PARAMETERIZATION OF IN VIVO
LEUKEMIC CELL POPULATIONS

S. I. RUBINOW, J. L. LEBOWITZ, *and* ANNE-MARIE SAPSE

From the Biomathematics Division, Cornell University Graduate School of Medical Sciences, New York 10021, the Sloan-Kettering Institute for Cancer Research, New York 10021, and the John Jay College of the City University of New York, New York 10010

ABSTRACT   A quantitative mathematical formalism which was previously intro-
duced has been utilized to obtain the cell kinetic parameters which characterize the
in vivo leukemic myeloblast cell populations in two patients studied by Clarkson
and his coworkers. The principal tentative conclusions are: (*a*) all cells which are
actively proliferating must enter the resting state following cell division; (*b*) about
90% of the cells are in the resting state; (*c*) the generation time of the cells in the
active state is about 25 hr and is essentially the same as the generation time of
normal myeloblast cells.

INTRODUCTION

A quantitative mathematical formalism has recently been introduced (1) that de-
scribes grain-count distributions observed by autoradiography of a cell population
which is exposed to radioactive label. The theory was formulated in the context of
the model of bone marrow cells proposed by Lajtha, Gilbert, Porteous, and Alex-
anion (2). The connection between the theory and various observable quantities of
interest such as median grain count, labeling index, labeled mitotic index, etc. as
functions of the time was worked out in detail.

We have utilized this theory to analyse the autoradiographic observations of
Clarkson, Ohkita, Ota, and Fried (3) of in vivo leukemic myeloblast cell populations
in two patients. We find that we are able to simulate virtually all the qualitative
features of the observations with the aid of an appropriate choice of the values of the
parameters of the model. The biological implications of this choice are discussed.

THE MODEL

The model treated in reference 1 is illustrated in Fig. 1. The reader is referred to
reference 1 for mathematical details of the theory and the manner in which the cal-
culations are made. The principal properties of the model are repeated here from
that work, for convenience.

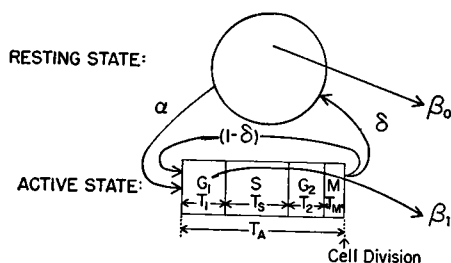The model supposes that there are two cellular compartments, in one of which all

175

FIGURE 1 ⌐ Schematic representation of the mathematical model of leukemic myeloblast proliferation. The system comprises an active state in which cells mature and divide, and a resting or "G_0" state in which cells are dormant and merely age. For each cell division, a fraction δ of the daughter cells enter the resting state and the remaining fraction $(1 - \delta)$ reenter the active state. In addition, cells may enter the beginning of the active state from the resting state at a fractional rate α. The fractional loss rates per unit time from the two states are β_0 and β_1.

cells are actively proliferating, the active state, and in the second of which cells do not proliferate, the resting state. Cells from either compartment may die or disappear at characteristic fractional loss rates β_0 and β_1, respectively, from the resting and active states. The time a cell spends in the active state is denoted by T_A, and this interval is assumed to be the same for all cells in the active state. This time interval T_A is conveniently divided into the time intervals T_1, T_S, T_2, and T_M, representing, respectively, the durations of the G_1, S, G_2, and M phases of the active cell cycle, where these symbols have their usual meaning (4). Thus, $T_A = T_1 + T_S + T_2 + T_M$. After a cell completes the mitotic (M) phase of the cell cycle, it divides into two cells. Of these newborn cells, a fraction δ goes into the resting state, while the remaining fraction $(1 - \delta)$ goes back to the beginning of the active state. In addition, cells may enter the beginning of the presynthetic (G_1) phase of the active state from the resting state at a fractional rate α per unit time. All cells in the resting state are assumed to have an equal probability of entering the active state at any time.

Altogether, it is seen that there are essentially five parameters completely characterizing the system: α, δ, β_0, β_1, and T_A, but that T_A is decomposed further into T_1, T_S, T_2, and T_M. It is also assumed that the system is in a steady state, so that the total population remains constant with time. In vivo, the population may actually be expanding or, if the patient is in a state of remission, contracting. The steady-state assumption is expected to be a valid approximation so long as the time scale over which the population is doubling (or halving) is large compared to the mean lifetime of a cell. For example, if the population is in exponential growth at a fractional rate ϵ, than the aforementioned presumptions imply that the nondimensional quantity ϵT_A is small compared to unity. Dimensional considerations which may be substantiated by explicit calculations further imply that quantities calculated from the theory such as the labeling index suffer only a small change of order ϵT_A from their steady-state values. At time $t = 0$, the system is exposed to a pulse of radioactive

thymidine. This exposure is believed to label all cells in the DNA synthesis (S) phase, and no others.

As has been previously indicated, cells in G_1 phase or the resting state, also called the G_0 phase (2), are at the present time operationally indistinguishable from each other. Therefore, an essentially equivalent interpretation of the model is that the cell population consists of a single proliferative compartment in which all members have the same time intervals assigned to the S, G_2, and M phase, but have a time interval assigned to the G_1 phase which is variable from cell to cell. This time interval has a distribution which is zero up to a time T_1 when it jumps to a maximum and has an exponential tail thereafter. A certain number of G_1 cells enter the S phase per unit time, and a certain number are lost. The fractional loss rate is different (as long as $\beta_0 \neq \beta_1$) for cells in G_1 from the loss rate for cells in the other phases.

Cell properties observed in autoradiography are determined by the cell density function $n'(x, \mu, t)$ where $n'(x, \mu, t)\, d\mu\, dx$ represents the number of cells in the interval $d\mu$ surrounding the maturation level μ and containing an amount of radioactive label between x and $x + dx$ at time t. The maturation level is any cell parameter (such as cell volume) which can continuously characterize the various stages in the cell cycle. In the present application it is taken to be cell age, where the age of a given cell is the time elapsed from birth. The cell density function is completely determined by the theory once the parameters and the initial labeled cell distribution are given. Because of the assumptions regarding the nature of the pulse labeling process we set

$$n'(x, \mu, 0) = f(\mu)\varphi(x), \tag{1}$$

where

$$f(\mu) = \begin{cases} \dfrac{N_A}{T_A}, & T_1 < \mu < T_1 + T_S \\ 0, & \text{otherwise.} \end{cases} \tag{2}$$

Here N_A is the number of cells in the active state and N_A/T_A represents the total cell density in the active compartment in the steady state when $\beta_1 = 0$, which we assume for simplicity. The distribution of radioactive material among the cells following injection is given by $\varphi(x)$ which is assumed to have the functional form of a γ-distribution,

$$\varphi(x) = \frac{c^{\gamma+1} x^\gamma e^{-cx}}{\Gamma(\gamma + 1)}, \tag{3}$$

where c and γ must be given. The function $\varphi(x)$ is normalized so that $\int_0^\infty \varphi(x)\, dx = 1$. The γ-distribution is a skewed unimodel distribution which is known empirically to

give a good representation of the generation time distribution of different kinds of cells (5).

The assumption that the system is in a steady-state imposes the following constraint among the parameters α, β_0, and δ:

$$\alpha = \beta_0(2\delta - 1), \tag{4}$$

with $1 \geq \delta \geq \frac{1}{2}$.

If the parameters are given, some important quantities of interest may be calculated from them. Thus, the average time T_0 spent by cells in the resting state is

$$T_0 = \frac{1}{\alpha + \beta_0}, \tag{5}$$

and the average lifetime of a cell measured from birth to its subsequent division is $T_0 + T_A$. The fractions of cells N_0/N, and N_A/N in the resting state and active state, respectively, are given by the expressions

$$\frac{N_0}{N} = \frac{\delta}{\delta + T_A[\alpha + \beta_0(1 - \delta)]}, \tag{6}$$

$$\frac{N_A}{N} = \frac{T_A[\alpha + \beta_0(1 - \delta)]}{\delta + T_A[\alpha + \beta_0(1 - \delta)]}, \tag{7}$$

where $N \equiv N_0 + N_A$ is the total cell population.

APPLICATION TO IN VIVO MEASUREMENTS OF LEUKEMIC CELL POPULATIONS

Clarkson et al. (3) made what are probably the most extensive measurements of kinetic quantities in two patients with acute myeloblastic leukemia, R. R. and M. T. The experimental procedure was to inject intravenously some tritiated thymidine at time $t = 0$. Bone marrow aspirates were taken at subsequent intervals from which smears and then autoradiographs were prepared. Cells having less than five grains were classified as unlabeled. From the autoradiographs, the following quantities of interest could be determined: (a) the fraction of labeled cells $\bar{p}(k, t)$ with an observed count of k grains at time t; (b) the mitotic index as a function of time $L_M(t; j)$, which is the fraction of cells in mitosis that are labeled at time t; $L_M(t; j) = \sum_{k=j}^{\infty} \bar{p}_M(k,t)$, where $\bar{p}_M(k,t)$ is the fraction of cells in mitosis with an observed count of exactly k grains, and j is the threshold for counting a given cell as labeled, taken equal to five; (c) the labeling index as a function of time $L(t; j) = \sum_{k=j}^{\infty} \bar{p}(k,t)$

which is the fraction of cells that are labeled at time t. (d) the grain count $\xi(t; \nu)$, which is that grain count above which a specified fraction ν of the cells are labeled at time t; it is defined by the equation $L(t; \xi) = \nu L(t; j)$ where $\xi \geq j$. The grain count $\xi(t; \nu)$ also depends on j.

The experimental results of Clarkson et al. are shown in Figs. 2 a–5 a for patient R. R., and Figs. 2 b–5 b for patient M. T. We have assumed that the leukemic cell populations in these two patients were in a steady state. The fact that the total leukocyte count and the mitotic index were fairly constant in both patients over the time course of the observations (2) supports this supposition.

We have utilized these measurements to guide us in determining what appear to be reasonable values of the parameters of the model. As previously indicated (1), more sophisticated models could be constructed, for example, by permitting the other phases of the cell cycle to be variable in addition to G_1 (6). Experience indicates that such assumptions lead to a "rounding out" of such theoretical curves as those presented in Figs. 3–5. However, we feel that the reader must be cautioned at the outset that, because of the unavoidable errors in observation, models which are more complex than the simplistic one presented herein do not appear to be justified at the present time. Our present aim is to simulate the features of the data in a qualitative manner and in a quantitative manner, to the extent permitted by the data. We hope that this work will stimulate further quantitative observations of the kind made by Clarkson et al.

The Initial Grain-Count Distribution $\varphi(x)$

The predictions of the theory utilize a continuous function representation of the initial thymidine distribution, e.g., the function $\varphi(x)$. Let x be expressed in units such that a unit amount of radioactive material produces on the average one grain on the photographic plate of the autoradiographic process. As shown in reference 1, under reasonable assumptions about the uptake of thymidine by the cells, the expected fraction of cells with a grain count k is directly proportional to $\varphi(x)$ evaluated at $x = k$. This result requires that k be large compared to unity and is expected to be valid for k greater than the threshold of observation of five grains. Explicitly,

$$\bar{p}(k, \tau) = \frac{\varphi(k)}{\int_0^\infty \left[1 - e^{-x} \sum_{m=0}^{4} \frac{x^m}{m!} \right] \varphi(x) \, dx} . \tag{8}$$

Here τ is any time between $t = 0$ and $t = T_2 + T_M$ when cell division begins to occur. The observations of Clarkson et al. of $\bar{p}(k, \tau)$ are displayed as histograms in Fig. 2 a for R. R. and Fig. 2 b for M. T. The histograms are based on observations made at $\tau = 0.33$ hr for R. R., and $\tau = 2$ hr for M. T. It should perhaps be emphasized that the continuous function $\varphi(x)$ represents an idealization of the histogram that would result if a very large number of cells were observed. Practical limitations of the pains-
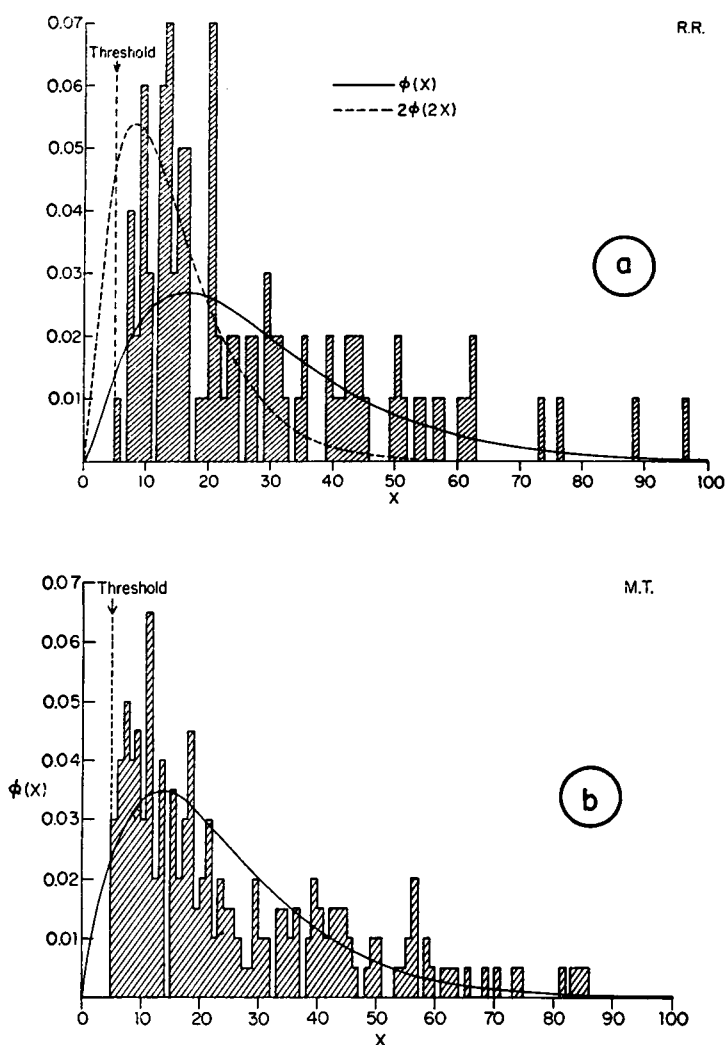
FIGURE 2   The solid line represents the assumed initial form of the (normalized) distribution function representing the fractional number of cells with a given amount of labeled material as represented by grain counts x. The continuous curve is based on a least square fit to the observed (3) distribution of fractional cell number vs. grain counts which is displayed as a histogram. The designation R.R. or M.T. in the upper right-hand corner denotes the name of the patient in which the observations were made. The dotted curve in Fig. 2 a represents the labeled material distribution function which is expected in the second generation, on the assumption that each daughter of a labeled cell receives exactly one-half the labeled material in the parent.

taking nature of the autoradiographic process govern the size of the observed cell samples. Assuming then that $\varphi(x)$ has the form given in equation 3, we used the method of least squares to determine the values of c and γ in each case. The calcu-
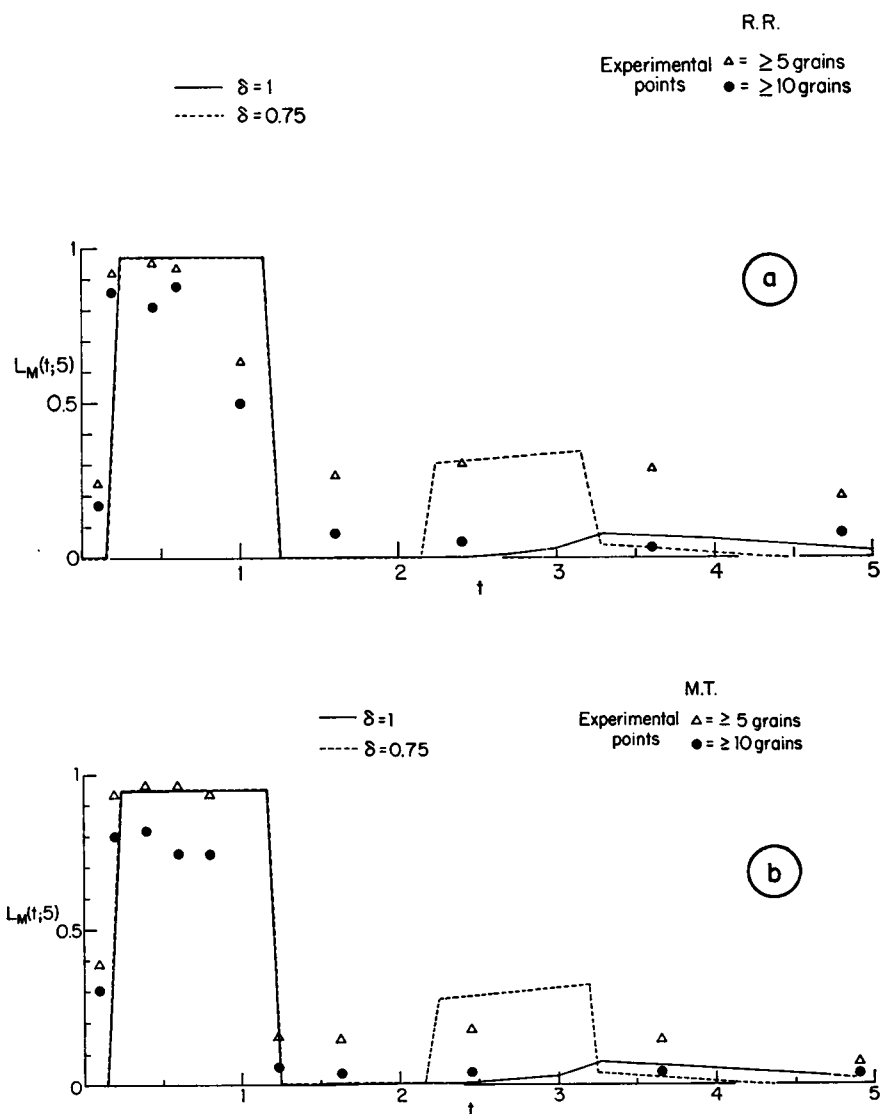
FIGURE 3   The fractional number of labeled cells in mitosis is shown as a function of the time t. The unit of time is 20 hr. The theoretical curve for $\delta = 0.75$ displays a second wave which is not a property of the observations, especially if 10 grains is taken as the threshold of observation for denoting a cell as labeled.

lations were simplified by setting the mean value of the grain count obtained from $\varphi(x)$, $\int_0^\infty x\varphi(x)\,dx$, equal to the observed value. This constraint imposes a relation between γ and c so that only one parameter need be determined by the least square method. According to the theory developed in reference 1, the experimental value $\bar{k}$, which is obtained by averaging only over those cells whose grain count is larger
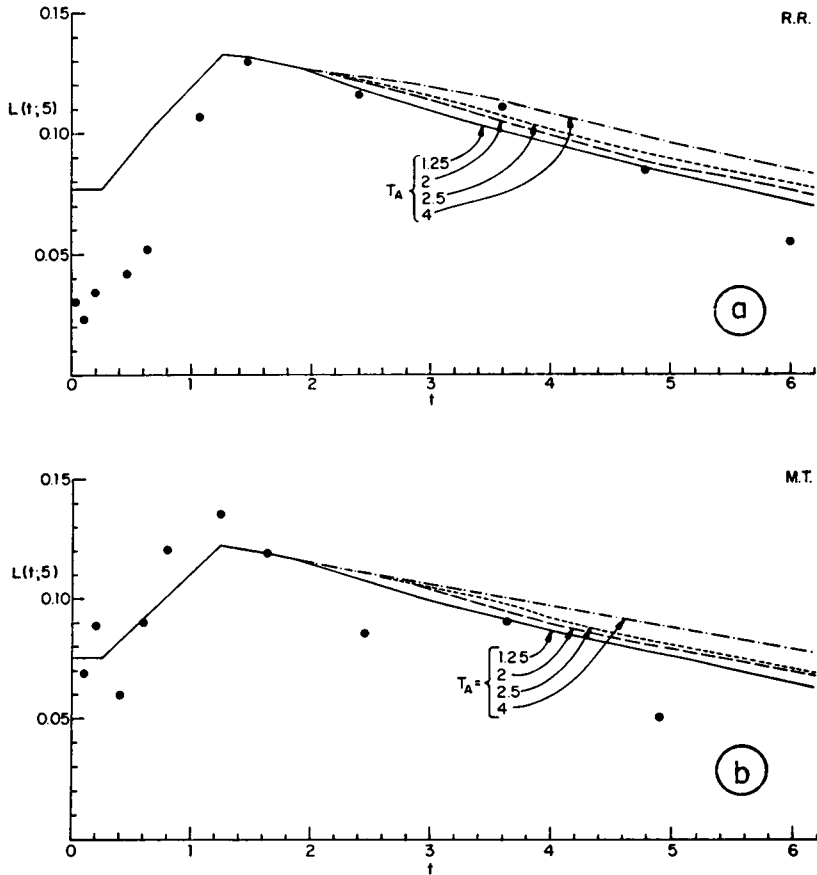
FIGURE 4 The labeling index $L(t; 5)$ is shown as a function of the time t. Four theoretical curves are shown based on different assumed values for T_A. The solid circles represent the experimental observations of Clarkson and his coworkers for the patients R.R. and M.T. In Fig. 4 a, the value of $L(0; 5)$ was adjusted so as to make the theoretical maximum agree more closely with experiment.

than four, should be larger than that obtained from averaging over $\varphi(x)$ for all $x > 0$. This difference as well as the fact that the proportionality constant in equation 8 is larger than one make a difference of only a few per cent in the present case where $\bar{k} \gg 5$ and have been ignored in our computations. The histogram for R. R. based on observations of 100 labeled cells has a value of $\bar{k} = 28.2$ and yields $\gamma = 1.36$ and $c = 0.0836$. The histogram for M. T. based on observation of 200 labeled cells has a value of $\bar{k} = 24.3$, and yields $\gamma = 1.20$ and $c = 0.090$. The theoretical distributions are also shown in Figs. 2 a and b. It is obvious by inspection that the continuous function $\varphi(x)$ is a better "fit" to the histogram for 200 observed cells (Fig. 2 b) than it is for 100 observed cells (Fig. 2 a). However, as we shall see, the quantities subsequently
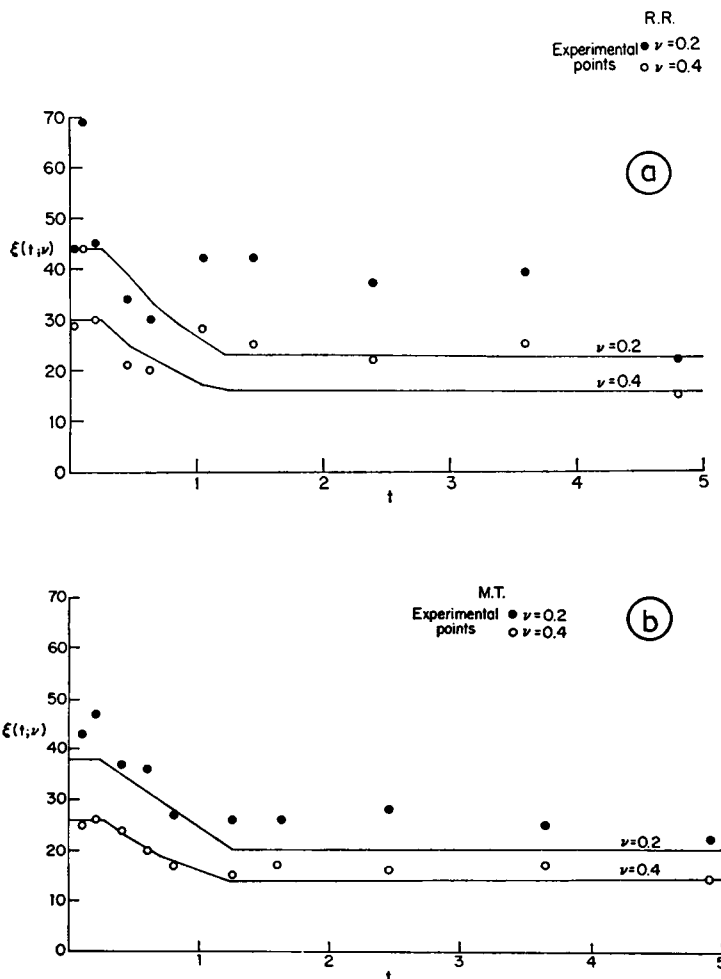
FIGURE 5 The minimum grain count ξ of that fraction of the cells ν which are most highly labeled is shown as a function of the time for two assumed values of ν. The theoretical curves were calculated on the assumption that $T_A = 2$. Their form is rather insensitive to the value T_A over the range of values studied.

derived from $\varphi(x)$ are not sensitive to the particular parametric choice that defines it.

In Fig. 2 *a* we also display the grain count distribution function $2\varphi(2x)$. This function is needed for the subsequent calculations (for details, see reference 1). This curve represents the normalized distribution of grain counts among the second generation of labeled cells. This is peaked at a smaller value of x than is $\varphi(x)$, because the amount of label of each cell is reduced by one-half as a result of division. Note that many more cells are falling below the threshold level in the second generation as

compared with the first generation. Naturally, this effect is even more pronounced as the generation number increases.

The Labeled Mitosis Function $L_M(t; 5)$

The labeled mitosis observations are the most significant for determining the kinetic parameters of the leukemic cell population. The width of the first peak in the labeled mitosis curve is given theoretically as T_S. This width is reported somewhat unambiguously in these two patients as being 19–22 hr (3). Therefore we set $T_S = 20$ hr and chose T_S as out unit of time. Thus in Figs. 2–5 the time t is nondimensional, with $t = 1$ representing 20 hr in dimensional time. In other words, we introduce non-dimensional primed quantities $t' = t/T_S$, $T_1' = T_1/T_S$, $T_2' = T_2/T_S$, $T_A' = T_A/T_S$, $T_0' = T_0/T_S$, $\alpha' = \alpha T_S$, $\beta_0' = \beta_0 T_S$, and subsequently drop the primes. During the G_2 interval following initial labeling, the theoretical curve for $L_M(t; 5)$ is zero, and the interval during which the curve rises to its maximum is T_M. We have chosen $T_2 = 0.15$ and $T_M = 0.1$, although the experiments could support the values for T_2 and T_M which are about one-half the above values. The reason that the theoretical L_M does not attain the value of unity at its maximum is that some "labeled" cells in the initial distribution are below the threshold of observation (see Fig. 3), and therefore no longer count as labeled cells.

The most strikingly unusual feature of the labeled mitoses observations is the lack of a second wave. Although the experiments appear to indicate a long "tail," it is seen that this tail is for the most part comprised of very lightly labeled cells, i.e., it largely disappears when the threshold for grain counting is increased to 10 grains. Inasmuch as there is some indication that some cells not in S phase (presumably in G_1 or G_0 phase) may become lightly labeled initially (7), we believe that one is on safer grounds in lending greater credence to the observations of L_M based on the 10 grain threshold. It is not consistent with theory to maintain that the lightly labeled cells beyond the first wave in Figs. 3 a and b are second generation labeled cells, because the theory indicates as shown by the curve of $2\varphi(2x)$ in Fig. 2 a that the vast majority of such cells that are above the threshold still have a grain count ≥ 10 grains.

As regards the theoretical calculations, it is apparent that as long as a nonnegligible number of cells at division are permitted to return to the beginning of the active phase, a second wave in the labeled mitoses curve result. This is illustrated by the curves labeled $\delta = 0.75$ in Figs. 3 a and b. Thus, a compelling consequence of this observation of L_M is that $\delta = 1$. This means that after division, all daughter cells enter the resting state.

Because there is no second wave, the theoretical curve for $L_M(t; 5)$ is for all practical purposes independent of the value of T_A. More particularly, the first wave is determined entirely by T_2, T_S, and T_M, and is independent of the choice of T_1.

The Labeling Index $L(t; 5)$

The theoretical value of $L(0; 5)$ is given by the expression

$$L(0; 5) = \frac{\beta_0}{1 + \beta_0 T_A} \left\{ 1 - \int_0^\infty e^{-x} \sum_{m=0}^{4} \frac{x^m}{m!} \varphi(x) \, dx \right\} \qquad (9)$$

in nondimensional parameters. Consequently, knowledge of $L(0; 5)$ may be utilized as an additional constraint on the parameters. In the investigation of patient R. R., a difficulty of interpretation is encountered because the first maximum of $L(t; 5)$ is approximately four times as large as the initial value. On theoretical grounds it is apparent that this maximum value can only be twice as large as the initial value (assuming all labeled cells after division remain in the system and do not fall below the threshold of observation). Therefore, we have deemed it more desirable to make the theoretical and experimental first maximum values of the curve $L(t; 5)$ agree. As a consequence, the quantity $L(0; 0) = \beta_0/(1 + \beta_0 T_A)$ was assigned a value of 0.080 for both cases, which makes $L(0, 5)$ equal to 0.075 for M. T., and 0.077 for R. R. Even so, such a value is abnormally low when compared with normal patients. Such a low value of the initial labeling index appears to be a very characteristic feature of leukemic cell populations (8–11).

The position on the time axis of these first maxima are found to be in agreement with observation. Theoretically, the position of the first maximum depends only on the value of $T_A - T_1$ which as we have seen is already determined by the labeled mitoses data as equal to 1.25 (equal to $T_S + T_2 + T_M$). Thus, the interpretations of the labeling index and labeled mitoses curves are in agreement with respect to the value of $T_A - T_1$. It is seen from the figures that the behavior of $L(t; 5)$ for t greater than the value for the first maximum is insensitive to the assumed value of T_A. This is really not surprising from a qualitative point of view because the principal fate of the labeled daughter cells resulting from division is to enter the resting state and slowly disappear from there. This fate is virtually independent of T_A. (It is slightly dependent on T_A because of the steady-state requirement.) The important parameter determining the form of labeling curve is $(\alpha + \beta_0)$ which equals $2\beta_0$ when $\delta = 1$. Because $\beta_0 = [1/L(0; 0) - T_A]^{-1}$ and $1/L(0; 0)$ is 12.5 for both R. R. and M. T., the relative insensitivity of β_0 and hence the labeling curve to the choice of T_A over the range 1.25–4 is readily understandable. If the comparison between theory and experiment is to be taken perhaps more seriously than is warranted, the experiments would appear to support the minimum possible value of $T_A = 1.25$, which is to say that $T_1 = 0$.

The Minimum Grain Count of the Most Highly Labeled Cell Fraction, $\xi(t; \nu)$

In Fig. 5 we display the theoretical curves $\xi(t; \nu)$ for $\nu = 0.2$ and $\nu = 0.4$ when $\delta = 1$, $T_A = 2$, and the other parameters are as previously indicated. These curves were cal-

culated for other values of T_A, but the effect of the different choices of T_A was negligible and therefore we have not displayed them. The most characteristic features of the theoretical curve are the relatively sharp decline during the initial time interval between $t = 0$ and $t = T_A - T_1$, and the relative slow decline subsequently. The large scatter in the observations for the case R. R. precludes any inferences being drawn about the comparison with the theory. However, in the case M. T., there is a reasonable agreement between theory and experiment. Once again, the observations tend to confirm the value of $T_A - T_1$, but say nothing about the value of T_1.

In the grain-count halving method (12), the curve of $\xi(t; \nu)$ is assumed to be an exponential, and the time for ξ to reduce to one-half of its initial value is interpreted as the mean generation time of the population. The time for ξ to reduce to one-half of its initial value when $\nu = \frac{1}{2}$ has been called the median generation time (13). Both Clarkson et al. (3) and Fried (13) utilized this method to estimate the mean generation times in the cases R. R. and M. T. They estimated that, for patient M. T., $T \sim 80$ hr. The estimates of T for patient R. R. were rather smaller than this value. It is recognized (13) that the interpretation of the halving time of $\xi(t; \nu)$ as a generation time depends on the observations extending over several generations at least. On the basis of the model presented herein, neither the latter criterion nor the exponential form of ξ is satisfied. Consequently, the inference we make regarding the mean generation time (defined as mean lifetime T) of the cells differs from the inferences of these authors in this respect.

Thus, we find that, depending on the choice of T_A, the values of β_0 and hence T_0 and T are as shown in Table I. These values are the same for both patients. In dimensional time, we find that a minimal estimate of the mean generation time is $T \sim 140$ hr. This occurs when $T_1 \sim 0$ and $T_A \sim 25$ hr. The corresponding value of β_0 denotes a fractional loss from the resting state $(\alpha + \beta_0)$ equal to 0.0088/hr, half of which goes to the active state, and half of which disappears or dies. The table also gives the relative fraction of cells in the resting and active states. As is expected on the basis of the small value of the initial labeling index, the majority of the cells are seen to be in the resting state. The remaining entries in the table indicate that the general nature of these inferences are not modified very greatly for other choices of T_A.

TABLE I

VALUES OF β_0, α, T_1, T_0, T, N_0/N AND N_A/N WHEN $\delta = 1$ AND T_A IS CHOSEN AS SHOWN IN FIRST COLUMN

The unit of time is 20 hr. These results are the same for both M. T. and R. R.

T_A	β	α	T_1	T_0	T	$\dfrac{N_0}{N}$	$\dfrac{N_A}{N}$
1.25	0.088	0.088	0	5.68	6.93	0.901	0.099
2	0.095	0.095	0.75	5.25	7.25	0.840	0.160
2.5	0.100	0.100	1.25	5.00	7.50	0.800	0.200
4	0.117	0.117	2.75	4.25	8.25	0.681	0.319

DISCUSSION AND CONCLUSIONS

We have shown that the grain-count theory previously presented (1) may adequately represent with a proper choice of parametric values all the features of the in vivo myeloblast cell populations observed in two patients with acute myeloblastic leukemia (3). These are the only observations extant which are extensive enough to permit the parameters of the model to be determined relatively unambiguously. These observations have undetermined experimental errors associated with them, and these are only two sets of them. Therefore, the conclusions reached about them must necessarily be considered tentative. Nevertheless, it is somewhat gratifying that the qualitative features of the kinetic behavior of the cells were the same in both patients. Furthermore, the values of the parameters chosen to represent the data were the same for both patients with the exception of the parametric values entering into the initial grain-count distribution function $\varphi(x)$.

The model assumes that the cells are in a steady state and that there are two states in which a cell may exist, a resting state, and an active state. We find that, on the basis of the model, the progeny of all cells which are actively proliferating and dividing must enter the resting state. The data favors the interpretation that there is no minimal time that a cell must spend in G_1 phase, i.e., $T_1 \sim 0$. The time spent in DNA synthesis is $T_S \sim 20$ hr, the time for G_2 phase is $T_2 \sim 1.5$–3 hr, the time for mitosis is $T_M \sim 1$–2 hr, the time spent in the active phase is $T_A \sim 25$ hr, the mean lifetime of cells in the resting state is $T_0 \sim 115$ hr, and the mean time between cell birth and cell division or mean generation time is $T \sim 140$ hr. Approximately 90% of the cells are in the resting state, and the remaining 10% are actively proliferating.

The least firm of these conclusions is the value of T_A (and hence T_1). However, the above estimate of T_A is in remarkable agreement with the inferred value of 25 hr for the generation time of normal proliferating myeloblasts (14), based on observations of the differential count of marrow granulocytes made by Killman, Cronkite, Fliedner, and Bond (15). The grain-count halving time of normal proliferating myeloblast cells is 31 hr (16). The possibility that actively proliferating leukemic myeloblast cells do indeed have the same generation time (defined as mean time spent in the active state) as normal proliferating myeloblast cells may be of importance in the chemotherapy of this type of acute leukemia.

REFERENCES

1. LEBOWITZ, J. L., and S. I. RUBINOW. 1969. *J. Theor. Biol.* **23**:99.
2. LAJTHA, L. G., C. W. GILBERT, D. D. PORTEOUS, and R. ALEXANIAN. 1964. *Ann. N. Y. Acad. Sci.* **113**:742.
3. CLARKSON, B., T. OHKITA, K. OTA, and J. FRIED. 1967. *J. Clin. Invest.* **46**:506.
4. HOWARD, A., and S. R. PELC. 1953. *Heredity.* **6**:261.
5. POWELL, E. O. 1955. *Biometrika.* **42**:16.
6. TRUCCO, E., and P. J. BROCKWELL. 1968. *J. Theor. Biol.* **20**:321.
7. PELC, S. R. 1963. *In* Cell Proliferation. L. F. Lamerton and R. J. M. Fry, editors. Blackwell and Mott Ltd., Oxford, England. 94.
8. GAVOSTO, F., G. MARAINI, and A. PILERI. 1960. *Blood J. Hematol.* **16**:1555.
9. MAUER, A. M., and V. FISHER. 1963. *Nature (London).* **197**:574.
10. CLARKSON, B., K. OTA, T. OHKITA, and A. O'CONNOR. 1964. *Proc. Amer. Ass. Cancer Res.* **5**:43.
11. KILLMAN, S. A. 1965. *Acta Med. Scand.* **178**:263.
12. KILLMAN, S. A., E. P. CRONKITE, T. M. FLIEDNER, and V. P. BOND. 1962. *Lab. Invest.* **11**:845.
13. FRIED, J. 1968. *Biophys. J.* **8**:710.
14. RUBINOW, S. I. 1969. *J. Cell Biol.* **43**:32.
15. KILLMAN, S. A., E. P. CRONKITE, T. M. FLIEDNER, and V. P. BOND. 1962. *Blood J. Hematol.* **19**:743.
16. CRONKITE, E. P., V. P. BOND, T. M. FLIEDNER, and S. A. KILLMAN. 1960. *In* Ciba Foundation Symposium on Haemopoiesis. G. E. W. Wolstenholme and M. O'Connor, editors. Churchill Ltd., London. 70.