CELL GROWTH AND DIVISION

III. CONDITIONS FOR BALANCED EXPONENTIAL GROWTH IN A MATHEMATICAL MODEL

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ABSTRACT In a previous paper, we proposed a model in which the volume growth rate and probability of division of a cell were assumed to be determined by the cell's age and volume. Some further mathematical implications of the model are here explored. In particular we seek properties of the growth and division functions which are required for the balanced exponential growth of a cell population. Integral equations are derived which relate the distribution of birth volumes in successive generations and in which the existence of balanced exponential growth can be treated as an eigenvalue problem. The special case in which all cells divide at the same age is treated in some detail and conditions are derived for the existence of a balanced exponential solution and for its stability or instability. The special case of growth rate proportional to cell volume is seen to have neutral stability. More generally when the division probability depends on age only and growth rate is proportional to cell volume, there is no possibility of balanced exponential growth. Some comparisons are made with experimental results. It is noted that the model permits the appearance of differentiated cells. A generalization of the model is formulated in which cells may be described by many state variables instead of just age and volume.

INTRODUCTION

In a previous paper (Bell and Anderson, 1967—hereafter referred to as I) we proposed a model for cell growth and division wherein it was assumed that the division probability of a cell and its growth rate were determined by the cell's age and volume.[1] In I some preliminary attempts were made to determine the average growth rates and division probabilities for populations of mammalian cells in balanced exponential growth and subsequently these measurements have been substantially improved.[2] As these data become available, it is of interest to inquire what sorts of

[1] As far as the mathematics is concerned one can everywhere substitute for "volume," any quantity which is divided equally between the daughter cells during mitosis. For example, the quantity of dry mass, protein, or nucleic acid could be used. We prefer to work in terms of volume because of its experimental accessibility.

[2] Anderson, E. C., G. I. Bell, D. F. Petersen, and R. A. Tobey. Data to be published.

growth rates and division probabilities are required in order that a state of balanced exponential growth can be achieved by a cell population. While we have, to date, achieved only partial results in this inquiry, some of them seem to be general enough to be worth reporting.

Let us first recall a few general features of our model. We have confined our attention to large populations of cells so that fluctuations from the mean could be ignored. We therefore described the population by $N(t, \tau, V)\, d\tau\, dV$, the number of cells which at time t have ages between τ and $\tau + d\tau$ and volumes between V and $V + dV$. We assumed that a cell of age τ and volume V will in time dt have a volume increment or growth given by $F(\tau, V)\, dt$ and probabilities of division or death given by $P(\tau, V)\, dt$ and $D(\tau, V)\, dt$. It was further assumed for simplicity, that cell division produces two daughter cells of precisely equal volumes, and this assumption is well-supported.[2] Under these assumptions the evolution of the population was shown in I to be governed by the following two equations:

$$\frac{\partial N(t, \tau, V)}{\partial t} + \frac{\partial N}{\partial \tau} + \frac{\partial FN}{\partial V} = -(P + D)N \qquad \tau \neq 0 \tag{1}$$

and

$$N(t, 0, V) = 4 \int_0^\infty P(\tau, 2V) N(t, \tau, 2V)\, d\tau. \tag{2}$$

For a variety of mammalian suspension cultures in exponential growth it has been found[2] that the average growth rate of a cell is approximately proportional to cell volume. We noted in I, that for this particular growth rate ($F \sim V$) the population is uniquely sensitive to its initial conditions. One motive for the present inquiry is a desire to better understand this sensitivity.

In this paper, many of our results are simply embellishments of the following obvious theme: *In order that a population of cells may achieve balanced exponential growth an average cell must double in volume during its lifetime.* We wish to understand, in the context of our model, what sorts of coupling between the cell growth cycle and the cell division cycle are necessary or sufficient for achieving a state of balanced exponential growth.

We will begin by converting Equation (1) to an integral equation which may then be combined with Equation (2) to yield a single integral equation for either $N(t, 0, V)$ or $N(t, \tau, V)$. This appears to be a convenient formulation for investigating the solution.

We will generally be considering the mathematical initial value problem. We assume that $N(0, \tau, V)$ is given and seek properties of the solutions to Equations (1) and (2) at later times. If a condition of balanced exponential growth is achieved at late times we take this to mean

$$N(t, \tau, V) \xrightarrow[t \to \infty]{} e^{\alpha t} N(\tau, V) \tag{3}$$

and a central question (not really answered herein) is for what functions F, P, and D will the solution approach Equation (3) and what will be α and $N(\tau, V)$? From the biological point of view we may wish to restrict our attention to populations which have no cells of arbitrarily large or small volumes so that we may require

$$N(t, \tau, V) = 0 \qquad \left.\begin{matrix} V = 0 \\ V = \infty \end{matrix}\right\} \begin{cases} 0 \leq t \\ 0 \leq \tau \end{cases} \tag{4}$$

THE INTEGRAL EQUATIONS

We convert Equation (1) to an integral equation by the method of characteristics (Courant and Hilbert, 1962). Thus rewrite Equation (1) as:

$$\frac{\partial N}{\partial \tau} + \frac{\partial N}{\partial t} + F\frac{\partial N}{\partial V} = -\left(P + D + \frac{\partial F}{\partial V}\right) N. \tag{5}$$

If we regard the left side as a total derivative along some trajectory $(t(\tau); V(\tau))$ we have

$$\frac{dN}{d\tau} = \frac{\partial N}{\partial \tau} + \frac{\partial N}{\partial t}\frac{dt}{d\tau} + \frac{\partial N}{\partial V}\frac{dV}{d\tau} \tag{6}$$

which on comparison with Equation (5) gives

$$\frac{dt}{d\tau} = 1$$

$$\frac{dV}{d\tau} = F(\tau, V) \tag{7}$$

with solutions

$$t = \tau + t_0 = t(t_0, \tau)$$

$$V = V(V_0, \tau) \tag{8}$$

where t_0 is the time of birth of a cell (time at age zero) and V_0 is its birth volume. Of course for a general $F(\tau, V)$ one will not be able to solve Equation (7) for $V(V_0, \tau)$ in closed form. However, if F is taken to be a function of V only then

$$\int_{V_0}^{V} \frac{dV}{F(V)} = \tau \tag{9}$$

and for simple choices of $F(V)$, this may be solved for $V(V_0, \tau)$. If, for example, $F(V) = f_1 V$, then $V = V_0 e^{f_1 \tau}$.

We may now integrate Equation (5) along a characteristic curve or trajectory with fixed V_0 and t_0, taking care to let t and V vary with τ according to Equation (8). To

make this clear we may rewrite Equation (5) as

$$\frac{1}{N}\frac{d}{d\tau}N(t(t_0,\tau),\tau,V(V_0,\tau)) = -\{P + D + F'(\tau,V(V_0,\tau))\} \quad (10)$$

where we have denoted $\partial F/\partial V$ by F'. For $t_0 \geq 0$ we may start the integration at $\tau = 0$ obtaining

$$N(t,\tau,V(V_0,\tau)) = N(t-\tau,0,V_0)\exp\left[-\int_0^\tau \{P + D + F'(\tau',V(V_0,\tau'))\}\,d\tau'\right] \quad \text{for} \quad t \geq \tau \quad (11)$$

where each of the functions P, D, and F' is a function of τ' and $V(V_0,\tau')$.

For $t_0 < 0$ we are considering cells born before time zero so that we should integrate back to time zero and use the initial condition, obtaining

$$N(t,\tau,V_1(V_0,\tau;\tau-t)) = N(0,\tau-t,V_0)\exp -\int_{\tau-t}^{\tau}(P + D + F')\,d\tau' \quad \text{for} \quad \tau > t \quad (12)$$

where $V_1(V_0,\tau;\tau-t)$ is the volume of a cell at age τ which had volume V_0 at age $\tau - t$.

Equation (11) says that a group of cells born at time $t - \tau$ with volume V_0 will grow up to volume V at age τ and at time t subject to depletion (in the exponential) by division and death. The term $\exp -(\int F'\,d\tau')$ takes into account that if the cells start out in a volume interval dV_0, they will end up at age τ in a volume interval dV which is larger by the factor $\exp(\int F'\,d\tau')$. To see this, consider some cells at volume V and some at $V + \Delta V$. Then

$$\frac{d\Delta V}{d\tau} = F(V+\Delta V,\tau) - F(V,\tau) = F'(V,\tau)\Delta V. \quad (13)$$

Integrating we have

$$\Delta V = \Delta V_0 e^{\int_0^\tau F'\,d\tau'} \quad (14)$$

as claimed. We may thus rewrite Equation (11) in a somewhat more transparent form:

$$N(\tau + t_0,\tau,V(V_0,\tau))\,dV = N(t_0,0,V_0)\,dV_0\exp -\int_0^\tau (P + D)\,d\tau'. \quad (15)$$

If the volume spectrum of dividing cells were measured at some time t_0, one

would thereby obtain $N(t_0, 0, V_0)$. Equation (15) could then be used to obtain the age and volume distribution at later times, provided that P, D, and F' were known. In Paper I and the forthcoming paper by Anderson et al.,[2] values of P and F were found which are averages over cell age for exponentially growing cells. In particular, we determined

$$f(V) = \frac{\int N(\tau, V)F(\tau, V)\, d\tau}{\int N(\tau, V)\, d\tau}, \qquad p(V) = \frac{\int N(\tau, V)P(\tau, V)\, d\tau}{\int N(\tau, V)\, d\tau}. \tag{16}$$

$N(\tau, V)$ is the spectrum in exponential growth as in Equation (3). The death rate, D, was assumed to be negligible. One can inquire whether from $f(V)$ and $p(V)$, only, enough information can be found to solve Equation (15) for the age dependence of N. The answer is no. However, if one is willing to assume something about the age dependence of F and P (and D) solutions can be found. The simplest case is if we take $P(\tau, V) = p(V)$ and $F(\tau, V) = f(V)$ in which case V_0 can readily be found and Equation (15) can be solved. However, this simple choice is by no means unique and is not attractive from a biological point of view.

Equation (2) gives the spectrum of dividing cells as an integral over the spectrum of all cells, while Equation (11) gives the spectrum of all cells in terms of the dividing cells. The two equations may be combined to give an equation for the spectrum of dividing cells:

$$\begin{aligned} N(t, 0, V) &= 4\int_0^t P(\tau, 2V)N(t - \tau, 0, V_0(2V, \tau)) \exp\left\{-\int_{\tau'=0}^{\tau} (P + D + F')\, d\tau'\right\} d\tau \\ &+ 4\int_t^{\infty} P(\tau, 2V)N(0, \tau - t, V_0(2V, \tau; \tau - t)) \\ &\qquad \cdot \exp\left\{-\int_{\tau'=\tau-t}^{\tau} (P + D + F')\, d\tau'\right\} d\tau, \end{aligned} \tag{17}$$

where Equation (12) was used in obtaining the second term. In this equation $V_0(2V, \tau)$ is the birth volume of a cell which reaches volume $2V$ at age τ and $V_0(2V, \tau; \tau - t)$ is the volume which a cell had at age $\tau - t$, in order to reach volume $2V$ at age τ. The second term in Equation (17) represents the cells which have just divided after developing without division from those initially present and we denote this term by $I(t, V)$. It is a function of the initial conditions. If we assume that $D = 0$, use Equation (14) to remove F' (obtaining $dV_0/2dV$), and define

$$\tilde{P}(\tau, 2V) = P(\tau, 2V) \exp - \left\{\int_{\tau'=0}^{\tau} P(\tau', V'(V_0(2V, \tau), \tau'))\, d\tau'\right\}. \tag{18}$$

Then Equation (17) may be written

$$N(t, 0, V) = 2\int_0^t \tilde{P}(\tau, 2V)N(t - \tau, 0, V_0(2V, \tau))\frac{dV_0}{dV}\,d\tau + I(t, V). \quad (19)$$

The function $\tilde{P}dt$ in Equation (18) is the probability that a cell grows up to volume $2V$ and age τ without division (this is the exponential) and thereupon divides in time dt. Thus Equation (19) says that those cells which are born at time t with volume V must have had mothers which were born at some time $t - \tau$, with volumes $V_0(2V, \tau)$ such that at age τ they had attained volume $2V$. If all cells divide, then for fixed V_0,

$$\int_0^\infty \tilde{P}(\tau, V(V_0, \tau))\,d\tau = 1 - \exp -\left\{\int_0^\infty P(\tau', V(V_0, \tau))\,d\tau'\right\} = 1 \quad (20)$$

$\tilde{P}$ is always a nonnegative function, though it is presumably zero for small values of τ.

We will presently exploit Equation (19) to obtain some information about solutions. But first we note that the integral in Equation (19), which is over the ages of dividing cells, can be transformed to an integral over the birth volumes of the mother cells. In making the transformation it is important to remember that every dividing cell has to arrive at volume $2V$ at time t. Thus let $\tau(V_0, 2V)$ be interpreted as the age at which a cell of birth volume V_0 reaches volume $2V$. We find

$$N(t, 0, V) = 2\int_{V_{\min}}^{2V} \tilde{P}(\tau(V_0, 2V), 2V)N(t - \tau(V_0, 2V), 0, V_0) \cdot\left(-\frac{d\tau}{dV_0}\right)\left(\frac{dV_0}{dV}\right)dV_0 + I(t, V). \quad (21)$$

Here the derivative $(-\,d\tau/dV_0)$ must be evaluated for a constant final volume. For example, suppose that $F(V, \tau) = f_1V$. Then for cells of final volume $2V$, we have $2V = V_0e^{f_1\tau}$ so that $(-\,d\tau/dV_0) = (f_1V_0)^{-1}$. We may also note that dV_0/dV must be evaluated at constant τ so that for this example $dV_0/dV = 2e^{-f_1\tau} = V_0/V$.

If one tries to solve Equations (19) or (21) in a Neumann series (Courant and Hilbert, 1962), then it is clear that a term in the series represents the contribution due to dividing cells of a particular generation. If, in particular, we try a solution of Equation (21) of the form

$$N_1(t, 0, V) = I(t, V) \quad (22\,a)$$

$$N_{n+1}(t, 0, V) = 2\int_{V_{\min}}^{2V} \tilde{P}\left(-\frac{d\tau}{dV_0}\right)\frac{dV_0}{dV}N_n(t - \tau, 0, V_0)\,dV_0 \quad (22\,b)$$

$$N = \sum_{n=1}^{\infty} N_n. \quad (22\,c)$$

Then N_n is the volume spectrum of cells which are born in the nth division after the population was started at $t = 0$.

In addition, if all cells divide, then it is easy to see that successive generations will differ in the number of dividing cells by exactly a factor two. This means

$$\int_0^\infty \int_0^\infty N_{n+1}(t, 0, V)\, dt\, dV = 2 \int_0^\infty \int_0^\infty N_n(t, 0, V_0)\, dt\, dV_0$$

which is most easily shown by starting from Equation (19), interchanging the t and τ orders of integration, and then using Equation (20).

THE EIGENVALUE PROBLEM

At first glance, Equations (19) or (21) resemble Volterra integral equations which are well-known not to possess eigenvalues (Courant and Hilbert, 1962). However, in Equation (19), the arguments of N involve τ in a complicated manner, whereas in Equation (21) the upper limit on V_0 is $2V$ instead of V as in the usual Volterra equation. This makes all the difference.

Let us consider the possibility of solutions of Equations (19) or (21) which at late times behave as

$$N(t, 0, V) = e^{\alpha t} m(V) \tag{23}$$

If at late times $I(t, V)$ becomes negligible we may investigate solutions of Equation (19)

$$m(V) = \beta \int_0^\infty K_1(\tau, 2V) m(V_0(2V, \tau))\, d\tau \tag{24}$$

where

$$K_1(\tau, 2V) = 2\tilde{P}(\tau, 2V) e^{-\alpha\tau} \frac{dV_0}{dV} \tag{25}$$

or of Equation (21)

$$m(V) = \beta \int_{V_{\min}}^{2V} K_2(V_0, V) m(V_0)\, dV_0 \tag{26}$$

where

$$K_2(V_0, V) = 2\tilde{P}(\tau(V_0, 2V), 2V) e^{-\alpha\tau(V_0, 2V)} \left(-\frac{d\tau}{dV_0}\right) \left(\frac{dV_0}{dV}\right). \tag{27}$$

In both Equations (25) and (27) we have introduced an auxiliary eigenvalue β. The reason is that the actual eigenvalue of interest, α, occurs in a complicated way in K_1 or K_2. We hope that, for fixed α, it may be possible to show the existence of β. Then

by varying α one may find the actual α as that which gives $\beta = 1.0$. This method has been successfully used, for example, in neutron transport theory (Wing, 1962).

The kernels, K_1 and K_2, may presumably be taken to be nonnegative. Further, we are seeking solutions for which $m(0) = m(\infty) = 0$.

It has been shown[3] that for certain kernels, $K_2(V_0, V)$, Equation (26) does have eigenvalues. Indeed, if one increases the upper limit of integration in a Volterra integral equation from V to $(1 + \epsilon)V$, eigenvalues become a possibility. The kernels considered to date do not, however, include any of obvious biological interest.

We will see, by considering a simple case, that the range of possibilities for Equations (25) and (27) must be quite rich. Thus for certain choices of F and P eigenvalues and eigenfunctions will exist while for other choices they will not. Moreover, it may not be possible to reach an eigenfunction from the initial condition.

A SIMPLE EXAMPLE

From measurements of the lifetimes of mammalian cells (Puck and Steffen, 1963; Anderson and Petersen, 1965; Dawson, Madoc-Jones, and Field, 1965) one knows that most cells in a population divide at roughly the same age. It is, therefore, tempting to try out the hypothesis that all cells divide at exactly the same age which we denote by τ_0. This means that

$$\tilde{P}(\tau, 2V) = \delta(\tau - \tau_0) \tag{28}$$

with δ the Dirac delta function. With this assumption, the integral in (24) can immediately be found and we have, for $\beta = 1$,

$$m(V) = 2e^{-\alpha\tau_0} \left(\frac{dV_0}{dV}\right)_{\tau=\tau_0} m(V_0(2V, \tau_0)) \tag{29}$$

First of all, by integrating Equation (29) over all volumes ($\int_0^\infty dV$) we see that if α exists, then $\alpha = \ln 2/\tau_0$ and hence we may rewrite Equation (29),

$$m(V) = \left(\frac{dV_0}{dV}\right)_{\tau=\tau_0} m(V_0(2V, \tau_0)) . \tag{30}$$

If there exist values of $V = V^*$ such that

$$V^* = V_0(2V^*, \tau_0) \tag{31}$$

then a possible solution is to have $m(V)$ entirely concentrated at V^*, that is

$$m(V) = M\delta(V - V^*) \tag{32}$$

[3] Pimbley, G. H. 1967. Private communication.

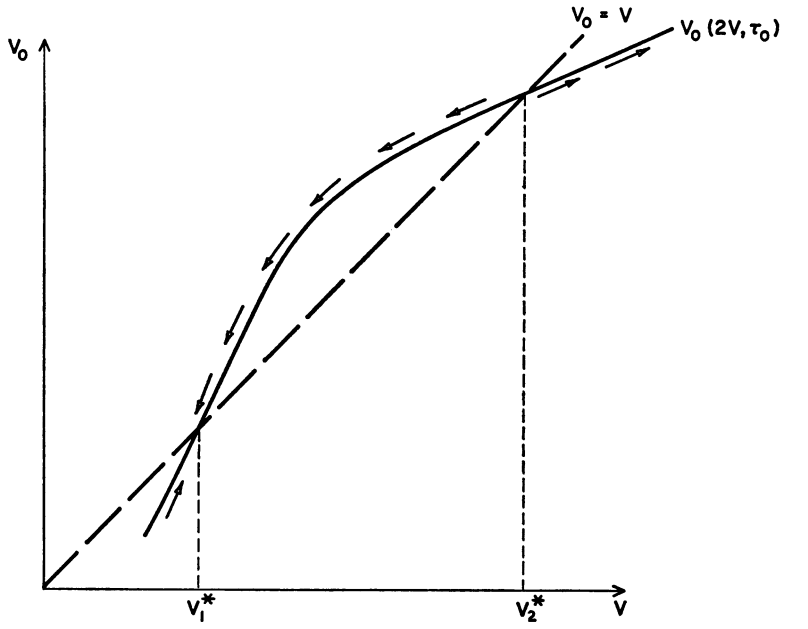

FIGURE 1 The relation between cell birth volumes in successive divisions, when all cells divide at age τ_0. $V_0(2V, \tau_0)$ is the birth volume of a cell which will divide upon reaching volume $2V$. The possible solutions for balanced exponential growth will find all cells born at volumes V_1^*, or V_2^*, as explained in the text. When they are born at V_1^*, the solution is stable and when born at V_2^* it is unstable as is indicated by the arrows.

where M is an arbitrary constant. In this case all cells divide at the same volume V^*, and the same age, τ_0. (The factor dV_0/dV is just what is needed to satisfy $\int_0^\infty \delta(V - V^*)\, dV = \int_0^\infty \delta(V_0 - V^*)\, dV_0$.) Moreover, this will in general be the only kind of acceptable solution, as may be seen as follows. We suppose that the curve $V_0(2V, \tau_0)$ is continuous and monotonic as sketched in Fig. 1.

Suppose that $m(V)$ were finite in a region of V and V_0 somewhat greater than V_1^*, where $dV_0/dV > 1$. Then in this region, from Equation (30), $m(V) > m(V_0)$ while $V < V_0$. Indeed, one can use Equation (30) to find values of $m(V)$ for V close to V_1^*. Let $dV_0/dV)_{V=V_1^*} = \gamma > 1$. Then near V_1^*, if $V_0 = V_1^* + \Delta V$, $V = V_1^* + \frac{\Delta V}{\gamma}$ and from Equation (30)

$$m\left(V_1^* + \frac{\Delta V}{\gamma}\right) = \gamma m(V_1^* + \Delta V).$$

Repeating this equation we have

$$m\left(V_1^* + \frac{\Delta V}{\gamma^n}\right) = \gamma^n m(V_1^* + \Delta V)$$

so that m must increase without limit as V approaches V_1^*. Indeed if we let $x = V - V_1^*$ and $m(V) = g(x)$, we see that near $x = 0$, $x = \Delta V/\gamma^n$ or $\gamma^n = \Delta V/x$ so that $g(x) \sim 1/x$. Thus $m(V)$ not only diverges near $V = V_1^*$, but it is not integrable. By extending this argument to other regions of V it is clear that $m(V)$ cannot be finite except where $V = V^*$.

By considering how the cells are developing, it is easy to see that a solution such as V_1^* in Fig. 1, for which $dV_0/dV > 1$, is a stable solution while a solution such as V_2^* for which $dV_0/dV < 1$ is unstable. To see this, consider some cells born with V_0 in the region $V_1^* < V_0 < V_2^*$ in Fig. 1. These will divide with values of V which can be read from the curve and which will have $V < V_0$. Hence as generations pass the volumes will shrink; approaching V_1^*. More generally cells born with volumes near to V_1^* will divide leaving daughters closer to V_1^*. The opposite is true for cells born with volumes near V_2^*. Upon division their volumes will be farther from V_2^*. Roughly speaking the cells are trying to seek out either a larger or a smaller birth volume so that in time τ_0 they can exactly double in volume.

It is perhaps worth summarizing our conclusions for this simple case in the form of a theorem.

Theorem. When all cells divide at age τ_0, then $\alpha = \ln 2/\tau_0$ and possible eigensolutions are $m(V) = M\delta(V - V^*)$ with V^* a solution of $V^* = V_0(2V^*, \tau)$. If $(dV_0/dV)_{V=V^*} \gtreqless 1$, the solutions are respectively stable, neutral, or unstable.

Of course, merely because we have an eigensolution does not mean that we can get there from the initial conditions. If, for example, in Fig. 1 all the first generation cells were born with $V > V_2^*$ then as generations passed the cell volumes would simply increase without limit (assuming no values of $V^* > V_2^*$). In addition, it is clear that to get an exponentially increasing population, one would have to start with very special initial conditions. Any trace of synchrony in the initial population would persist forever.

Let us now see how some simple growth laws, $F(\tau, V)$, fit these conclusions. Suppose that the growth rate is a function of V only, $F(\tau, V) = f(V)$. Then from Equation (14)

$$\frac{dV_0}{d2V} = e^{-\int_0^{\tau_0} \frac{df}{dV} d\tau}.$$

But $dV/d\tau = f(V)$ so that the exponent is simply $-\int df/f = -\ln f$. Thus

$$\frac{dV_0}{dV} = 2\frac{f(V_0)}{f(2V)}. \tag{33}$$

In addition an equation for V^* may be found by integrating $d\tau = dV/f(V)$,

$$\int_{V^*}^{2V^*} \frac{dV}{f(V)} = \tau_0. \tag{34}$$

Thus in balanced exponential growth all cells will have birth volumes V^* given by Equation (34) and a solution will be stable if $f(V^*) > f(2V^*)/2$, neutral if $f(V^*) = f(2V^*)/2$, and unstable if $f(V^*) < f(2V^*)/2$. This stability criterion could have been anticipated by the following simple argument. Consider a cell born at volume $V^* + \Delta V$. This volume would have been reached by a cell born at V^* after time $\Delta t = \Delta V/f(V^*)$. Hence our cell will be able to grow for this additional time, Δt, after reaching volume $2V^*$ and in this time it will grow by volume $\Delta t f(2V^*)$. Upon dividing, the daughters will differ in volume from V^* by

$$\frac{1}{2}\Delta t f(2V^*) = \Delta V \frac{f(2V^*)}{2f(V^*)}$$

so that they are farther from V^* if $f(2V^*)/2f(V^*) > 1$ which is the condition for instability.

If, for example, the growth rate were constant, $f(V) = f_0$, then $V^* = f_0\tau_0$ and the solution is stable.

If $f(V) = f_s V^s$ with f_s a constant and s not equal to 1, then

$$V^* = \left(\frac{f_s \tau_0(1 - s)}{2^{1-s} - 1}\right)^{1/1-s}. \tag{35}$$

The solution will be stable for $s < 1$, and unstable for $s > 1$.

If $f(V) = f_1 V$, the situation is entirely different. There can exist an eigensolution if and only if $f_1\tau_0 = \ln 2$. If this condition is satisfied, $V = V_0(2V, \tau_0)$ for all V and hence there is no special value V^*. If it is not satisfied, then there is no value of V for which $V = V_0(2V, \tau_0)$. We thus see once more that the situation is quite anomalous when growth is simply proportional to volume.

QUALITATIVE EXTENSIONS

For more general nonnegative kernels in the integral equations (24) and (26) it is possible to draw some conclusions by suitable extensions of the theory of Krein and Rutman (1948). Preliminary results have been obtained by Pimbley.[3] Without undertaking the detailed mathematical analysis, it would still seem that some qualitative conclusions could be drawn by considering straightforward generalizations of the preceding simple case.

Suppose that $\tilde{P}(\tau, V)$ were a function of τ only; not a delta function but a function peaked near τ_0. We believe that the results obtained in the previous section can be qualitatively extended to cover this more general situation. Instead of a single curve, $V_0(2V, \tau_0)$, which relates the birth volumes of mother and daughter, we must consider a family of such curves, identified by τ as a parameter. The important members of the family will be those for which $\tilde{P}(\tau)$ is relatively large and we expect that birth volumes will become concentrated around a value of V^* given by the intersection of such a curve with the line $V = V_0$. The stability of such solutions will also be

determined much as before by the sign of $(dV_0/dV)_{V=V^*} - 1$. The case of growth proportional to volume ($F(\tau, V) = f_1 V$) will again be a special one inasmuch as it leads to no favored value of V^* and to no balanced exponential growth unless f_1 and τ_0 are appropriately related. Even when they are appropriately related, however, an eigensolution is not possible within a bounded volume range inasmuch as cells can keep drifting to larger and smaller volumes on each division. Thus if one started with a sharp volume distribution, the spectrum of dividing cells would take on a Gaussian shape with a half-width increasing as $\sqrt{t}$ at late times, and no eigensolution would be possible except a constant at all volumes. This result can be rigorously proved by the central limit theorem of probability (Feller, 1957). We let x be the ratio of the birth volume of a daughter cell to the birth volume of its mother. Then $y = \ln x$ may be considered as a random variable assumed to have zero mean and finite variance. The probability distribution of the sum of n such random variables will give the probability distribution of the logarithm of the birth volume after n generations to initial birth volume. But the central limit theorem states that this sum will, as n becomes large, approach a Gaussian shape with half-width $\sim \sqrt{n}$.

If P were a function of cell volume as well as of cell age, the situation could be quite different. If, in particular, there were a critical volume range within which cells preferred to divide (i.e. $\tilde{P}(\tau, V)$ large in this volume range), then the dividing cells would tend toward this volume range, more or less independently of $F(\tau, V)$. The possibility of stable populations in balanced exponential growth would then be expected, even for growth laws which lead only to unstable or neutral solutions if P is a function of τ only.

Indeed, if $P(\tau, V)$ and $F(\tau, V)$ were both functions of V only, the implied tight coupling between growth and division cycles would appear to make for a rather trivial attainment of balanced exponential growth. However, this mathematical possibility does not appear reasonable from a biological viewpoint. Since mitosis is apparently a final event in a more or less orderly sequence of biochemical reactions, we expect that the division probability $P(\tau, V)$ is a strong function of cell age.

If one assumed that both F and P were functions of τ only, then there would be nothing in the model (except for initial conditions) to determine the volume scale. The possibility of balanced exponential growth would require a relation between F and P such that on the average cells doubled in volume before dividing.

COMPARISON WITH EXPERIMENTS

In I, we have determined average growth rates and division probabilities for a variety of mammalian suspension cultures in exponential growth and these measurements have been refined.[2] In this paper, the averages were denoted by $f(V)$ and $p(V)$ and defined in Equation (16). It was found that to first approximation $f(V) = f_1 V$. Thus the special case of neutral stability is of particular interest.

In addition it was found that the growth rate for large cells falls off precipitously. One might think that this was a stabilizing influence which partly determined the

volumes at which cells were dividing. However, it appears that the falloff occurs only for volumes which are appreciably larger than those of most dividing cells. Only around 10% of the cells get to such large volumes. Therefore, the stabilizing influence would not seem to be effective in determining division volume and it may be that the large cells are simply abnormal. In addition for the large cells $p(V)$ is also decreasing with V, suggesting that these cells are too large to divide or otherwise abnormal.

If $F(\tau, V) = f_1 V$, then $P(\tau, V)$ cannot, according to the previous section, be a function of τ only (except for a Dirac delta function which is hardly possible biologically). Thus the existence and stability of the actual populations must be caused by deviations of $F(\tau, V)$ from $f_1 V$ and/or by a volume dependence of $P(\tau, V)$. Superficially it would appear that the deviations of $F(\tau, V)$ from $f_1 V$ are so slight as not to confer much stability (if any) on the population. It is, therefore, attractive to conjecture that $P(\tau, V)$ depends on cell volume so as to favor the division of cells in a critical volume range.

GENERALIZATIONS

Mathematically, our model can be generalized to admit a description of the state of a cell by more than the two variables, τ and V. Thus suppose that the state of a cell is specified by τ and by some variables X_i, $(i = 1, 2, \cdots, I)$ where, for example, X_1 might be the mass of DNA, X_2 the total mass of ribosomes, X_3 the mitochondrial mass, etc. The structure of our equations remains much the same, independent of I, provided that the following assumptions are satisfied: (*a*) The division probability is determined by the present state of the system (i.e. division is a *Markov* process (Feller, 1957)). We may then let $P(X_1, X_2, \cdots X_I, \tau) = P(\mathrm{X}, \tau)$ be the division probability per unit time. (*b*) All state variables are divided equally between the two daughter cells. Thus, on division 2X becomes X. (*c*) The rate of increase of any variable is *determined* by the state of the system. This might seem to be quite a restrictive condition, but if one postulated enough variables and then looked at only a few variables, the presence of the unexamined hidden variables could make the cell's apparent behavior undetermined or statistical. At any rate, this assumption means we can write

$$\frac{dX_i}{dt} = F_i(\mathrm{X}, \tau) \, . \tag{36}$$

With these assumptions, equations for the development of a population of cells are

$$\frac{\partial N}{\partial t}(t, \tau, \mathrm{X}) + \frac{\partial N}{\partial \tau} + \sum_{i=1}^{I} \frac{\partial}{\partial X_i} \{F_i(\mathrm{X}, \tau) N\} = - \{P(\mathrm{X}, \tau) + D(\mathrm{X}, \tau)\} N \tag{37}$$

$$N(t, 0, \mathrm{X}) = 2^{I+1} \int_0^\infty d\tau' P(2\mathrm{X}, \tau') N(t, \tau', 2\mathrm{X}) \tag{38}$$

One would then like to know for what sorts of F_i and P will balanced exponential growth be possible.

It is clear that these models include the possibility of the appearance of differentiated cells. Even the two variable model which we have considered admits this possibility. For example, one could postulate that if cells reached some large age without division or if they were born with too small volume, they would then cease to grow ($F(\tau, V) = 0$) or divide ($P(\tau, V) = 0$). These cells would then be quite different from the bulk of the population but they would be present even in balanced exponential growth.

Note Added in Proof. Reference should also be made to the closely related work of Fredrickson, Tsuchiya, and collaborators, described in Fredrickson, A. G., D. Ramkrishna, and H. M. Tsuchiya, 1967, *Mathematical Biosciences*, **1**:327, and other references given therein. I am indebted to Dr. E. Trucco for calling my attention to this work.

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REFERENCES

ANDERSON, E. C., and D. F. PETERSEN. 1965. *Exptl. Cell Res.* **36**:423.

BELL, G. I., and E. C. ANDERSON. 1967. *Biophys. J.* **7**:329.

COURANT, R., and D. HILBERT. 1962. Methods of Mathematical Physics. Interscience Publishers, Inc., New York. **1**.

DAWSON, K. B., H. MADOC-JONES, and E. O. FIELD. 1965. *Exptl. Cell Res.* **38**:75.

FELLER, W. 1957. An Introduction to Probability Theory and Its Applications. John Wiley & Sons, Inc., New York. 2nd edition. **1**.

KREIN, M. G., and M. A. RUTMAN. 1948. *Usp. Mat. Nauk.* **3**:3; *Am. Math. Soc. Transl., No. 26.*

PUCK, T. T., and J. STEFFEN. 1963. *Biophys. J.* **3**:379.

WING, G. M. 1962. An Introduction to Transport Theory. John Wiley & Sons, Inc., New York. Chapter 8.