Continuous maturation of proliferating erythroid precursors

M. C. Mackey and P. Dörmer*

*Department of Physiology, McGill University, Montreal, Canada, and *Institut für hamatologie der GSF, Munich, Federal Republic of Germany.*

Abstract. This study examines published steady state cell kinetic (mitotic and DNA synthesis phase) data from the recognizable proliferating erythroid precursors in humans, rats, and guinea-pigs, and human neutrophilic precursors, for consistency with a continuous maturation-proliferation model of the cell cycle. We find that these data are completely consistent with the hypothesis that maturation between morphological compartments may take place at any point in the cell cycle. A number of compartmental parameters are derived under this assumption.

Many cell kinetic studies of the recognizable proliferating erythroid and neutrophilic precursors have utilized a sequential maturation-proliferation model implicitly for experimental design and explicitly for data analysis. Within the framework of such a scheme it is assumed that all cells undergo DNA synthesis, mitosis, and cytokinesis at least once within each morphological compartment, and that cells pass from one morphological compartment to the next immediately after cytokinesis. However, it has been noted before (Cronkite, 1964; Rubinow, 1969; Rubinow & Lebowitz, 1975; Mackey & Dörmer, 1981; Mary, 1981) that the available data are inconsistent with this hypothesis.

Other workers (Lajtha & Oliver, 1960; Tarbutt, 1967; Tarbutt & Blackett, 1968; and others, *cf.* Discussion) have postulated that proliferating haematopoietic precursor cells may pass between morphological compartments at any point in the cell cycle (a continuous maturation-proliferation hypothesis). Here we show that the Sprague–Dawley rat erythroid precursor data of the previous paper (Dormer *et al.*, 1981), the cell kinetic data from human erythroid (Dormer, 1973; Rondanelli *et al.*, 1969) and neutrophilic (Rondanelli *et al.*, 1967) precursors, and the August-Marshall rat (Roylance, 1968; Tarbutt & Blackett, 1968) and Hartley guinea-pig (Starling & Rosse, 1976) erythroid precursors are completely consistent with a continuous maturation-proliferation hypothesis. All of these data were previously shown to be inconsistent with a sequential maturation-proliferation hypothesis (Mackey & Dörmer, 1981).

MATERIALS AND METHODS

The Model

Consider a population of recognizable proliferating erythroid precursors, and assume each cell within the population may be characterized by an age a and a maturation level μ (*cf.* Fig. 1).

Correspondence: Dr M. C. Mackey, Department of Physiology, McGill University, 3655 Drummond, Montreal, P.Q., Canada H3G 1Y6.
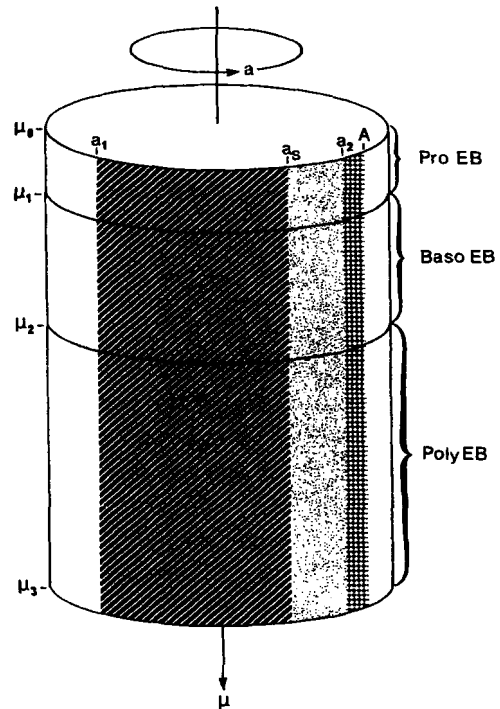
Fig. 1. A schematic representation of the continuous maturation-proliferation model. Each cell is characterized by an age a and a maturation level μ. Age a ranges from $a = 0$ the birth of a cell to $a = A$ at cytokinesis. Thus, the location of any cell with respect to (a, μ) can be located on the surface of a cylinder with the maturation variable along the cylinder axis. See the text for further details.

Cellular age a is related to the position of a cell within the cell cycle. Immediately after cytokinesis, cells have age $a = 0$. Cells with an age a between $a = 0$ and $a = a_1$ are classified as G_1 phase cells, while DNA synthesis (S) phase cells have ages between $a = a_1$ and $a = a_S$. G_2 and mitotic (M) phase cells are characterized by ages a between a_S and a_2 and a_2, and A, respectively. A is the maximum age of a cell for at age $a = A$ mitosis is complete, cytokinesis occurs, and two daughter cells of age $a = 0$ are born. Here is is assumed that cells progress through the cell cycle at a velocity V_a independent of position in the cell cycle, though not necessarily independent of maturation level μ. Thus, the times required for a cell to complete the G_1, S, G_2 and M phases of the cell cycle are $t_1 = a_1/V_a$, $t_S = (a_S - a_1)/V_a$, $t_2 = (a_2 - a_S)/V_a$, and $t_M = (A - a_2)/V_a$, respectively. The intermitotic time is given by $T = A/V_a$.

The cellular maturation level μ is related to an observable cellular characteristic, e.g. haemoglobin content, that changes in an orderly manner as cells mature. In this study the proerythroblasts (Pro EB, $i = 1$) are characterized by a maturity between μ_0 and μ_1, the basophillic erythroblasts (Baso EB, $i = 2$) by a maturation level between μ_1 and μ_2, and the polychromatic erythroblasts (Poly EB, $i = 3$) by a maturation μ between μ_2 and μ_3. If cells mature at a velocity V_μ, the transit time through a particular morphological compartment is given by $\tau = (\mu_F - \mu_1)/V_\mu$, where μ_1 and μ_F are, respectively, the initial and final maturation levels defining the given morphological compartment.

The complete steady state analysis of this continuous maturation-proliferation model is presented in Appendix 1. In Appendix 2 it is demonstrated how the intermitotic time (T) may be calculated given only the fraction of cells in mitosis (f_M) and t_M (equation A.2.1). Further, given the absolute or relative cellularity (Σ) of a given morphological compartment, the absolute or relative cellular efflux from that compartment (M_0), and T for the compartment

then it is straightforward to calculate the cellular input flux (M_I) for that compartment (equation A.2.3). Having M_I, M_0, and T the compartmental transit time τ may then be determined from Equation A.2.4.

In many circumstances one does not have the mitotic data (f_M, t_M) available for a given class of cells, but only the S phase data (f_S, t_S). Appendix 3 illustrates how the intermitotic time T may be estimated from S phase data, though with less precision than if based on accurate M phase data. Once T is estimated from S phase data, all of the compartmental parameters may be determined as outlined above.

The data

From the foregoing it is clear that ideally one should have available mitotic phase data for the recognizable proliferating precursors, though the analysis may be carried out with DNA synthesis phase data. To minimize variations between laboratories as much as possible, the analyses of this paper have been restricted to data sets containing (Σ_i, f_{Mi}, t_{Mi}) or (Σ_i, f_{Si}, t_{Si}), $i = 1, 2, 3$, all collected from the same laboratory at the same time. Though this criterion for selection for analysis is limiting, it is felt to be essential.

With this requirement in mind, we have analysed the status of the human erythroid precursor, using the mitotic phase data of Rondanelli *et al.* (1969) and compared these results with the predictions of the S phase data of Dörmer (1973). Further we have analysed the erythroid precursor status in the 50, 100, 150, and 300 g Sprague–Dawley rat using the S phase data of the previous paper (Dörmer *et al.*, 1981). The 100 g August-Marshall rat erythroid S phase data of Roylance (1968) and the 200–250 g August-Marshall rat data of Tarbutt & Blackett (1968) have also been analysed. Finally we have applied our analysis to the Hartley guinea-pig S phase data of Starling & Rosse (1976). Though our principal aim was to examine erythroid maturation and proliferation, an analysis of the human neutrophilic recognizable proliferating precursors, using the M phase data of Rondanelli *et al.* (1967), was included.

RESULTS

Human erythropoiesis

To complement the mitotic data of Rondanelli *et al.* (1969) the human erythroid production rate (EPR), which is assumed to be identical with the output flux (M_0) for the polychromatic erythroblasts, and the total cellularity of the recognizable proliferating erythroid precursors are required. The data of Wintrobe (1976) for red blood cell numbers ($5 \cdot 2 \times 10^{12}$/l), blood volume (60 ml/kg) and an erythrocyte lifespan of 120 days gives an estimated human EPR = $1 \cdot 1 \times 10^8$ cells/kg per hr. Dörmer (1973) gives a total cellularity of $2 \cdot 74 \times 10^9$ cells/kg.

The results of the analysis of the M phase data of Rondanelli *et al.* (1969) are presented in Table 1. They predict intermitotic times for the three proliferating precursor compartments of (T_1, T_2, T_3) = ($13 \cdot 7$, $18 \cdot 8$, $30 \cdot 2$) hr. Once the relevant fluxes between compartments have been determined, the compartmental transit times are calculated as (τ_1, τ_2, τ_3) = ($2 \cdot 1$, $11 \cdot 4$, $24 \cdot 8$) hr to give a total mean transit time of $38 \cdot 3$ hr or $1 \cdot 6$ days through the proliferating erythroid precursor compartment in the human. Finally, the total cellular amplification within these three compartments is about 3, corresponding to about $1 \cdot 6$ divisions.

The availability of M and S phase data for the human erythroid precursors offers an opportunity to compare the estimates of the intermitotic time based on S phase data and the analysis of Appendix 3 with the more accurate methods of Appendix 2 based on M phase data. From the S phase data of Dörmer (1973) and equations A.3.6 and A.3.7, for the proliferating recognizable human erythroid precursors the minimum intermitotic times (T_{LB1},

Table 1. Analysis of the status of the recognizable proliferating human erythroid precursors with a continuous maturation-proliferation hypothesis and the mitotic phase data of Rondanelli *et al.* (1969)

Quantity	Proerythroblast ($i = 1$)	Basophilic erythroblast ($i = 2$)	Polychromatic erythroblast ($i = 3$)
Intermitotic time (T, hr)	$13 \cdot 7 \pm 1 \cdot 5$	$18 \cdot 8 \pm 2 \cdot 7$	$30 \cdot 2 \pm 3 \cdot 9$
Input flux (M_i, 10^7 cells/kg per hr)	$3 \cdot 7$	$4 \cdot 1$	$6 \cdot 2$
Output flux (M, 10 cells/kg per hr)	$4 \cdot 1$	$6 \cdot 2$	$11 \cdot 0$
Compartmental transit time (τ, hr)	$2 \cdot 1$	$11 \cdot 4$	$24 \cdot 8$
Amplification	$1 \cdot 1$	$1 \cdot 5$	$1 \cdot 8$

T_{LB2}, T_{LB3}) are ($11 \cdot 8$, $14 \cdot 7$, $29 \cdot 6$) while the maximum inter-mitotic times. (T_{UB1}, T_{UB2}, T_{UB3}), are ($14 \cdot 1$, $18 \cdot 4$, $41 \cdot 9$) hr. Thus, for each cell type the range of allowed intermitotic times is in satisfactory agreement with the intermitotic times calculated from M phase data (*cf.* Table 1).

Taking the mean intermitotic time for each cell type determined from S data gives ($\bar{T}_1$, $\bar{T}_2$, $\bar{T}_3$) = ($12 \cdot 9$, $16 \cdot 5$, $35 \cdot 7$) hr which are close to the values calculated from M phase data. Using this set of S phase determined intermitotic times gives compartmental transit times of ($3 \cdot 1$, $7 \cdot 3$, $24 \cdot 9$) hr for a total of $35 \cdot 3$ hr through the recognizable proliferating portion of the human erythron. These data further predict $1 \cdot 4$ mitoses throughout these recognizable compartments. Thus, on the basis of all of the above compartmental parameters the conclusions derived from M phase data seem to be in accord with those derived from the independently determined S phase data.

Human granulocytopoiesis

In the analysis of the available M phase data of Rondanelli *et al.* (1967) for the human myeloblasts (MB), promyelocytes (Pro) and myelocytes (Mye), a total proliferating cellular density of $2 \cdot 11 \times 10^9$ cells/kg and a granulocyte production rate (GPR) of $3 \cdot 63 \times 10^7$ cells/kg per hr (Dancey *et al.*, 1976) were used. The GPR was taken to be equal to the myelocyte output flux.

From this analysis of the neutrophilic precursor data the intermitotic times (T_1, T_2, T_3), based on the Rondanelli *et al.* (1967) mitotic phase data, are ($22 \cdot 2$, $47 \cdot 3$, $94 \cdot 6$) hr. The individual compartmental transit times (τ_1, τ_2, τ_3) are ($8 \cdot 0$, $16 \cdot 9$, $53 \cdot 6$) hr to predict a total transit time through the recognizable proliferating neutrophilic precursors of $78 \cdot 5$ hr, or $3 \cdot 27$ days, in the human. Finally an estimated $1 \cdot 3$ mitoses take place throughout these three compartments, corresponding to an overall amplification of about $2 \cdot 4$.

Rat erythropoiesis

The Sprague–Dawley rat data of the previous paper (Dörmer *et al.*, 1981) include (Σ_i, f_{si}, t_{si}), $i = 1, 2, 3$ and these were supplemented with the EPR determinations of Ganzoni (1970) in Sprague–Dawley rats as a function of weight. As before it was assumed that the EPR was identical to the cellular efflux from the polychromatic erythroblast compartment.

The major results of this analysis are presented in Table 2 and several points are noteworthy. Generally, there is a trend to a progressive increase in the intermitotic times for a given compartment with increasing weight (maturity). This general trend is also seen in progressive increases in compartmental transit times as maturation progresses. The total

Table 2. Sprague–Dawley (SD) and August-Marshall (AM) rat erythroid cell kinetic data analysed with the continuous maturation-proliferation model. SD data from the previous paper (Dörmer *et al.*, 1981), and 100 g and 200–250 g AM data from Roylance (1968) and Tarbutt & Blackett (1968), respectively. All analyses assumed the output from the polychromatic erythroblasts is equal to the EPR as determined by Ganzoni (1970). From Ganzoni EPR (50) = 11·7, EPR (100) = 6·71, EPR (150) = 4·68, EPR (200–250) = 2·83, and EPR (300) = 3·04 where the EPR is in units of 10^7 cells/100 g body weight per hr

Weight (g)	Mean intermitotic times (hr) $(\bar{T}_1, \bar{T}_2, \bar{T}_3)$	Transit times (hr) (τ_1, τ_2, τ_3)	Total transit time (hr)	Total amplification
Sprague–Dawley				
50	(8·6, 9·5, 18·5)	(0·9, 2·1, 5·5)	8·5	1·6
100	(7·8, 9·0, 18·6)	(1·0, 2·2, 6·1)	9·4	1·7
150	(8·8, 10·1, 21·2)	(0·9, 2·2, 6·6)	9·7	1·5
300	(9·8, 12·0, 26·8)	(1·2, 2·5, 7·8)	11·5	1·5
August-Marshall				
100	(7·7, 9·1, 18·5)	(0·5, 2·1, 6·7)	9·3	1·9
200–250	(10·7, 12·5, 26·1)	(1·1, 4·4, 11·0)	16·6	1·9

transit time through all of the proliferating erythroid precursors shows a definite increase with increasing maturation, rising from 8·5 hr in the 50 g animals to 11·5 hr in the 300 g animals. As for the human erythroid and neutrophilic precursors, and predicted amplification within the recognizable proliferating erythroid cells is very small (about 1·5).

In addition, for comparison the data of Roylance (1968) and Tarbutt & Blackett (1968) from the proliferating erythroid precursors of the August-Marshall rat were analyzed. Lacking any definitive estimate of the EPR for these two weight categories of the August–Marshall rat it was assumed that the EPR was given by the values determined for the Sprague–Dawley by Ganzoni (1970).

The results of this analysis are presented in Table 2. The calculated intermitotic and compartment of transit times for the 100 g August-Marshall rat are in agreement with those of the 100 g Sprague–Dawley rat. However, in the 200–250 g rats these values seem to be elevated over those of the Sprague–Dawley series. For both weight classes the predicted over amplification is 1·9.

Guinea-pig erythropoiesis

The cell kinetic data of Starling & Rosse (1976) for the proliferating erythroid precursors of the Hartley guinea-pig includes $(\Sigma_i, f_{Si}, t_{Si})$, $i = 1, 2, 3$ and again an estimate of the EPR is required to carry out the analysis of these data. Starling & Rosse (1976) give an orthochromatic erythroblast (Ortho EB) density of $1·23 \times 10^5$ cells/mm³ of bone marrow and an Ortho EB transit time of 17·7 hr to predict an EPR in the guinea-pig of $6·95 \times 10^3$ cells/mm bone marrow per hr.

The results of the analysis of these guinea-pig cell kinetic data are presented in Table 3. The data are consistent with mean intermitotic times $(\bar{T}_1, \bar{T}_2, \bar{T}_3) = (13·8, 13·5, 26·5)$ hr and compartmental transit times of $(\tau_1, \tau_2, \tau_3) = (4·5, 8·6, 21·2)$ hr to predict a total transit time through the recognizable proliferating erythroid precursors of 34·3 hr. The data also predict an amplification of 3·4 corresponding to about 1·8 mitoses throughout. Finally, the fraction of cells in mitosis data (f_{M1}, f_{M2}, f_{M3}) published by Starling & Rosse (1976), in conjunction

Table 3. The state of the guinea-pig proliferating erythroid precursors determined from the continuous maturation-proliferation hypothesis and the data of Starling & Rosse (1976)

Quantity	Proerythroblast ($i = 1$)	Basophilic erythroblast ($i = 2$)	Polychromatic erythroblast ($i = 3$)
Minimum intermitotic time (T_{LB}, hr)	12·1	11·9	20·7
Maximum intermitotic time (T_{UB}, hr)	15·5	15·2	32·3
Mean intermitotic time ($\bar{T}$, hr)	13·8	13·5	26·5
Input flux (M_1, 10^3 cells/mm^3 BM/hr)	2·1	2·6	4·0
Output flux (M_0, 10^3 cells/mm^3 BM/hr)	2·6	4·0	7·0
Compartmental transit time (τ, hr)	4·5	8·6	21·2
Amplification	1·3	1·6	1·7

with the calculated mean intermitotic times of Table 3, predict mitotic times of (t_{M1}, t_{M2}, t_{M3}) = (0·9, 0·9, 1·1) hr for the proliferating guinea-pig erythroid precursors.

DISCUSSION

One of the earliest formulations of a continuous maturation-proliferation model was that of Lajtha & Oliver (1960) for the erythropoietic precursors. Based on a number of earlier observations (Lajtha & Suit, 1955; Suit et al., 1957; Lajtha, 1959; see also Alpen & Cranmore, 1959a,b; Alpen et al., 1962) they suggested that the transition between morphologically distinguishable erythroid cell types was not dependent on mitosis and might occur throughout the cell cycle (the continuous maturation-proliferation hypothesis). They further postulated a connection between the haemoglobin content of erythroid precursor cells and their ability to divide, hypothesizing that no further divisions can take place once the cellular haemoglobin content reaches a critical threshold value. It was noted that this scheme would predict a variable number of divisions dependent on the rate of haemoglobin synthesis.

The morphological classification of erythroblasts residing in interphase or mitosis depends on subjective criteria, including nuclear and cellular size, chromatin texture and density, and cytoplasmic staining. However, whereas the haemoglobin content increases during the cell cycle, giving the cell a more reddish and mature appearance, nuclear size also increases by roughly a factor of two from early G_1 to late G_2 (Yataganas et al., 1970). Thus, on the basis of nuclear size a cell appears to become more immature as the cell cycle progresses. A coincidence of subjectively classified cells with functional cellular compartments can hardly be expected. The continuous maturation proliferation hypothesis therefore does not primarily anticipate biological events, but rather is a way of relating artificially compartmentized data to the actual events of proliferation and maturation in the erythroid system. The actual interrelationship between proliferation and maturation in erythropoiesis is insufficiently understood (Bessis & Brecher, 1975).

The concept of continuous maturation throughout the cell cycle has been utilized in cell kinetic studies of the erythroid precursors in the embryonic (Wheldon et al., 1974), young (Roylance, 1968), and mature (Hanna & Tarbutt, 1971; Tarbutt, 1967; Tarbutt & Blackett, 1968) rat and the mouse (Covelli et al., 1972). Other workers have employed this hypothesis in theoretical studies of erythropoiesis and granulopoiesis (Creekmore et al., 1978; Mary, 1978; Mary et al., 1980; Lasota & Mackey, 1981).

In the studies by Tarbutt (1967), Roylance (1968), Tarbutt & Blackett (1968) and Covelli

et al. (1972), the labelling index of the proerythroblasts was followed as a function of time following the administration of ^{55}Fe. Under the assumptions that: (1) all Pro EB are initially labelled; (2) there is no influx of ^{55}Fe labelled cells into the Pro EB compartment; and (3) there is no significant reutilization of label by the Pro EB, they then determined the compartment transit time for the Pro EB to be the time for the ^{55}Fe labelling index to go to zero. This procedure yields a value for the Pro EB transit time of 25–30 hr in the rat which is considerably greater than that determined in this study.

Prothero *et al.* (1978) have pointed out that this transit time for the Pro EB, and indeed those subsequently determined for the Baso and Poly EB, are inconsistent with the observed grain count data of Starling & Rosse (1976). They suggest that reutilization of radioactive iron may be the source of the discrepancy.

To avoid these possible errors in the ^{55}Fe labelling data, we have chosen to work backwards (from the Poly EB to the Pro EB) in our estimation of compartmental parameters. This procedure yields a consistent picture of the status of the proliferating erythroid precursors in man, the Sprague–Dawley and August-Marshall rat, and the guinea-pig, and the human neutrophilic precursors. The surprising feature common to all of these analyses is the relatively small predicted level of amplification within the recognizable proliferating precursor compartments. However, this must be viewed as a preliminary conclusion since the standard errors reported in pooled data from a number of animals are so large that they preclude an accurate assignment of the likely ranges of various calculated compartmental parameters.

Given that: (1) a sequential maturation proliferation scheme is unable to account for existing cell kinetic data (Mackey & Dörmer, 1981); and (2) a continuous maturation-proliferation scheme provides a unifying view of these data; it seems clear that the time has come for a concerted experimental examination of the continuous maturation-proliferation hypothesis. At the very least, such a study must determine $(\Sigma, f_M, t_M, f_S, t_S)$ for each recognizable proliferating precursor category, as well as the absolute production rate, in individual animals. Further, it would be helpful if another more precise determinant of cellular maturation status, e.g. haemoglobin content, were available in addition to the crude morphological classification presently available.

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APPENDIX 1

The continuous maturation-proliferation model

Consider a population of cells, each of which may be characterized by an age a as well as a maturational level μ at any time t (*cf.* Fig. 1). Cellular age a is related to the status of a cell within the cell cycle, and it is assumed that immediately following cytokinesis cells have age $a = 0$. For cells with ages between 0 and a_1, this corresponds to the G_1 portion of the cell cycle. The DNA synthesis (S) phase of the cell cycle corresponds to cells with age between a_1 and a_S; the G_2 phase to ages between a_S and a_2; and mitosis (M) to ages between a_2 and A. At age a A mitosis is complete and cytokinesis takes place. Cells are assumed to move through the cell cycle with a velocity V_a which, in general, may not be constant.

Cellular maturation is related to some observable quantity, e.g. haemoglobin content, morphological characteristics, that changes in an orderly fashion as the cell matures. Cells are assumed to mature with a velocity V_μ which may depend on a number of intrinsic or extrinsic factors. Here we assume that a certain class of cells may be defined by an initial maturity level μ_I and a final maturation level, μ_F.

Let $p(t, a, \mu)$ denote the density of cells of age a and maturation level μ at time t. Then if there is no loss from the population $p(t, a, \mu)$ satisfies the partial differential equation

$$\frac{\partial p}{\partial t} + \frac{\partial (V_a p)}{\partial a} + \frac{\partial (V_\mu p)}{\partial \mu} = 0 \qquad (A.1.1)$$

which expresses conservation of cell numbers. The solution of equation A.1.1 requires the specification of an initial condition

$$p(0, a, \mu) = g(a, \mu) \qquad (A.1.2)$$

and a boundary condition

$$p(t, 0, \mu) = 2p(t, A, \mu) \qquad (A.1.3)$$

which accounts for the cell doubling effect of cytokinesis.

Given the density of cells $p(t, a, \mu)$ from (A.1.1) in conjunction with (A.1.2, A.1.3) a number of quantities of interest to the experimentalist may be computed.

The total number of cells of all ages between maturational levels μ_I and μ_F is given directly by

$$\Sigma(t) = \int_{\mu_I}^{\mu_F} \int_0^A p(t, a, \mu) \, \mathrm{d}a \, \mathrm{d}\mu \qquad (A.1.4)$$

Further, the flux of cells out of the compartment, across the $\mu = \mu_F$ boundary, is given by

$$M_0(t, \mu_F) = \int_0^A V_\mu \, p(t, a, \mu_F) \, \mathrm{d}a \qquad (A.1.5)$$

while the input flux to the compartment at the $\mu = \mu_I$ boundary is

$$M_I(t, \mu_I) = \int_0^A V_\mu \, p(t, a, \mu_I) \, \mathrm{d}a \qquad (A.1.6)$$

The fractions of cells engaged in DNA synthesis and mitosis within a given cellular compartment are given directly by

$$f_S(t) = \frac{1}{\Sigma} \int_{\mu_I}^{\mu_F} \int_{a_1}^{a_S} p(t, a, \mu) \, \mathrm{d}a \, \mathrm{d}\mu \qquad (A.1.7)$$

and

$$f_M(t) = \frac{1}{\Sigma} \int\limits_{\mu_I}^{\mu_F} \int\limits_{a_2}^{A} p(t, a, \mu) \, da \, d\mu \qquad (A.1.8)$$

In a steady state situation when the velocities of ageing and maturation are respectively independent of cellular age a and maturation μ, then (A.1.1) takes the form

$$V_a \frac{\partial p}{\partial a} + V_\mu \frac{\partial p}{\partial_\mu} = 0 \qquad (A.1.9)$$

The steady state density $p(a, \mu)$, for constant V_a, V_μ, is given by

$$p(a, \mu) = p_0 \, e^{-Ca} \, e^{K(\mu_F - \mu_I)} \qquad (A.1.10)$$

wherein $C = A^{-1} \ln 2$ and $K = C(V_a/V_\mu)$.

With the steady state distribution of (A.1.10) the cellular density between μ_I and μ_F is

$$\Sigma = \frac{p_0}{2CK} (2^{\tau/T} - 1) \qquad (A.1.11)$$

where $T = A/V_a$ is the cell cycle time and $\tau = (\mu_F - \mu_I)/V_\mu$ is the compartment transit time. Further, the output flux is

$$M_0(\mu_F) = \frac{V_\mu p_0}{2C} 2^{\tau/T} \qquad (A.1.12)$$

while the input flux is

$$M_I(\mu_I) = \frac{V_\mu p_0}{2C} \qquad (A.1.13)$$

and thus

$$M_0(\mu_F) = M_I(\mu_I) \, 2^{\tau/T} \qquad (A.1.14)$$

Further, with these relations (A.1.11) may be written as

$$\Sigma = \frac{T(M_0 - M_I)}{\ln 2} \qquad (A.1.15)$$

Finally, note that in a steady state the fractions of the total compartment cell density Σ in S and M are, respectively,

$$f_S = 2 \cdot 2^{-t_I/T} [1 - 2^{-t_S/T}] \qquad (A.1.16)$$

and

$$f_M = 2^{t_m/T} - 1 \qquad (A.1.17)$$

where $t_1 = a_1/V_a$ is the time spent in G_1 by a cell, $t_S = (a_S - a_1)/V_a$ is the time spent in S, and $t_M = (A - a_2)/V_a$ is the time required for a cell to complete mitosis.

APPENDIX 2

Estimation of compartment parameters from M phase data

In the event that the absolute cellularity Σ and cellular output, M_0, for a compartment are known and f_M, t_M, f_S and t_S are also known then it is especially easy to estimate certain

properties of the compartment. Thus given f_M and t_M then the cell cycle time T is given, from (A.1.17), by

$$T = \frac{t_M}{\log_2(1 + f_M)} \qquad (A.2.1)$$

Given an estimate of T from (A.2.1), then with f_S and t_S it is straightforward to calculate the time spent in G1 by solving (A.1.16) for t_1:

$$t_1 = T \log_2 \{2[1 - (1 + f_M)^{-t_S/t_M}]/f_S\} \qquad (A.2.2)$$

To calculate the cellular input, M_I, into the compartment given Σ and T from (A.2.1), solve (A.1.15) for M_I to give

$$M_I = M_0 - \frac{\Sigma \ln 2}{T} \qquad (A.2.3)$$

Once the compartmental input is calculated from (A.2.3) then the compartment transit time τ is available from (A.1.14); viz,

$$\tau = T \log_2 (M_0/M_I) \qquad (A.2.4)$$

Finally, the effective number of cellular divisions within the compartment, m, is given by

$$m = \tau/T \qquad (A.2.5)$$

Any of the above relations (A.2.1, 2, 5) may be converted from base 2 logarithms to natural (base e) logarithms by using $\log_2 x = (\ln x)/\ln 2$.

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APPENDIX 3

Estimation of compartmental parameters from S phase data

From equation A.2.1, the availability of mitotic phase data (f_M, t_M) is crucial for the calculation of cell cycle time T. The cell cycle time, in turn, is needed to compute M_I and τ. However, in many situations M phase data are not available and only S phase data are given. How may we proceed to calculate T under such circumstances?

First note that, since $T = t_1 + t_S + t_2 + t_M$,

$$0 \le t_1 = T - t_S - t_2 - t_M \le T - t_S$$

so

$$0 \le \frac{t_1}{T} = 1 - \frac{t_S}{T} - \frac{(t_M + t_2)}{T} \le 1 - \frac{t_S}{T}$$

and thus

$$1 \ge 2^{-t_1/T} = 2^{(t_S - T)/T_2 \alpha} \ge 2^{(t_S - T)/T} \qquad (A.3.1)$$

where $\alpha = (t_M + t_2)/T$. Using (A.1.16), equation A.3.1 becomes

$$1 \ge \frac{f_S}{2(1 - 2^{-t_S/T})} = 2^{(t_S - T)/T_2 \alpha} \ge 2^{(t_S - T)/T} \qquad (A.3.2)$$

and an evaluation of the left and right hand inequalities allows one to specify lower and upper bounds for the cell cycle time T:

$$\frac{t_S}{\log_2(1 + f_L)} \le T = \frac{t_S}{\log_2 1 + 2^{-\alpha} f_S} \le \frac{t_S}{\log_2[2/(2 - f_S)]} \qquad (A.3.3)$$

However, note also that

$$M_I = M_0 - \frac{\Sigma \ln 2}{T}$$

and since it is required that $M_I \ge 0$ then it is clear that

$$T \ge \frac{\Sigma \ln 2}{M_0} \qquad (A.3.4)$$

Combining (A.3.3) with (A.3.4) we have

$$T_{LB} \le T \le T_{UB} \qquad (A.3.5)$$

where

$$T_{LB} = \max \begin{cases} \dfrac{t_S}{\log_2 (1 + f_S)} \\ (\Sigma/M_0) \ln 2 \end{cases} \qquad (A.3.6)$$

and

$$T_{UB} = \frac{t_S}{\log_2 [2/(2 - f_S)]} \qquad (A.3.7)$$

Often, instead of treating the range of intermitotic times defined by (A.3.5) we will use the mean intermitotic time T given by

$$\bar{T} = (T_{LB} + T_{UB})/2 \qquad (A.3.8)$$