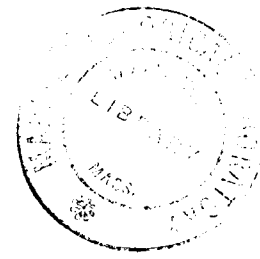

STATE VECTOR DESCRIPTION OF THE PROLIFERATION OF MAMMALIAN CELLS IN TISSUE CULTURE

I. Exponential Growth

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ABSTRACT Proliferation of mammalian cells, even under conditions of unlimited growth, presents a complex problem because of the interaction of deterministic and stochastic processes. Division of the cell cycle into a finite number of parts establishes a multidimensional vector space. In this space an arbitrary culture can be represented by a vector called the state vector. The culture's subsequent growth is represented mathematically as a series of transformations of the state vector. The operators effecting these transformations are presented in matrix form and their relationship to the distribution of cell generation times is described. As an application of the model, the growth of an initially synchronized culture is considered and an unambiguous measure of the degree of synchrony is proposed. Results of a computer simulation of such a culture show the behavior with time of the degree of synchrony, the total cell number, and the mitotic index. In particular the importance of the magnitude of the coefficient of variation of the generation time distribution is illustrated.

INTRODUCTION

A cultured cell population, although it may initially have been derived from a single cell, is not homogeneous with respect to a variety of cellular parameters. Mutations are manifestations of the lack of stability of the genotype. However, it appears that most randomization (in time) of cellular events occurs because of the statistical fluctuations in the very large number of reactions necessary for cellular reproduction (Peterson and Anderson, 1964). Since each individual reaction involves relatively few molecules, stochastic effects become very important. Cells picked at random from a culture vary in content of protein, DNA, or RNA. Their ages differ and they occupy different positions in the functionally defined cell cycle (Fig. 1), to name only a few of the variable parameters. If such cells are exposed to radiation (e.g., Terasima and Tolmach, 1963) or cytotoxic drugs (Walker and

Helleiner, 1963), they show varied responses, depending on their position within the cell cycle. Thus, the cell population is in fact, at any instant of time, a heterogeneous group of cells which individually reflect a spectrum of the range of values taken on by the various physical quantities determining the behavior of the culture. Because of the dynamics of cellular proliferation there is a more or less constant shift of cells from one subpopulation to another. A mathematical model that

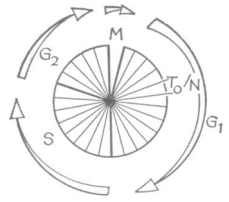

FIGURE 1 Life cycle of mammalian cells. M is the mitotic phase, G_1 the postmitotic (or presynthetic) phase, S is the phase of DNA synthesis, G_2 the premitotic (or postsynthetic) phase. The overall duration of the cycle is T_0. For the purposes of analysis, the cycle is subdivided into N intervals, each of duration T_0/N.

attempts to describe the dynamics of proliferation must incorporate both the stochastic effects and the deterministic aspects of cellular kinetics.

Of primary interest in many experiments is the change with time of the cell number. Growth curves of cell populations are usually characterized by three distinct periods: lag, exponential, and stationary (Fig. 2). During exponential growth, the cell number $n(t)$, measured at times t_1 and t_2 is related approximately by:

$$n(t_2) = n(t_1)2^{(t_2-t_1)/T_d} \tag{1}$$

where T_d is the doubling time of the cell population (under the particular environmental conditions of the experiment). Of course, the 2 occurs in equation (1) because mammalian cells increase their cell number almost entirely through binary fission. Equation (1) represents the simplest model of proliferation; it does not, however, demonstrate the fundamentally discontinuous aspects of cell division.

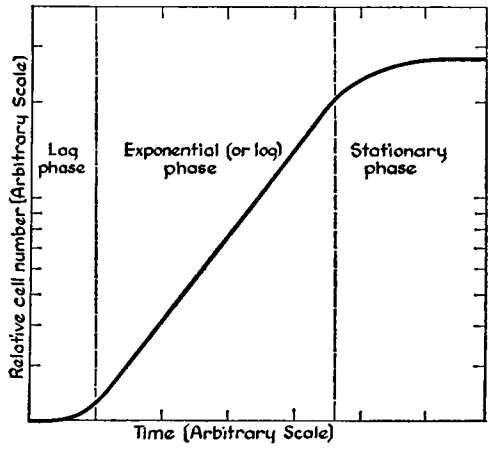

FIGURE 2 Growth curve of random mammalian cell culture as determined by daily counts on Coulter counter. Lag phase, logarithmic phase, and stationary phase are approximately as indicated. The present paper applies to logarithmic (or exponential) phase only.

Indiscriminate use of equation (1) may lead to serious error, particularly if it is applied to synchronous cultures. Furthermore, T_d, the doubling time, has little fundamental biological significance. T_d may be shorter, equal to, or longer than T_o, the mean generation time of the cell population. (T_o is defined as the ensemble average of generation times of those cells which will go on to division.)

At the other end of the spectrum of complexities of proliferation models are the stochastic models based on the theory of branching processes. These have been reviewed by Harris (1963). Unfortunately, to apply these models to specific cell systems requires knowledge of cellular parameters not obtainable by present-day techniques.

Both the simple deterministic equation (1) and the stochastic models treat time as a continuous variable. However, all information about a cell system is obtained by a more or less periodic sampling process. One can therefore regard the cell culture as a discrete time system. This is the point of view taken in the present paper. The resulting equations of the growth process are similar in many respects to the equations governing linear sample data systems, and use can be made of the considerable body of mathematical techniques developed for treating such systems. [For a survey of such techniques presented at the level appropriate here see Freeman (1965).] The description in terms of matrix equations is particularly convenient for computer simulation. The approach taken in constructing the model is essentially phenomenological. Care is taken to introduce only those parameters which can either be measured directly or obtained inferentially. The stochastic aspects of proliferation are described in average numbers only. Therefore the model is applicable only to cultures containing large numbers of cells (say, more than 10^4). Fluctuation phenomena are not considered.

BASIC ASSUMPTIONS

1. The heterogeneous population can be subdivided arbitrarily into a finite number of subpopulations. Within each of the subpopulations the parameters of interest are relatively constant and can be approximated by the mean values within the particular group. The values of the parameters define a cell state. A subpopulation consists of those cells occupying a cell state. We also speak of the status of the culture. This is a measure of the relative number of cells occupying the cellular states. For instance a perfectly synchronized cell population describes the status of a culture in which all cells occupy one specific cellular state.

2. The response of the entire culture can be determined from a linear combination of the responses of the subpopulations.

3. The probability density function of generation times has a standard deviation small compared with its mean.

4. The probability that a cell changes from state i to state $i + 1$ is not a function of its previous history nor a function of the value of i.

5. The probability distributions associated with the growth process are time invariant.

The first of these assumptions can be made valid by making the number of subdivisions sufficiently large. To determine what is mean quantitatively by "sufficiently large," one can either appeal to the sampling theorem (Oliver, Pierce, and Shannon, 1948), or argue in terms of measurement accuracy. In most tissue culture experiments resolution of time events (inferred from measurements of cellular quantities) to closer than ± 30 min is difficult, if not impossible. Therefore, for a cell line having a mean generation time of T_o hr, a reasonable number of subpopulations is: $T_o \leq N \leq 2T_o$. Here N is the number of cellular states (or subpopulations).

The concept of linearity (assumption 2), is accepted a priori by some investigators (Whitmore et al., 1965). To do so denies the possibility of interactions among cells. Available experimental evidence does indicate that in most tissue culture experiments interactions among cells is small if it exists at all. (Elkind and Sinclair, 1965; Tolmach, Terasima, and Phillips, 1965.) However as a caution against accepting linearity as a general principle it may be pointed out that it is common experimental experience that mammalian cells in suspension culture will grow only if the initial cell inoculum exceeds a specific minimum number. This is therefore a highly nonlinear threshold phenomenon.

The data available in the literature are consistent with assumption 3. Coefficients of variation cited by Dawson, Madoc-Jones, and Field (1965) range from 9.02% for HeLa S3 cells of Puck to 26.04% for kitten lung cells (Sisken and Kinosita, 1962). For Chinese hamster cells Peterson and Anderson (1964) quote a value of 13%.

Transition probabilities are impossible to measure directly; inferential measurements are very difficult to obtain. The recent work of Killander and Zetterberg (1965) indicates that transition probabilities, for L cells at least, are in fact not constant throughout the cell cycle. However in the absence of quantitative detail we assume that the process of randomization is independent of the position of the cell in the cell cycle. The last assumption, time invariance of the distribution functions, can be interpreted loosely as requiring that the moments of the distribution show no measurable change during the experiments. In tissue culture experiments this is usually the case.

THE MODEL[1]

The mean generation time, T_o, of the population is subdivided into N subintervals of uniform length. Mean cell age or position in the cell cycle is the criterion which

[1] Preliminary aspects of this work have been presented earlier (Hahn et al., 1965; Hahn, 1965). Furthermore, Engelberg (1964*b*) and Hirsch and Engelberg (1965) have treated much the same subject matter discussed here. However, there are some fundamental differences between

defines the cell state. In the usual representation of the cell cycle, the division is carried out as in Fig. 1. Clearly the criterion of mean cell age is arbitrary. Any single valued function of the cell cycle, e.g. mean RNA contents, could have been used with equal justification. However, in kinetic problems time appears as a natural variable; cell mean age is linearly related to time and hence a convenient variable to use. The zero of cell age is taken as the time of division of mother into daughter cells. This allows a simple way of enumerating the states. Cells with mean cell age $T_0/2N$ occupy state 1, cells with mean age $3T_0/2N$ state 2, and cells with mean age $(2i\text{-}1)T_0/2N$ occupy state i. The total cell population is obtained by summing over the subpopulations occupying the cellular states. The $1 \times N$ array of subpopulations, ordered according to mean age, defines a vector.

This vector is a measure of the distribution of the cells among the states at any instant of time; it is therefore called the state vector. Kinetics of cellular proliferation is represented in the model as a transformation of the initial state vector into a new one. An operator must be defined which effects the desired transformation. Only operators which leave the mean generation time T_0 invariant will be discussed.

First some important state vectors are examined. Conceptually, the simplest vector is one representing a culture having the same number of cells in each subpopulation:

$$S_{\text{uniform}} = P/N \begin{bmatrix} 1 \\ 1 \\ 1 \\ \\ 1 \end{bmatrix}$$

where P is the total cell population.

Such a vector does not represent any culture found eyperimentally. In steady-state conditions cells appear to be bunched in specific phases of the cell cycle. The environmental influence responsible for cessation of proliferation determines in which phase (or states) the cells accumulate. However the vector is a useful concept for comparison with vectors representing actual or realizable cultures.

If all the cells are in one cell state, the culture is perfectly synchronized. For the case of synchrony immediately following mitosis (all cells in state 1), the vector is

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their approach and the one of this paper. Perhaps the most important difference relates to the definition of state. In Hirsch and Engelberg's model the fundamental quantity is the distribution of generation times; a state is defined as relating to this distribution. In the present paper, the cell state is defined with respect to a measurable quantity: mean cell age, mean RNA content etc. Furthermore, the mathematical techniques used in the two developments differ markedly; Hirsch and Engelberg's model is a continuous one as opposed to the discrete time system presented here.

$$S_{sync} = P \begin{bmatrix} 1 \\ 0 \\ \\ \\ 0 \\ 0 \end{bmatrix} \tag{2}$$

Again, such a vector represents an idealization. However, it can be approached arbitrarily closely. For instance Puck (1964) describes a double-thymidine-block technique which in his hands can give rise to populations having 90% of their cells in a portion of the life cycle of about 5% of its length.

Perhaps the most important state vector is one describing an exponentially growing culture. This vector has the form

$$S_{exp} = \frac{P}{\sum_i 2^{-i/N}} \begin{bmatrix} 2^{-1/N} \\ 2^{-2/N} \\ \\ 2^{-i/N} \\ \\ 2^{-1} \end{bmatrix} \tag{3}$$

The terms inside the brackets arise from taking into account the age distribution (Hoffman, 1949). This vector has a variety of interesting properties. One of these is the fact that except for the common multiplicative factor the components of the vector are strictly invariant to deterministic kinetic events and approximately so to stochastic variations. The only effect of growth is an exponential increase with time of the multiplying constant. It is shown in a later section that after a time interval of T_o/N time units this vector transforms as follows:

$$S'_{exp} \cong 2^{1/N} S_{exp} , \tag{4}$$

where S is the vector at some time $t = t_o$, S' the vector T_o/N time units later. Another interesting property is the following: due to stochastic variations in generation times, any vector (with N sufficiently large) approaches the exponential vector as $t \to \infty$. This occurs independently of the original makeup of the culture or the exact form of the distribution of generation times. Thus the vector appears to be analogous to a fixed point probability vector of a Markov chain (Nahikian, 1964).

TRANSFORMATION MATRICES

Suppose a state vector is determined at some time $t = t_o$; what is its form at $t = t_o + T_o/N$? Examine the question in two parts. First, ignore stochastic effects and assume that T_o is the generation time of each and every cell. In that case the subpopulations

undergo a cyclic permutation of states. The population occupying the first state is now in the second, that in the i^{th} in the $i + 1^{st}$. Those cells in the last state, just prior to mitosis, undergo division, double in number, and appear in the first state; $S \rightarrow S'$ where

$$S = \begin{bmatrix} a \\ b \\ \\ k_{-1} \\ k \end{bmatrix} \rightarrow S' = \begin{bmatrix} 2k \\ a \\ b \\ \\ k_{-1} \end{bmatrix} \tag{5}$$

The matrix which effects this transformation is called the unit time shift operator, Δ_1. Its form is the following:

$$\Delta_1 = \begin{bmatrix} 0, 0, \cdots\cdots 0, 2 \\ 1, 0, \cdots\cdots 0, 0 \\ 0, 1, \cdots\cdots 0, 0 \\ \\ 0, 0, \cdots\cdots 1, 0 \end{bmatrix}. \tag{6}$$

Operating with Δ_1 on the three illustrative vectors yields

$$S' = \Delta_1 S_{\text{uniform}} = P_0/N \begin{bmatrix} 2 \\ 1 \\ 1 \\ 1 \\ 1 \end{bmatrix}, \tag{7}$$

where P_0 is the population at $t = t_0$;

$$S'_{\text{sync}} = \Delta_1 S_{\text{sync}} = P_0 \begin{bmatrix} 0 \\ 1 \\ \\ 0 \\ 0 \end{bmatrix} \tag{8}$$

and

$$S'_{\text{exp}} = \Delta_1 S_{\text{exp}} = \frac{P_0}{\sum_i 2^{-i/N}} 2^{1/N} \begin{bmatrix} 2^{-1/N} \\ 2^{-2/N} \\ \\ 2^{-1} \end{bmatrix} = 2^{1/N} S_{\text{exp}} \tag{9}$$

The factor 2 in the upper right-hand corner of expression (6) implies that each and every cell divides and in turn gives rise to two viable offsprings. However it is possible that only a fraction of the newborn cells will ultimately divide; the others presumably are then born without proliferative capability. It has been suggested (Elkind, Han, and Volz, 1963) that the plating efficiency in tissue culture experiments measures the percentage of cells having proliferative capacity. In that case the 2 should be replaced by 2 × plating efficiency (%)/100. Applying Δ_1 N times to S_{exp} yields

$$S'_{exp} = (\Delta_1)^N S_{exp} = 2S_{exp} . \tag{10}$$

This equation shows that in the absence of stochastic considerations, and if all cells maintain their proliferative capacity, the doubling time of the culture and the cells' mean generation time coincide.

The stochastic effects on the growth kinetics are represented by defining another matrix operator, called the unit dispersion matrix, δ. In growth problems, this dispersion matrix has meaning only when used with the time shift matrix; conversely, the time shift matrix must have the dispersion matrix associated with it. Furthermore, because the time shift matrix and the dispersion matrix do not commute, the order of applying the operators is important. The reason for keeping the matrix operators δ and Δ_1 separate rather than defining one operator (δ Δ_1) is that agents such as radiation or certain drugs interrupt the normal cell cycles. Then we still need the dispersion operator δ, but have to redefine the unit time shift operator.

The time shift matrix, Δ_1, permutates all the cells uniformly. However, there are cells in state i which move slower than the mean rate through a portion of the cell cycle. A fraction of these will, at time $t_0 + T_0/N$, not have taken on the characteristics which define state $i + 1$. They should therefore still be counted in state i. There are cells which were in state i at $t = t_0$ which at the later time should be counted with state $i + 1$ or even state $i + 2$ or $i + 3$. However, we assume that the probability of a rapidly moving cell "skipping" more than one state is vanishingly small.

If we start with the synchronized vector, equation (2), the final state vector after T_0/N time units would have the form

$$S'' = \begin{bmatrix} \beta \\ 1 - \alpha - \beta \\ \alpha \\ 0 \\ \\ 0 \end{bmatrix} \equiv (\delta\Delta_1) S_{sync} . \tag{11}$$

Here α is the probability that a cell advances two states in time T_0/N, while β is the probability that it does not advance at all. Brackets have been placed around the

product of the two operators δ and Δ_1 to emphasize their connection and the order of their application.

Utilizing this, and the basic assumption that transition probabilities are independent of the position of the cell in the cell cycle, the dispersion matrix δ is clearly,

$$\delta = \begin{bmatrix} 1-\alpha-\beta, & \beta, & 0, \cdots \alpha \\ \alpha, & 1-\alpha-\beta, & \beta, \cdots 0 \\ 0, & \alpha, & 1-\alpha-\beta, \cdots 0 \\ \\ \beta, & 0, & 0, \cdots \alpha, 1-\alpha-\beta \end{bmatrix} \tag{12}$$

Of interest is the result of operating with δ on S_{exp}: the ith component of the resulting vector S'_{exp} is

$$S'_i \cong \frac{P}{\sum_i 2^{-i/N}} \left[2^{-i/N} + \frac{\alpha-\beta}{N} \ell n\, 2 + 0\left(\frac{1}{N^2}\right) \right] \quad (i \neq 1, N) \tag{13}$$

For $\alpha \cong \beta$ (and both small compared with 1) and N large, $S'_i \cong S_i$. For $i = 1$;

$$S'_1 \cong \frac{P}{\sum_i 2^{-i/N}} [2^{-1/N} - \alpha/2] \tag{14}$$

with a somewhat similar result for $i = N$.

In practice the small departure from S_{exp} is negligible. For $N = 20$, $\alpha = \beta = 0.1$, a computer simulation showed that the mean square deviation of S'_{exp} from S_{exp} did not exceed 0.002 over a period of 10 doubling times [i.e., 200 multiplications of S'_{exp} by $(\delta\ \Delta_1)$].

The parameters α and β are not directly measurable. However, they are related to the distribution of generation times. This distribution is measurable by time lapse cinematography. The relationship is quite simple if the distribution is approximately Gaussian. Then $\alpha = \beta$ and it is readily shown that:

$$\alpha = N \frac{\sigma^2_{T_0}}{2}, \tag{15}$$

where $\sigma^2_{T_0}$ is the variance of the distribution of generation times in units of T_0^2. It can readily be seen that if $\alpha = \beta$ the distribution simulated by the model is also very nearly Gaussian. The distribution of generation times can be considered to result from the N-fold application of the dispersion operator to S_{sync}. The generation time as a random variable, therefore can be regarded as the sum of N equal random variables each of the latter resulting from a single operation with δ. The distribution of the generation times is therefore the N-fold convolution of the individual distributions (Parzen, 1960). One can either appeal to extensions of the central limit theorem or actually carry out the indicated convolutions to show that for N as low as 5 the distribution is indistinguishable from the Gaussian. The data of Dawson, Madoc-Jones,

and Field (1965) indicate that the measured distribution of generation times (at least for some cell lines) can be approximated by a Gaussian without too great an error.

APPLICATIONS OF THE MODEL

Degree of Synchrony. While it is easy to define a vector which in an idealized sense describes a completely synchronized culture, obtaining such a culture in practice is another matter. In fact, all experimental synchronization procedures yield populations which are parasynchronized; in addition to a large number of cells in one or two adjacent states there is usually a background of cells distributed over many states. One would like to obtain an index which in an unambiguous fashion establishes the quality of synchrony achieved. A variety of such indices has been proposed. Some of the indices depend on point estimates. The growth of the culture is compared by measurements at two points in the cycle, and from these measurements conclusions regarding the degree of synchrony are drawn. Such point estimate indices are due to Zeuthen (1958) and to Scherbaum (1959). They are simple to use, particularly for systems having short generation times, so that in practice the cycle cannot be resolved into many subintervals. However, such indices say nothing about what constitutes an asynchronous population. A more rigorous criterion was proposed by Engelberg (1961). Engelberg starts by defining an asynchronous population as one whose normalized rate of growth, $1/P\ dP/dt$, is independent of time and equal to a constant, K_e. For any culture not growing exponentially the value of $1/P\ dP/dt$ at any one instant of time will be below or above the value of K_e, depending upon the time of measurement. Engelberg defines per cent synchronization as 100 times the ratio of the area under the curve of $1/P\ dP/dt$ when $1/P\ dP/dt > K_e$ to $\int_0^{T_e} 1/P\ dP/dt$; i.e., the total area under this curve during one cell doubling time. This definition yields a value of 100 for a culture represented by a synchronous vector, and 0 for one represented by an exponential vector at least in the limit $N \to \infty$. As Engelberg points out (Engelberg, 1964*a*), however, 100% synchronization is also assigned to cultures whose growth curve shows more than one sharp rise in cell number during one cell doubling time. Such cultures are represented by state vectors of the type:

$$S = P\begin{bmatrix} \frac{1}{2} \\ 0 \\ \frac{1}{2} \\ 0 \\ \\ 0 \end{bmatrix}; \quad \text{or} \quad P\begin{bmatrix} \frac{1}{3} \\ 0 \\ \vdots \\ 0 \\ \frac{1}{3} \\ 0 \\ \vdots \\ 0 \\ \frac{1}{3} \end{bmatrix} \quad \text{etc.,} \tag{23}$$

provided again that we go to the limit as $N \to \infty$. It is contrary to one's intuitive concept of synchrony to call all such cultures 100% synchronized.

A measure of synchrony which is consistent with the formalism of this model and which removes the ambiguities of Engelberg's index, is to compare the state vector representing the particular culture with the state vector for an exponentially growing population.

Let n_i be the number of cells in state i of the vector whose degree of synchrony we are characterizing and $n_{i(\exp)}$ be the number of cells in state i of an exponential vector of the same total cell population and same value of N. The mean square difference (MSD) between n_i and $n_{i(\exp)}$, after proper normalization, yields an unambiguous measure of the synchrony of the culture:

$$\text{MSD} = k_N \sum_{i=1}^{N} (n_i - n_{(\exp)\,i})^2. \tag{24}$$

The normalization constant, k_N, is determined to yield a maximum MSD of 100. The maximum value of MSD will occur when all the cells of the culture are in state N; then

$$\begin{aligned} n_i &= 0 \qquad i \neq N \\ &= P \qquad i = N \end{aligned} \tag{25}$$

It is readily shown that

$$k_N = 100 \frac{k_{\exp}^2}{P^2} \left[\sum_{i=0}^{N-1} 2^{-2i/N} + (\tfrac{1}{2} - k_{\exp})^2 \right]^{-1} \tag{26}$$

where

$$k_{\exp} \equiv \sum_{i=1}^{N} 2^{-i/N}$$

The experimental determination of the state vector involves approximately the same measurements as are required for the $1/P\, dP/dt$ curve. The necessary information can be obtained from growth measurements, periodic sampling of the mitotic index, or by periodically counting the accumulation of metaphases after colchicine or colcemide treatment (Puck and Steffen, 1963). Independently of the technique used, a time sequence of measurements over one cell doubling period is required. From these an estimate of the initial state vector can be obtained.

Decay of Synchrony and the Time Behavior of the Mitotic Index. The decay of synchrony can be determined by following the behavior of the state vector as a function of time. Suppose a culture is perfectly synchronized (all cells in one state) at time t_0; what is the degree of synchrony at time $t = t_0 + T_0$? The rate of decay is determined by the distribution of generation times of the individual cells making up the culture; in the model it is determined, for fixed N, by the value of α (assuming $\alpha = \beta$).

In one simulation the state vector of an initially perfectly synchronized state vector

of 20 components was followed on the computer for 10 doubling times. The normalized mean square deviation from the exponential state vector was calculated, as was Engelberg's per cent synchrony, at each doubling of the cell population. The values of α (or β) used were 0.03, 0.1, 0.3; the corresponding standard deviations in the approximation of equation (15) are 5.5%, 10%, and 17.3%. These quantities are listed in Table I. Table II shows the normalized vector components for the case $\alpha = 0.1$ for

TABLE I

DECAY OF SYNCHRONY; ENGELBERG'S (1961) PER CENT SYNCHRONY AND MEAN SQUARE DEVIATION FROM EXPONENTIAL VECTOR AS A FUNCTION OF DOUBLING TIMES

Doubling times	Synchrony	Normalized mean square deviation from exponential
1. $\alpha = \beta = 0.03$; $\sigma_{T_0} = 5.5\%$;	%	
0	100	100.00
1	52.2	27.57
2	41.04	16.37
3	34.4	11.90
4	29.7	9.35
5	27.7	7.66
10	20.3	3.61
2. $\alpha = \beta = 0.1$; $\sigma_{T_0} = 10\%$;		
0	100	100
1	33.3	11.06
2	24.4	5.88
3	19.2	3.68
4	15.7	2.43
5	12.7	1.63
10	4.9	0.237
3. $\alpha = \beta = 0.3$; $\sigma_{T_0} = 17.3\%$;		
0	100	100
1	21.2	4.22
2	11.4	1.27
3	6.6	0.433
4	4.2	0.176
5	3.1	0.0945
10	2.2	0.0519
$N = 20$		

the first 4 population doublings. The latter table shows in detail how the decay of synchrony manifests itself in terms of redistribution of cells among the cellular states.

In another calculation the growth of a culture represented again initially by a synchronized vector was simultated on the computer. The quantities determined were the relative cell number and the mitotic index. The time dependence of these variables is presented in Figs. 3 and 4 respectively. Parameters used in the calculations were: $T_0 = 24$ hr; $N = 24$, $\alpha = \beta = 0.1$, 0.2 and 0.3 (see Table III). The corresponding

TABLE II

REDISTRIBUTION OF COMPONENTS OF STATE VECTOR AFTER VARIOUS DOUBLING TIMES

Last column is obtained by dividing first column by total cell population. The value 14.2 for the cell population occurs because it is the sum of subpopulations for an exponential state vector for $N = 20$ and $P = 1$.

Doubling times	Component number																				Maximum value of mitotic index
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	
0	14.2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	100
1	3.7	3.1	2.0	1.0	0.4	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.1	0.2	0.6	1.0	1.8	26
2	2.6	2.4	1.9	1.3	0.8	0.5	0.2	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.1	0.2	0.3	0.5	0.8	1.0	1.4	18.4
3	2.2	2.0	1.7	1.3	1.0	0.6	0.4	0.2	0.1	0.1	0.0	0.1	0.1	0.2	0.3	0.4	0.6	0.8	0.9	1.2		14.8
4	1.9	1.8	1.5	1.3	1.0	0.7	0.5	0.3	0.2	0.1	0.1	0.1	0.2	0.3	0.4	0.5	0.6	0.8	0.9	1.0		13.4

$\alpha = \beta = 0.1$; $N = 20$. Components of state vector (normalized to 14.2).

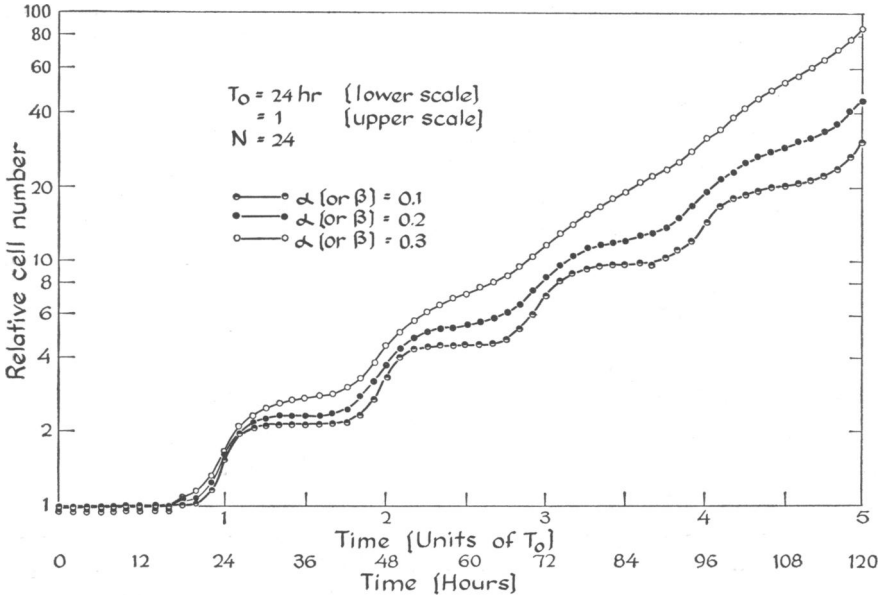

FIGURE 3 Theoretical growth curves of initially synchronized cell cultures having common mean generation times, but different coefficients of variation. The time average of the cell number increases exponentially according to $n(t) = n(0)2^{t/T_d}$. The fine structure of the growth behavior, as well as the value of the doubling time, T_d, is determined by the distribution of generation times.

values of σ_{T_0} just about span the values reported in the literature. Fig. 2 illustrates two facts: first, the graphs represent the optimum growth patterns (in the sense of maintaining synchrony) that can be expected from synchronized cultures. Actual cultures are only parasynchronized; the degree of synchrony achievable depends upon the synchronization technique used. Therefore the actual culture approaches exponential growth even more quickly than the idealized populations of Fig. 2. Another interesting point illustrated is that in the approximation considered here, where each

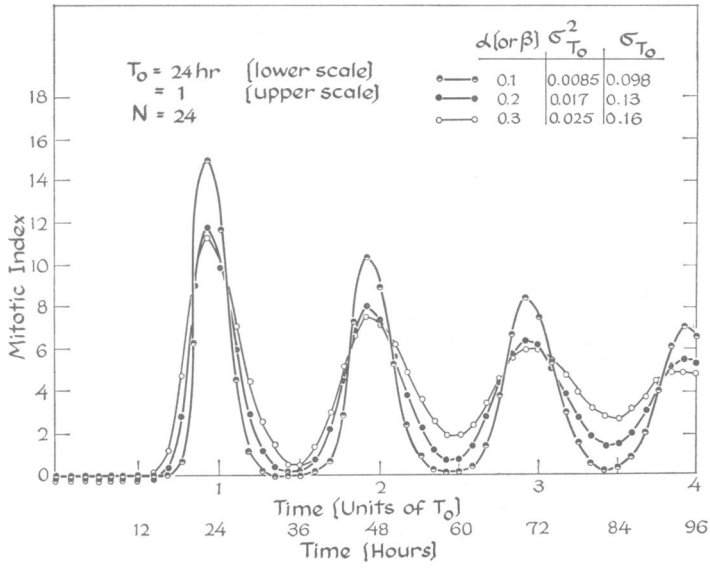

FIGURE 4 Mitotic index for initially synchronized cultures having common mean generation times but different coefficients of variation. The indices approach the value 0.693 M/T_d asymptotically. The location of the maxima is essentially independent of the coefficient of variation and is therefore a measure of the mean generation time. However the decay of the amplitudes of successive maxima is determined by the coefficient of variation.

cell gives rise to two viable new cells (and assuming symmetric distributions of generation times), the doubling time T_d is always equal to or less than the mean generation time, T_0. The relationship between the two is determined by the magnitude of the variance of the distribution $\sigma^2_{T_0}$, for those situations when equation (15) is at least approximately correct. Large values of σ_{T_0} give rise to short doubling times. This is illustrated in Table III for the curves of Fig. 3. α is the forward (or backward) parameter; σ_{T_0} the calculated standard deviation of generation times, T_0 the mean generation time and T_d the doubling time. All values correspond to the growth curves of Fig. 3.

The time behavior of the mitotic index, Fig. 4, is another way of presenting information similar to that contained in the growth data. In fact, if the duration of the mitotic phase were very small compared with the mean generation time, Fig. 3 would be a plot

TABLE III

COEFFICIENTS OF VARIATION, MEAN GENERATION TISSUES, AND DOUBLING TIMES OF ALL POPULATIONS

α (or β)	σ_{T_0}	T_0	T_d
0.1	0.09	24	23.6
0.2	0.13	24	21.1
0.3	0.16	24	18.2

of the normalized derivatives of the growth curves. The asymptotic values of the mitotic index for large t are, in each case

$$(M\ I)_{t\to\infty} \to 0.693\ \frac{M}{T_d} \tag{27}$$

where M is the duration of the mitotic phase. Equation (27)[2] represents the mitotic index of an exponentially growing population, showing again that exponential growth represents the limiting form of the growth behavior.

SUMMARY

A formalism has been presented which permits the calculation of kinetic parameters of arbitrary mammalian cell cultures under conditions of unlimited growth. The development starts with the definition of the status of a culture in terms of the population density of cellular states, and the definition of the state vector which is the quantitative description of the cell culture. Two operators are then defined which determine the growth behavior of the culture. These are (*a*) the unit time shift operator which accounts for the deterministic aspects of growth, and (*b*) the dispersion operator which then incorporates the effects of stochastic variation, at least in terms of average values. The structure of these operators is determined by the distribution of generation times of the cell line. Knowing the initial value of the state vector and the distribution of generation times suffices to predict the behavior of the culture for as long a period as the conditions of unlimited growth prevail.

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[2] In the literature (Harris, 1963; Hoffman, Metropolis, and Gardiner, 1956) the limiting mitotic index is given as $0.693\ M/T_0$. However that formulation appears to be inapplicable in the present case.

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