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ENIGMATIC HEMOPOIESIS

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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[1-3] that significant discrepancies exist between the predictions of such models and the available cell kinetic data.

Similar problems were encountered in an attempt to analyze the data of Dörmer et al.,[4] and these prompted us to conduct an in depth study of what we feel to be the best sets of cell kinetic data (as defined in the Methods section) on human and nonhuman erythroid and neutrophil proliferating precursor cells. We have analyzed these data within the framework of a 'classical' sequential model, and an ineffective hemopoiesis model. The data are inconsistent with both models. We conclude that there are either significant but unknown errors in almost all published erythroid and neutrophil cell kinetic data, or the usual concept of the relation between cellular maturation and proliferation in these two systems is inaccurate.

METHODS

Abbreviations

The following notation will be used: Pro EB = proerythroblast, Baso EB = basophilic erythroblast, Poly EB = polychromatic erythroblast, Ortho EB = orthrochromatic 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 PR, while the specific known erythroid and neutrophilic production rates are designated by EPR and GPR respectively (production rate units are cells/unit body weight/hr). Theoretically determined (c.f. Models, below) 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).

Models for data analysis

In this paper available cell kinetic data for proliferating recognizable erythroid and neutrophil precursors are analyzed with two different models and examined for consistency with known EPR's and GPR's.

In any examination of a set of cell kinetic data within the context of a given model connecting cellular proliferation with cellular transitions between morphological compartments, agreement between steady state data and the corresponding model relations is necessary, but not sufficient, for model validation. Once agreement between steady state data and a particular model is obtained, further confirmation rests on a demonstration that time dependent data (such as the time course of labeled cells after a pulse of label) is consistent with the time dependent behaviour of the model. Throughout this study our considerations have been restricted to steady state situations.

A sequential model. In this model (Figure 1a) 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 re-enter G1 in the same compartment.

This model is analyzed in APPENDIX 1 where it is shown that, given a set of cell kinetic data (σ_i, f_{Li}, t_{Si}) or (σ_i, f_{mi}, t_{mi}), consisting of differential counts, labeling (or mitotic) indices, and S (or M) phase durations, it is possible to define a range of relative production rates (T-RPR) consistent with the data (equation 1.13 et.seq.). (The T-RPR is the efflux from the most mature and final proliferating compartment, i.e., Poly EB or Mye). Given this T-RPR range consistent with the data, an estimate for the absolute cellularity

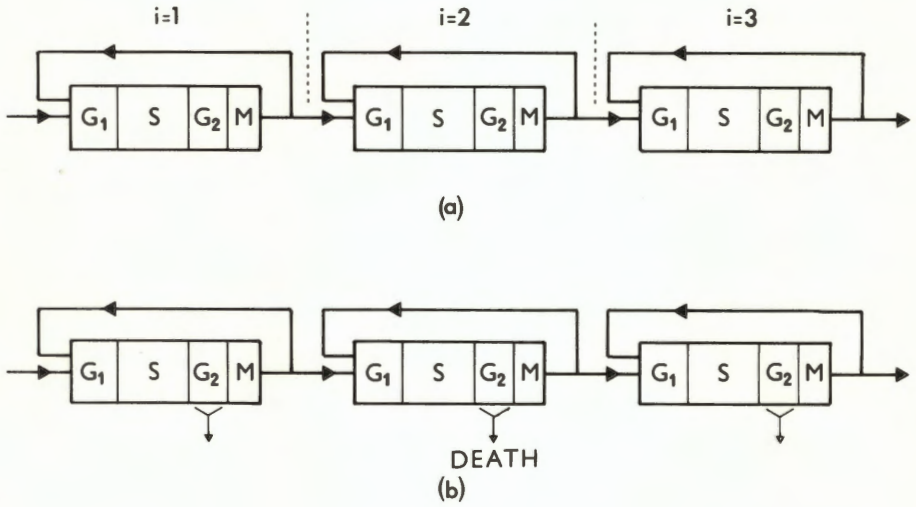

Fig. 1. Diagrammatic representation of the relation between the cell cycle and morphological compartments for the sequential (a) and ineffective hemopoiesis (b) models, i=1 corresponds to the most immature recognizable morphological category (proerythroblast or myeloblast), i=2 to the basophilic erythroblast or promyelocyte, and i=3 to the last proliferating compartment (polychromatic erythroblast or myelocyte). The cellular input to the i=1 category is presumed to come from the appropriate stem cell compartment, while the cellular efflux from the last proliferating compartment (i=3) is taken as the cellular production rate.

Σ of the recognizable proliferating precursor population, and the assumption of negligible cell loss in the post-proliferative maturation compartments, a range of theoretical absolute production rates (T-APR) may be calculated: T-APR = (Σ).(T-RPR). This T-APR is then compared with the known EPR or GPR and the level of significance (P) of the difference between the two is determined.

Ineffective hemopoiesis model. The sequential nature of the first model, coupled with the assumption of no in-cycle cellular loss, implies that the flux of cells through DNA synthesis and mitosis must be identical within a given morphological compartment. Since this relation is not obeyed by the data (c.f. RESULTS, below) a second model has been used to analyze the data. This ineffective hemopoiesis model is illustrated in Figure 1b.

This model, analyzed in APPENDIX 2, 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. The analysis of APPENDIX 2 gives well defined bounds on the T-RPR's allowed by the data (equations 2.10 - 2.12).

Data

To minimize variations in data as much as possible, the analysis of this paper has been restricted to data sets which contain $(\sigma_i, f_{Li}, t_{Si})$ or $(\sigma_i, f_{mi}, t_{mi})$, 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. Eight sets of data were identified for analysis with this requirement in mind[4-11].

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.[12] have measured the GPR 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[13] for red blood cell numbers $[(5.2 \pm 0.4) \times 10^{12}/\ell$, n=12], blood volume [60 ± 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).

Ganzoni[14] has determined the EPR in Sprague-Dawley rats as a function of weight, and these values have been used in the analysis of the rat erythroid cell kinetic data[4,9,11].

The data of Starling and Rosse[10] for the Hartley guinea pig give an Ortho EB density of $(1.23 \pm 0.10) \times 10^5$ cells/mm^3 of bone marrow and an Ortho EB transit time of approximately 17.7 hours to give an EPR for the guinea pig of $(6.95 \pm 0.57) \times 10^3$ cells/mm^3 bone marrow/hr (n=8).

RESULTS

The sequential model

Inconsistencies between S and M phase data. For the sequential model, in every proliferating compartment the flux of cells through DNA synthesis and mitosis must be identical, i.e., $(f_{Li}/t_{Si}) = (f_{mi}/t_{mi})$, i=1,2,3. In Table 1 (f_L/t_S) is compared with (f_m/t_m) for each proliferating erythroid and neutrophilic precursor stage in humans. In every category of cells, $(f_L/t_S) > (f_m/t_m)$ and with a threshold significance level of 5.00×10^{-2} the difference is significant in every case.

TABLE 1

A COMPARISON OF THE RELATIVE CELLULAR FLUXES THROUGH S AND M IN HUMANS

For DNA synthesis phase data[5,6], n=5; mitotic phase data[7,8], n=10

Cell Type	$(f_L/t_S) \times 10^2$	$(f_m/t_m) \times 10^2$	P
Pro EB	8.12 ± 1.21	5.22 ± 0.82	4.84×10^{-5}
Baso EB	6.37 ± 1.24	3.81 ± 0.77	1.28×10^{-4}
Poly EB	2.97 ± 0.59	2.36 ± 0.43	1.95×10^{-2}
MB	5.62 ± 1.22	3.17 ± 0.14	3.12×10^{-4}
Pro	5.28 ± 0.80	1.48 ± 0.12	5.62×10^{-10}
Mye	1.97 ± 0.65	0.77 ± 0.24	7.64×10^{-4}

The human neutrophilic and erythroid data are further analyzed in Tables 2a and 3a, which give the relative production rates consistent with the DNA synthesis and mitotic phase data calculated from the sequential model.

TABLE 2

ASSESSMENT OF HUMAN NEUTROPHILIC CELL KINETIC DATA WITH THE SEQUENTIAL MODEL

a. Comparison of the theoretically allowed relative human neutrophil production rates predicted from DNA synthesis phase[5] and mitotic phase data[7]. T-RPR expressed as cells/10^3 proliferating neutrophilic precursors/hr.

Data	n	T-RPR	P
S	5	30.01 ± 12.57	2.72×10^{-3}
M	10	11.08 ± 2.71	

b. Comparison of predicted and measured absolute neutrophil production. The production rates (T-APR) are based on the relative production rates of (a), and an absolute proliferating neutrophil precursor density[12] of 2.11×10^9 cells/kg. The T-APR's are compared with the actual production rate[12] (GPR) determined using H^3Tdr. All production rates in units of 10^7 cells/kg/hr.

Quantity	Data	n	Production Rate	P
T-APR	M	10	2.34 ± 0.57	5.21×10^{-4}
GPR	^{3}HTdr	5	3.63 ± 0.54	
T-APR	S	5	6.33 ± 2.65	2.80×10^{-2}

TABLE 3

EVALUATION OF HUMAN ERYTHROID CELL KINETIC DATA WITH THE SEQUENTIAL MODEL

a. Comparison of the T-RPR's from DNA synthesis phase data[6] and mitotic phase data[8]. T-RPR's expressed as cells/10^3 proliferating erythroid precursors/hr.

Data	n	T-RPR	P
S	5	42.64 ± 16.86	3.98×10^{-2}
M	10	29.65 ± 9.91	

b. A comparison of predicted and measured absolute erythroid production. The T-APR's are based on the T-RPR's of (a), and an absolute[6] proliferating erythroid precursor density of 2.74×10^9 cells/kg. The actual production rate (EPR) is calculated as detailed in METHODS. All production rates in units of 10^8 cells/kg/hr.

Quantity	Data	n	Production Rate	P
T-APR	M	10	0.81 ± 0.27	7.91×10^{-3}
EPR		12	1.10 ± 0.24	
T-APR	S	5	1.17 ± 0.46	.38

From these results we conclude that: *i. 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 larger than those consistent with mitotic phase data.* This conclusion, it must be noted, is independent of any estimate of proliferating precursor cell densities or actual production rates.

What might be the source of this discrepancy? The first obvious possibility is that there exists a consistent error(s) in the DNA synthesis and/or mitotic phase data. These could be: 1) (f_L/t_S) is too large in every morphological category (due, e.g. to an overestimation of f_L or underestimation of t_S); and/or 2) (f_m/t_m) is consistently too small in each compartment because of an underestimation of f_m or values of t_m that are too large.

A second possibility which might account for these discrepancies is that the data are correct but that the sequential model is an incorrect representation of the movement of cells through the compartments of the proliferating pool.

The first possibility (data error) is examined below by independently analyzing the mitotic phase and DNA synthesis phase data for consistency with actual neutrophil and erythroid production rates. Later, the second possibility (incorrect model) is explored by analyzing the S and M phase data within the

framework of the ineffective hemopoiesis model.

Implications of the mitotic phase data alone. Here it has been assumed that the available DNA synthesis phase data are unreliable and the mitotic phase data are examined, using the sequential model, for agreement between the T-APR and the actual production rates.

From the calculations presented in Tables 2b and 3b, it is clear that: *ii. In humans, the T-APR's calculated from the sequential model with neutrophilic and erythroid mitotic phase data are significantly less than the GPR and EPR respectively.*

Thus, based on these analyses, it seems that conclusion *i* of the previous section cannot be resolved by simply assuming that human neutrophilic and erythroid S phase data are unreliable.

Implications of the DNA synthesis phase data alone. In contrast to the previous section, here it is assumed that the mitotic phase data are suspect and the DNA synthesis phase data alone are examined with the sequential model for consistency between the T-APR and the known production rates.

From the results of our calculations presented in Tables 2b, 3b, and 4 it is clear that: *iiia. For the human erythroid series, conclusion i above can be resolved by assuming the mitotic phase data is incorrect and working with the available DNA synthesis phase data which, with the sequential model, predicts an erythrocyte production rate not significantly different from the actual value;* and *iiib. For the human neutrophilic series, and the erythroid series of the Sprague-Dawley rat, the August-Marshall rat, and the Hartley guinea pig, the T-APR's calculated from the sequential model with DNA synthesis phase data differ significantly from known production rates.*

The discrepancy noted in the human neutrophilic series between the T-RPR's based on DNA synthesis and mitotic phase data (Table 2a), coupled with the failure of either set of data (DNA synthesis or mitotic) to singly predict the correct GPR (Table 2b), implies that if the problem is due to data error alone it is not confined to a single set of data (DNA synthesis phase, mitotic phase, or proliferating neutrophilic precursor cell density). On the other hand, the success of the human erythroid series DNA synthesis phase data in predicting the EPR (Table 3b) would implicate the erythroid mitotic phase data if the discrepancy in the T-RPR's from DNA synthesis and mitotic phase data (Table 3a) is due to data errors.

Though at this point errors in the human erythroid mitotic phase data cannot be ruled out, it is reasonable to examine models other than the sequential model for their potential ability to embrace all of the existing DNA synthesis

TABLE 4

SPRAGUE-DAWLEY (SD) AND AUGUST-MARSHALL (AM) RAT ERYTHROID CELL KINETIC DATA ANALYZED WITH THE SEQUENTIAL MODEL

SD cell kinetic data from Dörmer et al.,[4] and 100 gm and 200-250 gm AM data from Roylance[9] and Tarbutt and Blackett[11] respectively. All T-RPR in units of cells/10^3 proliferating erythroid precursors/hr; absolute production rates (T-APR and EPR) in 10^7 cells/100 gm/hr; proliferating precursor densities in 10^8 cells/100 gm.

Weight	Quantity	T-RPR	Precursor Density	n	Production Rate	P
50 gm SD	T-APR	80.37 ± 26.51	8.38	7	6.74 ± 1.89	1.89×10^{-3}
	EPR	-	-	4	11.70 ± 2.32	
100 SD	T-APR	77.65 ± 16.78	5.22	7	4.05 ± 0.88	1.71×10^{-3}
	EPR	-	-	11	6.71 ± 1.91	
150 SD	T-APR	69.63 ± 15.68	3.82	7	2.66 ± 0.60	1.83×10^{-4}
	EPR	-	-	13	4.68 ± 1.45	
300 SD	T-APR	55.30 ± 13.50	2.96	7	1.64 ± 0.40	1.95×10^{-7}
	EPR	-	-	10	3.04 ± 0.28	
100 AM	T-APR	84.48 ± 31.43	5.22	10	4.41 ± 1.64	4.17×10^{-3}
	EPR	-	-	11	6.71 ± 1.91	
200-250 AM	T-APR	60.71 ± 21.40	3.71	10	2.25 + 0.80	2.99×10^{-2}
	EPR	-	-	9	2.83 ± 0.41	

HARTLEY GUINEA PIG ERYTHROID DATA ANALYZED WITH THE SEQUENTIAL MODEL

Data from Starling and Rosse[10]. T-RPR in same units as above. T-APR (10^3 cells/mm^3 bone marrow/hr) based on a proliferating erythroid precursor density of 1.51×10^5 cells/mm^3 bone marrow.

	T-APR	50.94 ± 6.94	-	8	7.70 ± 1.05	4.8×10^{-3}
	EPR	-	-	8	6.95 ± 0.57	

and mitotic phase data.

Since it is a ubiquitous finding that, within a given morphological compartment, the relative flux of cells through DNA synthesis is greater than through mitosis (Table 1) it is reasonable to argue that some cells may leave a given morphological compartment during G2. In the event that these cells die, then the ineffective hemopoiesis model (c.f. METHODS) analyzed in the next section is appropriate.

Ineffective hemopoiesis

This model is described in the Methods section above, illustrated in Figure 1b, and analyzed in APPENDIX 2. As the formulae of APPENDIX 2 indicate, when taking standard deviations of the data into account the calculated lower bound on the T-RPR may be negative, in which case the actual lower bound is set equal to zero.

TABLE 5

HUMAN NEUTROPHIL AND ERYTHROID PRODUCTION RATES FROM THE INEFFECTIVE HEMOPOIESIS MODEL

a. Calculations based on the neutrophilic data of Brinkman and Dörmer[5] and Rondanelli et al.,[7] and an absolute proliferating neutrophilic precursor density[12] of 2.11×10^9 cells/kg. T-APR and GPR in units of 10^7 cells/kg/hr.

Quantity[a]	T-RPR[b]	n	Production Rate	P[c]
T-APR[A]	6.00 ± 6.00	5	1.27 ± 1.27	2.53×10^{-3}
GPR	-	5	3.63 ± 0.54	
T-APR[B]	5.73 ± 5.73	5	1.21 ± 1.21	1.76×10^{-3}

b. Erythroid cell kinetic data from Dörmer[6] and Rondanelli et al.[8] and an absolute[6] proliferating erythroid precursor density of 2.74×10^9 cells/kg.

Quantity[a]	T-RPR[b]	n	Production Rate	P[c]
T-APR[A]	20.05 ± 10.05	5	0.60 ± 0.60	1.16×10^{-2}
EPR	-	12	1.10 ± 0.24	
T-APR[B]	24.88 ± 24.88	5	0.68 ± 0.68	3.58×10^{-2}

[a] [A] corresponds to a use of differential counts from S phase data, while [B] involved using M data differential counts.

[b] All relative production rates in units of cells/10^3 proliferating precursors/hr.

[c] The t values and significance levels were computed under the assumption that the T-APR came from 5 samples (n=5 for the S phase data) in spite of the fact that n=10 for the M phase data.

The result of analyzing the available DNA synthesis and mitotic phase data for the human neutrophil precursors is presented in Table 5a. Analogous calculations based on the human erythroid precursor DNA synthesis and mitotic phase data are presented in Table 5b.

From these results it is clear that: *iv. Ineffective hemopoiesis is not a sufficient explanation for the discrepancies noted in conclusion i because ineffective hemopoiesis predicts T-APR's for human neutrophilic and erythroid series cells significantly less than the known GPR and EPR respectively.*

DISCUSSION

Cronkite[1] and Rubinow[2] pointed out some of the same inconsistencies noted here when hemopoietic cell kinetic data are examined within the context of a sequential proliferation-maturation model. Later, Rubinow and Lebowitz[3] analyzed DNA synthesis and mitotic phase data for human neutrophil precursors, noting the same qualitative types of inconsistencies as in this paper, and ended their analysis with "We conclude that the present day estimates of the kinetic parameters of the proliferative neutrophil precursor pools are not consistent with any given theoretical scheme of proliferation".

In his examination of the extant data of 1964, Cronkite[1], mindful of variations between subjects and possible inconsistencies between laboratories, suggested that "...one probably needs to measure mitotic time, DNA synthesis time, and labeling and mitotic indices in the same individual". Though Cronkite's suggestion has not yet been implemented, the present study goes some way in this regard since cell kinetic data differential counts, cell cycle indices (labeling or mitotic) and phase durations (DNA synthesis or mitotic times) from the same subjects, collected by the same laboratories at the same time, have been used. In spite of the availability of presumably better data for the present study, the inconsistencies noted some 17 years ago remain. The availability of erythroid and neutrophilic DNA synthesis phase data on normal humans from the same laboratory, and mitotic data from another laboratory offers a unique opportunity for comparison of the two.

Here, it was a consistent finding that: 1) The flux of cells through S phase is significantly greater than the flux of cells through mitosis for every morphological category; and 2) Within the context of the sequential model, theoretical relative production rates based on DNA synthesis phase data are always significantly greater than the relative production rates

calculated on the basis of mitotic data for both the erythroid and neutrophilic series. These observations point out inconsistencies between S and M cell kinetic data, independent of any absolute cellular density estimates, or estimations of erythroid and neutrophil production rates.

When absolute proliferative precursor densities were used in conjunction with the theoretical relative production rates calculated from DNA synthesis and mitotic data, the human theoretical absolute production rate based on DNA synthesis data was always greater than the measured erythroid or neutrophil production rates, while the theoretical absolute production rates based on mitotic data were less than the measured production rates. For the human neutrophilic series all of the differences are significant, but for the erythroid series it is only the difference between the theoretical absolute production rate based on M phase data and the measured EPR that is significant. If these differences are interpreted to reflect cell kinetic data errors, then we would conclude that neutrophilic DNA synthesis and mitotic data are incorrect, as are the erythroid mitotic data, but that the erythroid DNA synthesis data is free of errors. This, however, is difficult to explain as all of the human DNA synthesis phase cell kinetic data were collected in the same laboratory using the same techniques, as were the Sprague-Dawley rat DNA synthesis phase data.

In conclusion, from our sequential model analysis it seems possible that there may exist large systematic errors in published cell kinetic data based on DNA synthesis and mitotic phase studies, though there is no conclusive evidence one way or another on this point. However, this observation should serve as a challenge to experimentalists to re-examine their techniques in an attempt to validate them.

As an alternative to assuming the existence of errors within the available cell kinetic data, other models for the relation between cellular maturation and proliferation have been examined.

Patt and Maloney[15] studied neutrophilic precursor kinetics in dogs. They noted that their S phase data, analyzed within the context of a sequential model, predicted an efflux from the myelocyte compartment much greater than the predicted cellular influx into the metamyelocytes. To account for this difference, they postulated the existence of a myelocyte sink into which a fraction of myelocytes was lost to death.

Since (Table 1) human cell kinetic data implies that the cellular flux through DNA synthesis is always greater than the flux through mitosis within a given compartment, it seems reasonable to generalize the Patt and Maloney

hypothesis to allow for the possibility of cellular loss from every morphological stage. This led to the formulation of the ineffective hemopoiesis model, which would qualitatively account for these observations. However, as is illustrated in Table 5, the consequences of the ineffective hemopoiesis hypothesis are not quantitatively consistent with the data. The ineffective hemopoiesis model always predicts a theoretical absolute production rate significantly less than the actual production rate for both the human erythroid and neutrophilic series cells. Also, it is clear that such a hypothesis could not account for the failure of the sequential model to correctly predict the theoretical absolute production rates in rats, as the calculated values are all significantly less than the measured values.

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. Lajtha and Oliver[16] advanced a speculative model for maturation and proliferation within the erythron to explain a number of clinical and laboratory observations. They suggested that 1) cells may pass between morphological compartments at any point in the cell cycle; and 2) 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, and would al-ow for cellular transition between morphological compartments without intervening mitosis. Stohlman and coworkers (c.f. Stohlman, et al.[17] for a summary and further references) have made similar suggestions.

It seems quite possible that transitions between compartments might take place continuously throughout the cell cycle, perhaps at a rate that varies from point to point within the cell cycle. In a general consideration of cellular proliferation Quastler[18] proposed such a scheme, which was analytically formulated by Carnie et al.[19,20] and applied to an analysis of cell kinetic data from rat intestinal epithelial tissue.

Extensive experimental work on the erythroid[9,11,21-23] and neutrophilic[24] systems of the August-Marshall rat, and the erythroid system of the mouse,[25] has employed such a random maturation-proliferation scheme for data interpretation. This work, coupled with the conclusions of this paper and the recent demonstration by Mackey and Dörmer[26] that all of the steady state data analyzed here is consistent with a random transition model, lends strong support to a random maturation-proliferation scheme. Further insight into this problem awaits a

consideration of time dependent data within the framework of such a model.

APPENDIX 1. THE SEQUENTIAL MODEL

In this model it is assumed that following mitosis daughter cells may go to the next morphological stage (efflux F^o) or reenter cycle within the same stage (feedback flux F^f). There is a cellular influx F^i from the previous morphological stage. The total number of cells within a given compartment is given by T.

For such a model, the flux of cells through S (F^s) should be identical with the flux of cells through M: $F^s=F^m$. Since $F^s=Tf_L/t_S$ and $F^m=Tf_m/t_m$ this reduces simply to

$$\frac{f_L}{t_S} \equiv \frac{f_m}{t_m} \tag{1.1}$$

at each morphological stage.

In a steady state, compartment size must be constant which requires that fluxes balance. Thus

$$2(F^i + F^f) = F^o + F^f \tag{1.2}$$

and

$$F^s = F^m = F^i + F^f \tag{1.3}$$

Since $F^s=F^m$, henceforth set $F^s=F^m=F$. Equations 1.2 and 1.3 give

$$F^f = 2F - F^o \tag{1.4}$$

and

$$F^i = F^o - F \tag{1.5}$$

Noting that $F^f \geq 0$ and $F^i \geq 0$ in conjunction with (1.4) and (1.5) yields

$$F \leq F^o \leq 2F \tag{1.6}$$

These relations must hold for any proliferating sequential morphological compartment. Consider now a series of three such compartments, as shown in Figure 1, with compartment 1 supplying cells to 2, and 2 to 3. Then for compartment 1, by (1.6)

$$F_1 \leq F_1^o \leq 2F_1 \tag{1.7}$$

However, $F_1^o = F_2^i$ and $F_2^i = F_2^o - F_2$ by (1.5) so (1.7) becomes

$$F_1 + F_2 \leq F_2^o < 2F_1 + F_2 \tag{1.8}$$

Equation 1.6 also gives

$$F_2 \leq F_2^o < 2F_2 \tag{1.9}$$

and (1.8) in conjunction with (1.9) gives

$$\max \begin{cases} F_1 + F_2 \\ F_2 \end{cases} = F_1 + F_2 \leq F_2^o \leq \min \begin{cases} 2F_1 + F_2 \\ 2F_2 \end{cases} \tag{1.10}$$

Next note that $F_2^o = F_3^i$ and carry out the same sequence of steps to arrive at

$$F_1 + F_2 + F_3 \leq F_3^o \leq \min \begin{cases} 2F_1 + F_2 + F_3 \\ 2F_2 + F_3 \\ 2F_3 \end{cases} \tag{1.11}$$

a relation which the fluxes F_i (i = 1,2,3) and F_3^o must obey for a serially connected system of the type shown in Figure 1.

If T_i for a given morphological compartment is equal to σ_i, the fraction of the total proliferating (i = 1,2, and 3) population in the i the compartment, then

$$F_i = \frac{\sigma_i f_i}{t_i} \tag{1.12}$$

where $f_i = f_{Li}$ or f_{mi} and $t_i = t_{Si}$ or t_{mi}, and the inequality (1.11) will give upper and lower bounds for the theoretical relative production rate (T-RPR), F_3^o, expressed in units of cells/proliferating precursor/hr.

If, on the other hand, T_i is given by $(\sigma_i \Sigma)$ [where Σ is the absolute density of all recognizable proliferating precursors, usually in cells/kg body weight] then the inequality (1.11) gives upper and lower bounds for the theoretical absolute production rate (T-APR), usually in units of cells/unit body weight/hr. Clearly, given lower and upper bounds for the T-RPR it is sufficient to multiply both by Σ to obtain the corresponding bounds for the T-APR.

In using (1.11) in conjunction with data to calculate the T-RPR allowance must be made for variations in the data when known. Throughout, the range of values given by (1.11) has been *maximized* (giving the data the greatest possible latitude) by taking

$$L_B \leq F_3^o \leq U_B \tag{1.13}$$

wherein

$$L_B = F_1^- + F_2^- + F_3^- \tag{1.14}$$

$$U_B = \min \begin{cases} 2F_1^+ + F_2^+ + F_3^+ \\ 2F_2^+ + F_3^+ \\ 2F_3^+ \end{cases} \tag{1.15}$$

and

$$F_i^{\pm} = \frac{(\sigma_i \pm \Delta\sigma_i)\ (f_i \pm \Delta f_i)}{(t_i \mp \Delta t_i)} \tag{1.16}$$

When T-RPR's are quoted as determined from the data, the mean T-RPR is taken to be

$$\text{T-RPR} = \frac{L_B + U_B}{2} \tag{1.17}$$

and the standard deviation of the T-RPR is taken as

$$\Delta(\text{T-RPR}) = \frac{U_B - L_B}{2} \tag{1.18}$$

APPENDIX 2. THE INEFFECTIVE HEMOPOIESIS MODEL

In this formulation of the proliferative activity within a given morphological compartment, it is assumed that a discrepancy of the form $F^s > F^m$ is a reflection of an irreversible loss of cells (flux $F^d = F^s - F^M$) from the G_2 phase of the cell cycle. The notation is identical with that used in APPENDIX 1, but it is no longer assumed that $F^s = F^m$.

In a steady state the fluxes must obey the relations

$$2F^m = F^f + F^o \tag{2.1}$$

$$F^d = F^s - F^m \tag{2.2}$$

and

$$F^s = F^i + F^f \tag{2.3}$$

From (2.1) and (2.3)

$$F^i = F^o + F^s - 2F^m \tag{2.4}$$

and

$$F^f = 2F^m - F^o \tag{2.5}$$

so the restrictions $F^d \geq 0$, $F^i \geq 0$, and $F^f \geq 0$ reduce to

$$F^m \leq F^s \tag{2.6a}$$

and

$$2F^m - F^s \leq F^o \leq 2F^m \tag{2.6b}$$

If three morphological compartments of this type are connected serially, then arguments of the type employed in APPENDIX 1 lead to the relation

$$\Gamma_1 \leq F_3^o \leq \Gamma_2 \tag{2.7}$$

between the individual fluxes F_i^s and F_i^m $(i=1,2,3)$ and F_3^o, where

$$\Gamma_1 = \max \begin{cases} 2(F_1^m + F_2^m + F_3^m) - (F_1^s + F_2^s + F_3^s) \\ 2(F_2^m + F_3^m) - (F_2^s + F_3^s) \\ 2F_3^m - F_3^s \\ 0 \end{cases} \tag{2.8}$$

and

$$\Gamma_2 = \min \begin{cases} 2(F_1^m + F_2^m + F_3^m) - (F_2^s + F_3^3) \\ 2(F_2^m + F_3^m) - F_3^s \\ 2F_3^m \end{cases} \tag{2.9}$$

When taking data variations into account the range defined by (2.7) has been maximized by taking

$$L_B \leq F_3^{o} \leq U_B \tag{2.10}$$

where

$$L_B = \max \begin{cases} 2\,(F_1^{m-} + F_2^{m-} + F_3^{m-}) - (F_1^{s+} + F_2^{s+} + F_3^{3+}) \\ 2\,(F_3^{m-} - F_3^{m-}) - (F_2^{s+} + F_3^{s+}) \\ 2\,F_3^{m-} - F_3^{s+} \\ 0 \end{cases} \tag{2.11}$$

and

$$U_B = \min \begin{cases} 2\,(F_1^{m+} + F_2^{m+} + F_3^{m+}) - (F_2^{s-} + F_3^{s-}) \\ 2\,(F_2^{m+} + F_3^{m+}) - F_3^{s-} \\ 2\,F_3^{m+} \end{cases} \tag{2.12}$$

For both sets of data examined where both S and M phase data are available, $L_B = 0$ and the mean T-RPR and standard deviation of the T-RPR are defined as before.

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REFERENCES

1. Cronkite, E.P. (1964) Fed. Proc., 23, 649-658.
2. Rubinow, S.I. and Lebowitz, J.L. (1975) J. Math. Biol., 1, 187-225.
3. Rubinow, S.I. and Lebowitz, J.L. (1975) J. Math. Biol., 1, 187-225.
4. Dörmer, P., Militzer, H., Dahr, P. and Ruppelt, W. (1981) Preprint (submitted to Cell Tissue Kinet.).
5. Brinkmann, W., Dörmer, P. (1976) in Erkrankungen der Myelopoese (eds. A. Stacker, P. Höcker), Urban & Schwarzenberg, München, pp. 31-33.
6. Dörmer, P. (1973) Prog. Histochem. Cytochem., 6, 1-83.
7. Rondanelli, E.G., Magliulo, E., Giraldi, A., Cario, F.P. (1967) Blood, 30, 557-568.
8. Rondanelli, E.G., Magliulo, E., Verona-Rinati, M., Petrocini, S. (1969) Nouv. Rev. Fr. d'Hemat., 9, 347-364.
9. Roylance, P.J. (1968) Cell Tissue Kinet., 1, 299-308.
10. Starling, M.R. and Rosse, C. (1976) Cell. Tissue Kinet., 9, 191-204.
11. Tarbutt, R.G. and Blackett, N.M. (1968) Cell Tissue Kinet., 1, 65-80.

12. Dancey, J.T., Deubelbeiss, K.A., Harker, L.A. and Finch, C.A. (1976) J. Clin. Invest., 58, 705-715.

13. Wintrobe, M.M. (1976) Clinical Hematology, Lea & Febiger, Philadelphia.

14. Ganzoni, A.M. (1970) Kinetik und Regulation der Erythrocytenproduktion: Experimentelle Untersuchungen an der normalen und anämischen Ratte. Springer-Verlag, New York, pp. 1-94.

15. Patt, H.M. and Maloney, M.A. (1964) Ann. N.Y. Acad. Sci., 113, 515-522.

16. Lajtha, L.G. and Oliver, R. (1960) in Ciba Foundation Symposium on Haemopoiesis (eds. G.E.W. Wolstenholme, M. O'Connor) J. & A. Churchill Lts., London, pp. 289-314.

17. Stohlman, F. Jr., Ebbe, S., Morse, B., Howard, D. and Donovan, J. (1968) Ann. N.Y. Acad. Sci., 149, 156-172.

18. Quastler, H. (1960) Ann. N.Y. Acad. Sci., 90, 580-591.

19. Cairnie, A.B., Lamerton, L.F. and Steel, G.G. (1965) Exp. Cell. Res. 39, 528-538.

20. Cairnie, A.B., Lamerton, L.F. and Steel, G.G. (1965) Exp. Cell. Res. 39, 539-553.

21. Wheldon, T.E., Kirk, J., Orr, J.S., Paul, J. and Conkie, D. (1974) Cell. Tissue Kinet., 7, 181-188.

22. Hanna, I.R.A. and Tarbutt, R.G. (1971) Cell Tissue Kinet., 4, 47-59.

23. Tarbutt, R.G. (1967) Exper. Cell. Res., 48, 473-483.

24. Constable, T.B. and Blackett, N.M. (1972) Cell Tissue Kinet., 5, 289-302.

25. Covelli, V., Briganti, G. and Silini, G. (1972) Cell Tissue Kinet., 5, 41-51.

26. Mackey, M.C. and Dörmer, P. (1981) Preprint (submitted to Cell Tissue Kinet).