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UNRAVELLING THE CONNECTION BETWEEN HUMAN HEMATOPOIETIC CELL PROLIFERATION AND MATURATION

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INTRODUCTION

Since the introduction of radioactive tracer techniques in the study of cell kinetics, there have been a number of studies examining the proliferative characteristics of recognizable erythroid and neutrophil precursors. Almost uniformly, these studies have utilized a conceptual analysis framework in which cells are assumed to go through mitosis at least once in each recognizable proliferating morphological category. However, it has been recognized for some time (Cronkite, 1964; Rubinow and Lebowitz, 1975) that significant discrepancies exist between the predictions of such models and the available cell kinetic data.

This paper summarizes the recent work of Mackey and Dörmer (1980) attempting to resolve these discrepancies, and then offers an alternative model for the connection between proliferation and maturation in recognizable erythroid and neutrophil precursor cells of humans.

PRELIMINARIES

Abbreviations

The following notation will be used: Pro EB = proerythroblast, Baso EB = basophilic erythroblast, Poly EB = polychromatic erythroblast, Ortho EB = othrochromatic erythroblast; MB = myeloblast, Pro = promyelocyte, Mye = myelocyte; S = DNA synthesis, M = mitosis. The index i=1,2,3 is used to designate morphological compartments,

with i=1 corresponding to the most immature but recognizable cell type of a particular cellular series (i.e. MB or Pro EB). The fraction of proliferating and recognizable precursor cells in the i th compartment is σ_i ($\sigma_1 + \sigma_2 + \sigma_3 = 1$); f_{Li} and f_{mi} are the labeling and mitotic indices of the i th compartment, and t_{si} and t_{mi} are the durations of S and M respectively. The total population density (cells/unit body weight) of recognizable proliferating precursor cells (e.g. MB+Pro+Mye) is denoted by Σ.

A known production rate for the erythroid or neutrophil series is denoted by EPR or GTR respectively (production rate units are cells/unit body weight/hr). Theoretically determined relative production rates (cells/1000 proliferating precursors/hr) are denoted by T-RPR, while T-APR denotes theoretically determined absolute production rates (cells/unit body weight/hr.

Data

To minimize variations in data as much as possible, the analysis of this paper has been restricted to data sets which contain (σ_i, f_{Li}, t_{si}) or (σ_i, f_{mi}, t_{mi}), 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. The data sources that were identified for analysis with this requirement in mind are: Brinkman and Dörmer (1976), Dörmer (1973), Rondanelli et al. (1967, 1969).

Cellular Production Rates

Comparison of the T-APR's consistent with the available cell kinetic data with known production rates is used to gauge the validity of the models. Using ^{3}HTdr in humans, Dancey et al. (1976) have measured the GTR and find it to be $(3.63 \pm 0.54) \times 10^7$ cells/kg/hr (n=5). To define the normal human EPR, the data of Wintrobe (1976) for red blood cell numbers [$(5.2 \pm 0.4) \times 10^{12}/\ell$, n=12]. Blood volume [60 $\pm$ 8.6 ml/kg, n=12], and an erythrocyte lifespan of 120 days was used to give an estimated EPR = $(1.10 \pm 0.24) \times 10^8$ cells/kg/hr (n=12).

A SUMMARY OF RECENT RESULTS

Mackey and Dörmer (1980), hereafter denoted M-D, have exhaustively examined available sets of cell kinetic data [(differential counts, labeling indices, and DNA synthesis times) or (differential counts, mitotic indices and mitotic times)] in conjunction with estimates of absolute precursor cellularity in humans and rodents for consistency with the sequential cell cycle model shown in Fig. 1a.

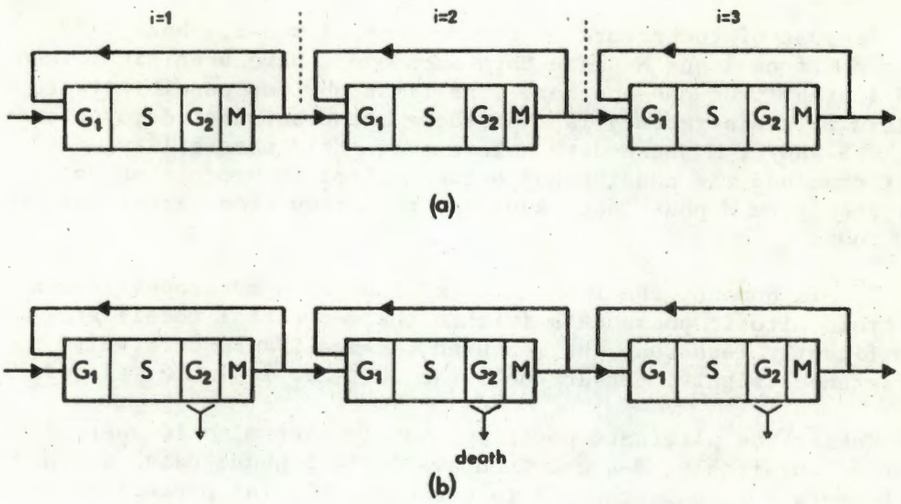

Fig. 1. (a). In this sequential model it is assumed that within each morphological category of proliferating cells, cells may either enter cycle from the previous compartment or after mitosis in the compartment in question. Cells progress through cycle (G1→S→G2→M) in a completely sequential fashion, i.e., first cell in is the first cell out. Following mitosis, some daughter cells pass to the next morphological compartment while others reenter G1 in the same compartment; (b) The ineffective hemopoiesis model is equivalent to the sequential model with the additional assumption that some cells are irreversibly removed from the G2 phase of each morphological compartment. It is assumed that these cells do not reappear elsewhere in the system, and thus this removal from G2 is equivalent to cellular death.

Using the techniques developed in M-D, it was possible to calculate the relative and absolute production rates allowed by the data, which were then compared with actual measured production rates.

The first observation of M-D was:

1. On the basis of the sequential model, in the human neutrophilic and erythroid series the T-RPR's consistent with DNA synthesis phase data are significantly greater than those consistent with mitotic phase data (difference is significant at the 2.7×10^{-3} level).

Because of the nature of the sequential model, these T-RPR's estimated from S and M phase data should not have been significantly different from one another. The first obvious possibility to explain this discrepancy is that there are consistent data errors in the S and/or M phase data. To examine this possibility, M-D first examined the consistency between absolute production rates estimated from M phase data and measured production rates, only to find that:

2. In humans, the T-APR's consistent with neutrophilic and erythroid mitotic phase data (within the sequential model) are significantly less than the measured GTR and EPR respectively (differences significant at the 5.2×10^{-4} and 7.9×10^{-3} levels).

Taking the alternate position, namely that mitotic phase data might be unreliable, M-D examined available S phase data from humans and rodents for consistency with measured cellular production rates. They found that:

3. In humans, the T-APR calculated from erythroid S phase data using a sequential model is not significantly different from the measured erythrocyte production rate; but

4. For the human neutrophilic series and the erythroid series of the Sprague-Dawley rat (four different weights), the August-Marshall rat (two different weights), and the Hartley guinea pig, the T-APR's calculated from S phase data using the sequential model were significantly different from measured rates in every case.

Since the S phase data for the human neutrophil and erythroid precursors, as well as for the four weight categories of the Sprague-Dawley rats, were all collected in the same laboratory using the same techniques, M-D concluded that the agreement noted in (3) above might be fortuitous. It was further noted (Mackey and Dormer, 1980, Table 2) that within a given morphological compartment, in the human erythroid and neutrophilic precursors it was a consistent finding that the relative flux of cells through S was always greater than through M. They argued that a reasonable hypothesis might be that some cells leave a given morphological compartment during G2, and on this basis analyzed the human S and M cell kinetic data within the context of two alternate models.

The first of these alternate models, the ineffective hemopoiesis model, is illustrated schematically in Figure 1b. Within this scheme the discrepancy between S and M phase cellular fluxes is removed by assuming that a fraction of cells die during G2. This is an extension of the Patt and Maloney (1964) hypothesis. In spite of the removal of the discrepancy between S and M phase data, however, M-D found that:

5. Ineffective hemopoiesis must be rejected as an adequate model, since the T-APR predicted for both the human erythroid and neutrophilic series was significantly less than the measured production rates (differences significant at the 1.2×10^{-2} and 2.5×10^{-3} levels respectively).

The second alternate model utilized by M-D is illustrated in Figure 2. This model also reconciles differences between S and M phase fluxes, but by postulating that cells may pass between morphological compartments during G2 as well as during a postmitotic GO phase. Based on their analysis of the human cell kinetic data, Mackey and Dörmer concluded that:

6. Though the GO/G2 maturation model provides a sufficient explanation for the combined S and M phase data for both the human erythroid and neutrophilic precursors, it must be rejected on the grounds that it further implies that the flux of cells out of G2 at the myelocyte and polychromatic erythroblast stages is so large that significant fractions of metamyelocytes and orthochromatic erythroblasts with 4N DNA content would be present.

Mackey and Dörmer concluded from their study that either: 1) There exist large, and unknown, errors in all extant sets of cell kinetic data from human erythroid and neutrophilic precursors; or 2) The connection between hemopoietic cellular proliferation and maturation is quite different from any postulated model.

AN EARLY S PHASE MATURATION HYPOTHESIS

Yataganas et al. (1970) conducted a cytophotometric determination of DNA and hemoglobin (Hb) content in individual erythroid precursor cells from four normal human subjects. They found that within each of the recognizable proliferating morphological compartments (Pro EB, Baso EB, and Poly EB) Hb content increased during GO-G1 and the early part of S phase. Within each cell category Hb content remained approximately constant during middle and late S phase.

Taking this observation, and drawing the correspondence that increasing Hb content is in some fashion related to cellular maturation, I have analyzed the human erythroid cell kinetic data within the context of the model in Figure 3. As shown, I have assumed that maturation may take place during GO-G1 as well as an early phase of S, denoted by S1. In the remainder of S (denoted by S2) it is assumed that maturation may not take place. The equations necessary to determine the relevant fluxes in such a model are developed in the Appendix.

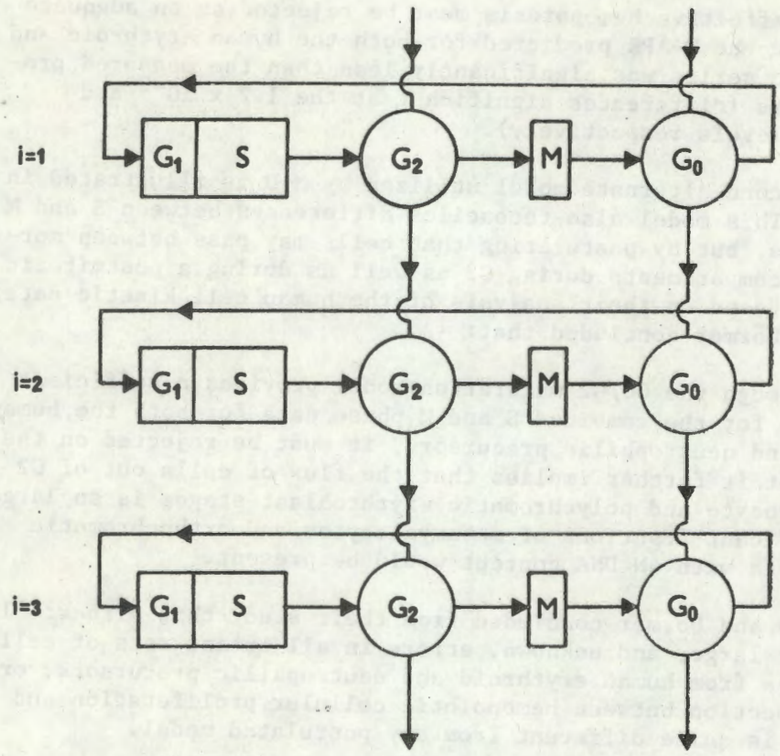

Fig. 2. This G_0/G_2 transition model tries to qualitatively account for the discrepancy between the flux of cells through S and through M by postulating that cells pass to the next recognizable morphological category out of G_2 as well as G_0. Thus, for example, a cell which completed DNA synthesis as a Pro EB might pass into the Baso EB compartment during G_2 and would probably go through mitosis in the Baso EB stage. However, if the transition rate was sufficiently large, it might not go through mitosis until it reached the poly EB stage.

The results of analyzing the human erythroid cell kinetic data with this model (cf. Table 1, Appendix) indicate that the cellular efflux from the S1 portion of S1 portion of S at the Poly EB level would comprise approximately 11% of the total EPR. Unfortunately, though it is possible to specify the magnitude of the fluxes between various compartments, since it is not possible to specify the size of S1 it is thus not possible to determine the likely range of DNA content that cells in S1 would possess).

Carrying this concept over to the human neutrophilic precursors, I have also analyzed the cell kinetic data from that system.

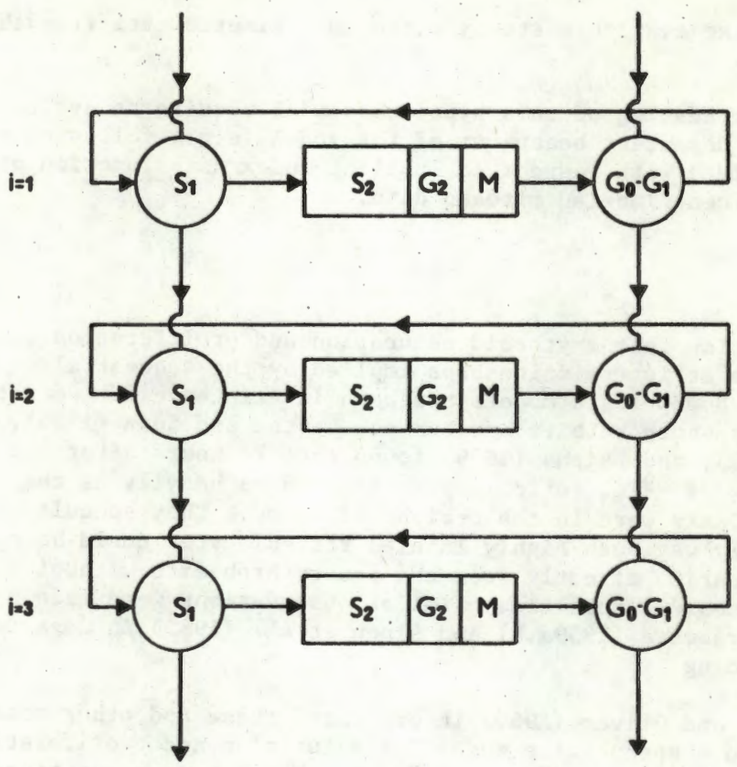

Fig. 3. In this model, it is assumed that cells may pass between morphological compartments during a postmitotic GO-G1 phase as well as during an early portion of S denoted by S1.

The detailed results are presented in the Appendix (Table 2), and they indicate that the cellular efflux from the Mye S1 cells would have to comprise about 52% of the GTR.

One of the most surprising predictions of such a model concerns the relative amplification to be expected within the entire recognizable proliferating compartments. Thus within the human erythroid precursors a total amplification of 3.13 is calculated, corresponding to 1.6 mitoses on the average. For the neutrophilic precursors the number of possible mitoses is estimated to be 1.3.

Though many questions of importance are unanswered at this stage, I tentatively conclude that:

7. The hypothesis that human erythroid and neutrophilic precursors may mature between compartments during GO-G1 as well as during an early portion of S phase offers a sufficient unifying

picture of the available steady state cell kinetic data from these two systems.

Further testing of this hypothesis will require an evaluation of the time dependent behaviour of the model (e.g., following a pulse of ^{3}HTdr) with respect to labeling index as a function of time and percent labeled mitoses data.

DISCUSSION

The notion that erythroid maturation and proliferation might not have the strict relationships implied by the sequential or ineffective hemopoiesis models has been in existence for some time. In studies in both rabbits and humans, Lajtha and Suit (1955), Suit et al. (1957), and Lajtha (1959) found that 24 hours after the administration of ^{59}Fe, reticulocytes labeled as heavily as the proerythroblasts were in the peripheral blood. They speculated that the only way such highly labeled reticulocytes could be present was if maturation directly from the proerythroblasts without intervening mitoses was possible. Similar observations were made by Alpen and Cranmore (1959a,b) and Alpen et al. (1962) in dogs following bleeding.

Lajtha and Oliver (1960) incorporated these and other observations into a speculative model for maturation and proliferation within the erythron. They suggested there exists a connection between the hemoglobin content of erythroid precursor cells and their ability to divide, postulating that no further divisions can take place once the hemoglobin content reaches a critical threshold value. They further pointed out that this scheme would predict a variable number of divisions dependent on the rate of hemoglobin synthesis.

Stohlman and coworkers (c.f. Stohlman, et al., 1960, for a summary and further references and Bessis and Brecher, 1975, Brecher et al., 1975 for more recent work) experimentally examined aspects of this hypothesis. Based on their experimental and clinical findings they hypothesized that: 1) Erythropoietin triggers hemoglobin synthesis; 2) The rate of hemoglobin synthesis is proportional to the concentration of erythropoietin; and 3) When the intracellular hemoglobin concentration reaches a critical level (corresponding to a MCHC of approximately 20%) nucleic acid synthesis terminates leading to a cessation of cellular division. [Subsequently, Yataganas et al. (1970) have shown that in humans, when the MCHC > 22% there are no cells in DNA synthesis or in G_2, though a causal connection has yet to be established]. Stohlman et al. (1968) noted that with these three assumptions and an assumed fixed cellular generation time, the rate of hemoglobin synthesis will determine the number of divisions within the erythron.

Specifically, increasing the hemoglobin synthesis rate with erythropoietin should decrease the time required to reach the critical hemoglobin concentration, decrease the number of divisions within the erythroid precursors, and result in the appearance of macrocytic reticulocytes. Conversely, decreasing hemoglobin synthesis rates by Fe deficiency should lead to an increase in the time required to reach the critical hemoglobin concentration, and result in microcytic reticulocytes. These effects were observed in their laboratories. Further, Mueller et al. (1973) have shown that in the normal human there is a variable number of divisions within the erythroid precursors, while in the anemic state the critical hemoglobin concentration seems to be decreased below normal and there is a consequent reduction in the number of erythroid mitoses. (Mueller et al., 1974).

In addition to these in vivo observations there exist a number of intriguing in vitro findings suggesting that cellular maturation may take place in the absence of DNA synthesis and/or mitosis. Thus Gallien-Lartigue and Goldwasser (1965) noted that mitotic block by colchicine in cultured rat marrow decreased the Hb synthesis stimulatory action of erythropoietin (EPO) but did not completely destroy it. Later, Bedard and Goldwasser (1976) demonstrated that cytosine arabinoside (Ara C) inhibition of DNA synthesis in cultured rat marrow cells did not destroy the ability of EPO to stimulate Hb synthesis. Similar results were obtained by Paul and Conkie (1973) using various inhibitors of DNA synthesis.

Benestad and Rytömaa (1977) utilized three different procedures to inhibit the proliferative activity of mouse bone marrow cells cultured in diffusion chambers. In every case inhibition of proliferative activity was accompanied by an increase in the maturation velocity of neutrophil precursors.

Ebert et al. (1976) have shown that in the T3-C12 line of Friend leukemia cells, inhibition of DNA synthesis by a number of agents (dimethyl sulfoxide, Ara C, hydroxyurea, mitomycin C and adriamycin) was accompanied by an increase in Hb synthesis. A similar observation was recently reported by Rovera et al. (1980), who showed that 12-O-tetradecanolphorbol-13-acetate (TPA) inhibits DNA synthesis in a human promyelocytic leukemia cell line (HL-60) at the same time it induces terminal differentiation into macrophage-like cells.

These in vivo and in vitro data strongly suggest that the connection between hematopoietic cell proliferation and maturation is a far more dynamic process than is usually conceived. They clearly indicate that under certain circumstances maturation may take place in the absence of DNA synthesis and/or mitosis in precursor compartments, and in some cases it would appear that there is a "tradeoff" between proliferative activity and maturation.

I think that it is probably more realistic to conceptually view this maturation/proliferation process as a continuous one in which maturation is described by a continuous variable (or perhaps a set of variables) while progression through the cell cycle is described by another. In the most general case, the velocities of progression through the cell cycle and maturation might well depend on maturation levels, position within the cell cycle, and on extrinsic regulatory factors, e.g. EPO. Work is currently underway analyzing continuous models like these to explore the implications of such a hypothesis. The GO-G1/S1 maturation model of this paper is a special case of such a continuous model, in which the velocity of maturation is assumed to be zero except in GO-G1 and S1.

APPENDIX. Analysis of the GO-G1/S1 maturation model

In this model it is assumed that the proliferating morphological cell compartments may be represented as shown in Figure 3. Let the total number of cells in the i th compartment be Σ_i, the mitotic index f_{mi}, and the mitotic time t_{mi}. Then the flux of cells through M is given by

$$F_i^m = \Sigma_i \, f_{mi}/t_{mi}$$

If there was no cellular efflux from S1, then the total flux of cells through S would be given by

$$F_i^s = \Sigma_i \, f_{Li}/t_{si}$$

where f_{Li} and t_{si} are the labeling index and DNA synthesis time. However, by assumption there is a flux out of S1, $F_i^{O,S1}$, to the next morphological compartment such that the flux of cells through S2 is equal to F_i^m. Thus the cellular efflux from S1 is given by

$$F_i^{O,S1} = F_i^S - F_i^m$$

The total output flux for the i th compartment to the next compartment is F_i^O, so we may immediately write that the efflux from the i th GO-G1 phase to the next is

$$F_i^{O,G} = F_i^O - F_i^{O,S1}$$

In a steady state all fluxes must balance to maintain constant cellular populations. Thus for the i th S1 phase we must have

Table 1. The relevant fluxes for the GO-G1/S1 maturation model calculated from the human erythroid data

Flux (cells/kg-hr)	Poly EB	Baso EB	Pro EB
F^{O}	1.10×10^{8}	5.96×10^{7}	4.37×10^{7}
$F^{O,S1}$	1.22×10^{7}	1.04×10^{7}	3.87×10^{6}
$F^{O,G}$	9.78×10^{7}	4.92×10^{7}	3.98×10^{7}
F^{f}	5.24×10^{7}	2.23×10^{7}	-
F^{I}	5.96×10^{7}	4.37×10^{7}	3.64×10^{7}
$F^{I,S1}$	1.04×10^{7}	3.87×10^{6}	-
$F^{I,G}$	4.92×10^{7}	3.98×10^{7}	-

Table 2. Human neutrophilic precursor fluxes determined from the GO-G1/S1 model

Flux (cells/kg-hr)	Poly EB	Baso EB	Pro EB
F^{O}	3.63×10^{7}	2.45×10^{7}	1.91×10^{7}
$F^{O,S1}$	1.90×10^{7}	1.38×10^{7}	3.10×10^{6}
$F^{O,G}$	1.73×10^{7}	1.07×10^{7}	1.60×10^{7}
F^{f}	1.70×10^{7}	1.61×10^{7}	-
F^{I}	2.45×10^{7}	1.91×10^{7}	1.48×10^{7}
$F^{I,S1}$	1.38×10^{7}	3.10×10^{6}	-
$F^{I,G}$	1.07×10^{7}	1.60×10^{7}	-

$$F_i^{O,S1} + F_i^m = F_i^{I,S1} + F_i^f$$

where $F_i^{I,S1}$ is the input to the i th S1 phase from the (i-1) S1 phase (clearly $F_i^{I,S1} = F_{i-1}^{O,S1}$) and F_i^f is the "feedback" flux from GO-G1 to S1. Balancing the fluxes for the i th GO-G1 phase in an analogous fashion gives

$$2F_i^m + F_i^{I,G} = F_i^{O,G} + F_i^f$$

where $F_i^{I,G}$ is the input flux from the (i-1) GO-G1 phase. Here, again, $F_i^{I,G} = F_{i-1}^{O,G}$. From these two balance equations for GO-G1 and S1 it is immediate that

$$F_i^I = F_i^O - F_i^M$$

Thus, with the above equations we may immediately determine all of the relevant fluxes in the model of Figure 3. However, it must be noted that for the most proximal compartment of the system it is not possible to determine $F^{I,S1}$, $F^{I,G}$ (though F^I may be determined) or F^f.

Table 1 shows the results of an analysis of the human erythroid S phase data of Dörmer (1973) and M phase data of Rondanelli et al. (1969), assuming $\Sigma = 2.74 \times 10^9$ cells/kg (Dörmer, 1973) and an EPR = 1.1×10^8 cells/kg-hr.

The analogous computation of Table 2 for the human neutrophilic precursors is based on the Brinkman and Dörmer (1976) S phase data and the Rondanelli et al. (1967) M phase data. Here $\Sigma = 2.11 \times 10^9$ cells/kg and GTR = 3.63×10^7 cells/kg-hr (Dancey et al., 1976).

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