A Model of Tumor Growth Based on Cell Cycle Kinetics

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ABSTRACT

This work describes a mathematical model of growth based on the kinetics of the cell cycle. A traditional model of the cell cycle has been used, with the addition of a resting ($G_0$) state from which cells could reenter the reproductive cycle. The model assumes that a growth regulatory substance regulates the transition of cells to and from the resting state. Other transitions between the phases of the cycle were modeled as a first order process. Cell loss is an important feature of growth kinetics, and has been represented by a general but tractable mathematical form. The resulting model forms a system of ordinary nonlinear differential equations. Analytic methods are employed first in the study of this system. Simplifying assumptions regarding cell loss give rise to special cases for which equilibrium solutions can be found. One special case, which assumes first order loss from all cell cycle phases at equal rates, is presented here. For small time values, approximations corresponding to exponential growth were developed. The equations describing an intrinsic growth rate were derived. Simulation methods were used to further characterize the behavior of this model. Parameter values were chosen based on animal tumor cell cycle kinetic data, resulting in a set of 45 model simulations. Several tumor treatment protocols were simulated which illustrated the importance of the intrinsic growth rate and cell loss concepts. Although the qualitative behavior regarding absolute and relative growth is reasonable, this model awaits data for model fitting, parameter estimation, or revision of the equations.

1. INTRODUCTION

This work concerns the mathematical modeling of two features of growth, the kinetics of reproducing cells and the growth of a cell population. While these concepts are not independent, it is clear that mathematical models of *Please address all reprint requests to Steven Piantadosi at above address.
growth need not include any implicit or explicit assumption about the kinetics of cellular reproduction. However, one would expect that a model describing the kinetics of replicating cells would contain, at least implicitly, a model of the growth of that cell population. The rationale for interest in cellular reproduction and growth is provided, in part, by oncology. The investigator is faced with an array of cancers, a variety of treatment modalities, and the general problem of optimizing treatment—to prolong survival of the host. Since it is impossible to perform all of the treatment experiments of interest, one is led immediately to the field of mathematical modeling as a means of designing and interpreting experiments and the possibility of tailoring treatment not only to the cancer type but also to the individual tumor. While this is an ambitious goal, the necessary tools are becoming available.

Several approaches to the modeling of cellular systems have been useful [1, 5, 26]. Because of the desire for mathematical simplicity and the use of available experimental or clinical data, the general approach using ordinary differential equations has been adopted here.

The current state of knowledge regarding the biology of cellular reproduction has been built around the events of the cell cycle, that sequence of biochemical steps through which presumably every cell must proceed in order to divide. Theory and conjecture about the kinetics of the cell cycle will form the foundations for this mathematical model of cellular reproduction and cell population growth. This work will describe the formulation of a mathematical model of the cell cycle, some analytic features of this model, and some empirical relationships.

A. THE CELL CYCLE

The phases of the cell cycle were first described by Howard and Pelc in 1953. Since the introduction of tritiated thymidine labeling [43] and its use in the kinetic study of a cell population [32], the value of the concepts of cell cycle kinetics has been evident. These concepts have remained largely intact to the present time [4], and much has been accomplished in understanding the biochemical mechanisms involved [29, 31]. One might define the cell cycle as the sequence of events between one mitosis and the mitosis of a daughter cell. Usually, this definition is expanded to include subcellular processes that are known to regulate proliferation.

In the process of division, a cell will be seen to proceed through several sequential phases, originally distinguished mostly on morphologic grounds. These phases are $G_1$, a brief resting period; $S$, the period of DNA synthesis; $G_2$, a short pause after the completion of DNA synthesis; and $M$, mitosis. Following mitosis, the cell enters $G_1$ to begin the cycle again. Out of convenience, the concept of a prolonged resting phase has arisen, since some
cells with the apparent capacity to reproduce do not do so for long periods of time. This prolonged resting phase has been termed $G_0$ [20], although this term is not universally accepted. The $G_0$ phase is evidently identical to the $G_1$ phase except for the reluctance of the cell to divide, although recently the possibility of distinguishing features has arisen [28]. For convenience in the formulation of this model, it will be assumed that cells may switch between the $G_1$ and $G_0$ phases by a mechanism to be outlined later. The travel of a cell is unidirectional through the cycle.

A cell population will contain numbers of cells in each phase of the cell cycle. All the cells in the $G_1$ phase will be referred to as the $G_1$ subpopulation, and similarly for the other phases and subpopulations. Over the lifetime of a cell population, the size of each subpopulation changes in accordance with time or the rate of cell proliferation (or equivalently the size of the population).

B. SOME IDEAS CONCERNING TUMOR CELL KINETICS

Intuitively, if the cell cycle is accurately modeled, the features of growth kinetics should be satisfied by default. However, it is useful to examine some of what is known about tumor growth kinetics, since this imposes constraints on any model of the cell cycle.

While early observers linked malignancy with high growth rates [24], it is known now that other factors are responsible for malignant change in a cell population. However, the idea that kinetic features are important in understanding tumor growth is valid, and several common ways of looking at cell population kinetics will be discussed here.

The mitotic index is defined as the number of mitoses per 100 cells. It is a function of the rate at which cells enter mitosis as well as the duration of mitosis. When stathmokinisis is used to determine the rate of cell entry into mitosis [21], wide variation is seen between and within tumor types. Doubling times, as measured from growth rates, show wide variation [39, 40], even within a single histologic type of tumor [6]. The labeling index, which is the percentage of cells synthesizing DNA, has frequently been shown to be high in tumors with rapid growth rates, but also varies greatly, even within a single specimen [34, 44]. One must also keep in mind that the labeling index is related to the ratio of the duration of the $S$ phase to the intermitotic time [17], and consequently may have unequal values for populations with equal cell cycle times.

Another commonly used kinetic technique is the determination of the percentage labeled mitosis (PLM) or fraction labeled mitoses (FLM). These data provide information on the duration of the phases of the cell cycle, but are of limited use in human tumors [27, 38]. Even so, there is no evidence to indicate that tumors have shorter cell cycles than other cell populations.
The determination of the growth fraction or proliferative fraction of a cell population is another commonly used kinetic measure [23]. Its limitations have been discussed, as well as a critique of the supposed correlation between growth rate and growth fraction [16]. In any case, there does not seem to be a basis for assuming that slower growing tumors have lower growth fractions.

C. CELL LOSS

Many of the conclusions of the previous section can be more easily rationalized if one realizes that cell loss is a major determinant of tumor growth. It is also one kinetic feature that is very hard to evaluate. Modes of cell loss include the following: (1) aging, (2) differentiation, (3) inadequate metabolic support, and (4) host immune defenses [9]. Clearly, these are not independent mechanisms for cell loss. Some or all of these modes might increase in importance as a cell population ages, and we might expect a variety of mathematical forms to occur in attempting to describe cell loss.

Steel [37, 39] has popularized the notion of the cell loss factor as a measure of cell loss from tumors. The cell loss factor is \( 1 - \frac{t_{\text{potential}}}{t_{\text{observed}}} \) the ratio of the potential doubling time to the observed doubling time. The potential doubling time can be estimated from the cell production rate (mitotic rate), provided some assumption about the growth rate of the population (e.g., exponential growth) is accepted. It is clear that cell loss in tumors can be very high, and there does not seem to be a connection between cell loss and malignancy. However, cell loss is an important feature of growth kinetics.

There are few guidelines as to the relative rates of cell loss in various phases of the cell cycle. If only young cells (with a low risk of dying) divide, one might expect cell loss to occur exclusively from the \( G_0 \) phase. However, if loss occurs predominantly through means independent of cell age, such as the host immune response or inadequate metabolic support, then equal relative rates in all cell cycle phases would be a reasonable assumption.

D. POPULATION GROWTH

The previous section has demonstrated the paucity of information distinguishing normal from malignant growth on kinetic grounds. In this section some additional ideas concerning growth are presented that might be relevant, despite the fact that much of this would not be mathematically explicit in a cell cycle model.

An early concept of tumor growth allowed for exponential growth of the tumor cell population until the resources of the host were exhausted, at which time death ensued. More recent and accurate assessments of tumor size indicate a definite plateau size for most tumors, and indeed, many tumors are not clinically detectable until late in their growth, at which time the size changes in a decidedly nonexponential fashion. More realistic growth models
contain a limiting size at large time values, with an early “exponential phase.” A number of growth models fit this billing.

The Gompertz growth equation [13] has been widely used for modeling tumor growth [49]. In fact, it is frequently implied in the literature that tumors grow in a Gompertz fashion. This idea has ample support [18, 41], but little attention has been given to other forms for growth curves that might prove equally useful.

One exception to this statement is the work of Gratton, Appleton, and Alwiswasy [14], in which the Gompertz and other growth equations were used to describe murine fibrosarcoma growth. The authors favor the Gompertz, although several curves fit the data adequately. Interestingly, the determination of growth rates is highly dependent on the growth model assumed.

Most tumor size studies involved the (caliper) measuring or weighing of tumor specimens, which are necessarily late in their growth phase, and consequently are less than definitive in modeling cell population sizes over the full range of growth. For this reason, one is forced to admit the possibility of other forms for population growth, and a number of options are available. For example, Goel, Maitra, and Montroll [11] allow for growth rates proportional to any monotonic function $G$ such that $G(\infty) = 0$, and Turner et al. [45] have described a “generic” model which contains many commonly used growth equations as special cases.

Other features of real tumor growth do not lend themselves to description by any monotonic function, because some individual tumors have been observed to grow in an irregular fashion. This type of behavior is usually ignored from the modeling point of view, but it is well documented [39]. The main point here is that it is not necessary to be bound beforehand to any particular form for the overall growth equation, or even an analytic solution to the growth rate equation, although qualitative behavior similar to known growth laws would be desirable.

2. A CELL CYCLE MODEL

This section will describe the formulation and behavior of a cell cycle model based on the previously outlined concepts. The model will be given a postulational basis, which is not unique, but is simple and appealing, and has a foundation in other work. The model will be examined analytically where possible; otherwise, model behavior will be explored by simulation.

In what follows, the “population” is all the viable cells present, and a “subpopulation” is the cells present in a particular phase of the cell cycle. $G_1$, $S$, $G_2$, $M$, and $G_0$ will represent labels for the subpopulations or time dependent functions whose values are the subpopulation sizes. The meaning will be clear from the context. $N(t)$ or $N$ is the total population size at time $t$. 
A. POSTULATES FOR A MODEL

The postulates presented here will focus on how cells accomplish the transition from a particular state or phase of the cell cycle to the succeeding one. The details of the mechanism of growth control in the cell cycle are not fully known, although evidence exists for control at the $G_1-G_0$ transition [29, 31]. Such regulation might take the form of an inhibitory substance [10, 47, 48] or a growth factor [29]. The mathematical formalism of a growth regulator which acts in an inhibitory fashion on the $G_1$ cell is adopted here.

The remaining transitions in the cell cycle apparently require the completion of large numbers of individual biochemical steps [31]. The variability of the duration of $S$, $M$, and $G_2$ is less than that of $G_1$, possibly due to $G_0$ cells being "seen" as $G_1$ cells. In any event, the assumption here is that the transitions follow first order kinetics.

As mentioned earlier, modeling cell loss is not entirely objective. It seems unreasonable to assume that cycling cells have no risk of dying, although they may have, by virtue of their age, a lower rate of death. Since, in most cases, a small growth or reproducing fraction supports a large quiescent fraction, the qualitative behavior of the two models is very similar [30]. The assumption that all subpopulations have equal relative death rates provides a certain mathematical ease, and has been adopted here.

The postulates for this model are now stated formally for future reference.

Postulate 1. Each $G_1$ cell is responsive to a regulatory molecule $I$, which attaches to a site, causing a $G_1$ cell to become a $G_0$ cell. A $G_0$ cell may lose its regulator and enter the $G_1$ phase.

Postulate 2. The regulator is secreted by all cells at a rate $P$ (mass $\cdot$ cell$^{-1}$ $\cdot$ time$^{-1}$) and degraded or eliminated at a rate $D$ (time$^{-1}$).

Postulate 3. There is some (constant) effective volume of distribution, $V$ (vol), for cells and regulator.

Postulate 4. The concentration of regulator equilibrates rapidly compared to the rate of growth of the cell population.

Postulate 5. Cells move from each subpopulation to the next one at a rate proportional to the number of cells present in the first subpopulation.

Postulate 6. Cells in the mitotic subpopulation divide into exactly two cells, subject to Postulate 5.

Postulate 7. Cells are lost from each subpopulation at equal relative rates with first order kinetics.

B. MATHEMATICAL DEVELOPMENT OF THE POSTULATES

For the time being, cell loss will be ignored in order to develop preliminary equations.
Postulate 1 may be expressed by the rate equation

\[ \frac{d[G_0]}{dt} = k_f[I][G_1] - k_2[G_0] \]  

(1)

where \([\text{\_}]\) denotes concentration and \(k_f\) has units of \(\text{vol} \cdot \text{mass}^{-1} \cdot \text{time}^{-1}\).

Using Postulates 2, 3, and 4, \([I]\) may be replaced to obtain

\[ \frac{d[G_0]}{dt} = k_f \frac{PN}{DV} [G_1] - k_2[G_0]. \]

(2)

Using Postulate 3 to replace concentrations and Postulate 7 to model cell loss yields

\[ \frac{dG_0}{dt} = \frac{k_7}{k_9} N G_1 - k_2 G_0 - K_1 G_0, \]

(3)

where

\[ \frac{k_7}{k_9} = \frac{k_f P}{DV}, \]

and \(k_9\) is in units of cells. Thus, (3) is the rate equation for the \(G_0\) subpopulation. Since \(G_0\) cells come only from \(G_1\) cells, (3) can be used with Postulates 5 and 6 to write

\[ \frac{dG_1}{dt} = k_2 G_0 - \frac{k_7}{k_9} G_1 N + 2k_6 M - k_5 G_1 - K_1 G_1. \]

(4)

In a similar manner, (3) and (4) can be extended using Postulates 5, 6, and 7 to write the complete system

\[ \dot{G}_0 = k_7 G_1 \frac{N}{N_e} - k_2 G_0 - k_1 G_0, \]

(5.1)

\[ \dot{G}_1 = k_2 G_0 + 2k_6 M - k_7 G_1 \frac{N}{N_e} - k_5 G_1 - k_1 G_1, \]

(5.2)

\[ \dot{S} = k_3 G_1 - k_4 S - k_1 S, \]

(5.3)

\[ \dot{G}_2 = k_4 S - k_5 G_2 - k_1 G_2, \]

(5.4)

\[ \dot{M} = k_5 G_2 - k_6 M - k_1 M, \]

(5.5)

\[ \dot{N} = k_6 M - k_1 N, \]

(5.6)
where \( N = G_0 + G_1 + S + G_2 + M \) and \( N_e = k_9 \). Figure 1 shows a schematic of the cell cycle with rate constants corresponding to the system (5). The parameters \( k_1 - k_7 \) are positive constants. Each has units of time \(^{-1}\). It can be seen by examination of (5) that transitions between subpopulations occur not at constant rates, but at constant relative rates.

3. ANALYTIC FEATURES

A. SUBPOPULATION SIZES AT EQUILIBRIUM

An important feature of the system (5) is the behavior near equilibrium. Equilibrium solutions can be found by setting the derivatives defined in (5) equal to zero and solving the resulting algebraic equations. The equilibrium values will be symbolized as \( G_{0e}, G_{1e}, S_e, G_{2e}, M_e, \) and \( N_e \), respectively.
The subpopulation equilibrium sizes are

\[ M_e = \frac{k_1}{k_6} N_e, \]  
\[ G_{2e} = \frac{k_1 (k_1 + k_6)}{k_6 k_5} N_e, \]  
\[ S_e = \frac{k_1 (k_1 + k_6)(k_1 + k_5)}{k_6 k_3 k_4} N_e, \]  
\[ G_{1e} = \frac{k_1 (k_1 + k_6)(k_1 + k_4)(k_1 + k_4)}{k_6 k_5 k_4 k_3} N_e, \]  
\[ G_{0e} = \frac{k_1 (k_1 + k_6)(k_1 + k_4)(k_1 + k_4)k_7}{k_6 k_5 k_4 k_3 (k_1 + k_2)} N_e. \]  

The asymptotic growth fraction is

\[ GF_e = 1 - \frac{G_{0e}}{N_e} = 1 - \frac{k_1 (k_1 + k_6)(k_1 + k_5)(k_1 + k_4)k_7}{k_6 k_5 k_4 k_3 (k_1 + k_2)}. \]  

Summing the system (6), dividing by \( N_e \), and rearranging terms yields a parameter constraint, which could be satisfied in a number of ways, but most simply may be solved for \( k_7 \) to yield

\[ k_7 = \frac{(k_1 + k_2) \{ 2k_3 k_4 k_2 k_6 -(k_1 + k_3)(k_1 + k_4)(k_1 + k_5)(k_1 + k_6) \}}{k_1 (k_1 + k_4)(k_1 + k_5)(k_1 + k_6)}. \]  

The values for \( k_7 \) used in the simulations were calculated from this equation. By obeying this constraint, it is assured that the system (5) has a positive equilibrium value equal to \( N_e \).

**B. INTRINSIC GROWTH RATE**

An additional analytic result can be obtained from (8). The expression in braces in the numerator must be positive to yield positive values for \( k_7 \). This focuses attention on the roots of

\[ 0 = 2k_3 k_4 k_5 k_6 - (k_1 + k_3)(k_1 + k_4)(k_1 + k_5)(k_1 + k_6), \]
which is also a fourth order polynomial in \( k_1 \),

\[
0 = k_1^4 + k_1^3(k_3 + k_4 + k_5 + k_6)
+ k_1^2(k_4k_6 + k_5 + k_4k_3 + k_5k_6 + k_4k_6 + k_3k_5)
+ k_1(k_4k_5k_6 + k_3k_4k_5 + k_3k_4k_5 + k_5k_3k_6) - k_4k_5k_6. \tag{9}
\]

By Descartes's rule of signs [25], this equation has one real positive root, say \( k_p \). If \( k_1 \geq k_p \), then \( k_7 \leq 0 \) and the system (5) will not have a positive equilibrium solution. Consequently, \( k_p \) is an "intrinsic growth rate" (IGR) in the sense that if the rate of cell loss exceeds \( k_p \), the system cannot grow. The IGR was calculated by finding the positive real root of (9) for each case.

C. APPROXIMATION FOR SMALL \( G_0 \)

An analytic approximation will prove itself useful later in understanding the behavior of this model. At small time values, the \( G_0 \) subpopulation is small. If \( G_0 = 0 \) the resulting system is linear, corresponding to early exponential growth. The system (5) reduces to

\[
\begin{align*}
\dot{G}_1 &= 2k_6M - (k_1 + k_3)G_1, \\
\dot{S} &= k_3G_1 - (k_1 + k_4)S, \\
\dot{G}_2 &= k_4S - (k_1 + k_5)G_2, \\
\dot{M} &= k_5G_2 - (k_1 + k_6)M.
\end{align*}
\tag{10}
\]

For sufficiently large times, the solutions of (10) can be approximated by

\[
\begin{align*}
G_1(t) &\approx c_{11}e^{\lambda_1 t}, \\
S(t) &\approx c_{21}e^{\lambda_1 t}, \\
G_2(t) &\approx c_{31}e^{\lambda_1 t}, \\
M(t) &\approx c_{41}e^{\lambda_1 t},
\end{align*}
\tag{11}
\]

where \( \lambda_1 \) is the largest root of the characteristic equation [6]. Differentiating (11) demonstrates that in each case the ratios \( \dot{G}_1/G_1, \dot{S}/S, \) etc., equal \( \lambda_1 \).

When \( G_0 \) is sufficiently small [so that \( (k_1 + k_2)G_0 \ll k_7NG_1 \)],

\[
\dot{G}_0 = Ae^{2\lambda_1 t}, \quad \text{where} \quad A = k_7c_{11}\sum_{i=1}^4 c_{1i}.
\tag{12}
\]

Consequently,

\[
G_0 \approx \frac{A}{2\lambda_1}e^{2\lambda_1 t}.
\]
and
\[ \frac{\dot{G}_0}{\dot{G}_0} = 2\lambda_1. \]

Finally, reconsider the characteristic equation for the linear system derived from (5). The characteristic equation can be written
\[ \text{Det}(\lambda I - B) = 0, \quad (13) \]
where \( I \) is the identity matrix and
\[
B = \begin{bmatrix}
-(k_1 + k_3) & 0 & 0 & -2k_6 \\
-k_3 & -(k_1 + k_4) & 0 & 0 \\
0 & -k_4 & -(k_1 + k_5) & 0 \\
0 & 0 & -k_5 & -(k_1 + k_6)
\end{bmatrix}.
\]

The polynomial defined by (13) is
\[ (\lambda_1 + k_1 + k_3)(\lambda_1 + k_1 + k_4)(\lambda_1 + k_1 + k_5)(\lambda_1 + k_1 + k_6) - 2k_3k_4k_5k_6 = 0. \]
\[ (14) \]
Since \( k_p \), the IGR, is defined to be the positive root of the fourth order polynomial (9),
\[ (k_p + k_3)(k_p + k_4)(k_p + k_5)(k_p + k_6) - 2k_3k_4k_5k_6 = 0. \]
\[ (15) \]
(14) and (15) are identical if
\[ \lambda_1 = \text{IGR} - k_1. \]
(16)

This last result suggests a simple relationship between the early growth rates of the subpopulations, the intrinsic growth rate (which can be calculated), and the cell loss parameter.

4. NUMERICAL SOLUTION OF THE EQUATIONS

In order to further investigate the behavior of this model, the system (5) was solved numerically. The algorithm used was a variable order Adams method [35]. All calculations were performed in double precision Fortran with the \( H \) level extended compiler on an IBM 4341 Model 2 Computer. Subsequently, all model fitting and graphics were done using the Statistical Analysis System [3]. In all cases, the simulation was stopped when the total population size changed by less than 0.1 percent on three successive steps.
A. SELECTION OF PARAMETER VALUES

Parameter values used for these simulations were chosen based on a fairly extensive compilation of animal tumor cell cycle kinetic data presented in Steel [39]. These data include the duration of the $G_2$, $S$, and $G_1$ phases, intermitotic time, mitotic index, labeling index, growth fraction, cell loss factor, and doubling time. The data presented relates to model parameters as follows:

$$\frac{1}{k_1 + k_3} = \text{duration of } G_1,$$
$$\frac{1}{k_1 + k_4} = \text{duration of } S,$$
$$\frac{1}{k_1 + k_5} = \text{duration of } G_2,$$
$$\frac{1}{k_1 + k_6} = \text{duration of } M$$

$$= \text{(intermitotic time)} - (\text{duration of } G_1 + S + G_2).$$

The cell loss constant $k_1$ can be crudely estimated if one is willing to accept the approximation of exponential growth. While this is not exact, it does allow a rough guess for $k_1$. Two useful relations from Steel [39] are

$$\frac{k_L}{k_p} = \text{CLF} \quad \text{(valid in general)}$$

and

$$\frac{\log 2}{T_d} = k_p (1 - \text{CLF}) \quad \text{(valid for exponential growth)},$$

where

$$k_L = \text{rate constant for cell loss},$$
$$k_p = \text{rate constant for cell production},$$
$$\text{CLF} = \text{cell loss factor},$$
$$T_d = \text{doubling time}.$$

These can be used to obtain

$$k_1 \approx k_L = \frac{\text{CLF}}{1 - \text{CLF}} \cdot \frac{\log 2}{T_d}.$$
TABLE 1

Parameter Values Used for Simulations

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Mean</th>
<th>Range</th>
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</thead>
<tbody>
<tr>
<td>k₁</td>
<td>1.60</td>
<td>± 1.45</td>
</tr>
<tr>
<td>k₂</td>
<td>1.60</td>
<td>± 1.60</td>
</tr>
<tr>
<td>k₃</td>
<td>31.04</td>
<td>± 25.98</td>
</tr>
<tr>
<td>k₄</td>
<td>12.50</td>
<td>± 7.18</td>
</tr>
<tr>
<td>k₅</td>
<td>58.22</td>
<td>± 41.30</td>
</tr>
<tr>
<td>k₆</td>
<td>257.80</td>
<td>± 241.7</td>
</tr>
<tr>
<td>k₇</td>
<td>Calculated from (8)</td>
<td></td>
</tr>
</tbody>
</table>

These equations were used to calculate values for \( k_1 \) and for \( k_3 \) through \( k_6 \), and the resulting ranges were covered with a rotatable design [7] using 45 sets of parameter values. The remaining parameters \( k_2 \), \( k_1 \), and \( N_e \) were given reasonable values. \( k_2 \) was given the following three values: 0.0, \( \bar{k}_1 \) (the mean of the \( k_3 \) range), and 2\( \bar{k}_1 \). The parameter \( k_7 \) was calculated from (8) because of considerations mentioned earlier. \( N_e \) was set to \( 1 \times 10^{10} \), corresponding to roughly 10 grams of tumor tissue. The relative sizes of the parameters obtained in this fashion were near those which were observed by trial and error to give reasonable growth curves. Parameter ranges resulting from the rotatable design are listed in Table 1. Initial conditions were chosen identically for all simulations. A single cell was assumed to be in the \( G_1 \) phase at the start of tumor growth.

B. SIMULATIONS AND QUALITATIVE BEHAVIOR

The results of simulations are most easily examined graphically. In most cases the ordinate is scaled by the equilibrium size and the abscissa is scaled by the intrinsic growth rate. Consequently, plots are \( N(t)/N_e \) versus time/IGR.

If one looks at the growth of the total population size, a range of behavior will be seen. Examples are shown in Figure 2, illustrating several points. Oscillation about the equilibrium value is common. This is most frequently seen as simple overshoot of the ultimate equilibrium, but a more extreme case is shown. A more traditional sigmoid shape can be obtained, as well as curves with a slower rise and no overshoot.

Other qualitative features of the system can be seen graphically. The samples shown here are illustrative but not exhaustive. The time course of subpopulation growth is shown in Figures 3 and 4 for two sets of parameter values. As expected, \( G_0 \) eventually dominates the subpopulation sizes. Under conditions of small cell loss (Figure 4), virtually all cells are in the resting phase. Figure 5 shows subpopulation fractions versus time for one simula-
Fig. 2. A sample of growth curves.

Curve A: $k_1 = 1.6, k_2 = 0, k_3 = 31.0, k_4 = 12.5, k_5 = 58.2, k_6 = 257.8, k_7 = 20.6$.

Curve B: $k_1 = 0.98, k_2 = 2.27, k_3 = 20.1, k_4 = 9.48, k_5 = 75.6, k_6 = 156.2, k_7 = 48.4$.

Curve C: $k_1 = 0.14, k_2 = 1.6, k_3 = 31.0, k_4 = 12.5, k_5 = 58.2, k_6 = 257.8, k_7 = 367.8$.

Fig. 3. Plot of relative subpopulation size versus time. $k_1 = 2.20, k_2 = 2.27, k_3 = 42.0, k_4 = 9.48, k_5 = 40.9, k_6 = 359.5, k_7 = 40.6$. 
FIG. 4. Plot of relative subpopulation size versus time. $k_1 = 0.14$, $k_2 = 1.6$, $k_3 = 31.0$, $k_4 = 12.5$, $k_5 = 58.2$, $k_6 = 257.8$, $k_7 = 367.8$.

FIG. 5. Plot of subpopulation fractions versus time. $k_1 = 2.21$, $k_2 = 2.27$, $k_3 = 42.0$, $k_4 = 15.5$, $k_5 = 40.9$, $k_6 = 156.2$, $k_7 = 49.7$. 
Fig. 6. Plot of growth fractions versus time.

Curve A: $k_1 = 0.985, k_2 = 0.925, k_3 = 47.0, k_4 = 9.48, k_5 = 40.9, k_6 = 359.5, k_7 = 60.3$.
Curve B: $k_1 = 0.985, k_2 = 2.27, k_3 = 20.1, k_4 = 9.48, k_5 = 40.9, k_6 = 359.5, k_7 = 16.6$.
Curve C: $k_1 = 0.985, k_2 = 0.925, k_3 = 20.1, k_4 = 9.48, k_5 = 40.9, k_6 = 156.7, k_7 = 27.66$.
Curve D: $k_1 = 2.21, k_2 = 0.925, k_3 = 20.1, k_4 = 9.48, k_5 = 40.9, k_6 = 359.5, k_7 = 12.0$.
Curve E: $k_1 = 2.21, k_2 = 2.27, k_3 = 20.1, k_4 = 9.48, k_5 = 40.9, k_6 = 156.2, k_7 = 16.6$.

Qualitatively, similar behavior would be seen for other parameter values as well. The $S$ fraction decreases over time, as do $G_1$, $G_2$, and $M$. The $G_0$ fraction is the only one that can show a sustained increase over time.

We may plot the growth fractions versus time for this model, as is done in Figure 6. Qualitatively similar behavior would be obtained for any set of parameter values.

It is neither practical nor desirable to characterize each individual simulation. Rather, some common behavior should be sought for all sets of parameter values. Recalling the results of Section 3.C, if the logarithm of each subpopulation size is plotted against the logarithm of the total population size, as seen in Figure 7, in all cases there is near linearity over 10 orders of magnitude. However, when a linear model is fitted to any curve in Figure 7, systematic error in the residuals demonstrates a downward curvature. However, in all cases the slopes for the log-log plots of $G_1$, $S$, $G_2$, and $M$ versus $N$ are near 1.0, and the slope for the log-log plot of $G_0$ versus $N$ is near 2.0.

The oscillatory behavior of this system is interesting. Aside from the abovementioned large scale oscillations, smaller oscillations are always seen
Fig. 7. Plots of log(subpopulation size) versus log(relative total population size). $k_1 = 1.60$, $k_2 = 1.60$, $k_3 = 31.0$, $k_4 = 12.5$, $k_5 = 58.2$, $k_6 = 499.6$, $k_7 = 41.5$.

near the equilibrium point. The Jacobian matrix of the system defined in (5) can be formed at each time step during the numerical simulation. The eigenvalues of the matrix provide a key to the local behavior of the system. All eigenvalues are real until a time near equilibrium, and subsequently become complex, demonstrating oscillations (usually when $G_0$ becomes "large"). These fine oscillations are not seen on the large time scale that characterizes most graphs presented here.

5. COMPARISON WITH MICROFLUOROMETRY DATA

If the fraction of cells in each phase is examined at each time point, there are some experimental data with which to compare model behavior. These data are obtained by the technique of cell microfluorometry [46]. This technique involves the staining of cells in suspension with a DNA binding fluorochrome. Individual cells can then be assigned to their place in the cell cycle on the basis of DNA content. Several excellent reviews of this method are available [15, 51]. DNA histograms obtained by microfluorometry can reveal accurate and useful cell kinetic data [50, 52].

Microfluorometry was used recently to gather data on the fraction of cells in each phase of the cell cycle for a hyperdiploid mammary carcinoma in mice [2]. The results show a fall in the $S$ fraction with gradual rise in the $G_1$
and \( G_2 + M \) fractions over time. Apparently the \( G_1 \) fraction is the sum of \( G_0 \) and cycling \( G_1 \) cells. The rise in \( G_2 + M \) is probably due to diploid or hyperdiploid \( G_0 \) cells being interpreted as \( G_2 \) cells. With this in mind, the behavior of this model is reasonable. If \( G_1 + G_0, S \) and \( G_2 + M \) are plotted as shown in Figure 8, the result is similar to that of Barfod and Barfod [2]. The difference, as mentioned above is probably attributable to the hyperdiploid nature of the experimental tumor.

These same authors also show a strong correlation between cell production rate and the fraction of cells in \( S \) phase. One might expect the fraction of cells actively cycling to decrease over time. This concept can be depicted in terms of growth fractions, a sample of which was seen in Figure 6. It should be emphasized that no data are available on growth fractions during the very early phases of growth. It is known that the growth rate declines in a nearly exponential fashion later in time [19]. If the growth rate is proportional to the size of the growing population, the growth fraction will be expected to decline qualitatively in the manner shown.

6. IMPLICATIONS FOR CANCER TREATMENT

The previous sections have outlined the basic analytic features and the behavior of a reasonable sample of simulations of the cell cycle model presented. It is reasonable to ask what implications this model holds concern-
ing the goal of cancer therapy—the complete elimination of tumor cells. This section will examine some preliminary conclusions in answer to this question.

Numerous studies have been done attempting to model mathematically the effects of therapy on the growth of tumors. Several common problems emerge regarding these results [42]. Implications of the study depend on the particular kinetic feature modeled (e.g., cell cycle time, thymidine index, or overall growth) as well as the particular model assumed for tumor growth and the action of the therapy.

Several problems are not adequately dealt with by these types of study. In particular, excision of a primary tumor can cause more rapid growth of metastases [33, 36]. There is evidence that drug resistant mutations in tumor cells arise spontaneously, like mutations in microbial populations [22]. Goldie and Coldman [12] have presented a mathematical model of this phenomenon. With these features of treated tumors in mind, one might pessimistically view therapy as the process of selecting a drug resistant cell population. Unless the last few tumor cells are killed, the problem becomes the regrowth of a new population.

The model presented here supports these conclusions. Even when a tumor cell population is attacked with a highly effective agent, the regrowth is rapid. Figure 9 shows a near-equilibrium cell population which was "treated" by
the removal of 99.99 percent of all cells, in a "log-kill" fashion. The rapid regrowth is obvious. Even if frequent repeated "treatments" are given (Figure 10), the results are similar.

One might try to improve upon this result by selectively eliminating cycling cells preferentially over resting cells (analogously to chemotherapy) and vice versa (perhaps analogously to surgery). Because of the ability of G\textsubscript{0} cells to reenter the cell cycle, the results are equally discouraging.

In spite of this, the model does give a clue to what might be a more effective tumor killing strategy. Recalling the concept of the intrinsic growth rate, and the requirement that the rate of cell loss be less than the IGR, we can see that if at any time in tumor growth the rate of cell loss exceeds the IGR, the size of the cell population will decline. Figure 11 shows this effect. Near equilibrium, some mechanism has increased the rate of cell loss (i.e., \( k_1 \)) above the IGR. Exponential decline in the population size results. What process would allow a sustained increase in the rate of cell loss? Clearly not repeated doses of drugs, but perhaps the chronic administration of a drug with low host toxicity, even if it has a lower antitumor activity. Enhanced host immune response might also be a process which would cause such an increase in the rate of cell loss.

This model suggests that methods of selecting chemotherapeutic (or other therapeutic) agents on the basis of high short term activity against tumor
cells, and probably long term activity against host cells, is not optimum. Perhaps less effective agents with lower toxicity should be examined.

8. SOME IDEAS FOR FUTURE STUDY

The behavior of this model could be used to draw other general conclusions about optimum tumor treatment. However, prior to this, more work would need to be done in several areas. These are outlined here.

No data have yet been available for actual fitting of this model. Some difficulties in a specific study were outlined in Section 7. Perhaps these problems could be overcome by a different form of the model or a more systematic gathering of data. This model would be shown to be inadequate by such data, or alternatively the parameters of the model could be estimated by model fitting.

The modeling of cell loss is an important determinant of the behavior of simulations as well as a key feature of the analytic properties of the model equations. Investigations of model behavior for cell loss other than first order have not been done. Perhaps other simple but flexible forms of cell loss would lend some analytic tractability to the system.

The oscillatory behavior of the model is interesting, but has not been characterized analytically. It is not clear under what circumstances, if any, these oscillations could be eliminated. Alternatively, if the model could be
written in terms of its smoothed behavior, it might map onto a multivariate growth scheme.

Along the same lines, overshoot of the equilibrium size is common and somewhat reassuring in view of known irregularities in the growth of individual tumors. However, real tumors may grow irregularly for different reasons, such as blood supply, host response, etc. Overshoot may be an artifact of the parameters chosen. It seems to be prominent when \( k_2 < k_1 \), that is, when \( G_0 \) cells are slow to return to the cell cycle relative to cell loss. This issue could be cleared up by a clearer experimental understanding of exactly how a \( G_0 \) cell can behave.

Finally, one could ask what features of this model account for the properties exploited here. Is the near-allometric behavior a consequence of the circularity of the flow of cells through the cycle? Would other formulations of the cell cycle demonstrate these same properties?

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GROWTH AND CELL CYCLE KINETICS


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