International Journal of Cell Cloning 2: 408-424 (1984)

The Kinetics of Granulopoiesis in Long-Term Mouse Bone Marrow Culture. Part II.

H.-Erich Wichmann[a], *Markus Loeffler*[b], *Ursula Reincke*[c]

[a]Medizinisches Institut f. Umwelthygiene, Duesseldorf; [b]Medizinische Universitaetsklinik, Cologne, West Germany; [c]Department of Radiation Therapy, Harvard Medical School, Boston, MA, USA

Key Words. Bone marrow · Long-term culture · Mathematical model · Granulopoiesis · Cell kinetics · Blast cells

Abstract. A mathematical model of mouse granulopoiesis in long-term bone marrow culture was constructed, based on established in vivo cell kinetic parameters. We applied the model to the cell kinetic experiment presented in Part I. Comparing model-predicted cell kinetics with the experimental data led to iterative testing of several hypotheses. In the final model, the cell kinetics of intact tissue culture flasks were reconstructed, using the experimental data from 10 days of tube culture. Among other things, our analysis suggests that the parameters of normal in vivo granulopoiesis apply to bone marrow culture.

48

Introduction

The purpose of this study was to construct a model of granulopoiesis in long-term marrow culture (LTMC) that could predict the outcome of a previously performed experiment when only the initial experimental data points were given. The experimental design and the observed results are described in Part I of this communication [1]. A powerful model could predict cell kinetics under conditions that are too complex for direct observation.

The experiment utilized the spontaneous stratification of the cultures into three layers. Stem cells and immature hematopoiesis are maintained within the stroma, cells are produced in a layer that loosely adheres to the stroma, and mature cells are suspended mostly in the supernatant medium. We subcultured the three layers separately in plastic tubes, thereby forcing the cells to remain unattached and to complete their life cycle without further supply of stem cells from the supporting stroma.

Correspondence: Dr. Markus Loeffler, Medizinische Universitaetsklinik, LFI-02, Joseph Stelzmann Str. 9, D-5000 Cologne 41 (West Germany).

Received October 3, 1983; accepted April 13, 1984.

0737-1454/84/$2.00/0

Under these conditions the granulopoietic precursors are presumed to pass through the successive stages of maturation, reach the functional stage, become senescent, and die. In a closed system with limited stem cell supply, the total cellularity should decrease slowly until all cells programmed to divide have done so. From then on, the senescent death rate should govern the cell disappearance curves. Given optimal conditions, one would expect the cell kinetics to be influenced by senescent deaths, the number of intervening divisions, and the available supply of precursor and progenitor cells. Under less than optimal conditions, however, presenescent deaths might occur, proliferative cells might fail to divide, and progenitor cells might fail to differentiate.

Mathematical analysis was performed to design a model of granulopoiesis in vitro for both subcultures in plastic tubes and intact LTMC in flasks, with the experiments in tubes testing the model's assumptions. Subsequently, the established model should quantitatively predict the production, migration, and death of granulopoietic cells in LTMC. These data cannot be obtained by direct measurements.

Materials and Methods

Experimental Methods

The experimental design and methods were detailed in Part I [1]. Briefly, we separated the substrata of LTMC and subcultured the cells from each layer in plastic tubes until the cells had disappeared. The upper layer consisted of the nonadherent (NA) cells suspended in culture medium. The middle layer consisted of the lightly adherent (LA) cells that could be shaken off the stroma after the medium was poured off. The bottom layer consisted of the remaining (REM) cells that had to be scraped off the floor of the tissue culture flask.

We determined [1] the absolute and differential cell counts for 8 to 10 days in each of the subcultured layers as well as the total cellularity and number of pluripotent stem cells [colony-forming units-spleen (CFU-s)] in each layer for one week.

Mathematical Methods

The final model was formulated based on established knowledge of murine hematopoiesis [2-5] and an evaluation of the cell disappearance curves shown in Part I.

The whole process of granulopoiesis can be compartmentalized as follows: pluripotent stem cells (S) represented by CFU-s; granulopoietic progenitors (C) to the CFU-c (colony-forming units-granulocyte/macrophage), but tentatively represented here by undifferentiated blast cells (BL); myeloblasts (G1); promyelocytes (G2); myelocytes (G3); and nonproliferative granulocytes (G4). For comparison with the number of mitotic figures found experimentally, the theoretical number of mitotic cells (MIT) also is considered.

In the mathematical simulation, four types of information were used as input for the model: cell kinetic parameters, initial experimental values, half-life values, and qualifying assumptions.

Table I. Parameters used in formulating the model

Compartment	Transit time[a]		Mitoses
	Maximum (hours)	Minimum (hours)	
S Stem cells	NS	NS	NS
C Progenitors	240	60	10
G1 Myeloblasts	24	6	1
G2 Promyelocytes	24	6	1
G3 Myelocytes	72	18	2
G4 Metamyelocytes	40	40	0
Banded cells			
Segmented cells			

[a]The minimum transit time is assumed immediately after feeding, the maximum value several days after feeding.
NS = not specified.

Cell Kinetic Parameters of Mouse Granulopoiesis

The sizes of undifferentiated and very immature spleen colonies (unpublished results) as well as the sizes of the largest granulopoietic colonies in vitro [6, 7] indicate that 10 to 15 successive mitoses may occur in the granulopoietic progenitor stage. Ten mitoses were arbitrarily assumed to occur in model compartment C. One mitosis each was assumed in both G1 and G2, two mitoses were assumed in G3, and none in G4 [4], resulting in a total of four mitoses for the precursor cells. This value has been determined for mice [8, 9] and also holds for humans [10-12].

The minimum transit times in the proliferative compartments were calculated as the product of the number of mitoses and the minimum cell cycle time. The minimum cell cycle time was assumed to be 6 h, based on measurements between 5.4 h (unpublished results) and 6 h [13]. For the postmitotic G4 compartment, a transit time of 40 h was assumed; this value is consistent with experimental values [8, 9]. We assumed a maximum cell cycle time of 24 h. This value was not taken from the literature but was derived indirectly from the model. No specific parameters were assumed for stem cells. The parameters are summarized in Table I.

In all subsequent calculations, the above parameters remained constant. The model curves do not depend very strongly on the particular choice of these parameters. Individual parameters may be varied by up to 20% without significantly changing the model results. The reaction of the model depends heavily on the initial values and on the qualifying assumptions described below.

Initial Values from Tube Subculture

As the starting point for model calculations, we used the initial experimental values determined on day 0, except for nonadherent proliferative cells, where some unexplained loss occurred. In these cells, the initial values for model calculations were adapted to the counts measured on day 1.

Disappearance of Stem Cells and Progenitors

The half-life values for stem cells and progenitors were from the disappearance curves of CFU-s and blast cells in tube subcultures [1]. From the half-lives the corresponding model parameters were derived in order to mathematically simulate the disappearance of these cells.

Qualifying Assumptions

We made two qualifying assumptions. First, the stimulatory "feeding" effect of replacing the medium was assumed to shorten the transit time to the minimum required for the cell cycle [14]. This effect was assumed in flasks and in tube subcultures. Second, presenescent cell death was assumed to occur after more than 7 days in culture, and to result in random loss of cells from all six compartments. This assumption is valid only for tube cultures.

We started with the "initial assumption," that (a) the parameters in vivo for mouse granulopoiesis were valid, (b) starting values were represented by the day 0 values, and (c) tube cultures died because the supply of stem and progenitor cells was depleted. However, this assumption was not sufficient to predict the outcome of the experiments [1]. Therefore, several additional hypotheses were tested until we arrived at a "final set of assumptions" combining the initial assumption with the qualifying assumptions as outlined above. The theoretical model curves, which correspond to these final assumptions, are shown in Figures 1-3 along with the observed results of our experiments [1].

To predict the cell kinetics in "reconstituted" flask cultures with a 7-day feeding interval, only the cellular parameters and feeding influences had to be considered. Such consideration led to the data presented in Table II and Figures 4-6, which characterize the situation in intact long-term bone marrow cultures.

Mathematical Description

The cellular compartments S through G4 are divided into 16 subcompartments, Y_i. In each proliferative subcompartment, only one mitosis occurs. The model equations for the subcompartments Y_i are:

$$Y_1' = a \times Y_1 - b \times Y_1 - k_1 \times Y_1, \quad (1)$$

$$Y_2' = b \times Y_1 - Y_2/T_2 - k_2 \times Y_2, \quad (2)$$

$$Y_i' = 2Y_{i-1}/T_{i-1} - Y_i/T_i - k_i \times Y_i, \text{ where } i = 3,\ldots,16. \quad (3)$$

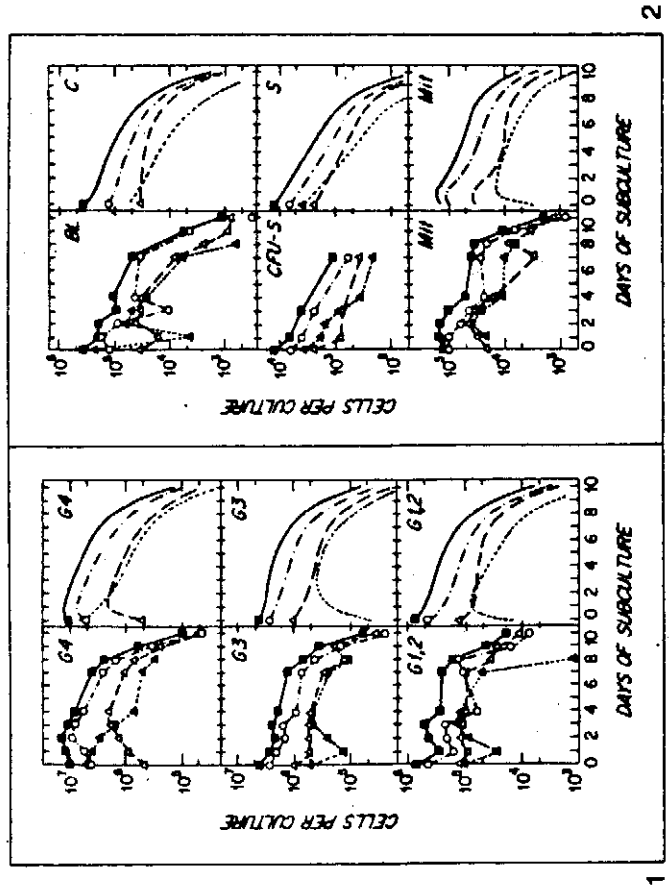


Fig. 1. Cell disappearance curves in tube subcultures. The left panel shows the observed data [1]; the right panel shows curves predicted by the mathematical model. The subcultured layers are: nonadherent cells (NA) = ▲-▲; lightly adherent cells (LA) = o---o; remainder cells (REM) = Δ--Δ; sum of NA + LA + REM (Total) = ■—■. Cell types are: myeloblasts and promyelocytes considered together (G1, G2); myelocytes (G3); and all postmitotic cells (G4).

Fig. 2. Cell disappearance curves in tube subcultures for stem cells (CFU-s, S), granulocytic progenitors (BL, C), and mitoses (MIT). Symbols are as in Figure 1. For the absolute number of CFU-s, the seeding factor of 0.2 was used.

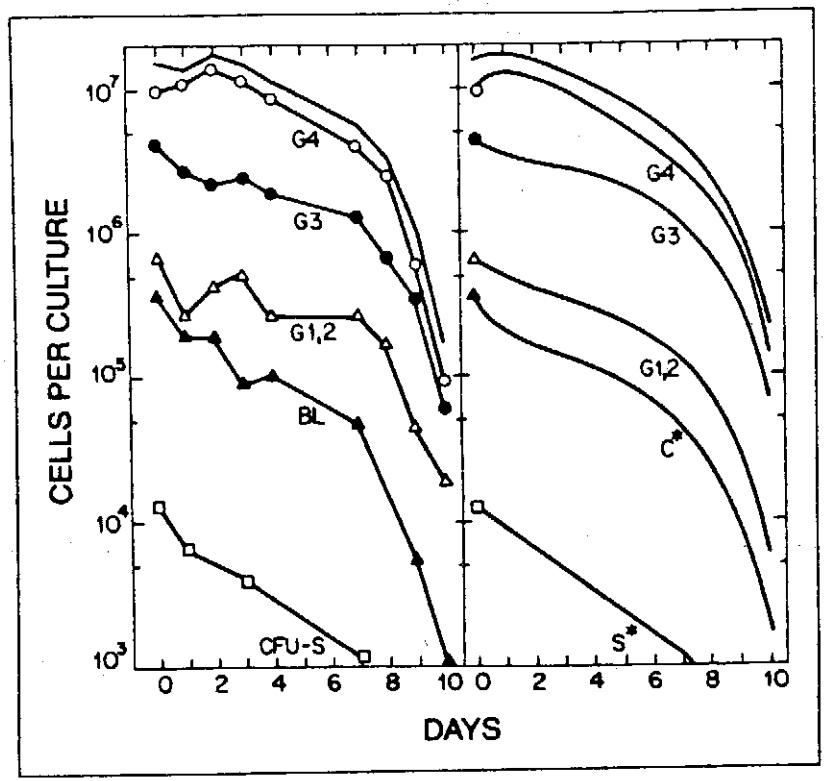
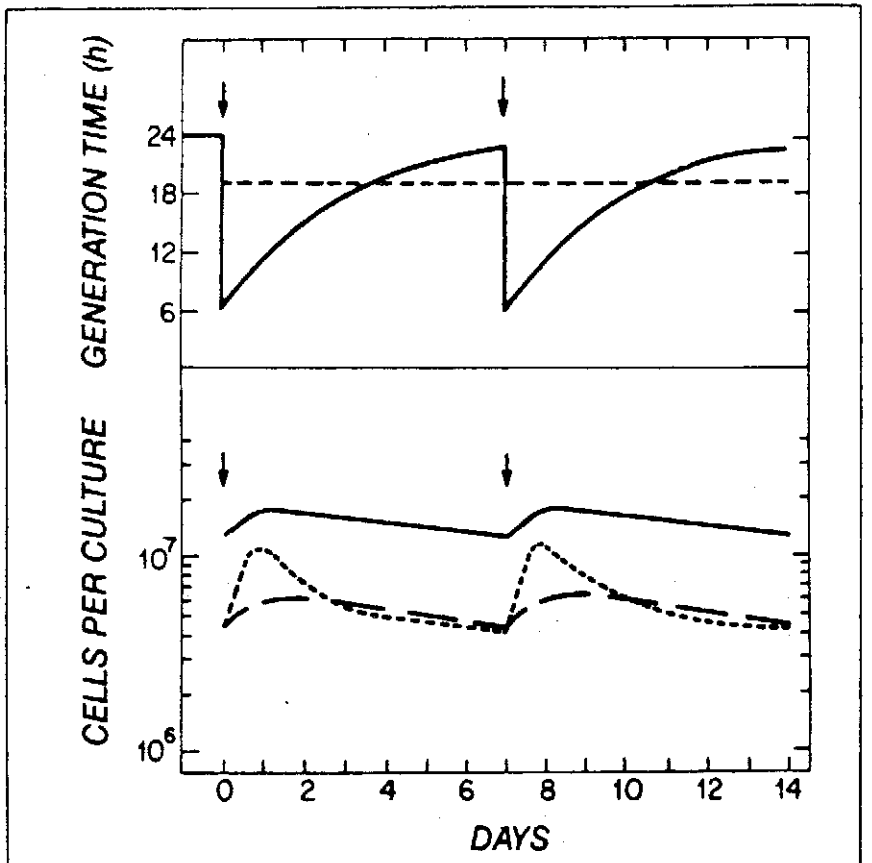


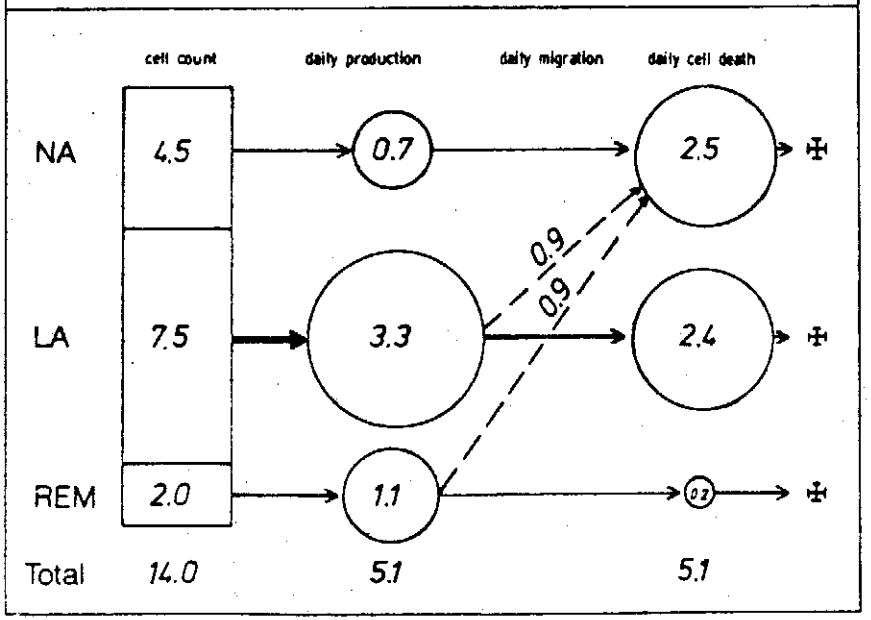
Fig. 3. Cell disappearance curves in tube subcultures for the total cell counts (NA + LA + REM). Data (left) and model (right) curves are reproduced from Figures 1 and 2 and shown together to illustrate more clearly the quantitative relationships of the different cell stages.

Table II. Average kinetics for the cell types in long-term marrow cultures as reconstructed by model analysis. These numbers are predicted from the model, and not experimental

Type of cell population	Cells/ reconstituted culture (1×10^6)	Daily number of mitoses (1×10^6/day)
C Progenitors	0.25	0.32
G1 Myeloblasts	0.25	0.32
G2 Promyelocytes	0.5	0.64
G3 Myelocytes	4.5	3.84
G4 Metamyelocytes	8.5	—
Banded cells		
Segmented cells		
Total	14.0	5.12



4



5

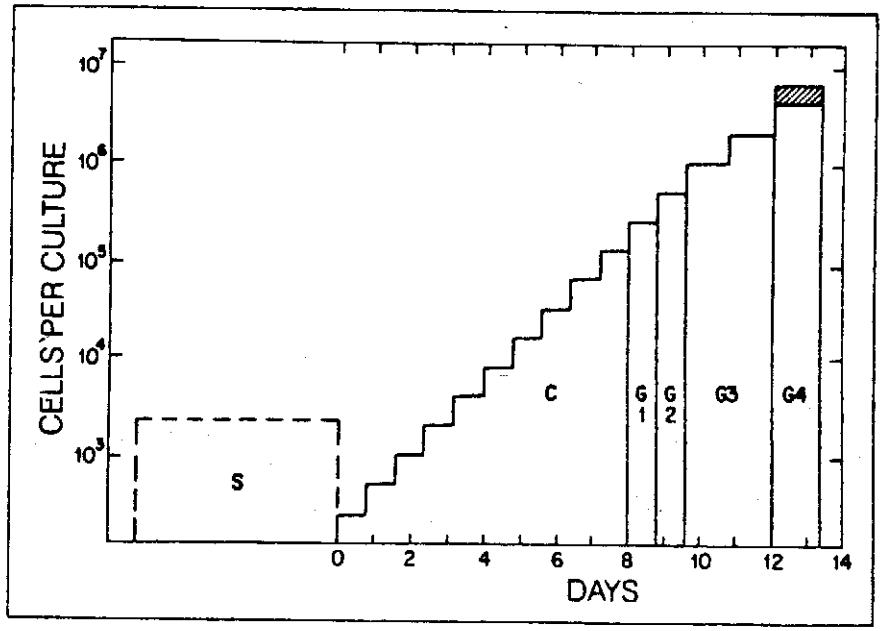


Fig. 6. Average flow of cells through granulopoiesis in long-term bone marrow cultures per mice as predicted by the model. The stem cells (S) are not further characterized. The shaded area in G4 results from the activation of proliferative cells due to feeding. Abbreviations are the same as those in Table I.

Fig. 4. Cell kinetics, predicted for long-term marrow cultures with weekly feeding, as derived from the model. Top: in C, G1, and G2, feeding (arrows) shortens the cycle time (——) from 24 h to 6 h. The average generation time (— —) is 19 h. In G3 the generation times are correspondingly longer (Table I), but their behavior is essentially the same. Bottom: periodical activation leads to fluctuations of the cell numbers. Total granulopoietic cell count (——); NA cell count (— —); daily production of granulocytes (G4) (– – –).

Fig. 5. Average kinetics in intact long-term marrow cultures as reconstructed by the model. In the different layers (NA, LA, and REM), total cell counts and the daily production, migration, and cell death of mature cells (G4) are shown (1×10^6). The values are averaged over a 7-day growth period between two feedings. For abbreviations, see Figure 1.

The coefficients for self-renewal and differentiation, a and b, are derived from the initial experimental values as $a = b = 1/12$ h.

The cell numbers for the six biological compartments are determined from the following subcompartments:

$$S = Y_1,\ C = \sum_{i=2}^{11} Y_i,\ G1 = Y_{12},$$
$$G2 = Y_{13},\ G3 = Y_{14} + Y_{15},\ G4 = Y_{16} \qquad (4)$$

From the cell compartment contents on day 0, $Y_i(0)$, cycle times T_i and the initial mitotic measurements on day 0 [MIT ($t = 0$)], the average duration t_m of the mitotic phase is calculated as 0.65 h from the formula

$$t_m = \mathrm{MIT}(t=0) \times \sum_{i=2}^{15} \frac{Y_i(t=0)}{T_i} \qquad (5)$$

With this t_m, the theoretical curves for the number of mitotic figures (MIT) (Fig. 2) can be calculated by

$$\mathrm{MIT} = \sum_{i=2}^{15} \frac{Y_i}{T_i} \times t_m \qquad (6)$$

To account for the effect of feeding in tubes and in flasks, the cell cycle times (T_i) were assumed to increase continuously from the minimum (directly after feeding) to the maxmium value. This process is described by

$$T_i = T_i(\max) - [T_i(\max) - T_i(\min)] \times \exp(-k_T \times t), \qquad (7)$$

where $i = 2,\ldots,15$

$1/k_T = 48$ h.

From the transit times in Table I, $T_i(\max)$ and $T_i(\min)$ can be calculated to be 24 h and 6 h for C, G1 and G2, 9h and 36h for G3 and 40 h for G4 [here $T_{G4}(\max) = T_{G4}(\min)$]. Only the value for k_T is chosen arbitrarily.

To account for the effects of presenescent cell death in tube subcultures, the loss coefficients k_i in equation (3) were assumed to be time-dependent

$$k_i = k_0 \times \exp(k_e \times t), \text{ where } i = 12,\ldots,16 \qquad (8)$$

$1/k_0 = 500$ h, $1/k_e = 24$ h.

k_0 and k_e were arbitrarily chosen such that the cell numbers drop only slightly in the first 7 days but by > 90% on day 10. An additional constant for premature loss (k_{ai}) was considered for stem cells and progenitors in tubes:

$$k_i = k_{ai} + k_0 \times \exp(k_e \times t), \text{ where } i = 1,\ldots,11 \quad (9)$$

The loss coefficients k_{a1} and $k_{a2} = \ldots = k_{a11}$ were derived separately from the half-lives of CFU-s and BL for each layer [1].

No premature cell loss was assumed if reconstituted flasks were considered:

$$k_i = 0, \text{ where } i = 1,\ldots,16. \quad (10)$$

Model Parameters

The minimum cell cycle times [T_i(min)] and number of mitoses were derived from granulopoiesis in vivo. The maximum cell cycle times [T_i(max)] were assumed to be 4 times the minimum values. Chosen arbitrarily, the factor of four is not very precisely defined since a factor of 2 or 6 would lead to similar results. Also the parameter k_T is free, as well as the loss coefficients k_e and k_0. The other parameters (a, b, k_{a1}, k_{a2}) were derived from the experiment [1]. In total, the model contains two qualifying assumptions with 4 free parameters, generating 18 theoretical time courses (for NA, LA, and REM in Figures 1 and 2; the curves in Figure 3 can be derived from these).

Results

The mathematical model was first applied to the tube cultures with limited stem cell maintenance, and the data could be interpreted, based on a comparison of the 18 theoretical and experimental curves, representing about 160 data points. These tube results have no important biological implications but are necessary to test the model's assumptions.

Next, the interpretation derived from the tubes was applied to intact long-term cultures in flasks. The cell kinetics in flasks were characterized, and cell production, migration, and death were predicted quantitatively.

Model Analysis of the Tube Cultures

Cell layers were subcultured in order to make possible a more detailed mathematical treatment (Fig. 1, 2). After considering the cellularity in the three layers with respect to the original flask, and correcting the counts for daily losses incurred by the sampling, the numbers in the three subcultures were summed to obtain the total cell counts shown in Figure 3. This represents the simulated situation in which all cells of one flask had been subcultured in one large tube.

The experimental curves showed three characteristics: a gradual decrease in proliferating cells during the first 7 days, an increase in postmitotic cells during the first 2 days, and a rapid terminal cell loss beginning on day 8.

Initial Assumption

Assuming that differentiated cells are depleted only due to the limited stem cell supply, one expects the number of cells to decrease slowly at the beginning and more rapidly thereafter, while never increasing. The data are not in agreement with this. Furthermore, after the stem cell reservoir is empty, one expects the immature cells to decrease first and the mature cells last. This also was not observed.

Final Set of Assumptions

To explain the difference between the initial assumption and the experimental findings, several hypotheses were formulated and their predictions were calculated. We assumed two additional influences in order to interpret the data.

The first additional assumption is that feeding shortens the transit times in proliferative compartments. The initial increase in end cells and in mitotic activity can be understood if one assumes that the proliferative burst, which follows the feeding, shortened transit through the compartment. This does not influence cell numbers in the proliferating compartments as long as influxes and effluxes are increased simultaneously by the same factor; only the number of postmitotic cells will rise because of increased influx without increased loss. In the model, we assumed that the generation times can be shortened by a factor 4.

Premature death after more than 7 days is the second additional assumption. The rapid and simultaneous terminal decline of all cell populations cannot be explained alone by the depletion of precursor cells, for such a decrease should be less pronounced and should occur first in G1 and G2, then in G3, and last in G4. An additional influence must have been present toward the end of the culture survival. Therefore, we assumed that cells in each culture began to die randomly in all compartments after 8 or more days.

Using the final set of assumptions (i.e., these two additional assumptions together with the initial assumption), we derived the final model that leads to the disappearance curves for cell populations in tube culture (Fig. 1-3). Since the predicted and observed data are similar, the observed data was interpreted in terms of the final assumptions on which the model was based.

Stem cells and progenitors (S, C) showed an exponential decrease that was more pronounced in S than in C, and that was steeper for NA cells and slower for REM cells. Because enhanced differentiation, premature cell death, and other influences might be responsible for this decrease, more than one interpretation could explain the curves.

To change the half-lives of stem cells according to self-renewal probabilities, additional information would be required. However, a shorter half-life corre-

sponds to a smaller self-renewal probability if all other influences are constant. On this basis, the self-renewal probability of stem cells and progenitors in the REM layer would clearly be higher than in the NA layer. On the other hand, different concepts of stem cell kinetics could lead to different interpretations.

The slow decrease of myeloblasts, promyelocytes, and myelocytes (G1, G2, G3) during the first 7 days can be explained satisfactorily by diminished influx from the progenitor level. Only after day 7 did additional cell death seem to contribute to the decrease. In the postmitotic population consisting of metamyelocytes, banded and segmented granulocytes (G4), we assumed the influx was increased by the shortened transit times through all precursor compartments, resulting from the day 0 feeding. This influx generated the initial increase of cell number in G4. After 3 days, the population began to decrease because the feeding effect disappeared and the precursor compartments diminished. The largest cell compartment, G4, determined the total number of granulocyte cells in the system.

Mitotic figures (MIT) increased initially, then decreased slightly between day 3 and day 7, and decreased rapidly thereafter; perhaps the feeding effect induced the proliferative cells to divide after a shortened intermitotic interval or from a "resting" state. The slight reduction in mitotic activity after 2 days corresponds to the end of the feeding effect, and the final slope reflects random death among proliferating cells.

Alternative Explanations

In general, a model analysis cannot prove the correctness of an assumption that is in agreement with the data. Therefore, alternative assumptions need to be examined.

If no additional cell death in the more mature populations is assumed, the observations after day 7 cannot be reproduced because the slopes of all precursor curves change simultaneously and abruptly at day 7. If missing influx alone accounted for the disappearance curves, then a change of slope should affect the latest population last and the earlier populations first.

Also, the initial increase in G4 could be reproduced if feeding induces one or two additional mitoses in the precursor compartments rather than shortening the transit times. However, additional mitoses would increase the number of cells in the compartments where such mitoses occur, as well as in subsequent compartments. Additional mitoses in stages before G4 contradicts the findings. Nonetheless, inducing mitosis in G4 could explain the initial increase of the G4 population; indeed mitoses in G4 cells have been observed occasionally (see Part I, methods section [1]). However, the experimental evidence for this explanation

is not sufficient to overrule the accepted notion that G4 cells are generally nondividing. Instead of intercurrent cell death, if proliferative cells are inactivated and enter a resting state, G1, G2, and G3 should not decrease. Such a prediction is contrary to the observed findings and to the ratio of mitotic figures/total proliferative cells remaining constant through day 10.

Extrapolation of the Model to Intact Long-Term Cultures

The model was applied to the cell kinetic behavior in the original flask cultures, and the resulting kinetics are displayed in Figure 4. We assumed that the difference between cells in flasks and tubes pertained to the undisturbed growth (maintenance) of stem cells and progenitors in flasks. Because the feeding period in intact LTMC was 7 days, no premature death was assumed. Due to feeding on day 0, most proliferating cells would be activated to divide, shortening the cell cycle times of progenitors (C) and early precursor cells (G1, G2) from 24 h to 6 h. The cell cycle time would return to 24 h within a week (7-day average of 19 h). For the late precursors (G3), cell cycle times would be somewhat longer (Table I) but show essentially the same behavior. The fluctuation of cell cycle times would lead to an increase in total cellularity with a peak on day 1 and 2 (Fig. 4); thereafter, the number of cells would gradually return to the starting value until the next feeding. Repeated experiments with long-term cultures in the plateau phase confirmed this prediction, although the starting levels are often exceeded or undercut (Table II)[15].

Without sacrificing a bone marrow culture, one cannot count its total cellularity but only that of the NA cells. In the present study, this number constituted 32% of granulopoiesis in the original flasks. Figure 4 shows that daily cell production, as calculated by the model, might be mirrored by the supernatant cell counts, and could be estimated by them.

Cell kinetics in the original flasks was reconstituted in even more detail, as shown in Table II. The average number of cells per flask was averaged from the experimental values on day 0. Then, from the average cell cycle times, the daily number of mitoses were calculated for the different cell populations.

For the three layers of a flask (NA, LA, and REM cells), the daily production, migration, and death of mature G4 cells were reconstituted (Fig. 5). Of the total count of 14×10^6 cells, 4.5×10^6 cells were in the NA layer, 7.5×10^6 were in the LA layer, and only 2×10^6 were in the REM layer. Cell production was small in NA (0.7×10^6 cells/day) and REM (1.1×10^6 cells/day), but large in LA (3.3×10^6 cells/day). However, the ratio of daily production to the cell count was maximum in REM (1.1×10^6 cells/day are formed by 2×10^6 cells), minimum in NA (0.7×10^6 cells/day are formed by 4.5×10^6 cells), and intermediate for LA.

There is a considerable migration of cells. In the NA layer, only 0.7×10^6 G4 cells were produced per day (from the G3 cells in that layer), while 1.8×10^6 cells migrated to the NA layer. These migrating cells came in equal parts from the two adherent layers where they are produced in excess. According to the model, 49% of all G4 cells in a flask were in the NA layer (where they die), while this layer represented only 32% of total hematopoietic cellularity.

Finally, Figure 6 is a comprehensive diagrammatic representation of the cell kinetics in long-term culture according to the granulopoiesis model. The picture (drawn in a way similar to that of *Cronkite and Vincent* [3]) reflects the consistency between our final assumptions and experimental findings, as well as the consistency between in vivo and in vitro granulopoiesis in the mouse.

Discussion

First, the model analysis suggested that presenescent cell death occurs in the second week of tube subcultures. This also was indicated experimentally by an increase in the frequency of necrotic cells (Part I) during constant phagocytosis. The causes of the presenescent death are uncertain, but are unlikely to be related to inadequate nutrition in view of the normal survival of cells not fed for 14 days (Part I). Accumulation of toxic substances, lack of serum supplementation at day 7, or failures resulting from the lack of communication with a stromal layer may account for the terminal death rate.

Second, the model analysis suggested that a stimulatory effect of feeding occurs on proliferative cells. The stimulatory effect of feeding on CFU-s and supernatant cell counts in long-term culture has been reported to last 3 to 4 days [16]. This study suggests that one of the mechanisms involved is a shortening of intervals between mitoses. This mechanism may be due to a shortened G_1-period, assuming all cells are cycling [17-19], or to extra cells being added to the cycle from the resting state [20]. Neither explanation affects predictions of the model.

Because it was impractical to measure the CFU-c, the number of undifferentiated blast cells was measured for the purpose of model calculations. Morphologically, these cells fit the description of the "hemocytoblast" given by *Bessis* [21]; therefore, we believe the hemocytoblasts in the differential count were probably identical to CFU-c, since no other cells were observed to differentiate in the culture system. But not all CFU-c are necessarily recognized as hemocytoblasts.

According to the model, 2×10^6 cells per day migrate from the adherent to the nonadherent layer to replace senescent cells which have died. This large proliferative reserve of the adherent layer has also been observed by *Dexter* et al. [22] for CFU-s, CFU-c, and total cellularity.

The high degree of cell turnover in culture is elucidated by further model calculations. If all nonadherent cells were removed every 24 h, the yield would fall from $4\text{-}5 \times 10^6$ to $2\text{-}3 \times 10^6$ cells after 5 days of daily harvesting. Harvesting every other day or at longer intervals would have no detectable influence; i.e., the yield would still be 4×10^6 to 5×10^6 at each harvest.

Stem cells or progenitors differed between the nonadherent and adherent layers. Stem cells in the REM layer showed higher self-renewal probability than those in the NA layer, while those in the LA layer were intermediate. Using a serial transplantation technique, *Mauch* et al. [23] found that the CFU-s in the adherent layer has greater self-renewal capacity than the nonadherent CFU-s. Consistent with the notion of (functional) stem cell heterogeneity [24, 25], this finding seems to be particularly related to reports of less self-renewal in circulating than in marrow-bound stem cells [26]. But whether such heterogeneity is inherent to the stem cells or is an environmental effect cannot be determined from our study.

Granulopoiesis in long-term culture appears to be influenced by periodic shortening of the generation times due to feeding. Although feeding is not found in intact mice, the cellular parameters such as the number of mitoses, generation times, etc., seem to be almost identical in vitro and in vivo. The latter result was not unexpected [16]; and it also encourages the further use of this culture system as a model of hematopoiesis within a self-controlling microenvironment but without the specific hormones and "poietins." Having predicted the behavior of several interdependent cell types in a detailed and consistent fashion, the granulopoiesis model may have the power to predict cell kinetics under conditions that are too complex for direct observation.

Acknowledgments

This study was supported by the Deutsche Forschungsgemeinschaft (WI 621/1+2), the Heinrich-Hertz-Stiftung and NIH grants P01 CA 12662 and 5R01 CA 10941.

We acknowledge the technical assistance of *S. Gontard*.

References

1 Reincke, U.; Loeffler, M.; Wichmann, H.E.; Harrison, B.: The kinetics of granulopoiesis in long-term mouse bone marrow culture. Part I. Int J Cell Cloning *2:* 394-407 (1984).

2 Cronkite, E.P.: Kinetics of granulopoiesis. Natl Cancer Inst Monogr *30:* 51-62 (1969).

3 Cronkite, E.P., Vincent, P.C.: Granulocytopoiesis. Ser Haematol *4:* 3-43 (1969).

4 Cronkite, E.P.: Myelopoiesis—normal biochemistry and physiology; in Root, Berlin, Physiological Pharmacology, pp. 291-296 (1974).

5 Potten, C.S.; Lajtha, L.G.: Stem cells versus stem lines. Ann NY Acad Sci *397:* 49-61 (1982).
6 Cronkite, E.P.; Carsten, A.L.; Bullis, J.: Colossal granulocytic and erythropoietic colonies and bursts formed by culture of regenerating bone marrow in plasma clot diffusion chambers (PCDC). Exp Hematol *7:* 92 (1979).
7 Korn, A.P.; Henkelman, R.M.; Ottensmeyer, F.P.; Till, J.E.: Investigations of a stochastic model of haemopoiesis. Exp Hematol *1:* 362-375 (1973).
8 Gerecke, D.W.; Wechselbeziehungen zwischen Zytostatikawirkung und Zellkinetik, untersucht an normalen and malignen haemopoetischen Zellsystemen. Habilitation thesis, University of Cologne, 1-166 (1979).
9 Smeby, W.; Benestad, H.B.: Stimulation of murine granulopoiesis. Blut *41:* 47-60 (1980).
10 Cronkite, E.P.; Bond, V.P.; Fliedner, T.M.; Killmann, S.A.: The use of tritiated thymidine in the study of haemopoietic cell proliferation; in Wolstenholme, O'Connor, Ciba Foundation Symposium on Haemopoiesis. Cell Production and Its Regulation, pp. 70-92 (J & A Churchill, London 1960).
11 Cronkite, E.P.; Fliedner, T.M.: Granulopoiesis. N Engl J Med *270:* 1347-1352 (1964).
12 Killman, S.A.; Cronkite, E.P.; Fliedner, T.M.; Bond, V.P.: Mitotic indices of human bone marrow cells. I. Number and cytologic distribution of mitoses. Blood *19:* 743-750 (1962).
13 Lajtha, L.G.; Gilbert, C.W.; Guzman, E.: Kinetics of haemopoietic colony growth. Br J Haematol *20:* 343-354 (1971).
14 Cameron, I.L.: Cell proliferation and renewal in the mammalian body; in Cameron, Thrasher, Cellular and Molecular Renewal in the Mammalian Body, pp. 45-85 (Academic Press, New York 1971).
15 Reincke, U.; Hannon, E.C.; Hellman, S.: Residual radiation injury exhibited in long-term bone marrow cultures. J Cell Physiol *112:* 345-352 (1982).
16 Dexter, T.M.; Allen, T.D.; Lajtha, L.G.; Krizsa, F.; Testa, N.G.; Moore, M.A.S.: In vitro analysis of self-renewal and commitment of hematopoietic stem cells; in Clarkson, Marks, Till, Differentiation of Normal and Neoplastic Hemopoietic Cells. Cold Spring Harbor Conference on Cell Proliferation, pp. 63-80 (Cold Spring Harbor, MA 1978).
17 Kretschmar, A.L.: Erythropoietin: hypothesis of action test by analog computer. Science *152:* 367-368 (1966).
18 Morse, B.S.; Rencricca, N.; Stohlman, F.: The relationship of erythropoietin effectiveness to the generative cycle of the erythroid precursor cell. Blood *35:* 761-774 (1970).
19 Stohlman, F.: Control mechanisms in erythropoiesis; in Gordon, Condorelli, Peschle, Regulation of Erythropoiesis, pp. 71-89 (Il Ponte, Milano 1972).
20 Lajtha, L.G.; Pozzi, L.V.; Laura, V.; Schofield, R.; Fox, M.: Kinetic properties of haemopoietic stem cells. Cell Tissue Kinet *2:* 39-49 (1969).
21 Bessis, M.: Blood Smears Reinterpreted (Springer-Verlag International, New York 1977).
22 Dexter, T.M.; Spooncer, E.; Varga, J.; Allen, T.D.; Lanotte, M.: Stromal cells and diffusible factors in the regulation of haemopoietic cell development; in Killmann, Cronkite, Muller-Berat, Haemopoietic Stem Cells, pp. 303-318, Alfred Benzon Symposium 18. (Munksgaard, Copenhagen, Denmark 1983).

23 Mauch, P.; Greenberger, J.S.; Botnick, L.; Hannon, E.; Hellman, S.: Evidence for structured variation in self-renewal capacity within long-term marrow cultures. Proc Natl Acad Sci USA *77:* 2927-2930 (1980).
24 Hellman, S.; Botnick, L.E.; Hannon, E.C.; Vigneulle, R.M.: Proliferative capacity of murine hematopoietic stem cells. Proc Natl Acad Sci USA *75:* 490-494 (1978).
25 Monette, F.C.; Stockel, J.B.: Immunological evidence for murine hematopoietic stem cell subpopulations differing in self-renewal capacity. Stem Cells *1:* 38-52 (1981).
26 Micklem, H.S.; Anderson, N.; Ross, E.: Limited potential of circulating haemopoietic stem cells. Nature *256:* 41-43 (1975).