CELL GROWTH AND DIVISION

I. A MATHEMATICAL MODEL WITH APPLICATIONS TO CELL VOLUME DISTRIBUTIONS IN MAMMALIAN SUSPENSION CULTURES

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ABSTRACT A mathematical model is formulated for the development of a population of cells in which the individual members may grow and divide or die. A given cell is characterized by its age and volume, and these parameters are assumed to determine the rate of volume growth and the probability per unit time of division or death. The initial value problem is formulated, and it is shown that if cell growth rate is proportional to cell volume, then the volume distribution will not converge to a time-invariant shape without an added dispersive mechanism. Mathematical simplications which are possible for the special case of populations in the exponential phase or in the steady state are considered in some detail. Experimental volume distributions of mammalian cells in exponentially growing suspension cultures are analyzed, and growth rates and division probabilities are deduced. It is concluded that the cell volume growth rate is approximately proportional to cell volume and that the division probability increases with volume above a critical threshold. The effects on volume distribution of division into daughter cells of unequal volumes are examined in computer models.

INTRODUCTION

Let us consider a population of cells, the individual members of which may grow and divide or possibly die without division. If the properties of the individual cells are known and if the factors governing their growth and division are understood, then one should be able to make predictions concerning the development of the population. Conversely, if sufficiently precise measurements are made on the development of a population in time, it should be possible to draw conclusions concerning the behavior of the individual cells, determining such properties as their lifetimes and rates of growth. Various new techniques have been developed in recent years for the characterization of cells growing in suspension cultures. In particular, with electronic cell counters of the Coulter type (Gregg and Steidley,

1965; Anderson and Petersen, 1967), one can determine cell concentrations to a precision of better than 1 % at very frequent intervals and, in addition, make simultaneous measurements of the cell volume distributions with high resolution and accuracy. As a result, it is profitable to look more closely than in the past at cell growth and division and to consider what sorts of things can be learned from the fine structure of the data.

It is well-known that the coupling between the cell division cycle and the cell growth cycle is a loose one, easily perturbed by environmental influences (Prescott, 1964; Schaechter, Maaloe, and Kjeldgaard, 1958). On the other hand, some feedback must exist between the two cycles in order to maintain the stability of the culture over periods of many generations. If a population is in balanced exponential growth (that is, if all parameters describing the instantaneous state of the population remain constant except only cell number), then there must exist a time-invariant dynamic balance between growth and division.

It is the aim of the present paper to develop a general mathematical model for cell growth and division in which cell age and volume are considered to be its fundamental parameters. Our choice of these two parameters for characterizing the cell is motivated primarily by their accessibility to experimental determination. We recognize that the medium in which a cell is placed will profoundly affect its growth and division, but if we confine our attention to a particular medium (constant in time), then characterization of a cell by its age and volume may be biologically reasonable as well as convenient. Some biochemical reactions (in particular, those leading to the replication and segregation of DNA and the synthesis of certain enzymes) seem to proceed sequentially during the cell life cycle, and the state of these reactions may be succinctly characterized by the cell age. Other reactions which take place throughout most of the cell life cycle, such as those leading to ATP and increase of structural materials, may depend less on cell age than on volume through such factors as diffusion times, surface-to-volume ratios, and number of ribosomes, mitochondria, and other cell organelles. Thus, cell age and volume may summarize its properties about as well as is possible with two parameters.

It is our intent to make the theory sufficiently general that it may be applied to the interpretation of a variety of different experiments. In the present paper, we use the theory to analyze the volume spectra of mammalian cells in exponential growth in suspension culture and to derive from experimental volume spectra (Anderson and Petersen, 1967) information on growth rates and division probabilities of individual cells. However, a considerable variety of other experiments is suggested by the model, and a discussion of some of these is given in the concluding section.

Previous workers have developed models for analysis of cell populations in which attention is focused on the number of cells in the population and on the time between divisions. We may mention the work of Rahn (1931–1932) in which it was postulated that a cell divides when all of a number of *independent* reactions have been completed, and that of Kendall (1948) who postulated division to ensue when a number of

successive steps have been completed. In some of these studies the fluctuations in a population were considered, as well as the mean or average growth, and general discussions of such random processes are given by Harris (1959, 1963). In addition, some studies have been reported in which the distribution of cell volumes in a population were considered, and the relationship of this distribution to growth and division of single cells was also considered. The distribution of cell sizes in a population of *Tetrahymena pyriformis* G_L was analyzed by Scherbaum and Rasch (1957). For a more or less exponentially growing population, they showed that the experimental size distribution was consistent with some assumed rates of volume growth for the individual cells and was inconsistent with other growth rates. In their most general considerations, they allowed the relative cell volume (the ratio of volume to birth volume) to be a function of the relative age (age to age at time of division). Koch and Schaechter (1962) considered a model in which cells divide upon reaching a critical volume and in which the rate of cell growth is proportional to cell volume (mass). Allowing for some fluctuations in cell size at division, they were able to obtain agreement with experiments on some bacterial populations. Collins and Richmond (1962) considered the volume (length) distribution of *Bacillus cereus* organisms in exponential growth and, from the stable length distributions, were able to deduce the mean rate of growth of single organisms. Powell (1964) has analyzed the model of Koch and Schaechter (1962) in more mathematical detail.

In the present paper we wish to develop a more general approach to the age and volume distribution of cell populations. When we come to apply the theory to analyze experimental volume distributions, it will be necessary to make simplifying assumptions. Nevertheless, we feel that it is important to have a fairly general theory available so that the implications of these assumptions can be more clearly understood and so that as a greater variety of data becomes available, it can be understood in a uniform context.

ASSUMPTIONS AND GENERAL EQUATIONS

Throughout this paper we shall confine our attention to populations which contain large enough numbers of cells that fluctuations from the mean or expected development of the population can be ignored. We assume that cells grow in volume and divide or die, where by death of a cell we mean its disappearance from the population by means other than division. Since in our model we characterize a cell by its age and volume, we assume that the growth rate of a cell and its probability per unit time of division or death are uniquely determined by its age and volume. It is interesting to note that the theory refers to a *probability* of division or death, while a *deterministic* rate of growth is assumed. Alternatively, one could contemplate a theory in which probabilities of various growth rates are allowed, but in the absence of experimental guidance this seems an unnecessary complication. In addition, it will be seen that for an exponentially growing or a steady-state population this deterministic assumption becomes unimportant.

Finally, we will generally assume that when a cell divides, there are born two daughter cells of precisely equal volumes. While in principle this assumption could readily be removed, we will usually retain it for reasons of mathematical simplicity. In some numerical work, to be reported in the section on Analysis of Experimental Volume Spectra, unequal daughters will be allowed.

Let $N(t, \tau, V)\, d\tau\, dV$ be the number of cells in the population at time t that have ages between τ and $\tau + d\tau$ and volumes between V and $V + dV$. For a cell of age τ and volume V, let $F(\tau, V)\, dt$ be the change in cell volume in time dt, let $P(\tau, V)\, dt$ be the probability that the cell divides in time dt, and let $D(\tau, V)\, dt$ be the probability of cell death in time dt.

We may now derive a differential equation for the cell population, $N(t, \tau, V)$, in terms of the functions F, P, and D. Let us first confine our attention to cells of finite age (that is, cells that are not just being born). Consider the number of cells, $N(t + dt, \tau, V)\, d\tau\, dV$, in the age-volume element $d\tau\, dV$ at time $t + dt$. Suppose that $dV \gg F(\tau, V)\, dt$. Then this number of cells equals the number of cells in the same age-volume element at time t and having ages $\tau - dt$ plus the number of cells which have grown into the volume element in time dt, minus the number of cells which have grown out in time dt, and minus the number of cells which have divided or died. Writing down these contributions mathematically, we have for $t \neq 0$:

$$\begin{aligned} N(t + dt, \tau, V)\, d\tau\, dV = {} & N(t, \tau - dt, V)\, d\tau\, dV + F(\tau, V)N(t, \tau, V)\, d\tau\, dt \\ & - F(\tau, V + dV)N(t, \tau, V + dV)\, d\tau\, dt \\ & - \{P(\tau, V) + D(\tau, V)\}N(t, \tau, V)\, d\tau\, dV\, dt. \end{aligned} \quad (1)$$

Passing to the limit $dt \to 0$, $dV \to 0$, we have:

$$\frac{\partial N(t, \tau, V)}{\partial t} + \frac{\partial N}{\partial \tau} + \frac{\partial (FN)}{\partial V} = -(P + D)N \qquad \tau \neq 0. \quad (2)$$

For cells of age 0, the situation is quite different. Cells of ages between 0 and $d\tau$ and volumes between V and $V + dV$ will be present at time $t + dt$ only due to the division of cells of any age and of volumes between $2V$ and $2V + 2dV$ during times from t to $t + dt$. Thus,

$$N(t + dt, 0, V)dt\, dV = \left[2 \int_0^\infty d\tau \cdot P(\tau, 2V)N(t, \tau, 2V)\right] dt \cdot 2dV. \quad (3)$$

Here the first factor of 2 on the right-hand side arises because of division into two daughter cells, and the second factor 2 is present because the cells in a volume element $2dV$ divide into a volume interval dV. Equation (3) gives to first order in dt:

$$N(t, 0, V) = 4 \int_0^\infty P(\tau, 2V)N(t, \tau, 2V) \cdot d\tau. \quad (4)$$

If one wished to allow daughter cells of unequal size, then the right-hand side of Equation (4) would involve an integration over volume as well as age, and a more general function than $P(\tau, 2V)$ would be required in the integrand.

Equations (2) and (4) are fundamental equations of our model. One can think of them as defining an initial value problem wherein the initial population, $N(0, \tau, V)$, is given and it is required to find the population at any later time. If necessary, it could be assumed that cells of very small volume are not present, $N(t, \tau, 0) = 0$. The sense of the equations is that Equation (2) describes how cells grow up, subject to loss through division and death, while Equation (4) describes how the population is regenerated by the division process.

For a "well-behaved" biological population of growing cells, one might expect that the cell population should increase exponentially with time.[1] Or perhaps it might be expected to approach a steady state with $\partial N/\partial t = 0$. In either case, at long times one might expect the solution of the initial value problem to behave as $e^{\alpha t}$, with α a constant, that is, $N(t, \tau, V) \underset{t\to\infty}{\longrightarrow} e^{\alpha t} N(\tau, V)$. In particular, α would be 0 for the steady state. Hence, the interesting mathematical question arises: What conditions must be satisfied by the functions F, P, and D in order that solutions of Equations (2) and (4) become exponential functions at large times? The answer to this question is apparently not known.[2]

One can readily think of functions, F and P, that do not permit exponential solutions. For example, suppose that P is such that all cells divide upon reaching a particular age, τ_0, while F depends on V only. If F were such that no cell could double its volume in time τ_0, then the cells in the population would become continuously smaller and a simple exponential behavior in time could not occur. However, we shall generally assume that, for biological populations of interest, the functions F, P, and D are such that exponential solutions of Equations (2) and (4) are permitted and, in particular, that the initial value problem can lead to exponential solutions at later times.

Equations (2) and (4) can be converted to an integral equation by introducing characteristic curves of Equation (2) (Courant and Hilbert, 1962). One interesting feature of such a development can be appreciated without formality: namely, if the rate of cell growth is proportional to cell volume, then the population will forever

[1] For any population wherein the dependence of N on age and volume is independent of time, it is easy to see that the dependence of N on time, if any, must be exponential. For, suppose $N(t, \tau, V) = T(t) \cdot N(\tau, V)$. Substitute in Equation (2) and divide by $N(t, \tau, V)$. Then, the only term involving time is $\frac{1}{T}\frac{\partial T}{\partial t}$, and this must equal a function of τ and V. This is only possible if the term equals a constant, $\frac{1}{T}\frac{\partial T}{\partial t} = \alpha$, whence $T = T_0 e^{\alpha t}$. This general feature of exponential solutions has probably been appreciated by all earlier students of these problems and was explicitly proved at length by Scherbaum and Rasch (1957).

[2] The much simpler problem in which cell volume is not considered has apparently just been solved by Nooney (1967), who used renewal theory to show that if the probability of division depends in a *continuous* manner on cell age then an exponential solution will be reached at large times.

be sensitive to its initial conditions. For suppose that the growth rate, $F(\tau, V) = cV$, with c a constant. Then a daughter cell, having just half the volume of the parent cell, will grow at just half the rate of the parent cell. It follows that if one starts with a group of cells of volume V, age τ, at time 0, then any daughter cell of this group, no matter when formed, will always have a volume equal to half the volume of an undivided cell in the group. There will then be no dispersion of cell volumes with time, and the population will consist at any time of a number of cell generations differing by just a factor of 2 in volume. For more general initial conditions, the population at late times will still reflect the initial state rather than simply growing exponentially in time.

Inasmuch as the growth rates of actual cells have frequently been deduced or assumed to be proportional to volume (for example, see Koch and Schaechter, 1962; Collins and Richmond, 1962), it is relevant to consider the peculiarities of such a growth law somewhat further. We have seen that such a growth law, combined with division into two precisely equal daughter cells, will not lead to any dispersion in cell volumes with the passing of time. However, if some dispersion of cell volumes is introduced either by allowing division into unequal daughter cells or by slight departures from $F = cV$, we may then expect that a population will become exponential as time passes and that the rate at which it approaches the exponential state will reflect the extent of the dispersion. In addition, it may be noted that even without any additional cell dispersion it may be possible to choose a particular initial population, $N(0, \tau, V)$, which will grow exponentially in time.

Correlations between the lifetimes of related cells are possible in our model. Thus, sister-sister correlations and mother-daughter correlations may be included through the volume dependence of $P(\tau, V)$. Suppose, for example, that $P(\tau, V)$ were chosen so that cells had to be larger than some minimum volume, V_m, before they could divide. Then a cell that is unusually small at birth would tend to live longer than the average cell, and there could be a positive correlation between the lifetimes of sister cells. Similarly, a mother cell which lived longer than the average cell and was larger than the average would give rise to large daughters with correspondingly shortened life expectancies, so that a negative mother-daughter correlation could be obtained.

If we integrate Equation (2) over all cell ages, $(0, \infty)$, the result may be combined with Equation (4). The term $\partial N/\partial \tau$ will give a contribution at $\tau = 0$, for which we may use Equation (4). We assume no cells to be present of infinite age. Then defining,

$$n(t, V) = \int_0^\infty N(t, \tau, V)\, d\tau$$

$$\bar{f}(t, V)n(t, V) = \int_0^\infty F(\tau, V)N(t, \tau, V)\, d\tau$$

$$\tilde{p}(t, V)n(t, V) = \int_0^\infty P(\tau, V)N(t, \tau, V)\, d\tau$$

$$\tilde{d}(t, V)n(t, V) = \int_0^\infty D(\tau, V)N(t, \tau, V)\, d\tau\,. \tag{5}$$

we find from Equations (2) and (4)

$$\frac{\partial n(t,V)}{\partial t} + \frac{\partial(\tilde{f}n)}{\partial V} = -\{\tilde{p}(t, V) + \tilde{d}(t, V)\}n(t, V) + 4\tilde{p}(t, 2V)n(t, 2V)\,. \tag{6}$$

Since the functions $\tilde{f}$, $\tilde{p}$, and $\tilde{d}$ depend on time, Equation (6) is generally less tractable than the previous Equations (2) and (4). However, for an exponentially growing population, $\tilde{f}$, $\tilde{p}$, and $\tilde{d}$ are independent of time and the situation is much simpler. Therefore, let us now consider such populations.

EXPONENTIAL POPULATIONS

By an exponential population, we mean one in which the time dependence is separable and exponential so that

$$N(t, \tau, V) = e^{\alpha t} N(\tau, V) \tag{7 a}$$

and

$$n(t, V) = e^{\alpha t} n(V). \tag{7 b}$$

The particular case of a steady state is found for $\alpha = 0$: For an exponential population, it is readily seen from the equations in (5) that the functions $\tilde{f}$, $\tilde{p}$, and $\tilde{d}$ are independent of time, and Equation (6) becomes

$$\alpha n(V) + \frac{d(f(V)n(V))}{dV} = -\{p(V) + d(V)\}n(V) + 4p(2V)n(2V) \tag{8}$$

where $n(V)$ was defined in Equation (7 *b*),

$$f(V) = \int_0^\infty F(\tau, V)N(\tau, V)d\tau/n(V) \tag{9}$$

and, as in Equation (5), $p(V)$ and $d(V)$ are similarly related to $p(\tau,V)$ and $d(\tau,V)$.

Equation (8) may be thought of as an eigenvalue problem: that is, it will have solutions only for certain definite values of α, eigenvalues, and for each there will be a definite volume distribution, $n(V)$, the eigenfunction (Margenau and Murphy, 1943). A particular form of Equation (8) has been given by Powell (1964) for the special case of growth rate proportional to volume [$f(V) = f_1 V$ and $d(V) = 0$]. In this case, we will see presently that $\alpha = f_1$.

Note that the only assumption we have used in deriving Equation (8) from Equations (2) and (4) is that the population is exponentially growing, as defined by Equation (7). Indeed, Equation (8) is more general than implied by our model. We believe that it is valid for any exponential population in which cells can only die or divide into two equal daughter cells. If a cell should really be characterized by many variables in addition to age and volume, nevertheless for a population in which the time dependence is separable and exponential, as in Eq. (7 *a*), the integral of the population over all variables except for volume should satisfy Equation (8). Note further that the simple presence of $f(V)$ in Equation (8) does not necessarily imply that cell growth depends on volume in any fundamental way. For example, $F(\tau, V)$ might depend only on cell age, (τ), and nevertheless from Equation (9) we see that $f(V)$ can depend only on cell volume. Similar remarks apply to $p(V)$ and $d(V)$.

Some interesting relations can be derived between the eigenvalue, α, and the eigenfunction, $n(V)$. If we integrate Equation (8) over all cell volumes, $(0, \infty)$, and assume that $f(V)n(V)$ vanishes at the limits of integration, we find

$$\alpha = \int_0^\infty \{p(V) - d(V)\}n(V)\, dV \Big/ \int_0^\infty n(V)\, dV. \tag{10}$$

This equation has the obvious interpretation that the rate of increase of the number of cells, $\alpha \int_0^\infty n(V)\, dV$, equals the birth rate minus the death rate.

A different relation can be obtained by multiplying Equation (8) by V and then integrating. The term involving $f(V)n(V)$ is integrated by parts with zero contribution assumed from limits so that

$$\alpha = \frac{\int_0^\infty f(V)n(V)\, dV - \int_0^\infty V\, d(V)n(V)\, dV}{\int_0^\infty Vn(V)\, dV}. \tag{11}$$

This equation evidently describes how the total volume of the population $[\int Vn(V)\, dV]$ changes with time. Note that for the special case considered by Powell, $f(V) = f_1 V$ and $d(V) = 0$, then $\alpha = f_1$.

We derived Equation (8) by integrating Equation (2) over all cell ages. Alternatively, we could have integrated Equation (2) over all cell volumes. For an exponential population and defining

$$\int_0^\infty N(t, \tau, V)\, dV = e^{\alpha t}\mathfrak{N}(\tau)$$

$$\int_0^\infty F(\tau, V)N(t, \tau, V)\, dV = e^{\alpha t}\mathfrak{F}(\tau)\mathfrak{N}(\tau)$$

$$\int_0^\infty P(\tau, V)N(t, \tau, V)\, dV = e^{\alpha t}\mathfrak{P}(\tau)\mathfrak{N}(\tau)$$

$$\int_0^\infty D(\tau, V)N(t, \tau, V)\, dV = e^{\alpha t}\mathfrak{D}(\tau)\mathfrak{N}(\tau), \tag{12}$$

we would find from Equation (2)

$$\frac{d\mathfrak{N}(\tau)}{d\tau} + \{\alpha + \mathcal{P}(\tau) + \mathfrak{D}(\tau)\}\mathfrak{N}(\tau) = 0 \tag{13}$$

while from Equation (4),

$$\mathfrak{N}(0) = 2\int_0^\infty \mathcal{P}(\tau)\mathfrak{N}(\tau)\, d\tau. \tag{14}$$

Equation (13) can be readily solved for $\mathfrak{N}(\tau)$ in terms of $\mathfrak{N}(0)$ and the result substituted in Equation (14) to yield

$$2\int_0^\infty \mathcal{P}(\tau)\{\exp - \int_0^\tau (\mathcal{P}(\tau') + \mathfrak{D}(\tau') + \alpha)\, d\tau'\}\, d\tau = 1 \tag{15}$$

which may be considered as an equation for determining α without explicit knowledge of $\mathfrak{N}(\tau)$. Such equations for the special case of $\alpha = 0$ have been discussed by von Forster (1959).

SIMPLE RESULTS FOR EXPONENTIAL POPULATIONS

In order to gain some insight into the expected volume spectra for exponential populations, it is useful to consider some especially simple cases. Suppose that there are no cell deaths and that all cells divide upon reaching the volume $2V_0$. This means that in Equation (8) $d(V) = 0$ and $p(V) = 0$ when $V < 2V_0$. Then (assuming $f(V) > 0$), the volume spectrum is confined to the region $V_0 \leq V < 2V_0$, and for the open interval $V_0 < V < 2V_0$, Equation (8) takes the form

$$\alpha n(V) + \frac{d}{dV}(f(V)n(V)) = 0 \tag{16}$$

which has the solution

$$n(V) = n(V_0)\exp - \int_{V_0}^{V} \frac{\alpha + df/dV'}{f(V')}\, dV'. \tag{17}$$

The eigenvalue α can be determined from the condition that twice as many cells are growing up from volume V_0 as are growing into volume $2V_0$. Inasmuch as $f(V)n(V)$ is the number of cells becoming larger than volume V per unit time, this condition may be stated as

$$2f(2V_0)n(2V_0) = f(V_0)n(V_0),$$

or by using Equation (17),

$$2f(2V_0)\exp - \int_{V_0}^{2V_0} \frac{\alpha + df/dV'}{f(V')}\, dV' = f(V_0). \tag{18}$$

If, for example, a cell's growth rate is independent of its volume, so that $f(V) = f_0$ with f_0 a constant, then from Equation (18) we find that $\alpha = (f_0/V_0)\ \ln 2$. Thus, from Equation (17)

$$n(V) = n(V_0)\, e^{(1-V/V_0)\ \ln 2} = n(V_0) 2^{(1-V/V_0)}. \tag{19}$$

More generally, if $f(V)$ is a linear function of V,

$$f = f_0 + f_1 V \tag{20}$$

we may find α from Equation (18)

$$\alpha = \frac{f_1 \ln 2}{\ln(f_0 + 2f_1V_0) - \ln(f_0 + f_1V_0)} \tag{21}$$

and $n(V)$ from Equation (17),

$$n(V) = n(V_0) \left(\frac{f_0 + f_1V_0}{f_0 + f_1V}\right)^{\frac{\alpha+f_1}{f_1}}. \tag{22}$$

For the special case where a cell's growth rate is proportional to its volume, $f_0 = 0$, $\alpha = f_1$, and

$$n(V) = n(V_0) \left(\frac{V_0}{V}\right)^2. \tag{23}$$

The simple results for zero order and first order growth, given by Equations (19) and (23) have been derived by many previous authors [see, for example, Koch and Schaechter (1962)]. Nevertheless, it is instructive to plot the resulting volume spectra in Fig. 1 so that they may be compared with some experimental volume spectra to be discussed shortly. From Fig. 1 we see that when $f(V) = f_1V$, the resulting volume spectrum falls more rapidly with increasing volume than does the spectrum for $f(V) = f_0$. This result is quite general in that a growth function, $f(V)$, that increases rapidly with V leads to a spectrum, $n(V)$, that falls rapidly with V. This feature is seen to be necessary when we recall that $f(V)n(V)$ is the number of cells which became larger than V per unit time.

We could now go on to consider more realistic forms for the cell division probability, $p(V)$, and to derive more complicated volume spectra. However, at this point we would like to turn to the experimental volume spectra which we will then try to interpret in the remainder of this paper. The spectra are those of a variety of mammalian cells in exponential growth in suspension culture.

ANALYSIS OF EXPERIMENTAL VOLUME SPECTRA

It is shown in the accompanying paper (Anderson and Petersen, 1967) that experimental volume spectra, obtained for various mammalian cells in exponential growth, bear a qualitative resemblance to the idealized spectra of Fig. 1. The most obvious difference is that the experimental spectra are broader, as would be expected if cells in a considerable volume range were dividing and if sisters were not of equal volume.

What can be learned from these volume spectra? In particular, can any of the

functions in our model be deduced from the volume spectra? Let us assume that we have truly exponential growth so that Equation (8) may be applied. Further, because of the expectation that few, if any, cells in the population are dying, let us set $d(V) = 0$. Then Equation (8) becomes

$$\alpha n(V) + \frac{d}{dV}\{f(V)n(V)\} = -p(V)n(V) + 4p(2V)n(2V). \qquad (24)$$

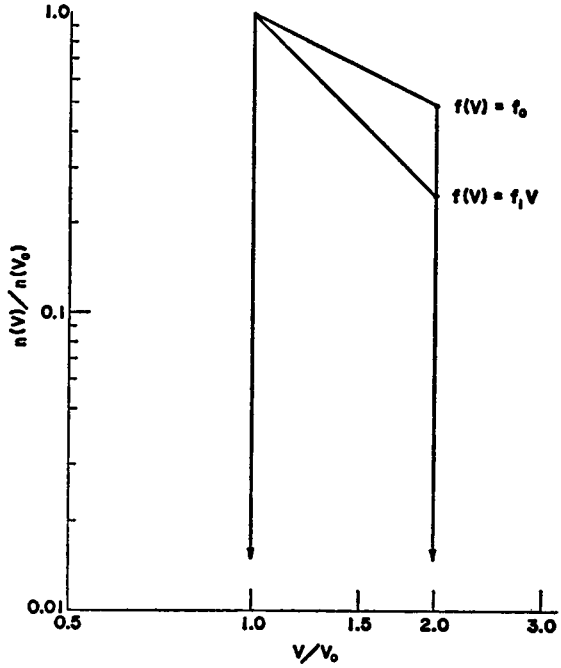

FIGURE 1 Idealized cell volume distributions for growth rate, $f(V) = f_0 + f_1V$, all cells assumed to divide in half on reaching volume $2V_0$. For $f_1 = 0$ (upper curve), the volume growth rate is constant (linear volume growth), while for $f_0 = 0$ (lower curve), the growth rate is proportional to volume (exponential volume growth). Note logarithmic scales.

Let us suppose that the cell volume spectrum, $n(V)$, is known from experiment. Evidently if the volume spectrum of dividing cells (first term on right-hand side) or the volume spectrum of newborn cells (second term on right-hand side) were known, then the right-hand side of Equation (24) would be known and one could solve the differential equation for $f(V)$.[3] Actually, an arbitrary constant of integration would be present in the solution, but it could be determined from α by using Equation (11).

[3] In Equation (24) we are assuming that cells divide into two precisely equal daughter cells so that, from the volume spectrum of dividing cells, the volume spectrum of newborn cells can be deduced. With unequal daughter cells, one would have to measure both volume spectra in order to determine the right side of Equation (24).

If, however, the right-hand side of Equation (24) is not known, then for any postulated division probability, $p(V)$, one could formally solve Equation (24) for a growth rate, $f(V)$. It is thus clear that if one knows only $n(V)$, it is impossible to determine uniquely $p(V)$ and $f(V)$ from Equation (24). On the other hand, for many choices of $p(V)$, it would be found that the growth rate, $f(V)$, is unreasonable (negative, for example) and hence many solutions could be ruled out as "unreasonable."

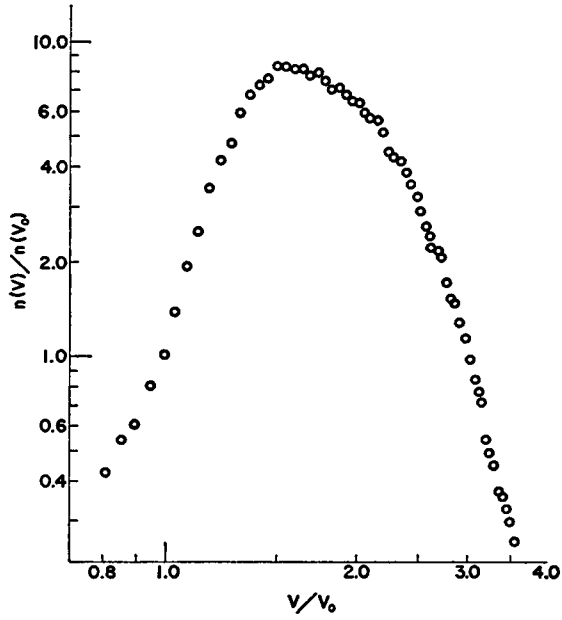

FIGURE 2 Experimental volume distribution spectrum of murine fibroblast (L cell) culture. Note that both scales are logarithmic so that the shape is independent of scaling factors (such as choice of V_0).

Let us consider a typical volume spectrum as shown in Fig. 2. First of all, the spectrum has quite a sharp lower edge; few cells are found with volumes less than a minimum volume, V_0. This implies that few cells divide with volumes less than $2V_0$, or $p(V) \simeq 0$, $V < 2V_0$. Hence, for volumes less than $2V_0$, cells are being born and are growing. For volumes larger than $2V_0$, cells begin to divide. However, if division of cells with volumes greater than $4V_0$ is negligible (as is the case for some but not all of the experimental volume spectra), then few cells are dividing into the volume region $2V_0 \leq V \leq 4V_0$. For these narrow volume spectra, the analysis is simplified as previously noted by Collins and Richmond (1962) and Powell (1964).

By a narrow volume spectrum we mean one in which $n(V)$ is appreciable only in the volume range $V_0 \leq V \leq 4V_0$. Then setting $p(V) = 0$ for $V < 2V_0$, Equation (24) may be written, for $V < 2V_0$

$$\alpha n(V) + \frac{d}{dV} f(V) n(V) = 4p(2V) n(2V) \tag{25a}$$

while for larger volumes

$$\alpha n(2V) + \frac{1}{2} \frac{d}{dV} f(2V) n(2V) = -p(2V) n(2V) \tag{25b}$$

where to find the derivative term in Equation (25 *b*) we have used

$$\left. \frac{d}{dx} f(x) n(x) \right|_{x=2V} = \left. \frac{d}{d2x} f(2x) n(2x) \right|_{x=V}$$

$$= \frac{1}{2} \frac{d}{dV} f(2V) n(2V) .$$

Adding Equation (25 *a*) and four times Equation (25 *b*), we obtain

$$\alpha \{n(V) + 4n(2V\} + \frac{d}{dV} f(V) n(V) + 2 \frac{d}{dV} f(2V) n(2V) = 0$$

$$V_0 \leq V \leq 2V_0. \tag{26}$$

Therefore, if $f(V)$ is known in *either* the interval $V_0 \leq V \leq 2V_0$ *or* the interval $2V_0 \leq V \leq 4V_0$, it can be determined throughout the interval $V_0 \leq V \leq 4V_0$. The division probability, $p(V)$, can then be found from Equation (25 *a*) or Equation (25 *b*). Suppose, for example, that $f(V)$ is known, or has been guessed, in the interval $V_0 \leq V \leq 2V_0$, then integrating Equation (26) from V_0 to V and solving for $f(2V)$, we have

(27)

$$f(2V) = \frac{2f(2V_0) n(2V_0) - \alpha \int_{V_0}^{V} \{n(V') + 4n(2V')\} dV' - f(V) n(V) + f(V_0) n(V_0)}{2n(2V)} .$$

If, on the other hand, $f(V)$ is known for $2V_0 \leq V \leq 4V_0$, we may integrate Equation (26) from V to $2V_0$ and solve for $f(V)$ to find

(28)

$$f(V) = \frac{f(2V_0) n(2V_0) + \alpha \int_{V}^{2V_0} \{n(V') + 4n(2V')\} dV' - 2f(2V) n(2V) + 2f(4V_0) n(4V_0)}{n(V)} .$$

Thus, we could try the following procedure for estimating "reasonable" growth and division rates, $f(V)$ and $p(V)$, from an experimental $n(V)$. First guess $f(V)$ for $2V_0 \leq V \leq 4V_0$. Compute $f(V)$ from Equation (28) for smaller volumes, $V_0 \leq$

$V \leq 2V_0$, and if it looks "reasonable," compute $p(V)$ from Equation (25). Alternatively, one could start by guessing $f(V)$ for volumes $V_0 \leq V \leq 2V_0$. In either case, if the resulting $f(V)$ and $p(V)$ are reasonable, by which we mean $p(V)$ and $f(V)$ nonnegative and smooth functions, then they are satisfactory solutions to the problem. Happily, we have found that only over a rather limited range are the solutions reasonable.

First of all, let us note that $f(V)$ is fairly well determined in the region $V \simeq 2V_0$, which is the volume region where cells are mostly just growing and not dividing or being born. To see this, consider Equation (27) evaluated at $V = 2V_0$ and multiplied by $2n(4V_0)$. Then, since for a narrow spectrum $n(V_0)$ and $n(4V_0)$ are very small compared to $n(2V_0)$ and assuming all the f's to be comparable, we may solve for $f(2V_0)$.

$$f(2V_0) \simeq \alpha \int_{V_0}^{2V_0} \{n(V') + 4n(2V')\}\, dV'/n(2V_0). \tag{29}$$

If a functional form for $f(V)$ is assumed, then this estimate may be further refined, but for the cases considered we found it good to about 10%.

Second, the slope of $f(V)$ can be estimated near $V = 2V_0$. This follows from Equation (24), where for $V \simeq 2V_0$ the right-hand side is near 0 and thus

$$f'(2V_0) = -\frac{f(2V_0)n'(2V_0)}{n(2V_0)} - \alpha \tag{30}$$

where $f'(2V_0) = d/dV\, f(V)|_{V=2V_0}$.

From $f(2V_0)$ and $f'(2V_0)$, we have linearly extrapolated $f(V)$ to larger V, computed $f(V)$ for smaller V from Equation (28), and then computed $p(V)$. In general, the resulting growth and division functions, $f(V)$ and $p(V)$, look reasonable, the chief exception being that $f(V)$ may become rather wild near $V = V_0$. We are inclined to attribute this difficulty to two causes: (*a*) At small volumes ($V \simeq V_0$) there is a background of small objects in the population. These are presumably not cells and should be subtracted from the spectrum. (*b*) Division into daughter cells of unequal volume will broaden the lower end of the volume spectrum. This aspect is further investigated in a following section.

We have also found that neither the slope nor the value of $f(V)$ at $V = 2V_0$ can be varied much from the values given by Equations (29) and (30) without leading to rather unsatisfactory behavior of $f(V)$ or $p(V)$. As an example, consider the population of murine fibroblast (L) cells whose experimental volume spectrum was given in Fig. 2. Three different slopes (1, 0, and 1.92) were assumed for $f(V)$ in the region $2V_0 \leq V \leq 4V_0$, corresponding to $f(V) = V$, $f(V) = f_0$, and $f(V) = f_2(V - V_0)$, respectively. In Fig. 3 A are shown the resulting values of $f(V)$ for $V \leq 2V_0$ calculated from Equation (28) compared with the extrapolations of the assumptions into this region. It appears that for $f(V) = V$, the agreement between points and line is

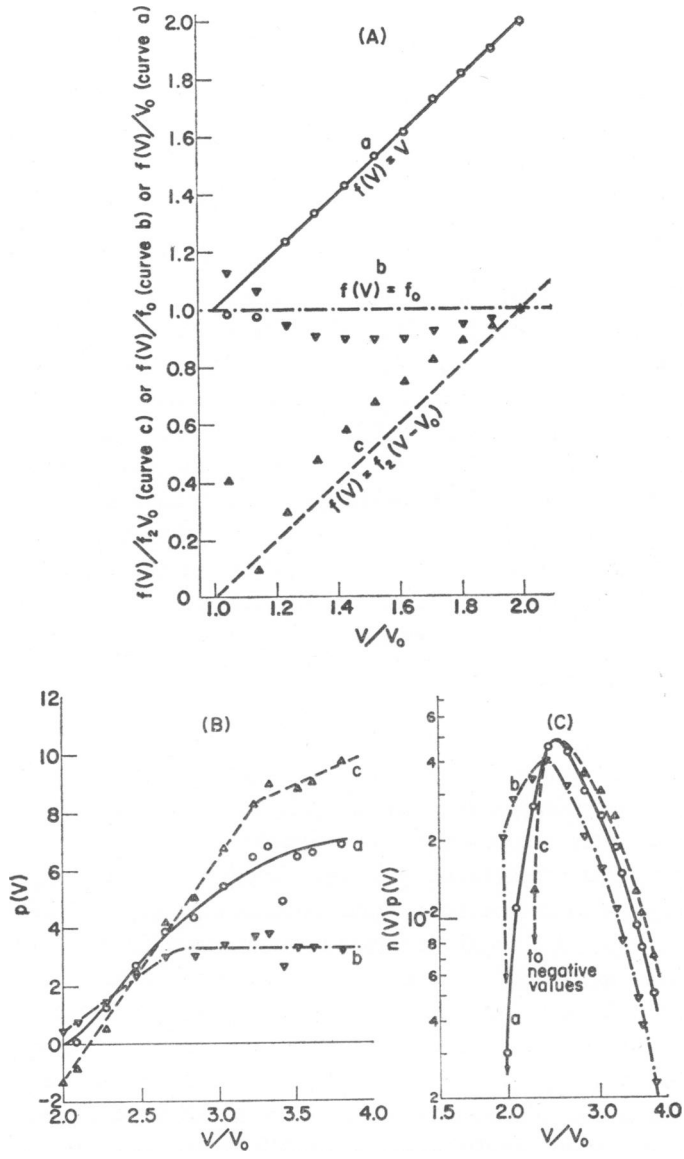

FIGURE 3 An analysis of the experimental volume spectrum for murine fibroblast (L) cells given in Fig. 2. A: Calculated values of the growth rate, $f(V)$, in the region $V_0 \leq V \leq 2V_0$ on the basis of three different assumptions for the region $V \geq 2V_0$: case *a*, $f(V) = V$ (exponential volume growth), ○; case *b*, $f(V) = f_0$ (linear volume growth), $f_0 = 37.8$, ▽; and case *c*, $f(V) = f_2(V - V_0)$, $f_2 = 1.92$, △. As noted on the ordinate label, $f(V)$ has been divided by a different constant in each case in order to separate the curves. The lines represent the assume growth laws, and the points represent the growth rates calculated from the smooth spectrum data. The constants f_0 and f_2 were chosen as noted in the text (following Equation (29)], and the units of time are such that $\alpha = 1$. Equation (28) was then used to compute $f(V)$ for $V < 2V_0$ with the results as shown. B: The division probability, $p(V)$, is here shown as computed from Eq. (25 *b*) for the three assumptions of $f(V)$. The lines approximate a smooth fit to the results and are without theoretical significance. Note that all three models give similar results: $p(V)$ rises monotonically at a decreasing rate. For case *c*, some negative values are calculated. C: The spectrum of dividing cells as calculated from the product $n(V) \cdot p(V)$. Note that in case *b* the spectrum has a discontinuity at $V = 2V_0$ and in case *c* the spectrum is negative for $V \simeq 2V_0$. Case *a*, $f(V) = V$, appears to be most reasonable. Changes in the assumed values of V_0 , f_0 , and f_2 did not alter this result.

significantly better than for either alternate assumption, indicating that a simple exponential growth law will better fit the spectrum over its entire range.

In Fig. 3 B we give the results of calculating the division probability, $p(V)$, on the basis of the same three assumptions for $f(V)$. In this figure the lines are without theoretical significance and are merely a smoothed fit to the data. There is little choice between the models, all indicating that $p(V)$ rises at a decreasing rate.

In Fig. 3 C the calculated volume spectra of dividing cells are shown. Here case *b* has a marked discontinuity at $V = 2V_0$, while case *c* gives negative values. It appears that case *a*, exponential volume growth, is somewhat more reasonable.

Volume spectra of dividing cells are accessible to direct determination using the method of Terasima and Tolmach (1963) to separate such cells by the shaking of monolayer cultures. Our preliminary results using this method have given spectra similar to those of Fig. 3 C. The widths of experimental spectra correspond to coefficients of variation ($\sigma_v/\bar{V}$) of about 0.11 compared with 0.14–0.16 for Fig. 3 C. Experimental spectra appear to be more nearly symmetrical than the calculated ones. Further study of the spectra of dividing cells is a promising approach to a more rigorous analysis since, as noted above in connection with Equation (24), this information would permit the solution of the differential equation for $f(V)$. One technical problem is that the dividing cells must be separated from a monolayer culture for which it is difficult to establish $n(V)$.

The results given in Fig. 3 A, 3 B, and 3 C are representative of those which we have found for a variety of mammalian cells in spinner culture. In general, the volume growth rates are roughly proportional to volume, although noticeable deviations from this proportionality in the direction of both larger and smaller values of $f'(2V_0)/f(2V_0)$ have been found. It is, of course, clear that $f(V)$ is really well determined only in the volume region around $V = 2V_0$, but for simplicity it is tempting to assume $f_1 = \alpha V$ for all cell volumes. The main feature of $p(V)$ seems to be a fairly rapid rise with increasing volume above $2V_0$, as shown in Fig. 3 B.

If the volume spectrum is not narrow, then we cannot neglect the division of cells having $V > 4V_0$. In that case, Equation (25 *b*) would contain a term, $4p(4V)n(4V)$, on the right-hand side. If division of cells with $V > 8V_0$ is negligible, one can write down another equation, similar to Equation (25 *b*), for $n(4V)$ and an appropriate linear combination of this new equation with Equations (25 *a*) and (25 *b*) will then have a right-hand side equal to 0. With this new equation corresponding to Equation (26), one can guess $f(V)$ for $2V_0 \leq V \leq 8V_0$, solve for $f(V)$ in the range $V_0 \leq V \leq 2V_0$, etc. In the next section we will see, for a simple case, how important such an extension of the volume interval may be.

SIMPLE CHARACTERIZATION OF VOLUME SPECTRA

We have seen that for a variety of mammalian cells the cell growth rate appears to be approximately proportional to cell volume. The division probability, $p(V)$, was found to increase with cell volume for volumes larger than some critical volume

$V = 2V_0$. In order to characterize possible volume distributions of cells in exponential growth, we have worked out solutions in which the division probability is assumed to be a linear function of volume.

We consider, therefore, solutions to Equation (24) for $f(V) = f_1 V$, $p(V) = 0$ for $V \leq 2V_0$, and $p(V) = p_1(V - 2V_0)$ for $V > 2V_0$. For a narrow volume spectrum, which as we will see means a large enough value of $p_1 V_0/f_1$, we may ignore cells larger than $4V_0$. Then we can easily solve Equation (24) for the volume range

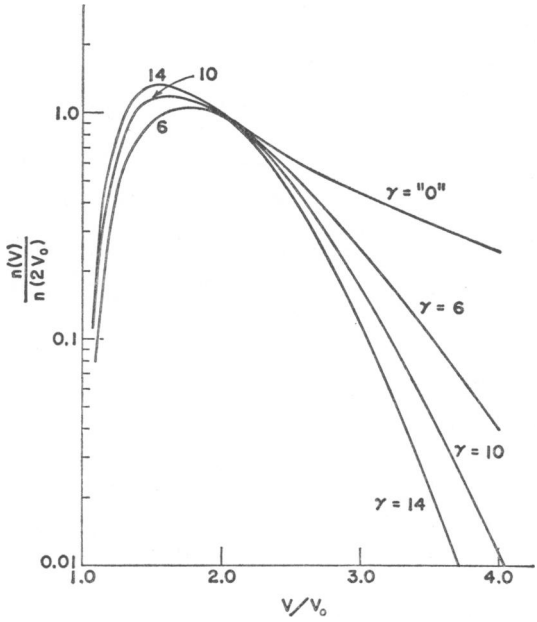

FIGURE 4 Calculated volume distribution spectra for cell population with $f(V) = f_1 V$ and $p(V) = p_1(V - 2V_0)$ for $V > 2V_0$. The parameter $\gamma = 2p_1 V_0/f_1$; γ = "0" corresponds to no division.

$2V_0 \leq V \leq 4V_0$. Assuming that all cells divide so that $\alpha = f_1$ and setting $2p_1 V_0/f_1 = \gamma$, the solution is

$$n(V) = n(2V_0)\left(\frac{V}{2V_0}\right)^{\gamma-2} \exp\left(-\gamma\left(\frac{V}{2V_0} - 1\right)\right) \qquad V \geq 2V_0. \tag{31 a}$$

The spectrum of dividing cells is then simply $p(V)n(V)$. For $V \leq 2V_0$, we may again solve Equation (24) to find

$$n(V) = 4n(2V_0)\left(\frac{V_0}{V}\right)^2 \gamma e^{\gamma} \int_1^{V/V_0} (x - 1)x^{\gamma} \exp(-\gamma x)\, dx/x \qquad V < 2V_0, \tag{31 b}$$

and the integral can be evaluated from tables of the incomplete gamma function

[Abramowitz and Stegun (1965), Table 26.7]. We have computed the spectra for $\gamma = 6$, 10, and 14, and the results are shown in Fig. 4.

When one evaluates $n(V)$ from Equation (31 *b*) as $V \rightarrow 2V_0$, he finds that the limit is less than $n(2V_0)$. The reason is that we have ignored cells larger than $4V_0$ and have, nevertheless, assumed that all cells divide. We are, therefore, assuming that all cells which reach $V = 4V_0$ thereupon divide. Hence, we have a discontinuity in $n(V)$ at $V = 2V_0$, amounting to all those cells which have not divided upon reaching $4V_0$. For the cases considered ($\gamma = 6$, 10, and 14), this discontinuity at $V = 2V_0$ is 17, 5, and 1%, respectively. In Fig. 4, however, we have simply removed the discontinuity by renormalizing $n(V)$ for $V < 2V_0$. In principle, the discontinuity should be removed by considering division of cells with $V > 4V_0$, but in practice renormalization will not seriously distort the volume spectrum for small discontinuities.

By comparing observed volume spectra with those in Fig. 4, we may estimate experimental values of γ. For example, the population in Fig. 2 has $\gamma \simeq 10$.

More generally, we note that all of the volume spectra in Fig. 4 have similar shapes near $V \simeq 2V_0$. Here the spectrum shape is primarily determined by $f(V) = f_1 V$ and is in reasonable agreement with many of the observed spectra. If an experimental spectrum is flatter than Fig. 4 for $V \simeq 2V_0$, then it will be found to have a growth rate, $f(V)$, which varies more slowly with volume than we have assumed. Conversely, if the observed spectrum is steeper than Fig. 4 for $V \simeq 2V_0$, this implies a more rapidly increasing $f(V)$.

Upon comparing Fig. 4 with experimental spectra, we find that in most cases the experimental spectra do not have such sharp lower edges. Evidently the division of cells into daughters of unequal volumes would lead to a spreading of the lower edge, and we have employed a computer model to investigate this dispersion mechanism.

COMPUTER MODELS

It is mathematically cumbersome to introduce into the analytical equations the option of sister cells being born with unequal volumes, and we have, therefore, written iterative computer programs to investigate the results of unequal division. In addition, the computer programs allow for the possibility of random variations in growth rates of individual cells. In these programs, cell volume is "quantized" into successive intervals, and the "cells" in a given interval are manipulated according to prescribed rules of growth and division [see "state vector" analysis of Hahn (1966)]. Models with either linear or logarithmic volume intervals have been used, the latter being more convenient for cases in which exponential volume growth is assumed. From 20 to 100 intervals have been assigned to the volume range V_0–$2V_0$, with sufficient additional intervals above and below to accommodate the developing spectrum. Thus, one iteration corresponds to a time interval of $\frac{1}{20}$–$\frac{1}{100}$ of the mean volume-doubling time.

In the terminology of Hahn (1966), we define the state of the population by a row vector with cell volume (rather than age) as our criterion of state. Our manipulations are equivalent to his transformation matrices for unit time shift and unit dispersion, although it is more economical of computer time to perform the transformations by programmed operations than by formal matrix multiplication (because of the large number of zeros in the matrices).

In addition to permitting the inclusion in the model of unequal sister cell volumes and of dispersive variations in growth rate, the computer models permit determination of the relaxation time of the system from an initial arbitrary state (spectrum) to stable equilibrium. However, in the present paper we will not consider any relaxation studies.

The computer models were checked where possible against the analytical formulation to establish that the former converge to the identical spectra given by the equivalent analytical solution. Such convergence occurred spontaneously in linear volume growth models, but with exponential volume growth, convergence occurred only when the computer model incorporated a dispersive mechanism, although the latter could be mild enough to product no detectable change in the final spectrum. (Convergence required 10–100 "generations," depending on the strength of the dispersion.)

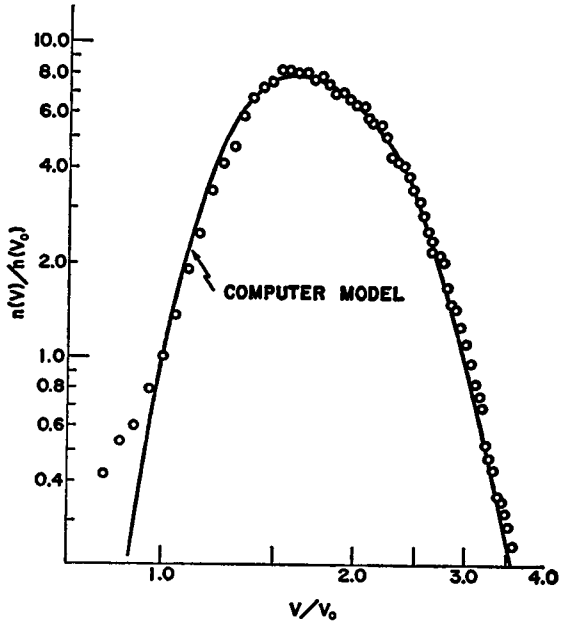

FIGURE 5 Comparison of spectrum generated by computer model with an experimental volume spectrum for murine fibroblasts (L cells).

The quality of agreement obtained between the computer model and the experimental data is shown in Fig. 5 for a culture of murine fibroblasts. (This is the same spectrum as that in Fig. 2.) The slope of the spectrum at large volumes (above 2.4) determines the division probability, while the slope below 1.4 determines the dispersion of sister cell volumes. The computer model plotted used $\alpha = 1, f(V) = V$, a coefficient of variation of 15% for the distribution of sister cell volumes, and a division probability $p(V) = p_0 \ln (V/2V_0)$ with $p_0 = 11.6$. By expanding the logarithm for V near $2V_0$, we see that p_0 corresponds to γ as defined in the previous section. The difference between the logarithmic $p(V)$ used in the computer model and the linear $p(V)$ used in the analytical formulation is not significant, as can be seen from the uncertainty in the deduced $p(V)$ as shown in Fig. 3 B. Note that in Fig. 5 an excellent fit to the data is obtained down to $V/V_0 = 1.0$. For precisely equal sister cells, it will be recalled from Fig. 3 A that a significant departure from exponential volume growth appeared below $V/V_0 = 1.2$. We conclude, therefore, that when allowance is made for unequal sister

cells, the data are consistent with exponential growth, $f(V) = f_1 V$, throughout the cell's life.

DISCUSSION AND SUMMARY

In this paper we have first of all formulated a mathematical model for development of a population of cells in which the individual cells may grow and divide, or die. A cell was characterized by its age (τ) and volume (V), and it was assumed that the age and volume of a cell determine both its growth rate, $F(\tau, V)$, and probabilities per unit time of division, $P(\tau, V)$, or death, $D(\tau, V)$. We have usually assumed that division leads to two daughter cells of precisely equal volumes. The model can include mother-daughter and sister-sister lifetime correlations. We have discussed the initial value problem in general and have noted that if cell growth rate is proportional to cell volume, then a population is forever sensitive to its initial conditions, unless an independent dispersive mechanism is operative.

We have seen that the general time-dependent problem is appreciably simplified when we restrict our attention to populations in the exponential phase or in a steady state. Further simplification arises if we integrate over all cell ages to obtain cell growth rates, $f(V)$, and division and death probabilities, $p(V)$ and $d(V)$, respectively, which depend on volume only (for a given population).

We then considered experimental volume spectra for some mammalian suspension cultures during stable exponential growth. By use of electronic volume sensing (Anderson and Petersen, 1967), we have been able to obtain much more precise and detailed volume spectra than those available to earlier workers who relied on optical observations of rather small samples from bacterial populations.

Our model was then used to interpret the observed volume spectra and, in particular, to try to deduce cell growth rates and division probabilities from observed volume spectra. At the outset we recognized the impossibility of uniquely determining $f(V)$ and $p(V)$ from the volume spectrum only. However, we feel that a number of qualitative features are quite clear. First of all, from the absence of cells with volumes less than a minimum volume, V_0, we conclude that few if any cells divide with volumes $<2V_0$. The cell growth rate can then be rather well determined for cells with volumes in the neighborhood of $2V_0$, both as to magnitude and slope. In general, we have found that growth rates roughly proportional to cell volume, $f(V) \sim V$, are suggested and that constant growth rates, $f(V) = \text{const}$, are inconsistent with the data. If we extrapolate this growth rate (linearly in V) to larger or to smaller cell sizes, thereby including either cells which are dividing or cells being born, we can then deduce the growth rate for cells of other volumes and also the division probabilities. When this is done, we find that $f(V)$ is roughly linear in V for all cell volumes and that $p(V)$ is quite reasonable, as is the volume spectrum of dividing cells. We interpret peculiar results for $f(V)$ at small volumes, $V \simeq V_0$, as due to our assumption of equal daughter sizes; in some numerical results in which daughter

cells of unequal size were allowed, much better agreement with the observed volume spectra at small volumes was found.

Our conclusion that $f(V) \sim V$ over a limited range of cell volumes means, of course, that for an individual cell in that volume range the volume will increase exponentially with time. The actual growth pattern probably varies with cell type and also with environmental conditions, as evidenced by the variety of results reported in the literature [see Prescott (1964) for a review] and by our observation of varying volume distributions (Anderson and Petersen, 1967). It is quite difficult to distinguish between linear and exponential growth on the basis of the measurement of volume or mass as a function of time; the maximum difference between the two alternatives is only 6%. A direct differential measurement of the rate of growth, on the other hand, should show a factor of two difference in the rates at the extremes of the life cycle for the exponential case.[4] Recent careful measurements by Zetterberg and Killander (1965) on the rate of protein synthesis by mouse fibroblasts show exponential growth, in agreement with their earlier results (1965) for dry mass increase of L cells.

While cell volume is an attractive parameter because of the ease and precision with which it can be measured, it is perhaps less closely linked with the basic synthetic rates of the cell than is dry mass or total protein. Thus, the results of some workers [Terasima and Tolmach (1963); Sandritter, Schiemer, Kraus, and Dorrien (1960)] suggest that a significant imbibition of water may occur in the premitotic period, causing cell volume to more than double the average birth volume for a brief portion of the life cycle. While we have found no evidence for an extreme effect of this sort in our measurements, we cannot eliminate the possibility that a varying degree of hydration could significantly influence the apparent rate law observed. This matter is under further study.

A valuable application of the knowledge of the shape of the stable cell volume spectrum is in selection of suitable cultures for life cycle analysis (Tobey, Petersen, Anderson, and Puck, 1966) and other studies requiring cells in biochemical balance with a known age distribution. The equilibrium steady-state spectrum is attained by a culture only after a number of generations of balanced growth. Failure of a culture to show the equilibrium spectrum will, therefore, demonstrate past variations in growth rate and will provide a sensitive test for transient disturbances which may have affected the biochemical balance, as well as the age distribution of the culture. One spectrum measurement may be as sensitive in this respect as the intensive monitoring of cell concentration for several generations previous to an experiment.

Evidently a number of other experiments could be helpful in determining the growth rates and division probabilities in our model for exponential populations. For one thing, if the volume spectrum of dividing and/or just divided cells were

[4] Note that the cell volume spectrum is similarly sensitive to the rate of cell growth, since the parameter $f(V) = dV/dt$ occurs in the equations [(see Equation (24) and Fig. 1].

known, one could accurately deduce the cell growth rate, $f(V)$, from the measured volume spectrum of all cells. However, to be useful, the volume spectrum of dividing cells would have to be quite accurately known. Preliminary experiments show this to be possible by using the Terasima and Tolmach (1963) method of isolating mitotic cells by washing off monolayers. As another possibility, if the growth rate of individual cells could be well enough determined, then $p(V)$ could be deduced from the volume spectra.

It may prove more fruitful, however, to consider populations which are not in exponential growth. In particular, for exploring the dynamic balance between growth and division in cell populations, it may be useful to follow the development of a population starting from particularly simple initial conditions. Thus, for example, with an appropriately synchronized culture, one could start with cells which all had the same age. Similarly, with a volume separator (Fulwyler, 1965), it would be possible to start with all cells having the same volumes. Finally, by volume separation of synchronized cultures, one could obtain an initial population of cells with identical volumes and ages. If one could follow the development of such a population, the growth function, $f(\tau, V)$, and division probability, $p(\tau, V)$, could be directly obtained, and moreover the validity of our characterization of cells by their ages and volumes could be assessed.

It should be noted that we have been considering a linear model for the development of a population. We mean by this that our fundamental equations are unaltered if the population is multiplied by some constant factor, so that the results are unaffected by the size (or concentration) of the population. Hence, the model could not, as formulated, treat such phenomena as, for example, the transition from exponential growth to a steady state in a population of cells. Nevertheless, by a simple elaboration of the model, such number- or concentration-dependent features could be considered. In particular, we have assumed that the growth, division, and death functions are determined by a cell's age and volume, *provided* that the cells are in a growth medium of fixed composition. However, as the concentration of cells in a population increases, the medium is presumably also changed (for example, due to depletion of important metabolites or elaboration of by-products). Thus, it is natural to think of allowing the growth, division, and death functions (F, P, and D) to be functions of cell concentration (or some similar parameter), as well as of cell age and volume. With the resulting nonlinear equations, one could readily consider a variety of number- or concentration-dependent situations.

This work was performed under the auspices of the U. S. Atomic Energy Commission.
Received for publication 16 January 1967.

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