

A MATURITY-TIME REPRESENTATION FOR CELL POPULATIONS

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ABSTRACT A maturity-time representation for the study of cell populations is introduced, which differs from the age-time representation suggested by von Foerster. A significant feature of the theory is the concept of maturation velocity. A solution to the fundamental equations of the theory is presented in terms of the individual generations which make up the population at any time. The problem of variability of generation time is considered from the differing viewpoints of the two representations, as well as that of an alternate theory due to Stuart and Merkle. The experimental observations of Prescott concerning the generation time distribution and population growth of *Tetrahymena geleii* HS cells appear to support best the theoretical formulation of the maturity-time representation. In particular, they suggest that memory of the maturation velocity or generation time of a given cell tends to persist from parent to daughter for several generations at least.

I. INTRODUCTION

An equation for the cell density function is presented that describes a population of cells in terms of the variables maturity and time. This is in contrast with the equation for the cell density function introduced by Scherbaum and Rasch (1) and studied by many authors (2-5) which describes cells in terms of the variables chronological age and time. The new equation is also different from that suggested by Stuart and Merkle (6), who, however, also utilized the variables maturity (called "physiological age" by them) and time. It has certain formal similarities to the modification of the Scherbaum-Rasch equation introduced by von Foerster (2). Nevertheless, there are basic differences both in the mathematical nature of the equation and the underlying biological implications of the different descriptions.

The new theory is formulated in section II. An important feature of it is the concept of "maturation velocity" which may or may not be variable, and in any case needs to be specified. An attempt is made to point out the major differences in the two representations for the cell density function, which we shall call the maturity-time representation and the age-time representation. Of particular significance is the different causes of variability in the observed cell generation times that is offered by

the various theories discussed. Thus, in the age-time representation, it is natural to specify the age at which mitosis occurs probabilistically. Then cells have no memory of the generation time of their parents. In the maturity-time representation, mitosis always occurs at the same maturity level. A possible reason that cells have variable generation times is that the population is heterogeneous with respect to cell maturation velocities. In that case the cells may remember the maturation velocity of their parents. A third point of view is that cells have different effective maturation velocities which are randomly determined as in the theory of Stuart and Merkle. In that theory, cells have no precise memory of the maturation velocity of their parents, but the mean maturation velocity of the population remains always the same. Probably none of these theories is exactly right, and ideally one would like to have a more fundamental theory in terms of the biochemical variables which make up the life process. In the absence of such a theory, however, one or another of the representations in terms of age or maturation may be more useful in describing kinetic aspects of cell populations.

In section III, a solution of the equations is presented for the case when the maturation velocity and the cell loss due to death or disappearance are functions of maturation level only. This solution is "physically" appealing in that the contribution of different generations is readily identifiable.

In order to illustrate the application of the two alternate representations to data obtained by Prescott (7) concerning the variable generation times of a population of *Tetrahymena geleii* HS cells, this same problem is solved explicitly in section IV, firstly with the age-time formalism, and secondly with the maturity-time formalism. The maturity-time formulation in the simplified version presented could be adapted so as to be presented completely within the framework of the age-time formalism. Even so, it would represent a different conceptual approach to the understanding of the experiments of Prescott. This problem was already treated by Stuart and Merkle (6). The different predictions made by each theory concerning the population growth are indicated. Such differences may serve as a test as to which formulation is more applicable for a given experimental situation.

In section V, comparison is made between the observed population growth of a number of *Tetrahymena geleii* HS cells under uniform conditions, and the predictions of the theories of the age-time representation, the maturity-time representation, and of Stuart and Merkle. In the maturity-time representation, the observed generation time distribution determines the theoretically postulated distribution directly. In the age-time formalism it determines the probability per unit time of mitosis. The prediction of the maturity-time representation is most closely in accord with Prescott's observations, and therefore the concept of memory of the generation time persisting from parent to offspring for several generations is supported. The predictions of the age-time representation and the Stuart-Merkle theory indicate a population growth that retains its synchrony to a greater degree than indicated by the data.

II. FORMULATION

Consider a population of living organisms which we shall take to be cells. The cells have a definite degree of maturation which increases with time but is variable from cell to cell.¹ At the point of maximum maturation they are generally assumed to undergo mitosis when p (usually 2) new daughter cells are produced from the old cell. The time is denoted by the variable t , and the degree of maturation is denoted by the variable μ . The population is to be described by the cell density function $n(\mu, t)$ where $n(\mu, t) d\mu$ represents the number of cells in the maturity interval μ to $\mu + d\mu$ at time t . We assume that

$$n(\mu + \Delta\mu, t + \Delta t) \left[\Delta\mu + \frac{\partial v}{\partial \mu} \Delta\mu \Delta t \right] = n(\mu, t) (1 - \lambda \Delta t) \Delta\mu. \quad (1)$$

This equation expresses the assumption that the number of cells of maturity $\mu + \Delta\mu$ at time $t + \Delta t$ equals the number of cells that have matured from an earlier level μ at time t less the number of cells that have been lost. The latter number is assumed to be proportional to n and the time interval Δt , with proportionality factor λ , i.e. the fractional loss is $\lambda \Delta t$. The bracketed expression on the left of equation 1 expresses the dilatation of the maturation element $\Delta\mu$ which takes place during the time interval Δt . Here $v = d\mu/dt$ is the time rate of change of maturity for cells of maturity μ at time t . If we divide equation 1 by $\Delta t \Delta\mu$ and take the limit as Δt and $\Delta\mu$ approach zero, there results the following equation for $n(\mu, t)$:

$$\frac{\partial n}{\partial t} + \frac{\partial}{\partial \mu} (vn) = -\lambda n. \quad (2)$$

This equation must be supplemented by an equation which specifies v which we shall call the "velocity of maturation." Thus, let

$$v = v(\mu, t, \beta) \quad (3)$$

This expresses the explicit functional dependence of v . Here β is a vector which represents the totality of parameters which can affect the velocity such as temperature, light, environmental nutrients, pH, etc. In usual biological circumstances, such quantities may be expected to depend on the time and perhaps on μ . It is also possible that v depends on n . If v were subject to stochastic variation, then this property could likewise be introduced into equation 3.

Equations 2 and 3 can be expected to possess a unique solution when supplemented by a boundary condition which specifies $n(0, t)$, or the manner in which cells are born, and an initial condition which specifies $n(\mu, 0)$ the initial maturation distribution.

¹ The possibility of utilizing a maturity-time description of a cell population was first pointed out to the author by Joseph B. Keller in private conversation.

By level of maturity is meant the various stages in the growth of the cell such as birth, onset of DNA synthesis, onset of mitosis, etc. These may or may not be readily observable. In fact it is difficult to say in what manner the maturity level of a cell should be determined. For bacterial cells such as *E. coli* in which DNA synthesis continues from the moment of birth, the amount of DNA in the cell could be utilized as a measure of cell maturity. Or μ could simply be considered to represent the amount of DNA in the cell. However, for many cells in which DNA synthesis is only a portion of the life cycle, such a measure is not completely satisfactory. Thus, at the present time even the dimensions of μ must be left unspecified. Another possibility is to let μ represent cell volume. In fact, the possibility of describing cell populations in terms of their volume distribution initially stimulated the work of Scherbaum and Rasch. More recently, Bell and Anderson (8) have proposed a cell density function which depends on volume, age, and time. The utilization of additional internal variables to describe the state of a cell population has also been suggested by Oldfield (9) and others (10).

If v is a constant, then μ is a linear function of the time. In that case, the time may sensibly be used as an indicator of maturity level for a given cell. Then μ would be measured in units of the time and could be called the "age" of the cell. More formally, we could change variables by setting $\mu = va$, where a is the age variable, and v is a constant. This is equivalent to setting $v = 1$ in equation 2. However, if v is not a constant, or if the cell population is not homogeneous, so that v varies among the different members of the population even though it is constant for any given member, then age and maturation are not equivalent variables. In such a case, only observation of intrinsic cellular properties is a true measure of maturation level. The variable μ then represents "cytological age," and is quite distinct from "chronological age" which is duration of time measured from the moment of birth.

Scherbaum and Rasch (1) originally introduced the equation for the cell density function which depends on age and time. Formally, their equation is the same as equation 2 with the age variable a replacing the maturity variable μ , $v = 1$, and $\lambda = 0$. This idea was developed further by von Foerster (2), who also pointed out the necessity of including a loss term $-\lambda n$ on the right-hand side of the equation. The solution and properties of von Foerster's equation have been discussed extensively by Trucco (3, 4) and Nooney (5).

Because of the formal similarity between von Foerster's equation and equations 2 and 3, it is important to understand some of the basic differences between the age-time description (von Foerster's equation) and the maturity-time description presented here. For example, in the von Foerster equation, the function λ takes into account loss of cells due to mitosis as well as cell death. Only in the case of a so-called "equivariant" population for which all cells undergo mitosis at the same age can the loss due to mitosis be removed from λ and accounted for by means of a boundary condition. In the maturity-time description, by contrast, the term λ represents cell

loss due to death or disappearance only. Since mitosis is assumed to always take place at the maximum value of the maturity variable, its effect is always represented by means of a boundary condition. This difference in mathematical structure leads to different formal solutions to the same problem. Of course these become equivalent when ν is constant for all cells.

In addition, in attempting to comprehend a cell population which has a variable generation time by means of the alternate representations for the cell density function, one is led in a natural way to making different assumptions regarding the underlying biological mechanism of cell variability. Thus, to account for the observed variability of cell generation times in a population which is maintained under constant environmental conditions, the age-time representation introduces a mitotic loss factor which is age dependent. Since each generation is subject to the same probabilistic rule governing mitosis, such a description asserts that each new cell has no memory of the generation time of its parent. In the maturity-time representation, mitosis occurs at a fixed value of the maturity variable. Variability in cell generation times may be introduced by assuming that the population is heterogeneous, consisting of cells with different generation times. This is equivalent to assigning different maturation velocities to different cells. Furthermore, if we assume there is no variation in ν following cell division, stochastic or otherwise, then the offspring of each cell has the same maturation velocity as its parent, i.e. each cell has perfect memory of the generation time of its parent. Thus, the two representations would make different predictions about the time evolution of a cell population.

Yet a third point of view is presented by Stuart and Merkle (6). These authors, too, utilize a cell density function which depends on maturity and time rather than age and time. They also assume that the cells have variable maturation velocities in order to account for variations in the observed generation times of a cell population. The variability about some mean velocity ν_0 is likened to a random walk in the variable μ , whatever the underlying cause. They consequently arrive at a diffusion-translation equation which in our notation is written as

$$\frac{\partial n}{\partial t} + \nu_0 \frac{\partial n}{\partial \mu} = D \frac{\partial^2 n}{\partial \mu^2}. \tag{4}$$

Here D is a “diffusion constant” which, together with ν_0 , are parameters which characterize a given cell population. Of course, a loss term $-\lambda n$ could also be added to the right-hand side of equation 4 just as in equation 2, although Stuart and Merkle did not do so.

This representation is somewhat between that of the two previously discussed representations: cells have a variable maturation velocity probabilistically determined about the mean value ν_0 . This means that the effective generation time is different from one cell to another, even though mitosis always takes place at a fixed value of μ . However, there is no memory from one generation to the next of the

parental generation time. An assumption of this model that is perhaps unattractive is that variability in the distribution of "effective" velocities is perforce symmetric about the mean value v_0 . An additional unsatisfactory feature of the model, recognized by the authors, is that some cells become less and less mature as time progresses. The differences in the various representations will be discussed further in section V.

III. A PARTICULAR SOLUTION

It is useful to give the solution to equations 2 and 3 in the special case when v and λ are functions of μ only. Let

$$n(\mu, 0) = g(\mu) \quad (5)$$

where $g(\mu)$ is a prescribed function, and assume that at the maximum maturation level which we choose to be the value $\mu = 1$, all cells complete mitosis and give birth to p new daughter cells. Therefore

$$n(0, t)v(0) = pn(1, t)v(1). \quad (6)$$

Then the solution to equations 2, 5, and 6 may be readily obtained in the following "physical" manner. The method of solution is akin to the "summation method" (reference 11) in the theory of optics by means of which a ray (incident on a plate, say) is traced through its multiple reflections and its amplitude determined at each reflection. Similarly, here we follow the initial cell distribution through succeeding generations, and correct for the effect of the new births as we go along.

We observe firstly that if we change variables from μ to $\eta = \int_0^\mu v^{-1}(\mu')d\mu'$, then equation 2 becomes the von Foerster equation in the variables η and t with an additional effective loss term $(-\partial v/\partial \mu)n$ appearing on the right-hand side. Since v is given explicitly in terms of μ , introduce $\mu = h(\eta)$, the inverse function to $\eta = \eta(\mu)$. It follows directly (reference 3) that a formal solution to equation 2 without regard to either boundary or initial conditions is given by the expression

$$n = f(\eta - t) \frac{v(h(b))}{v(\mu)} \exp \left[- \int_{h(b)}^\mu v^{-1}(\mu')\lambda(\mu') d\mu' \right], \quad (7)$$

where f and b are arbitrary functions of $(\eta - t)$. The initial generation, which satisfies equations 2 and 5 without regard to the boundary condition (equation 6), is designated as $n_1(\mu, t)$ and is obtained from equation 7 by setting $f(\eta) = g(h(\eta))$ and $b = \eta - t$:

$$n_1(\mu, t) = g(h(\eta - t)) \frac{v(h(\eta - t))}{v(\mu)} \exp \left[\int_{h(\eta-t)}^\mu v^{-1}(\mu')\lambda(\mu') d\mu' \right]. \quad (8)$$

The cells represented by n_1 give birth to the second generation $n_2(\mu, t)$ in accordance

with equation 6. Thus,

$$n_2(0, t) = pg(h(\eta_1 - t)) \frac{v(h(\eta_1 - t))}{v(0)} \exp \left[- \int_{h(\eta_1 - t)}^1 v^{-1}(\mu') \lambda(\mu') d\mu' \right]. \quad (9)$$

where $\eta_1 = \int_0^1 v^{-1}(\mu') d\mu'$. To obtain $n_2(\mu, t)$, we apply to equation 9 the translation operation $t \rightarrow t - \eta$ and multiply the result by $v(0)v^{-1}(\mu) \exp [- \int_0^\mu v^{-1}(\mu') \lambda(\mu') d\mu']$ which takes account of the presence of the terms $(\partial v / \partial \mu)n$ and λn in equation 2 without altering the birth condition equation 9. There results

$$n_2(\mu, t) = pg(h(\eta_1 + \eta - t)) \frac{v(h(\eta_1 + \eta - t))}{v(\mu)} \cdot \exp \left[- \int_{h(\eta_1 + \eta - t)}^1 v^{-1}(\mu') \lambda(\mu') d\mu' - \int_0^\mu v^{-1}(\mu') \lambda(\mu') d\mu' \right]. \quad (10)$$

If we continue in this manner for succeeding generations, the complete solution is obtained as

$$\begin{aligned} n(\mu, t) &= \sum_{j=1}^{\infty} n_j(\mu, t) \\ &= g(h(\eta - t)) \frac{v(h(\eta - t))}{v(\mu)} \exp \left[- \int_{h(\eta - t)}^\mu v^{-1}(\mu') \lambda(\mu') d\mu' \right] \\ &\quad + \sum_{j=2}^{\infty} p^{j-1} g(h((j-1)\eta_1 + \eta - t)) \frac{v(h((j-1)\eta_1 + \eta - t))}{v(\mu)} \\ &\quad \cdot \exp \left[- \int_{h((j-1)\eta_1 + \eta - t)}^1 v^{-1}(\mu') \lambda(\mu') d\mu' \right. \\ &\quad \left. - (j-2) \int_0^1 v^{-1}(\mu') \lambda(\mu') d\mu' - \int_0^\mu v^{-1}(\mu') \lambda(\mu') d\mu' \right]. \quad (11) \end{aligned}$$

Note that $g(x)$ is defined only in the interval $(0, 1)$ and is to be taken to be zero outside this interval. Therefore, at any given μ and t , only one term is nonzero in the series in equation 11. That is to say, at any time and for a given maturation level, only one generation can be observed; at any time and for all values of μ , two generations at most are observable. In the particular case when v is a constant, equation 2 becomes essentially the same as von Foerster's equation and the solution equation 11 simplifies to

$$\begin{aligned} n(\mu, t) &= \sum_{j=1}^{\infty} n_j(\mu, t) = g(\mu - vt) \exp \left[-v^{-1} \int_{\mu - vt}^\mu \lambda(\mu') d\mu' \right] \\ &\quad + \sum_{j=2}^{\infty} p^{j-1} g(j-1 + \mu - vt) \exp \left[-v^{-1} \int_{j-1 + \mu - vt}^1 \lambda(\mu') d\mu' \right. \\ &\quad \left. - (j-2)v^{-1} \int_0^1 \lambda(\mu') d\mu' - v^{-1} \int_0^\mu \lambda(\mu') d\mu' \right]. \quad (12) \end{aligned}$$

Equation 12 is an obvious generalization of Trucco's solution (reference 4) of the von Foerster equation for the problem of an equivariant population with $\lambda = 0$, and is also implicitly contained in his general solution (reference 3).

IV. APPLICATION TO A CELL POPULATION WITH VARIABLE GENERATION TIME

Prescott (7) has measured the generation times of a population of *Tetrahymena geleii* HS cells under uniform conditions. The observed generation times of 766 cells is shown in Fig. 1. The total observed population as a function of time of 50 cells which at $t = 0$ are newborn is shown in Fig. 2. It should be noted in Fig. 1 that the generation times appear to be skewed to the right so that they are not symmetric about the mean value as required by the Stuart-Merkle theory.

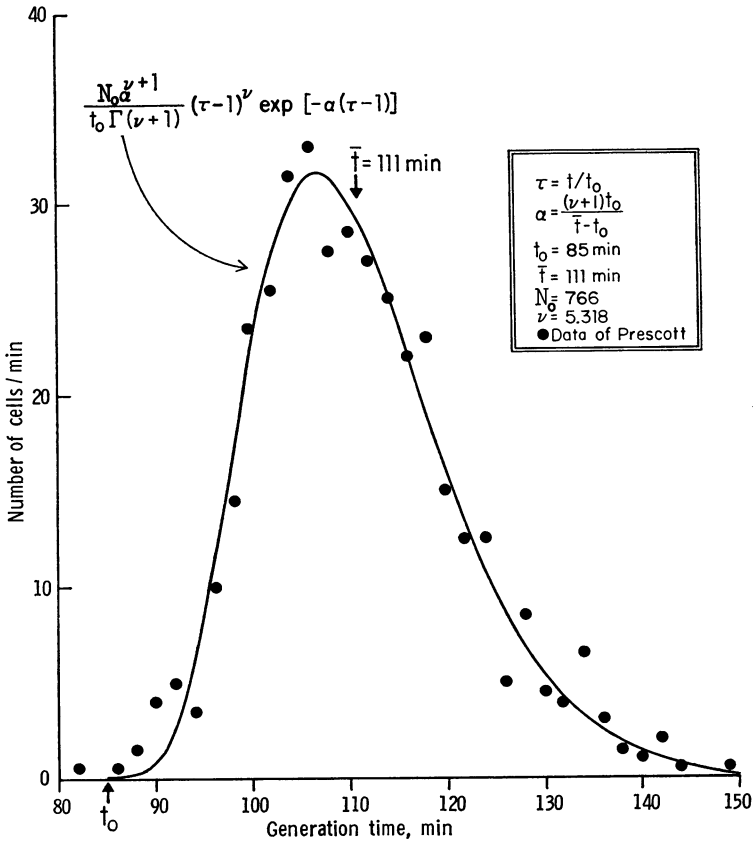


FIGURE 1 The data of Prescott (7) for the flux of cells of the HS strain of *Tetrahymena geleii* entering mitosis, as a function of time. The cells were grown under uniform conditions. All the cells are of age zero at time zero. The mean generation time is $\bar{t} = 111$ min. The gamma-distribution was constrained to have the mean generation time \bar{t} while the value of t_0 was arbitrarily chosen to be 85 min. The value of ν was determined by the method of least squares to fit the data.

Stuart and Merkle treated this problem theoretically according to equation 4. They presented graphically the total population as a function of time and this is shown in Fig. 5b. The quantities v_0 and D were determined by least-square fitting to Prescott's data as given in Fig. 1. In order to demonstrate in a concrete manner the differences in the various theories, we shall solve this same problem first in the age-time representation and next in the maturity-time representation of section II.

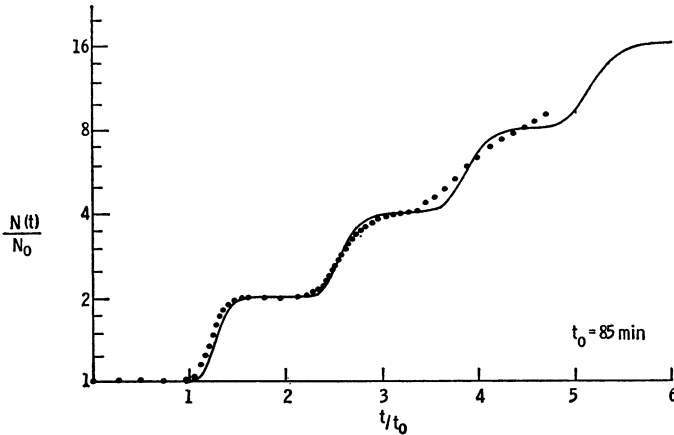


FIGURE 2 The data of Prescott (7) for the growth in population of an initially synchronized group of 50 cells of *Tetrahymena geleii* HS. The cells were grown under uniform conditions. A state of asynchronous growth appears to be rapidly reached within a few generations. The solid curve is the theoretically predicted growth curve for such a population in the age-time representation.

To represent Prescott's results from the standpoint of the age-time representation, we solve the von Foerster equation

$$\frac{\partial n}{\partial t} + \frac{\partial n}{\partial a} = -\lambda n \quad (13)$$

subject to the initial condition

$$n(a, 0) = N_0 \delta(a), \quad (14)$$

where $\delta(a)$ is the Dirac delta function, and N_0 is the total number of cells which constitute the initial population. This initial condition expresses the fact that there are N_0 cells of age zero at $t = 0$, in accordance with the manner in which the data were obtained. The age variable a is assumed to vary from 0 to ∞ . The loss function λ is assumed to consist of two parts: one part m due to mitosis, and a remainder due to other causes. The inclusion of mitosis as a cause of cell loss accounts for the variability in observed generation times. In Prescott's experiment, mitosis is the only cause of cell loss so that $\lambda = m$.

Cell birth is described by the boundary condition

$$n(0, t) = 2 \int_0^{\infty} m(a)n(a, t) da, \quad (15)$$

in which it is assumed that each cell in mitosis produces two daughters. The solution to equations 13–15 is readily obtained by the scheme described in the previous section. The result is

$$\left. \begin{aligned} n(a, t) &= \sum_{j=1}^{\infty} n_j(a, t), \\ n_1(a, t) &= N_0 \delta(a - t) \exp \left[- \int_{a-t}^a m(\xi) d\xi \right], \\ n_j(a, t) &= 2 \int_0^t y(\eta) n_{j-1}(a, t - \eta) d\eta, \quad j = 2, 3, 4, \dots \end{aligned} \right\} \quad (16)$$

where

$$y(t) = m(t) \exp \left[- \int_0^t m(\xi) d\xi \right]. \quad (17)$$

In equation 16, the first term $n_1(a, t)$ represents the contribution of the initial generation, the second term represents the contribution of the second generation, and so forth.

The number of cells which enter mitosis per unit time at any time t will be designated by $N_m(t)$ and is given by the expression

$$N_m(t) = \int_0^{\infty} m(a)n(a, t) da. \quad (18)$$

The number of cells of the initial generation entering mitosis per unit time is $N_{m1}(t)$ and is obtained by substituting $n_1(a, t)$ as given by equation 16 into equation 18, viz.,

$$N_{m1}(t) = N_0 y(t). \quad (19)$$

If $N_{m1}(t)$ is considered as experimentally determined by Fig. 1, we may utilize equations 17 and 19 to infer the function $m(t)$. Thus, by integrating equation 19, $\exp \left[- \int_0^t m(\xi) d\xi \right]$ and therefore $m(t)$ is easily found. The result is

$$m(t) = \frac{N_{m1}(t)}{N_0 - \int_0^t N_{m1}(t') dt'}. \quad (20)$$

By integrating $n(a, t)$ for all ages, we find from equation 16 the total population at any time t :

$$\left. \begin{aligned}
 N(t) &= \int_0^\infty n(a, t) da = \sum_{j=1}^\infty N_j(t), \\
 N_1(t) &= N_0 \exp \left[- \int_0^t m(\xi) d\xi \right], \\
 N_j(t) &= 2 \int_0^t N_{j-1}(t - \eta) y(\eta) d\eta, \quad j = 2, 3, 4, \dots
 \end{aligned} \right\} \quad (21)$$

We shall now solve this same problem by assuming that the observed variability in generation times is caused by an entirely different mechanism: the population consists of cells with variable maturation velocities. This concept has been utilized (12, 13) to explain curves of labeled mitoses that have been observed. As previously emphasized, the calculation that follows is equivalent to a calculation that utilizes the age-time formalism with $\lambda = 0$, a distribution of equivivant subpopulations, and a boundary condition to represent mitosis for each subpopulation. However, we shall not present it within the framework of the von Foerster equation partly because we wish to illustrate the maturity-time formalism and partly because we believe that it is useful conceptually and suggestive of an underlying biological mechanism to consider cells with variable generation times as having variable maturation velocities. Furthermore, a calculation based on the age-time formalism, unlike the following presentation, would preclude a possible extended treatment in which v is a variable function of maturation level for a given cell.

We shall utilize the theory presented in section II and assume that

$$n(\mu, t) = \int_0^\infty n_v(\mu, t) dv, \quad (22)$$

where $n_v(\mu, t) dv$ is that portion of the population density that is to be found with maturation velocities between v and $v + dv$. The function n_v is assumed to satisfy equation 2 with v constant and λ zero. The completion of mitosis occurs at the maximum maturity level $\mu = 1$. The result of mitosis is represented by the boundary condition

$$n_v(0, t) = 2n_v(1, t). \quad (23)$$

The initial condition is given by

$$n_v(\mu, 0) = N_0 w(v) \delta(\mu) \quad (24)$$

where $w(v)$ is the fractional density of the total initial population N_0 which have velocities between v and $v + dv$. Obviously, $w(v)$ satisfied the normalization condition

$$\int_0^\infty w(v) dv = 1. \quad (25)$$

It follows from equations 22, 24, and 25 that

$$n(\mu, 0) = N_0\delta(\mu) \quad (26)$$

which is similar to equation 14, the analogous condition for the solution via the von Foerster equation. However, it is to be noted that both the equation to be solved and the boundary condition are different and simpler than in the von Foerster case. The solution to equations 2, 23, and 24 is given by equation 12 with $\lambda = 0$, $p = 2$, and $g(\mu) = N_0w(v)\delta(\mu)$, or,

$$n_v(\mu, t) = N_0w(v) \sum_{j=1}^{\infty} 2^{j-1}\delta(j-1+\mu-vt). \quad (27)$$

The number of cells dividing in the time interval t to $t + dt$ is $N_m(t) dt$ where $N_m(t)$ is defined by

$$N_m(t) = \int_0^{\infty} vn_v(1, t) dv. \quad (28)$$

In particular, the number of cells of the first generation observed dividing per unit time is obtained from equations 27 and 28 as

$$N_{m1}(t) = \frac{N_0}{t^2} w\left(\frac{1}{t}\right). \quad (29)$$

Again, if we think of $N_{m1}(t)$ as experimentally determined, then equation 29 determines the weighting function w directly. Comparison with equation 19 makes clear the difference in interpretation of the experimental results which is presented by the two points of view.

By substituting equation 27 into 22 we obtain the complete solution

$$n(\mu, t) = \sum_{j=1}^{\infty} n_j(\mu, t) = N_0 \sum_{j=1}^{\infty} \frac{2^{j-1}}{t} w\left(\frac{j-1+\mu}{t}\right). \quad (30)$$

A further integration over μ yields the following expression for the total population as a function of time,

$$N(t) = \sum_{j=1}^{\infty} N_j(t) = N_0 \sum_{j=1}^{\infty} 2^{j-1} \int_{(j-1)/t}^{j/t} w(\xi) d\xi. \quad (31)$$

The j th term in the sum is identifiable as the contribution to the total population at time t of the j th generation, $N_j(t)$. For early times, it can be seen from the integration limits that the contribution made by later generations arises from cells with very large maturation velocities.

An alternative and perhaps intuitively clearer point of view is to think of the total

population as being made up of cells with different generation times rather than different maturation velocities. Thus if we set $T = 1/v$, then T may be called the generation time of a cell population whose velocity of maturation is v . The cell population as a function of generation times is represented by the distribution function $u(T)$ in place of $w(v)$. Instead of equation 22 we write

$$n(\mu, t) = \int_0^\infty n_T(\mu, t) dT. \quad (32)$$

The function $n_T(\mu, t)$ satisfies equation 2 with v constant and $\lambda = 0$, and the boundary condition (equation 23 with the subscript v replaced by T). The initial condition becomes

$$n_T(\mu, 0) = N_0 u(T) \delta(\mu), \quad (33)$$

where $\int_0^\infty u(T) dT = 1$. Then, as in equation 27,

$$n_T(\mu, t) = N_0 u(T) \sum_{j=1}^\infty 2^{j-1} \delta\left(j - 1 + \mu - \frac{t}{T}\right). \quad (34)$$

The number of cells dividing per unit time becomes

$$N_m(t) = \int_0^\infty \frac{1}{T} n_T(1, t) dT. \quad (35)$$

Substituting equation 34 into 35 and utilizing the relation $\delta[1 - t/T] = T\delta(T - t)$, there is obtained the following expression for the number of first generation cells completing mitosis per unit time.

$$N_{m1}(t) = N_0 u(t). \quad (36)$$

This shows that the distribution function u is directly measured by $N_{m1}(t)/N_0$. The above relation is easier to comprehend than the less transparent relation for the distribution function w given by equation 29. By comparing these two expressions it is seen that $u(T) = v^2 w(v)$.

From equations 32 and 34 we obtain

$$n(\mu, t) = \sum_{j=1}^\infty n_j(\mu, t) = N_0 \sum_{j=1}^\infty 2^{j-1} \frac{t}{(j-1+\mu)^2} u\left(\frac{t}{j-1+\mu}\right), \quad (37)$$

and

$$N(t) = \sum_{j=1}^\infty N_j(t) = N_0 \sum_{j=1}^\infty 2^{j-1} \int_{t/j}^{t/(j-1)} u(\xi) d\xi. \quad (38)$$

It should be emphasized that the results which arise from utilizing a generation

time distribution are completely equivalent to those which arise from utilizing a maturation velocity distribution. The generation time distribution has been introduced merely to facilitate the comprehension of the latter.

An important consequence of equation 38 is that the greatest contribution to $N(t)$ arises from, *ceteris paribus*, those cells with the shortest generation time. Such cells ultimately dominate the population. For example, imagine that initially the population consists of one cell with generation time $T_0/2$ and 10^3 cells with generation time T_0 . This means that $N_0u(T) = \delta(T - T_0/2) + 10^3\delta(T - T_0)$ where $N_0 = 1001$. From equation 38 it may be seen that ten generations later ($t = 10T_0 +$), the two types of cell have approximately the same number, and in another ten generations the fast growing cells outnumber the slow growing cells by a factor of 10^3 . Simultaneously the mean generation time decreases monotonically, approaching the value $T_0/2$.

V. COMPARISON WITH THE DATA OF PRESCOTT

To compare the two theories with the observations of *Tetrahymena geleii* HS cells under uniform conditions which were made by Prescott (7), we first determine a continuous mathematical representation of Prescott's data for the flux of cells per unit time completing mitosis which we have called $N_{m1}(t)$ (see Fig. 1). We assume that this can be represented by a gamma-distribution:

$$N_{m1}(t) = N_0 \frac{\alpha^{\nu+1}}{t_0 \Gamma(\nu + 1)} (\tau - 1)^\nu \exp [-\alpha(\tau - 1)]. \quad (39)$$

Such a choice is suggested theoretically by a particular stochastic birth process postulated by Kendall (14) and empirically by a statistical study of the generation times of various kinds of bacteria made by Powell (15). Here we have introduced the nondimensional time $\tau = t/t_0$. The quantities t_0 , α , ν are parameters to be determined. We set $t_0 = 85$ min more or less arbitrarily, but this value is not inconsistent with the data. We require the mean generation time defined by

$$\bar{t} = \frac{1}{N_0} \int_0^\infty N_{m1}(t)t dt \quad (40)$$

to have the value found by Prescott, $\bar{t} = 111$ min. Substituting equation 39 into 40, there results the expression

$$t = t_0 \left(1 + \frac{\nu + 1}{\alpha} \right), \quad (41)$$

which is a constraint on the parameters. Utilizing equation 41 to eliminate α from equation 39, we are left with the single parameter ν to determine. Its value as de-

terminated by the method of least squares applied to the data shown in Fig. 1 is $\nu = 5.318$. The resulting gamma-distribution is also shown in Fig. 1.

Equation 39 when combined with 20 determines $m(t)$, the fractional probability per unit time that a cell undergoes mitosis:

$$m(t) = \begin{cases} 0 & \tau < 1, \\ \frac{y(t)\Gamma(\nu + 1)}{\Gamma[\nu + 1, \alpha(\tau - 1)]} & \tau > 1. \end{cases} \quad (42)$$

Here $y(t)$ is defined by equations 19 and 39, Γ as a function of one argument is the complete gamma function, and Γ as a function of two arguments is the incomplete

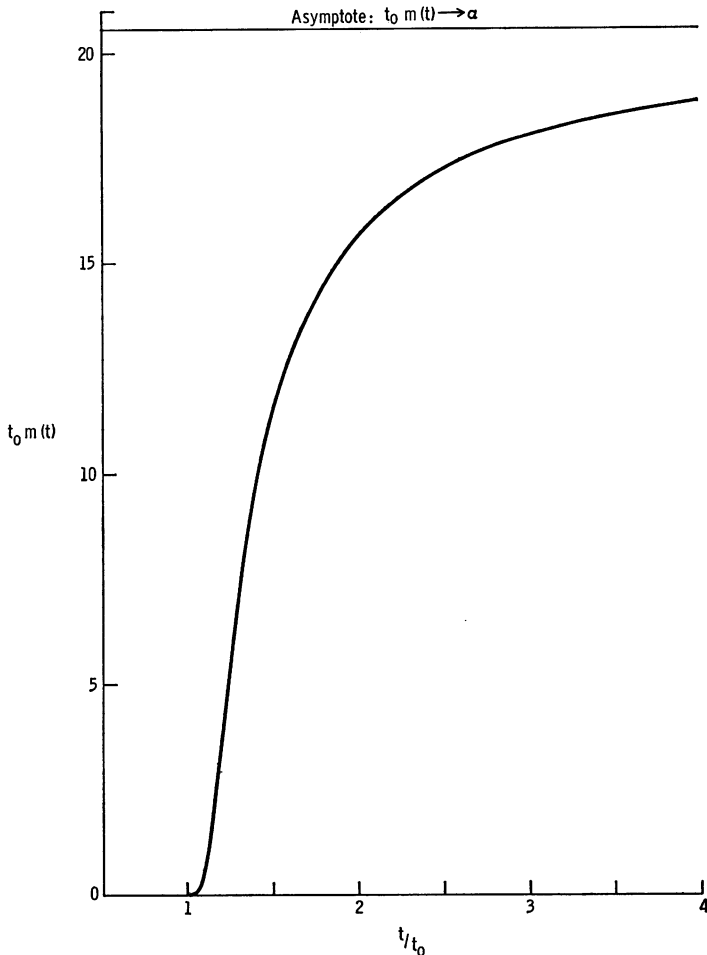


FIGURE 3 The function $m(t)$, which is the nondimensional probability per unit time that a cell undergoes mitosis, in the age-time representation. This function is determined by $N_{m1}(t)$, the solid curve shown in Fig. 1. The asymptotic value of $t_0 m(t)$ is $\alpha = 20.545$.

gamma function. The nondimensional form of $m(t)$ is shown plotted in Fig. 3. The time here is more properly thought of as age, i.e. time from moment of birth of a particular cell. As indicated in the figure, when $t \rightarrow \infty$, m approaches the limiting value α/t_0 .

Actually, the function $m(t)$ is not required for the subsequent calculation of $N(t)$. As is seen by equation 21, it is only necessary to know $y(t)$ and the function $N_1(t)$ which is given by the expression

$$N_1(t) = N_0 \begin{cases} 1 & \tau < 1, \\ \frac{\Gamma[\nu + 1, \alpha(\tau - 1)]}{\Gamma(\nu + 1)} & \tau > 1. \end{cases} \quad (43)$$

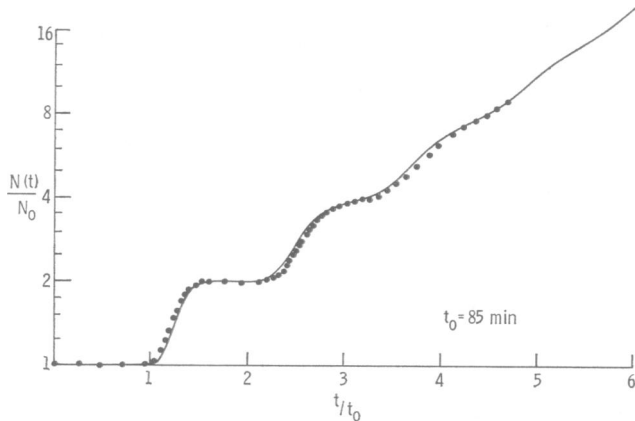


FIGURE 4. The total population as a function of time that is theoretically predicted by the maturity-time representation (shown as a solid line). The data of Prescott are repeated from Fig. 2.

The total population as a function of time as determined by equation 21 is shown as the solid line in Fig. 2. The agreement of this curve with the experimental data of Prescott can not be said to be too good. Therefore, the concept that an individual cell has no memory of the generation time of its parent but is subject to the same probability of mitosis as a function of age as every other cell is not supported.

We shall now apply the theoretical results of the maturity-time model discussed in the previous section to Prescott's data. In doing so we are naturally assuming that the initial cell population consists of a set of subpopulations each with different maturation velocities. These velocities are assumed to persist for a given cell line. If these assumptions represent the true state of the cells, one could validly question how such a population could arise, in view of the natural dominance that cells with the shortest generation time have over all others. A possible answer to this question is that the real cell population is a homogeneous one with a variety of maturation velocities which are the result of random variation, but on a time scale which is large compared to a generation time. Thus, over a few generations there would be persistence of memory of parental generation time but this memory would gradually

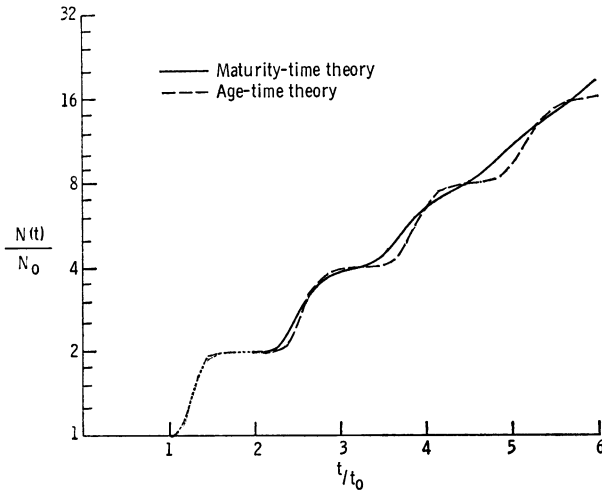


FIGURE 5a. The theoretical growth curves in the age-time and maturity-time representations are compared. Note the greater degree of synchrony that is retained by the cells according to the age-time theory.

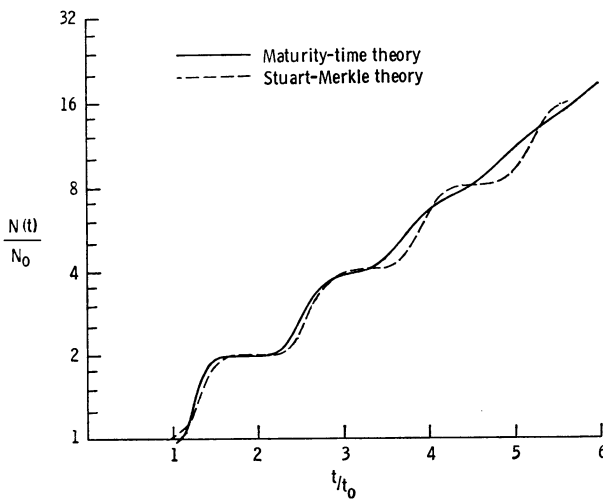


FIGURE 5b The theoretical growth curve according to the Stuart-Merkle theory (taken from reference 6) as compared to the growth curve for the maturity-time representation. The former curve is very similar to that of the age-time representation.

disappear on a longer time scale. Then the model may be considered to be an approximate treatment of such a homogeneous population which is only valid for a few generations. Another possibility is that, in the real cell population, the offspring of all cells with a given generation time have random generations which have a unimodal continuous distribution about a mean value which is the same as that of the parent generation. In such a case, too, the model presented may be expected to be a

simple yet more or less reasonable approximation to reality over a time span of a few generations or more.

In the maturity-time representation, $N_{m1}(t)$ as given phenomenologically by equation 39 determines the generation time distribution function $u(t)$, according to equation 36. With $u(t)$ known, the total population as a function of time $N(t)$ is obtained by means of equation 38. It is shown plotted as a function of time in Fig. 4. The data of Prescott as given in Fig. 2 are repeated here. These data appear to agree very well with the prediction of the theory. Note especially the rapid approach of the curve to what is nearly a straight line, i.e. the rapid loss of synchrony of the total population. Hence we conclude that these cells by and large do have memory of the generation

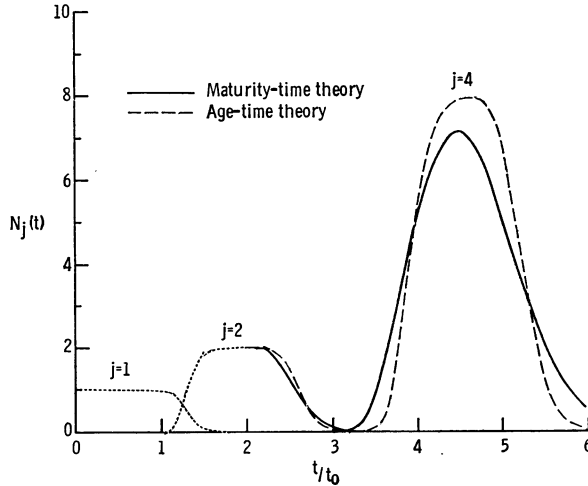


FIGURE 6 A comparison of the individual generation populations $N_j(t)$ according to the age-time representation, equation 20 and the maturity-time representation, equation 37. The function $N_1(t)$ is exactly the same in both theories. Note that for the maturity-time theory, the population for the case $j = 4$ shows more dispersion about the mean value than does the population for the age-time theory.

time of their parents, fast (slow) growing cells arising from fast (slow) growing parents.

This conclusion does not accord with that of Prescott, who considered the cells to be genetically equivalent. He suggested that the variation in generation times represents inequalities in the initial configuration of sister cells at birth: differences in the number of mitochondria, microsomes, etc., or perhaps cell weight. He supports this conclusion by data (reference 7, Table I) which indicates that rarely do two sister cells have the same generation time, although their generation times are highly correlated, according to Powell (15). Nevertheless, the agreement between theory and experiment shown in Fig. 4 suggests that there is in fact a high degree of correlation between the generation times of parent and offspring in these cells. This conclusion is also supported by the investigation by Hughes (16) of the generation times of successive generations of *E. coli* cells. He found that the growth rate of these bacteria is

inherited, by selecting fast and slow growing cells and observing the growth rate of their offspring. It should also be mentioned that Powell (17) found a positive association between the generation times of bacterial cells of various kinds which were related by descent, although the association was surprisingly weak between parent and daughter cells.

The two theoretical curves for $N(t)$ are repeated for comparison purposes in Fig. 5a. It is perhaps a little surprising that $N(t)$ in the age-time description is more "step-like," and therefore the population is more synchronous, than in the maturity-time description. The physical explanation for this is that the individual generations in the age-time description maintain the same average generation time, while in the maturity-time description, they do not. This point is also illustrated in Fig. 6, which shows the time dependence of some of the individual generations in the two representations.

Finally, we reproduce the theoretical curve for the total population as a function of time according to the theory of Stuart and Merkle, as taken from reference 6. This is shown in Fig. 5b, where we repeat for comparison the theoretical curve according to the maturity-time representation. It is noteworthy that the result of the Stuart-Merkle theory is virtually the same as that of the age-time description. Here, too, the fact that all cells according to the theory have the same average generation time produces a rather synchronized population in the early generations.

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REFERENCES

1. SCHERBAUM, O., and G. RASCH. 1957. *Acta Pathol. Microbiol. Scand.* **41**:161.
2. VON FOERSTER, H. 1959. In *The Kinetics of Cellular Proliferation*. F. Stohlman, Jr., editor. Grune & Stratton, Inc., New York. 382.
3. TRUCCO, E. 1965. *Bull. Math. Biophys.* **27**:285.
4. TRUCCO, E. 1965. *Bull. Math. Biophys.* **27**:449.
5. NOONEY, G. C. 1967. *Biophys. J.* **7**:69.
6. STUART, R. N., and T. C. MERKLE. 1965. Calculation of Treatment Schedules for Cancer Chemotherapy, Part II. Univ. of California E. O. Lawrence Radiation Laboratory Report UCRL-14505. Univ. of California, Berkeley, Calif.
7. PRESCOTT, D. M. 1959. *Exptl. Cell Res.* **16**:279.
8. BELL, G. I., and E. C. ANDERSON. 1967. *Biophys. J.* **7**:329.
9. OLDFIELD, D. G. 1966. *Bull. Math. Biophys.* **28**:545.
10. FREDRICKSON, A. G., D. RAMKRISHNA, and H. M. TSUCHIYA. 1967. *Math. Biosci.* **1**:327.
11. SOMMERFELD, ARNOLD. 1954. *Optics*. Academic Press, Inc., New York. 49.
12. BARRETT, J. C. 1966. *J. Natl Cancer Inst.* **37**:443.
13. LIPKIN, M., and E. DESCHNER. 1968. *Exptl. Cell Res.* **49**:1.
14. KENDALL, D. G. 1948. *Biometrika.* **35**:316; 1952. *J. Roy. Stat. Soc. Ser. B.* **14**:41.
15. POWELL, E. O. 1955. *Biometrika.* **42**:16.
16. HUGHES, W. H. 1955. *J. Gen. Microbiol.* **12**:265.
17. POWELL, E. O. 1958. *J. Gen. Microbiol.* **18**:382.