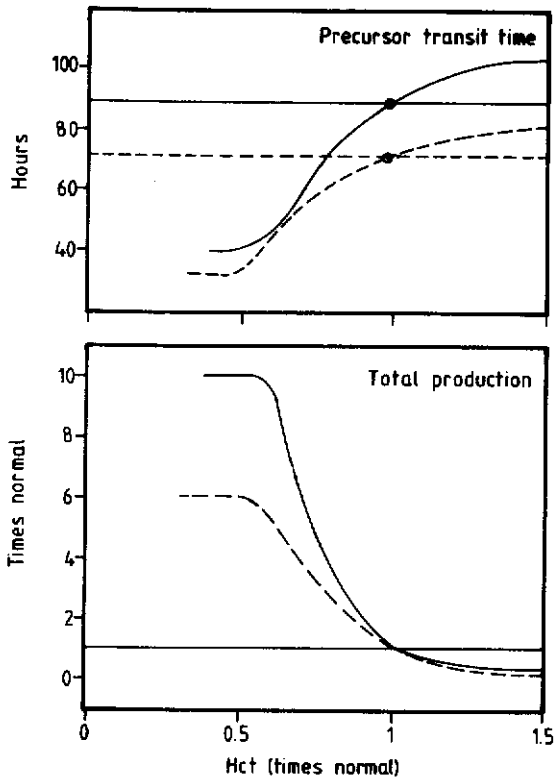


**Fig. 2.** Model curves for the precursor transit time and the total amplification rate of the erythropoietic bone marrow cells depending on the hematocrit (Hct) for rats (full) and mice (dashed).

Using these assumptions, one can run model simulations and compare them to experimental data. In Figure 3, an example is shown for the recovery of erythropoiesis in rats following acute blood loss. The data are taken from various reports. As a result of the low initial hematocrit, the CFU-E and erythroblasts become more numerous due to additional cell divisions. A wave of erythropoiesis begins that is regulated down as soon as the hematocrit recovers.

With an analogous concept, one can also describe thrombopoiesis in which the primary target of the feedback from mature platelets is the number of endomitoses in megakaryocytes [4, 5].

*Hypothesis III. Two Distinct Properties of the Stem Cells Are Regulated: Their Cyclic Activity and Their Self-Renewal. Both Are Under the Control of Stem Cell Autoregulation and Feedback from Progenitors and Precursors*

There are two elements involved in this hypothesis. One relates to the properties of stem cells, the other to the control of these properties.

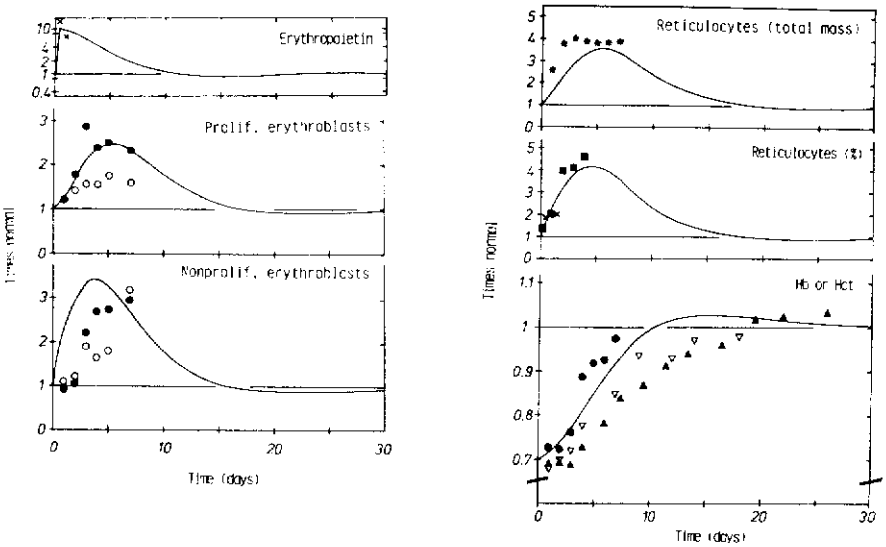


**Fig. 3.** Changes in erythropoiesis after an acute blood loss of 30% in rats. Model curves (——) Experimental data: × Epo-level, reticulocytes % [10]. ● Hct, erythroblasts [11]; ▲ total $Hb_{mass}$ [12]; ▽ Hb-concentration [13, 14]; ■ reticulocyte % in the blood [15]; * total mass of reticulocytes [16].

We assume that cyclic activity and the ability to increase or decrease their number are two distinct properties of stem cells that are regulated in different ways. The cyclic activity is measurable by the fraction of stem cells in cycle (i.e., not in $G_0$ phase), which shall be denoted by "$a_s$". In Figure 4, $G_0$ cells are illustrated by the large reservoir.

The concept of self-renewal of stem cells refers to the fact that individual stem cells can give rise to a number of subsequent cells of the same kind. Self-renewal is difficult to quantify on an individual cell basis, which has caused and still causes a great deal of debate. Therefore, we prefer to describe the behavior of a pool of many cells rather than the behavior of individual cells (although the sketch in Figure 3 may indicate the contrary). This leads to the probabilistic point of view and the concept of a self-renewal probability ("$p$"), making clear that the behavior of an individual cell cannot be predicted, but only the average behavior of the whole population. Roughly speaking, the concept of the self-renewal probability is similar to the concept of the growth fraction often used to describe tumor growth (Growth fraction $GF \approx 2p-1$). Under steady-state conditions, the self-renewal probability of stem cells is $p=0.5$, that is, after mitoses, one half of the daughter cells remain in the stem cell pool, while the second half enters differentiation to granulocytic or erythrocytic progenitors or other lineages. Under certain circumstances, this probability may be changed to become more or less than 0.5, such that the stem cell numbers increase or decrease.

The second element of the above hypothesis concerns the way by which these two stem cell parameters could be regulated. This is displayed by

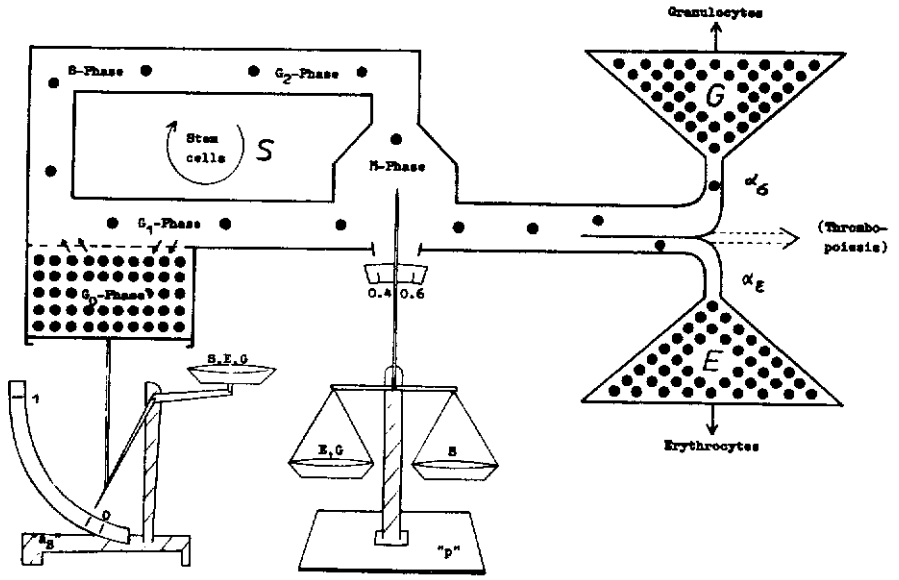


**Fig. 4.** Schematic representation of the model. Stem cells in normal steady state. The fraction $1-a_S$ of the stem cells $S$ is in a resting phase ($G_0$), whereas the fraction "$a_S$" is in active cell cycle. The self-renewal probability "$p$" equals 0.5, e.g.; after mitoses of the newly formed cells, 50% remains stem cells and 50% differentiate. Of the differentiating cells, the fractions $\alpha_E$ and $\alpha_G$ develop into the erythropoietic ($E$) and granulopoietic ($G$) progenitors and precursors. Thrombopoiesis is not considered.

the letter scale and the balance in Figure 4. The weights on them are considered to correlate with the demand for stem cells "S," erythropoietic "E," or granulopoietic "G" progenitors and precursors (see Fig. 1). Demand is defined as the difference between normal cell numbers and actual cell numbers. A demand (weight) can be positive (fewer cells present than required) or negative (more cells present than required).

If there is a demand for stem cells (lack of cells), the right scale in the balance for $p$ will be reduced and the self-renewal probability will increase. If there is a demand for differentiated cells (lack), the left scale will be reduced and $p$ will decrease below 0.5. Thus, there is an antagonism between stem cells and differentiated cells for the newly produced daughter cells. Depending on whether the need for one of these sides is more prominent, $p$ will increase or decrease.

In contrast to self-renewal, the fraction of stem cells in the cell cycle is influenced by stem cells and differentiating progenitors and precursors in a synergistic way: if there is a demand for either of these cells, more stem cells are activated. In normal steady state, only 15% of the stem cells are actively cycling, 85% being in a quiescent $G_0$ phase. For maximum stimulation, all cells may be activated.

Figure 5 shows how the activation of stem cells influences the hemopoietic system. Here, an isolated activation of the stem cells is assumed,

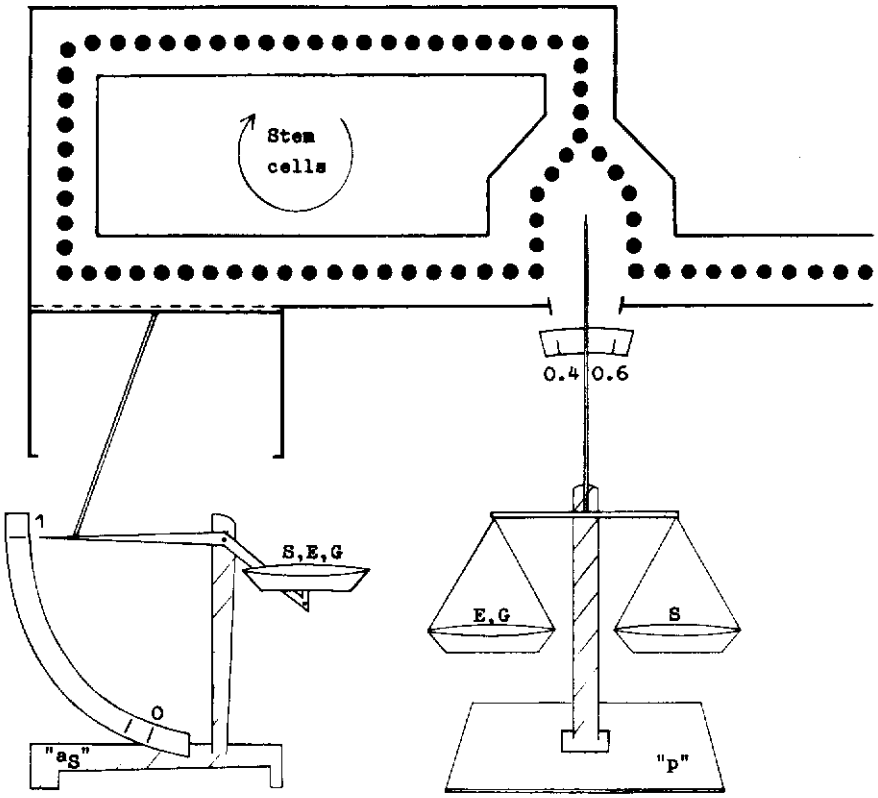


**Fig. 5.** Increased cyclic activity of stem cells with normal self-renewal probability. If all stem cells are in active cell cycle ($a_s = 1$), more new cells are formed per unit time. This leads to an increased flux of cells into differentiation and thus to an increase of the number of differentiated cells. However, the number of stem cells remains constant as long as the self-renewal probability is normal ($p = 0.5$).

with a normal self-renewal probability. As a consequence, the stem cell population has a much faster turnover than normal. However, its size remains constant although all previous $G_0$ cells are now in cycle. On the other hand, the number of cells entering differentiation per unit time is increased significantly. In total, isolated activation of stem cells (increase of $a_s$) leads to an increased flux of cells into differentiation without changing the stem cell number.

A different behavior is found if the self-renewal probability $p$ is varied (Fig. 6). If $p$ increases, on average more than 50% of the daughter cells remain in the stem cell compartment. Thus, the number of stem cells will increase exponentially. One important result of the sensitivity analysis performed with the model is that the range in which $p$ may vary is quite narrow, namely between 0.4 and 0.6 [3, 6]. As a consequence, a change of $p$ mainly influences the stem cell number but not the flux into differentiation.

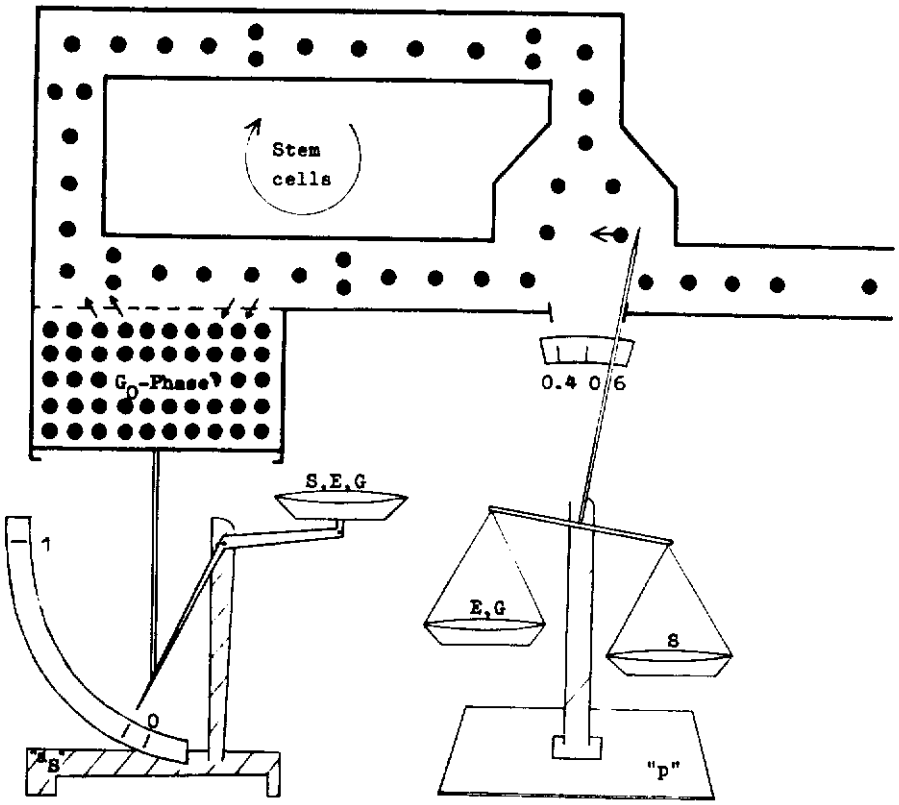


**Fig. 6.** Increased self-renewal probability with normal fraction of active cells. If the self-renewal probability is increased, the number of stem cells increases because more than 50% of the postmitotic cells remain stem cells.

Further details of the model are described elsewhere [3] and can be omitted here. Instead, some examples shall be discussed to illustrate the implications of this hypothesis. The following abbreviations will be used:

| Stem Cells | Progenitor Cells | | | Precursor Cells | |
|---|---|---|---|---|---|
| experiment: | | | | | |
| CFU-S, | BFU-E, | CFU-E, | CFU-GM | ERYPREC, | GRANPREC |
| model: | | | | | |
| S, | BE, | CE, | CG | E, | G |

where the last two terms denote all morphologically identifiable erythropoietic or granulopoietic precursors respectively.

In Figure 7, experimental data and model curves for the recovery of CFU-S after acute whole body irradiation are shown. In the model, the

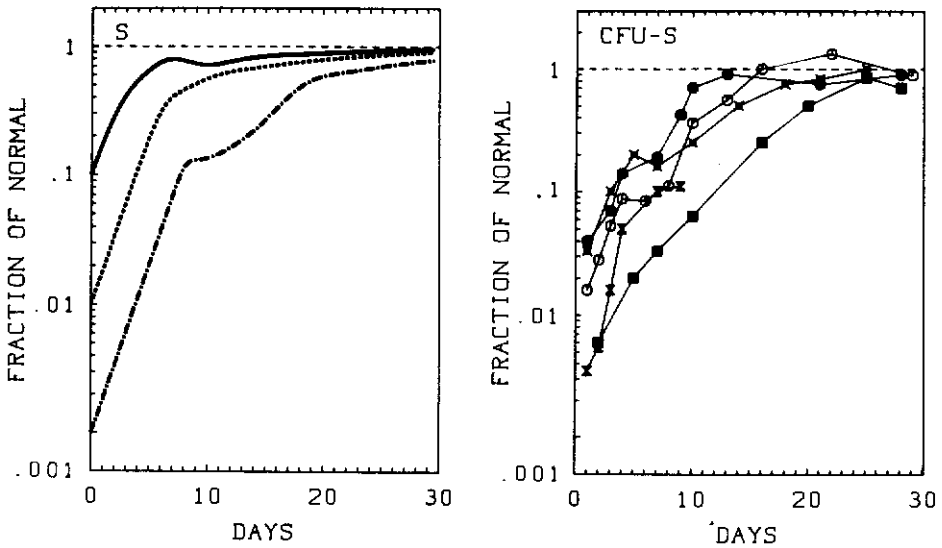


**Fig. 7.** Acute irradiation: comparison of model curves (left) and experimental data. In the three model calculations, the stem cells are reduced to initial values of 10% (——), 1% (– –), 0.1; (–·–·–). Experimental data from the bone marrow of mice. CFU-S: 3.0 Gy (○), [17]; 5.0 Gy (■), [18]; 4.0 Gy (⧗), [19]; 3.0 Gy (▣), [20]; 2.0 Gy (●), [21].

destruction of cells due to radiation damage is simulated by the reduction of the initial values in the affected cell compartments. Three different model calculations that are shown indicate the stem cell number reduced to 10%, 1%, or 0.1% respectively. Due to the overwhelming impact of the autoregulation process, the stem cells recover exponentially during an initial phase until their number has reached 10% or more of the normal values. Thereafter, the precursor stem cell control begins to gain some influence and the demand for stem cell differentiation becomes effective. The increase of the stem cells becomes slower and may even show an intermediate stop, before the final recovery starts.

As shown in more detail elsewhere [3, Vol. I, chap. 7], the above hypothesis also leads to a fairly satisfactory explanation of a large variety of recovery patterns observed after irradiation in other cell stages (e.g., BFU-E, CFU-GM, CFU-E, erythroblasts) and relates them to varying degrees of radiosensitivity. As shown, the autoregulation of stem cells may play a prominent role in the postirradiation recovery of stem cells.

In contrast, the reaction of stem cells to red cell hypertransfusion (a manipulation remote from the stem cells) may be an example where the precursor stem cell loop is more involved (Fig. 8). Immediately after red cell transfusion, the regulation is dominated by a process according to hypothesis II: the enlarged number of erythrocytes suppresses cell divisions and leads to the reduction of the CFU-E and the erythropoietic precursor cells. Consequently, the hematocrit will decline (not shown). In the meantime, the low number of erythropoietic precursor cells leads to an activation

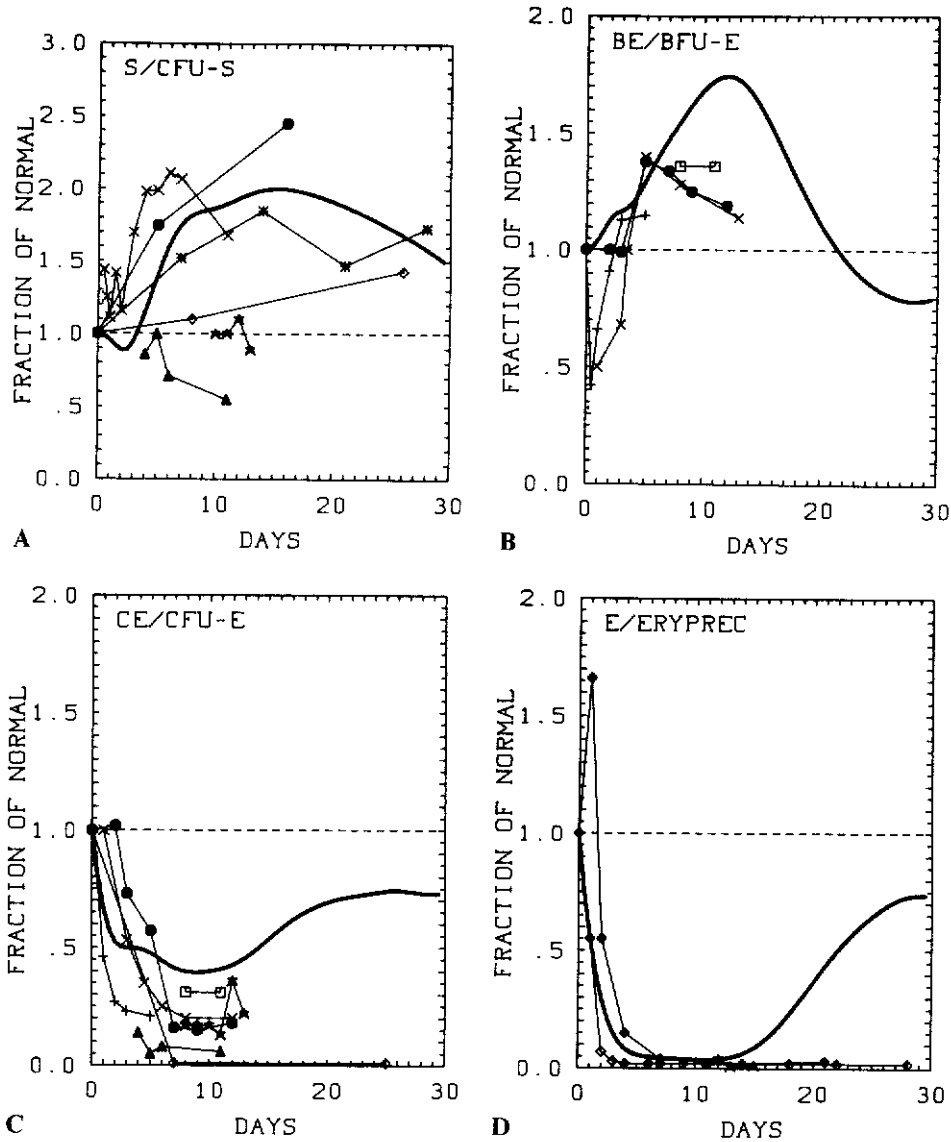


**Fig. 8.** Hypertransfusion. Comparison of model results (——) and data. The model simulations correspond to an initial hematocrit of 150% of normal, which normalizes within 25 days. The data are from the bone marrow of mice: (BFU-E, CFU-E, CFU-GM, □, [22]; (CFU-S, BFU-E, CFU-E, ●), [23, 24]; (CFU-S, CFU-E, CFU-GM, ▲), [25]; (BFU-E, CFU-E, +), [26]; (CFU-S, BFU-E, CFU-GM, ×) (CFU-GM, ◇), [27, 28]; (CFU-S, BFU-E, ERYPREC (original data: Fe uptake)), [29]; (CFU-S, CFU-E, CFU-GM, erythropoietic precursors, ★), [30, 31]; (CFU-S, ∗), [32]; (erythropoietic and granulopoietic precursors, ♦), [33].

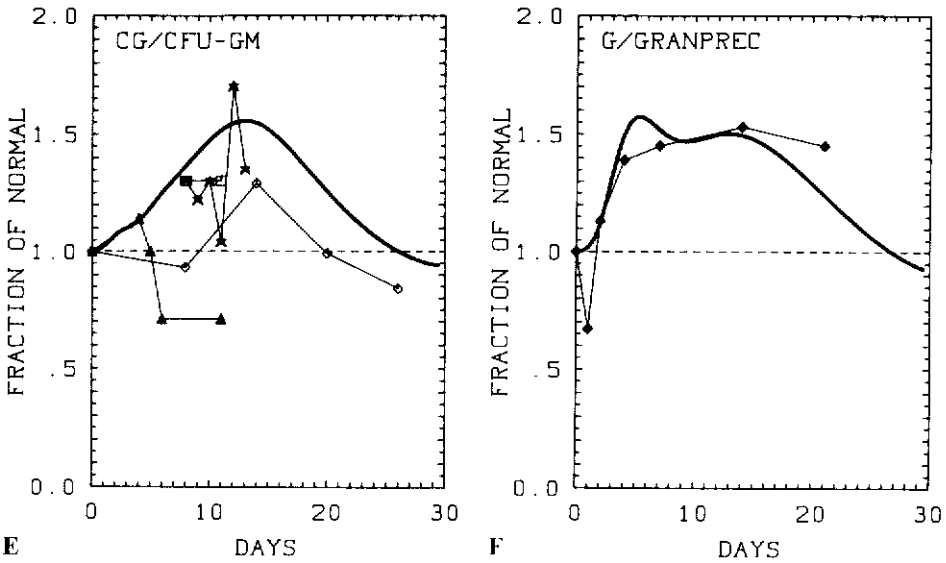


**Fig. 8** (continued)

of stem cell cycling. This enlarges the flux from CFU-S into differentiation, leading to a considerable increase in BFU-E, CFU-GM, and granulopoiesis. This overproduction of granulopoietic cells changes the status of the balances again. As granulopoietic precursors have a great impact on the stem cell self-renewal in the model, this parameter changes such that the number of CFU-S increases until stem cell autoregulation limits this process. We conclude with the following pattern: CFU-S and all differentiated non-EPO-sensitive cells (BFU-E, CFU-GM, myeloblasts) are increased, all EPO-sensitive cells are decreased. A similar response is found after termination of prolonged hypoxia [3, Vol. II, chap. 3; 7].

The concept of interaction between the autoregulation of stem cells and feedback from progenitors and precursors to stem cells has also been applied in the modeling of other experiments such as chronic irradiation, hypoxia, anemia, the administration of $^{55}$Fe or cytostatic drugs [3]. It seems to provide a consistent understanding of many different observations, in particular on the reaction of erythropoiesis and granulopoiesis and their relationship as it is mediated by stem cell regulation.

### *Hypothesis IV: A Large Variance of the Maturation Time From the Stem Cells to the Mature Cells Stabilizes the Control*

An example may illustrate the influence of the variance of the maturation time. Let us consider a narrow cohort of cells that enters granulopoiesis (Fig. 9). After some maturation time, the descendants will become mature neutrophils. If the variance in the marrow maturation time is small (e.g.,

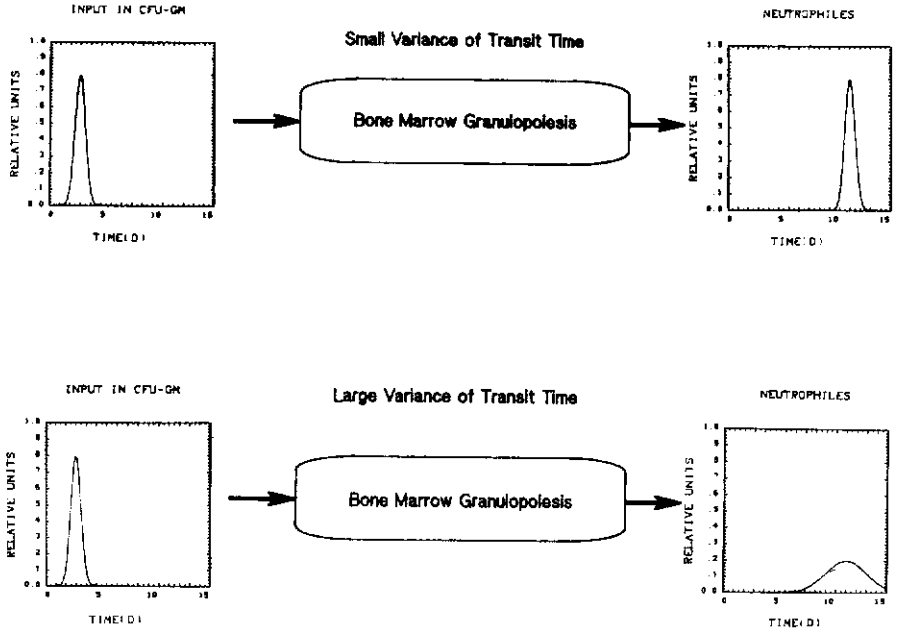


**Fig. 9.** Implication of the variance of transit times in granulopoiesis. A small variance of transit times causes a narrow wave at the beginning of granulopoiesis to stay narrow, whereas a large variance causes the same wave to broaden during its travel through granulopoiesis.

10%) the cells will mature in a synchronized way and leave the bone marrow as a narrow cohort (Fig. 9, top). If the variance in maturation time is large, the cohort will disperse as demonstrated by a broad and flat peak. If one starts to model a feedback loop in hemopoiesis, one will find that this variance is a sensitive parameter. We were unable to construct useful models with a small variance of the maturation time because the system began to oscillate. Only with a large variance (30% or more), as shown in Figure 9 (bottom), were we able to reproduce the responses to hemopoietic stress situations experimentally observed. A large variance of maturation time means that the individual cells do not mature with the same velocity, i.e., in a synchronized way, but that some mature more rapidly than others. We believe that the absence of a large variance in the transit time can be the cause of disease, as is illustrated in the following example.

Grey collies have an intrinsic defect of hemopoiesis, which leads to oscillations of all hemopoietic cell lineages with a stable period of 11–14 days [8]. To study this phenomenon by mathematical modeling, first a model of erythropoiesis and granulopoiesis in the normal dog had to be implemented using, in part, an earlier work by Steinbach and coworkers [9]. Using this model [34], it was possible to reproduce the oscillations by the

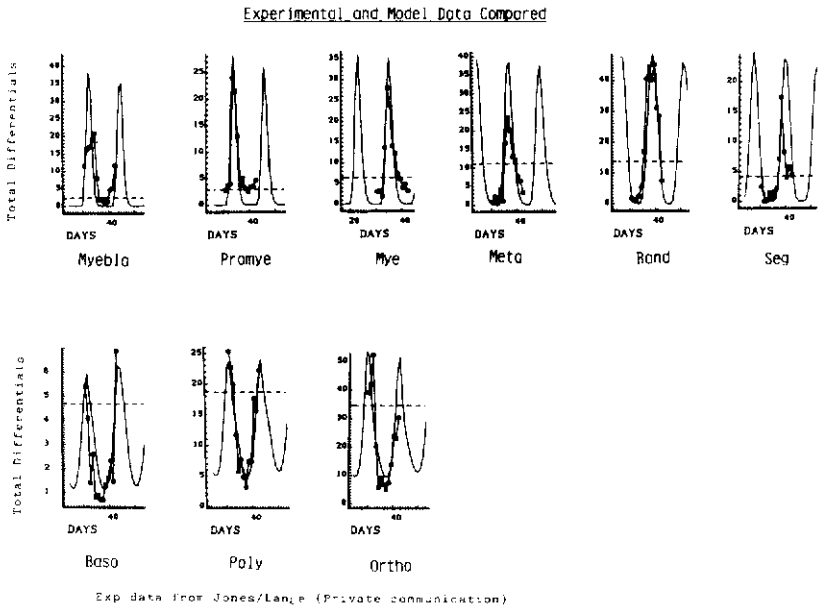


**Fig. 10.** Cyclic hemopoiesis in grey collie dogs. Comparison of model results (——) and experimental data. Experimental data from Jones and Lange [personal communication] for myeloblasts, promyelocytes, myelocytes, metamyelocytes, banded and segmented neutrophiles, basophilic, polychromatic, and orthochromatic normoblasts obtained in percent of marrow differentials.

hemopoietic cells to undergo subsequent maturation steps in a highly synchronized manner.

In this model, the synchronization of hemopoietic cells in grey collies is generated by a small variance of their transit times (Fig. 10). A detailed model-analysis shows that under this assumption, small fluctuations in granulopoiesis are amplified by the granulopoietic feedback loop from mature cells to the progenitor and precursor cells and that the oscillations are transferred from these to the stem cells by the corresponding feedback loop. As a secondary phenomenon, the erythropoietic lineage also begins to oscillate.

The model predicts, as is found experimentally, that neither anemia, hypoxia, nor hypertransfusion can eliminate the oscillations. However, most interestingly, the experimental result—that during chronic application of endotoxin the oscillations are extinguished—can be well understood within the model. The granulopoietic feedback loop from mature cells to progenitors and precursors is paralysed by endotoxin. A similar symptomatic cure should be expected if CSF was infused continuously. If CSF application is stopped the cycles should reappear. As the defect in grey collies is not related to the hemopoietic microenvironment but to the stem cells, one may conclude that the defect could be localized in the maturation program of all offspring, that is, of all hemopoietic cells. The defect may be called "synchronization." A more detailed analysis is given by Schmitz [34].

## DISCUSSION

In order to assess the relevance of biologic assumptions and explications on a quantitative basis, we tend to cast them into a mathematical shape such that their consequences can be calculated. In this respect, we try to go beyond the mere verbal explanations often given in biologic papers.

In the preceeding four hypotheses, we summarized our present view of the basic principles of hemopoietic regulation. They are based on biomathematical modeling of hemopoietic cell kinetics after a variety of perturbations.

The proposed concept of stem cell regulation may be challenged. Therefore, it may be helpful to relate it to some special concepts under present discussion.

In the model, a self-renewal probability is considered explicitly only for the stem cell population. For the progenitor and precursor cell stages, an age structure was used with a certain number of mitoses. However, instead of considering, for example, the CFU-E population as a pipeline with a given number of mitoses, one may take the equivalent view that CFU-E is a homogenous population with a certain self-renewal probability $p$ that must be below 0.5 because it is not a self-maintaining cell. Actually, five divisions would be roughly equivalent to a $p$ of 0.48 (for details see [3, Vol. I, chap. 3]). In this view, hypothesis II would take the form: *The feedback from mature cells to progenitors and precursors predominantly varies the self-renewal probability under the condition that it is always smaller than 0.5.*

In the model, stem cells are considered as a homogenous population. However, in the last years, subpopulations of stem cells with different properties have been discussed. Can both concepts be reconciled? Let us assume that the stem cells and progenitor cells are not distinct populations but rather sections of a continuum of cells with a decreasing stemness. This continuum would represent an age structure with cells having an inherent clock that counts generations. Each of the subpopulations within this series could have its specific self-renewal probability. If regulatory influences are considered, it is quite reasonable that the boundaries in which "$p$" may vary are different for the different subpopulations. There may be one group of (primitive?) stem cells that have a maximum "$p$" of 0.7 or more, and a different subgroup (late ones?) with a maximum "$p$" of 0.51. This may be compatible with an average maximum value of 0.6. As in the above model, only the average behavior is considered, the possibility of subpopulations with higher or lower values of $p$ is not excluded. However, the criterion of a stem cell pool would be that "$p$" can be larger than 0.5 in contrast to the committed cells. Within this frame, some readers might favor the view that hemopoietic maturation is expressed as a process of gradual loss of self-renewal probability from high-potential stem cells ($p$, very high) via low-potential stem cells ($p$, just above 0.5), and committed proliferative cells ($p$, below 0.5) to nonproliferative cells ($p=0$).

The four hypotheses imply mechanisms of hemopoietic regulation that guarantee a considerable stability and flexibility to cope with a broad range

of requirements. The separation of an overall feedback into three levels may be useful to stabilize the whole system and to enable a rapid response to a damage in whichever part it may occur. All these control processes seem weakly coupled. The correlation between cell division and cell maturation also does not seem to be strict under all circumstances. The more primitive the cell stages, the weaker appears the coupling.

The model proposed has implications for future experiments. First, it will be interesting to look for more direct proof of the stem cell autoregulation and the precursor–stem cell regulation. Although rapid progress is made in this respect with the identification of "growth factors" and their mode of action, little is known about the specific stimuli under which they are produced in vivo. More emphasis should be given to this question. Second, the injection of growth factors, for example, GM-CSF will lead via feedbacks to secondary changes in the stem cell pool and accompanying cell lines. The activation of feedback processes indirectly involved may not always be advantageous. Third, the model can be used to identify more specifically the effects of drugs on the hemopoietic system. Besides cytotoxic activity, a number of drugs may also exhibit modulatory activity on the feedback processes. Fourth, in the long run, quantitative modeling may support the design of chemotherapy or growth factor drug regimens.

*Acknowledgments.* Our work is supported by the grants Lo 342/1-2 and Wi 621/1-3 by the Deutsche Forschungsgemeinschaft.

We wish to thank Drs. J.B. Jones and R. Lange (Knoxville, Tennessee) for the permission to use unpublished material and for discussions. M.L. thanks Christopher Dunn, Willem Nijhof, Michael Mackey, and Jean Yves Mary for discussions on the topics that entered into this manuscript. We thank S. Gontard for help with the manuscript.

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## Discussion

CRONKITE—I appreciate your mathematical model but I would like to bring up some biologic questions. In our experiments on irradiation we never see the 10-day CFU-S return to preexposure levels. They only come up to about 80% of the control values. How do you explain this?

If persons are bled for many, many days by a small amount of blood, such as 10 ml per day, in a little while there will be a small increase in reticulocytes and the red cell number remains constant without changes in $O_2$ tension, delivery, or radioimmune assay of Epo. Thus, there is a mechanism other than lower $O_2$-dependent stimulation of Epo that regulates red cell numbers.

I have only seen two cases of cyclic neutropenia in my life. There are usually inflammatory lesions that develop before the return of the granulocytes. One has to incorporate this thinking at the biologic level. Inflammation may participate in stimulating the return of the granulocyte to normal.

WICHMANN—You mention the phenomenon that, after acute irradiation, CFU-S do not always return to normal. I think there are some experiments in which they return to normal, but there are others in which the CFU-S stay at a subnormal plateau. The reason for this behavior is not understood. One explanation might be that there is residual damage of the microenvironment that does not allow the system to return to normal. In our model we did not yet consider residual damage; it is constructed for situations in which hemopoiesis normalizes after the experimental perturbation has disappeared. The majority of experiments we have considered are of this type.

Experiments with a small loss of red cells due to continuous bleeding have not been analyzed with the model. Until now we have only looked at strong perturbations, such as severe bleeding, which obviously lead to stimulation via EPO. To keep the model simple, we have concentrated on the major pathways of regulation first, but obviously a refinement of the model is necessary in many respects. Before you can refine a concept, you must be sure that the concept itself is appropriate and that is what we have tried to do until now.

Finally, you mention inflammation as an important factor in cyclic neutropenia in man. I, too, think it is important in light of our analysis of cyclic hemopoiesis in grey collies. As I pointed out, for us the oscillations are started by some "random noise" in the granulopoietic cell numbers that is amplified via CSF. Because inflammation also stimulates the formation of CSF, I could imagine that inflammation in man takes over the role of "cell synchronization" I have described for the dogs to follow from a small variance of the transit times in the bone marrow. However, since we have not yet analyzed cyclic neutropenia in man, this statement is speculative.

FLIEDNER—Two comments. With respect to the radiation effects. We found that, in trying to simulate the effects of radiation injuries in man

and in dogs, we cannot simulate the peripheral events unless we assume that there is in the stem cell pool a group of stem cells that are dead but do not know it yet. In other words, there is the "injured cell" hypothesis telling us that some of these cells, on the basis of radiation injury, may have a limited number of cell replications and then die out; if this is a distribution of probability, one comes to a way of simulating what really happens. I think there is something in the body that responds to a number of cells and compartments, because apparently, the mechanisms recognize that there are cells, but when they are recruited they are not as efficient as normal cells. Therefore, you have a variation that should be considered. With respect to replication, I really feel that we should encourage everybody who has hidden material on self-replication of stem cells, fetal or whatever, to publish it. Although, you say it is unlikely, there is a replication rate of 95%.

WICHMANN – Second part first. It would be a misunderstanding if you take the message from our model description that self-renewal probability in general is limited to $p=0.6$. We only find that this value of 0.6 seems to be correct as an average of the whole CFU-S population. You will agree that there are clear indications for a substructure within the CFU-S compartment that is not considered in our simplified approach. I believe that the earlier CFU-S may have a higher maximum self-renewal capacity that may be close to $p=0.95$ or even $p=1$. The later the cells are, the more likely they experience a decrease in maximum self-renewal, and it may be close to 0.55 or even 0.5 for mature CFU-S. However, the average for all CFU-S, early and late, should be close to 0.6.

Your first question was related to the damage of stem cells by acute irradiation. I think Jim O'Kunewick was the first to favor the idea of an "abortive rise" in the recovery after irradiation due to a subpopulation of impaired cells that perform one or two mitoses before they die. As we have shown in detail in our book [1], in which Jim O'Kunewick has written one chapter [2, pp. 109–124], we are able to understand the experimental observations without this assumption, only by intramedullary feedback. That does not mean that we can rule out the mechanism you have described, but we do not need it for our interpretation.

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