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# Kinetic Analysis of the Growth of Bone Marrow Mononuclear Phagocytes in Long-Term Cultures

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The present work concerns a mathematical analysis of the growth of monoblasts, promonocytes, and macrophages in long-term bone marrow cultures in the presence of conditioned medium. For this purpose, use was made of the normalized data of four experiments, each done in triplicate. The computer program was based on a concept of hypothetical subcompartments within each developmental stage. The growth parameters were then determined experimentally or by trial and error after a series of computer simulations.

The mathematical results are in close agreement with the numbers of monoblasts, promonocytes, and macrophages obtained by counts in 21-day-old bone marrow cultures. This approach provides a means to understand the kinetic behaviour of mononuclear phagocytes.

**Key words:** monoblasts, promonocytes, macrophages, kinetic analysis, bone-marrow colonies

## INTRODUCTION

Bone marrow cells proliferate *in vitro* when appropriate growth factors are added to the medium. Among other constituents of the medium, such growth factors determine which of the cell lines originating from the pluripotent stem cell will proliferate. For example, colony stimulating factor in fibroblasts or L-cell conditioned medium favours the growth of the mononuclear phagocyte and granulocytic cell lineages [2,4]. In a liquid culture system with a glass surface for the cells to adhere to, two kinds of colonies develop, one composed of mature granulocytes and their precursors and the other comprising macrophages, promonocytes and monoblasts [2]. Mixed colonies consisting of cells from both cell lines were never observed.

In a later study it proved possible to lengthen the initial culture time from 4 days to about 21 days [7]. Cultures older than 9 days were found to consist entirely of mononuclear phagocyte colonies. In these long-term cultures counts of the total numbers of each type of cell were performed at various time-points. Since it is known

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that the sequence of development in vitro is monoblast, promonocyte, and macrophage [2], quantitative data allow a mathematical analysis of the proliferation of mononuclear phagocytes in vitro. This had been done previously for 4-day cultures with a simple calculation [1]. The present study concerns a rigorous kinetic analysis of mononuclear phagocytes grown in vitro for 21 days.

## MATERIALS AND METHODS

### Bone Marrow Cultures

Bone marrow cells from SPF-Swiss mice were cultured in medium consisting of Dulbecco's modified Eagle's medium containing 20% horse serum and 20% embryonic mouse fibroblast conditioned medium [colony stimulating factor (CSF-1)] in petri dishes provided with a glass coverslip [7]. The cultures were terminated after various periods of incubation.

For determination of the total number of mononuclear phagocytes during the first 4 days of culture, counts of these cells were performed on fixed coverslips; for counting at later time-points, the cells were carefully removed from the coverslip by placing the petri dish on crushed ice before scraping with a rubber policeman. The cells were counted in a hemocytometer. Next, for investigation of the cells remaining on the glass, the coverslips were fixed and stained. For calculation of the total number of cells in culture, a correction was made for the area of the petri dish surface not covered by the coverslip. Coverslips of duplicate cultures were rapidly dried in an airflow, fixed in absolute methanol, and stained with Giemsa stain. On these coverslips the distinction between granulocytic and mononuclear phagocyte colonies was made light microscopically. The cells of mononuclear phagocyte colonies were characterized as monoblasts, promonocytes, or macrophages as described elsewhere [2]. Monocytes cannot be identified in these colonies, because they stretch immediately after they are formed and then have a morphology indistinguishable from that of macrophages [2].

## RESULTS

### Quantitative Data of Long-term Bone Marrow Cultures

Approximately  $2.5 \times 10^3$  nucleated bone marrow cells/ml culture medium were incubated for a period of 21 days. To be able to apply quantitative data in a mathematical model permitting easy analysis, the data were normalized, the initial number of monoblasts being taken as one cell at time zero. An earlier study done in 4-day bone marrow cultures had shown that each colony originates from one monoblast [1,2]. Moreover, it was found that on day 4 there is one mononuclear phagocyte colony per  $10^3$  nucleated bone marrow cells plated [1,7]. The normalized data are presented in Table 1.

### Model of Mononuclear Phagocyte Proliferation In Vitro

The developmental sequence and proliferative behaviour of these cells of the mononuclear phagocyte cell line have been extensively studied both in vivo and in vitro in our laboratory [2,3,7]. The results of these investigations were used to construct the block diagram in Figure 1. In addition to proliferating cells, each subpopulation includes a subcompartment of nonproliferating cells. Such a model was

TABLE 1. Total Number of Monoblasts, Promonocytes, and Macrophages<sup>a</sup>

Day	Monoblasts $\times 10^3$	Promonocytes $\times 10^3$	Macrophages $\times 10^3$	Total $\times 10^3$
0	0.001			0.001
4	0.03	0.03	0.07	0.13
7	0.2	0.4	1.1	1.7
10	0.2	1.1	4.0	5.3
14	0.4	2.0	5.3	7.7
17	0.3	2.5	5.9	8.7
21	0.3	3.8	8.9	13.0

<sup>a</sup>Values are means of normalized data of four experiments, each done in triplicate [7]; the initial number of monoblasts is taken as one cell at day 0.

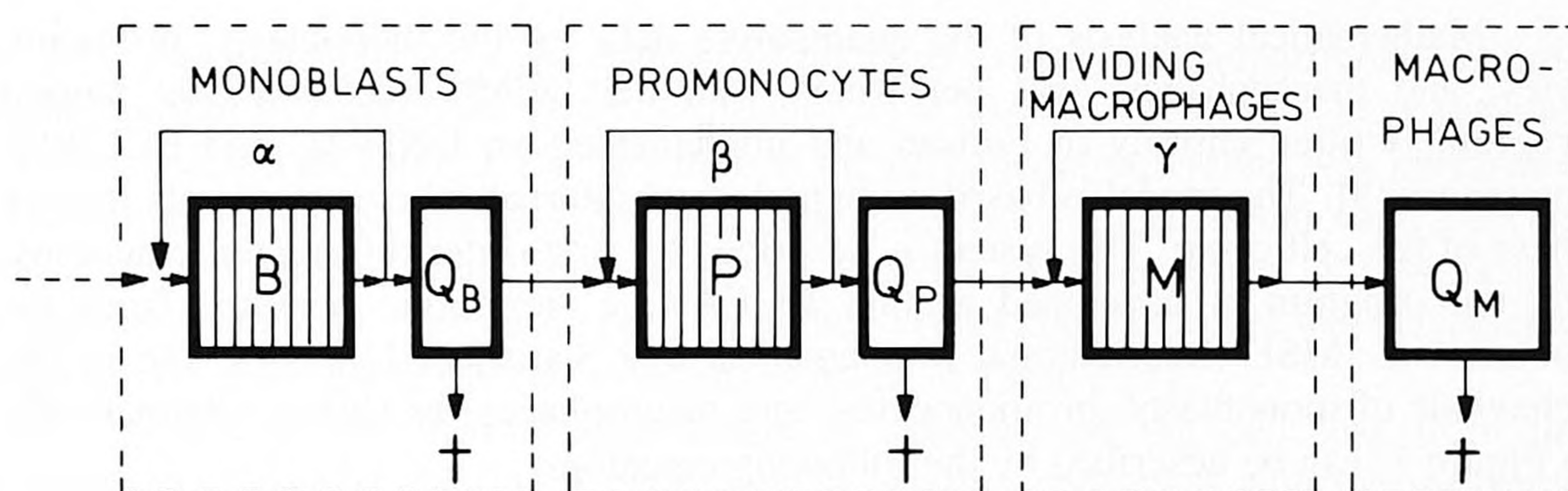


Fig. 1. Schematic presentation of the proliferation and maturation of cells of the mononuclear phagocyte cell line. B = monoblast; P = promonocyte; M = macrophage; Q = quiescent, nondividing cells;  $\alpha$ ,  $\beta$ , and  $\gamma$  = mean number of proliferative cells formed at mitosis; † = cell death.

TABLE 2. Labelling of Cells in Mononuclear Phagocyte Colonies<sup>a</sup>

Day	Labelled cells		
	Monoblasts (%)	Promonocytes (%)	Macrophages (%)
4	39	36	10
7	34	34	16
10	30	26	12
14	23	21	12
17	ND <sup>b</sup>	9	9
21	ND	6	4

<sup>a</sup>Incubated for 1 hour at 37°C in medium containing 0.1  $\mu\text{Ci/ml}$  [<sup>3</sup>H]thymidine.

<sup>b</sup>ND: not determined, because these cells cannot be identified with certainty.

thought to explain the course of the labelling indices (Table 2); and with the final parameter values (see below) selected in the present study, the labelling indices indeed showed the expected qualitative behaviour.

The letters B, P, and M in Figure 1 indicate monoblasts, promonocytes, and macrophages, respectively; Q refers to quiescent, nondividing, cells; and the possible cell loss is indicated by †. For this model we preferred the concept of quiescent (Q) cells to that of  $G_0$  cells; if a quiescent cell survives, it leaves this subcompartment randomly with parameter  $\lambda_Q$ . Parameters  $\alpha$ ,  $\beta$ , and  $\gamma$  represent the fraction of the daughter cells formed at mitosis that return to the cell cycle; thus  $2-\alpha$ ,  $2-\beta$ , and  $2-\gamma$  represent the numbers of new nondividing (Q) cells. It was previously assumed that mononuclear phagocytes beyond the promonocyte stage do not proliferate in vitro. However, the 1-hr [ $^3\text{H}$ ]thymidine labelling indices of the macrophages in liquid cultures are higher than 10% during the first 2 weeks of culture (Table 2); this indicates that there is some proliferative activity within this compartment. These cells were named dividing macrophages.

### Mathematical Analysis of Proliferation of Mononuclear Phagocytes

Mathematical analysis of the quantitative data for the monoblasts, promonocytes, and macrophages, was performed with the DEMO (deterministic model) program, written entirely in Fortran and implemented on UNIVAC and ECLIPSE computers [5]. The model is based on hypothetical internal subcompartments in each phase of the cell cycle. The system is described by first-order differential equations, and the program is developed around DGEAR, a subroutine obtained from the commercial IMSL (International Mathematical and Statistical Libraries, Inc.). The behaviour of monoblasts, promonocytes, and macrophages, as shown schematically in Figure 1, can be described by the following equations:

monoblasts:

$$\begin{aligned} \frac{dB_1(t)}{dt} &= -\lambda_1^{(B)}B_1(t) + \alpha\lambda_k^{(B)}B_k(t) \\ \frac{dB_i(t)}{dt} &= -\lambda_i^{(B)}B_i(t) + \lambda_{i-1}^{(B)}B_{i-1}(t), \quad i = 2, \dots, k \\ \frac{dQ_B(t)}{dt} &= -\lambda_Q^{(B)}Q_B(t) - \mu_Q^{(B)}Q_B(t) + (2 - \alpha)\lambda_k^{(B)}B_k(t) \end{aligned} \quad (1)$$

promonocytes:

$$\begin{aligned} \frac{dP_1(t)}{dt} &= -\lambda_1^{(P)}P_1(t) + \lambda_Q^{(B)}Q_B(t) + \beta\lambda_m^{(P)}P_m(t) \\ \frac{dP_i(t)}{dt} &= -\lambda_i^{(P)}P_i(t) + \lambda_{i-1}^{(P)}P_{i-1}(t), \quad i = 2, \dots, m \\ \frac{dQ_P(t)}{dt} &= -\lambda_Q^{(P)}Q_P(t) - \mu_Q^{(P)}Q_P(t) + (2 - \beta)\lambda_m^{(P)}P_m(t) \end{aligned} \quad (2)$$

dividing macrophages:

$$\frac{dM_1(t)}{dt} = -\lambda_1^{(M)}M_1(t) + \lambda_Q^{(P)}QP(t) + \gamma\lambda_n^{(M)}M_n(t)$$

$$\frac{dM_i(t)}{dt} = -\lambda_i^{(M)}M_i(t) + \lambda_{i-1}^{(M)}M_{i-1}(t), i = 2, \dots, n \quad (3)$$

macrophages:

$$\frac{dQ_M(t)}{dt} = -\mu_Q^{(M)}Q_M(t) + (2 - \gamma)\lambda_n^{(M)}M_n(t) \quad (4)$$

In these equations,  $B_i(t)$  is the number of monoblasts in a subcompartment  $i$  at time  $t$ ; the number of cells in the other compartments is similarly denoted. Other parameters are defined in Table 3. The solution of the differential equations was performed with initial values according to the real measurements; i.e., all initial values except those of monoblasts were zero (the total number of monoblasts at  $t = 0$  was normalized to unity). The parameters  $\lambda_i$  in the various compartments were calculated with the program using the cell cycle times given in Table 3 [5].

No direct experimental information is available on the cell loss from the population. However, to find a mathematical solution consonant with the measured behaviour of the subpopulations, it was necessary to reduce "flow" from monoblasts to promonocytes on the basis of cell loss. Macrophages in the population also had to be "killed" to keep their number at the observed level. Even then, there was a slight tendency of the calculated line to overshoot the data points (Fig. 2).

TABLE 3. Kinetic Parameters Used in the Simulation

Cell cycle times	
Monoblasts <sup>a</sup>	12.00 hr
Promonocytes <sup>a</sup>	12.00 hr
Dividing macrophages	12.00 hr
Mean number of proliferative cells formed at mitosis <sup>b</sup>	
$\alpha_0$	1.5
$\beta_0$	1.5
$\gamma_0$	2.0
Parameter for transition of quiescent monoblasts to promonocytes	
$(\lambda^{(B)})$	0.1 hr <sup>-1</sup>
Parameter for transition of quiescent promonocytes to dividing macrophages	
$(\lambda^{(P)})$	0.1 hr <sup>-1</sup>
Cell loss factors	
$\mu^{(B)}$	0.3 hr <sup>-1</sup>
$\mu^{(P)}$	0
$\mu^{(M)}$	0.03 hr <sup>-1</sup>

<sup>a</sup>Experimentally determined [1].

<sup>b</sup>Initial values of  $\alpha$ ,  $\beta$ , and  $\gamma$ .

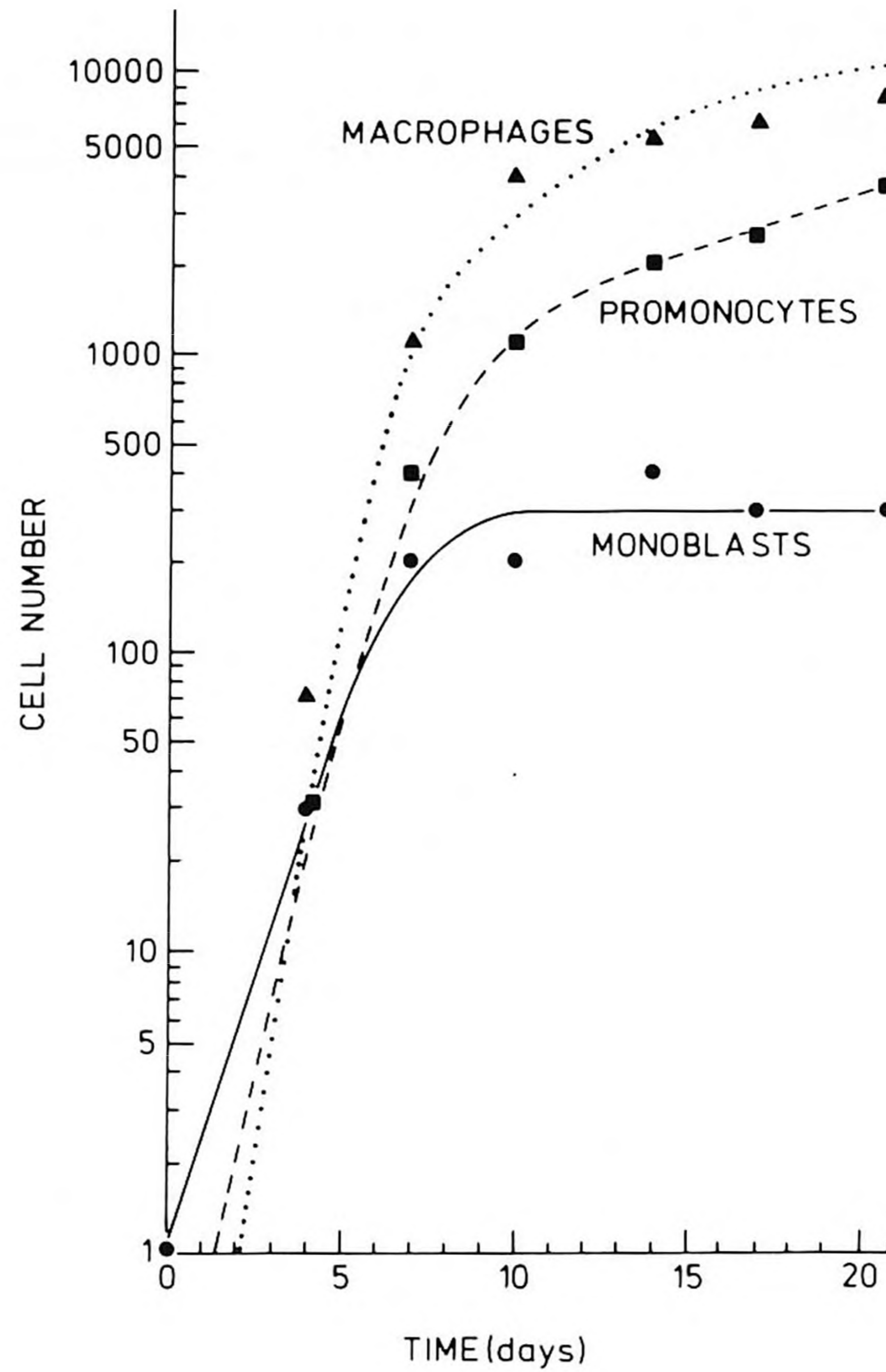


Fig. 2. Growth of monoblast, promonocyte, and macrophage populations in bone marrow cultures incubated for 21 days in the presence of CSF-1. Symbols refer to counted numbers of monoblasts (●), promonocytes (■), and macrophages (▲); the solid, dashed, and dotted lines represent the calculated growth pattern.

The feedback signal in the present model was derived from the number of monoblasts by progressively reducing parameters  $\alpha$ ,  $\beta$ , and  $\gamma$  in the following way:

$$\alpha = 1 + (\alpha_0 - 1)(1 - C(t)) \quad (5)$$

$$\beta = 1 + (\beta_0 - 1)(1 - C(t)) \quad (6)$$

$$\gamma = \gamma_0(1 - C(t)) \quad (7)$$

$$C(t) = \frac{\sum_i B_i(t) + Q_B(t)}{\sum_i B_i(\infty) + Q_B(\infty)} \quad (8)$$

These are arbitrary assumptions, and they may be (probably are) biologically unsound. From the mathematical point of view, however, the relevant assumption is

that  $\alpha$  and  $\beta$  approach unity when the population grows, and  $\gamma$  approaches zero. This last assumption means that dividing macrophages eventually evolve to a mere transit population, i.e., the cells divide just once and hence produce only nonproliferating end-cells (i.e., macrophages).

A series of computer simulations led to the selection of one set of parameter values apparently consistent with the experimental data. These parameters are shown in Table 3. The calculated curves shown in Figure 2 fit well with the observations. The kinetic analysis also indicated that the first promonocytes originating in vitro from monoblasts appear after about 36 hr and the first macrophages 16 hr after that.

## DISCUSSION

Several fates are possible for the daughter cells formed by mitosis. A daughter cell may proceed toward a new mitosis or revert to a resting or quiescent (Q) state, i.e., the mitotic cycle does not recur in the same subcompartment. Morphologically, a proliferative cell does not differ from a quiescent cell of the same type, but a quiescent daughter cell may mature to perform the function for which it was actually created. The final fate of any cell is death and disintegration, which can occur at any stage of maturity. The causes of cell death are not known.

The schematic presentation in Figure 1 may not reflect the true population's behaviour, because the growth kinetics of mononuclear phagocytes in vitro are complicated and at present there is too little detailed information to allow construction of an absolutely correct model. Some of the parameters used in this analysis can be determined experimentally (e.g., cell-cycle times [1]); other parameters given in Table 3 are determined by trial and error. For instance, the compartment of dividing macrophages was included because a small percentage of macrophages label after 1-hr incubation with [<sup>3</sup>H]thymidine. The present study provides support for, but does not prove, the existence of these proliferating cells. For instance, these cells could be labelled promonocytes showing morphologic features of macrophages due to the in vitro conditions. Nevertheless, it is possible that a proportion of the macrophages arising from dividing promonocytes continue to divide in vitro because CSF-1 is present [4]. CSF-1 is required for the survival, proliferation, and differentiation of mononuclear phagocytes in bone marrow cultures [6].

Feedback analyses raise special problems because sufficient qualitative and quantitative information is lacking. From the mathematical point of view, the only solution is to have the artificial population to grow in a manner resembling the true population behaviour. It is not at all clear that cell loss is the mechanism responsible for the retardation of the growth rate of the subpopulations. It is possible, and even more likely, that the growth parameters do not remain constant. A reduction in the growth of mononuclear phagocytes in bone marrow cultures may be due to a relative CSF-1 deficiency [6]. We reduced the growth rate progressively in the model by changing parameters  $\alpha$ ,  $\beta$ , and  $\gamma$ , i.e., the fraction of new proliferative cells; when the population grows,  $\alpha$  and  $\beta$  approach unity and  $\gamma$  approaches zero. This means that initially, dividing macrophages divide and return to the cell cycle, but eventually produce nonproliferating end-cells only.

The feedback signal could, of course, be produced in another way. For example, this signal could arise not from monoblasts, but from the cell density in general or from humoral inhibitors [e.g., monocyte production inhibitor (MPI) [8] or prostaglan-



dins [3]] whose concentration depends on the total number of (mature) cells. These signals could have a very complicated effect on the growth rate via changes in the cell cycle time. For example, it has been shown that in vivo a humoral factor [factor increasing monocytopoiesis (FIM)] has a stimulating effect on the monocyte production by shortening the cell cycle time of the promonocytes [9]. In principle, many reasonable alternatives can be proposed for the control of the cell proliferation process, but very little experimental evidence is available.

It is possible that the parameter values prevailing in vitro at the time when the system is approaching the steady state are those that reflect most closely the population behaviour under undisturbed in vivo conditions. The active growth period in vitro might indicate population behaviour in vivo during regeneration or expansion, e.g., as a response to functional demands.

Even if the correct block diagram and all of the feedback mechanisms were known, the mathematical description of the in vitro behaviour of mononuclear phagocytes would still depend on a large number of growth parameters that can be modified in different ways in the computer simulation to find apparently good agreement with experimental data. It is self-evident that most of these parameters must be determined experimentally; otherwise, the simulations remain of purely academic interest. However, a rigorous mathematical approach provides the means to find an explanation to the experimental results, and certainly constitutes one step toward a full understanding of the kinetic behaviour of the cells of the mononuclear phagocyte cell line.

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