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A Theory for the Age and Generation Time Distribution of a Microbial Population

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Summary

A theory is presented for the time evolution of the joint age and generation time distribution of a microbial population. By means of the solution to the fundamental equation of the theory, the effect of correlations between the generation times of mother and daughter cells may be determined on the transient and steady state growth stages of the population. The relationships among various generation time distributions measured under different experimental circumstances is clarified.

1. Introduction

A striking property of microbial populations, even when grown under constant environmental conditions, is the great variability of the generation times of individual members of the population, illustrated in Fig. 1. The cause of this variability remains an important unsolved biological problem. The variability is generally ascribed to intrinsic variable factors associated with cell growth and maturation, culminating in division [1].

Prescott [2] has suggested that the variability stems from variability in the initial state of a newborn cell, where initial state means cell weight, number of mitochondria, microsomes, etc. Variability in the initial state of the chromosome is likewise a fundamental feature of the Cooper-Helmstetter model for chromosome replication in E. coli [3]. In both of these views, the generation time is determined at birth.

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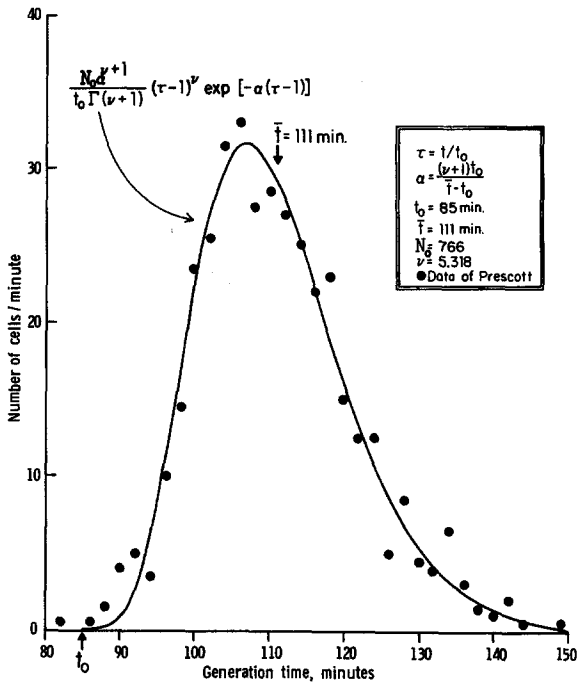

Fig. 1. The solid points represent the number of cells entering mitosis as a function of time, where $t=o$ represents cells of age 0, for 766 cells of the *HS* strain of *Tetrahymena geleii* cells observed by Prescott [2]. The solid line is a fitted curve of the form of equation (48), taken from reference 8. Such a skewed curve is a typical generation time distribution function for microbial populations

Rahn [4], on the other hand, viewed the division process as consisting of a large number of primitive biochemical steps, each step subject to probabilistic variation. This idea was further developed by Kendall [5]. In this view, all cells are initially identical, and variability is a consequence of the stochastic nature of the primitive steps. Essentially this latter viewpoint, that the generation time is inherently probabilistic and not determined at birth, underlies the age-time formalism [6, 7]. Here, cells may undergo division at any age a, with a certain probability independent of their history.

A different view of variability is presented in the maturity-time formalism [8], in which a cell density function is introduced which depends on maturity and time rather than age and time. In this formalism, the variability of generation times is ascribed to variability, whether stochastic or otherwise, in the velocity of maturation of different cells comprising the population, the final maturation state being exactly the same for all cells. This point of view has also been advocated by Kubitschek [9]. In the present work, we shall adopt the viewpoint that the generation time of a cell is an inherent characteristic which is essentially determined at the time of its birth.

Whatever the point of view adopted, the question naturally arises as to how the generation time of a parent cell is related to the generation times of its daughters. In the context of cell population theory, this question becomes the following: given a subpopulation of cells all with the same generation time, what is the distribution of generation times of their daughters? This problem has attracted the attention of many biologists. Thus, Powell [10] and Kubitschek [11] found, by looking at individual bacterial cells, that there was only a weak correlation between the generation times of mothers and daughters, although the data were not completely consistent [12, 13]. The existence of mother-daughter correlations can also be inferred by studying the development in time of a culture started under some specified conditions. Thus, Hughes [14] found that from a culture of E. coli *Br* cells derived from a single cell, it is possible to select out populations which grow either more or less rapidly than the parent strain, under the same environmental conditions. In other words, fast or slow growing mothers had, on the average, fast or slow growing daughters, respectively.

Similarly in the investigations of Prescott [2], the colonies originating from many different cells were followed in time. These measurements were then combined to describe the evolution of a single population by setting the time origin for each colony at the first division time of the mother cell. This yielded very precise measurements of the growth in time of a population of *tetrahymema geleii HS* cells. These measurements could also serve as a quantitative test of the relationship between the generation times of a cell population, and the generation times of its progeny.

In analysing this work, Rubinow [8] investigated the two following extreme hypotheses, as it were, concerning the generation-time relationship between mothers and daughters: 1. Cell generation times are random, and each newly formed cell is governed by the same probability density function for determining its generation time. In other words, there is no correlation between the generation times of mothers and daughters. With such a model, the probability for a newborn cell to divide when it has reached an age τ is the same for all cells at all times. 2. There exists a precise correlation between the generation times of mothers and daughters, in particular, the generation time of a daughter cell is exactly the same as the generation time of its mother. With this hypothesis, the heterogeneity in the generation times of the initial cell population determines the future distribution of generation times.

In the investigation, the distribution of generation times of the initial cell population was taken as given and the subsequent population growths predicted by the two hypotheses were compared with the observed growth. The somewhat surprising result of the comparison was that the second model agreed better with the observations of Prescott than the first model. Thus, it was concluded that the "maturation velocity" of a given cell tends to persist from mother to daughter for several generations at least. It was clear that this tendency could not persist forever, for if it did, the observed generation-time distribution function would not be an intrinsic property of the population. In fact, a generation time distribution would never be observed, because ultimately, the cells with the shortest generation time would dominate the population exclusively.

This conclusion, therefore, left unresolved an important theoretical question: how to introduce a birth law connecting generation times of mothers and daughters that would be compatible with both long-time and short-time kinetic behavior of cell populations. That is to say, what is the birth law which permits sufficient determinism between mothers and daughters so that their generation times may on the average be essentially the same, and sufficient indeterminism so that, after the establishment of long-time steady exponential growth, a distribution of generation times remains a characteristic invariant feature of the cell population.

In the theory we shall present, we assume that the cell population under consideration is characterized by the independent variables age a, time t, and the generation-time τ. The introduction of the variable τ permits relationships between the generation times of mothers and daughters that are not otherwise possible in the usual age-time formalism. Let $n(a, t; \tau)\, da d\tau$ denote the number of cells at time t whose age, measured from "birth", lies between a and $a+da$, and whose life-time (generation-time) lies between τ and $\tau+d\tau$. In other words, when the cell reaches the age τ it will undergo division producing two daughter cells of age zero. Clearly $n(a, t; \tau)$ is defined only for $0 \leqq a \leqq \tau$.

The time evolution of the density function $n(a, t; \tau)$ will depend on the distribution of generation times of newborn cells. We assume here that this distribution depends only on the generation time of the mother cells. This distribution is specified by giving the transition probability $K(\tau, \tau')$, where $K(\tau, \tau')\, d\tau$ is the fraction of cells born from mothers with generation time τ', which will have a generation time between τ and $\tau+d\tau$. The population is assumed to grow in some fixed environment which does not change with time, i.e. $K(\tau, \tau')$ is constant, independent of time, during the whole evolution.

This formulation enables us to clarify and present, from a unified point of view, the relationships between the different type of measurements which can be carried out on such populations. A comprehensive review of past work on the mathematics of microbial populations is found in the work of Painter and Marr [15]. Of particular interest are the steady state values of what Powell [16] calls the "generation time frequency" function $f(\tau)$ and the "population generation time distribution" function $P(\tau)$. We shall refer to the latter more simply as the generation-time distribution function, and denote it by $g(\tau)$. If there are correlations between mothers and daughters, then $K(\tau, \tau')$ is not a function of τ alone, and these distributions are different, a distinction indicated by Powell [16, 17].

These functions and other quantitative features of bacterial growth are defined and discussed in section 2. In section 3 we present the theory for the cell density function. We have solved the fundamental equation for n for both the long time and short time behavior, starting from a cell cohort of arbitrarily given ages and generation times. By means of this solution we are able to clarify further the meaning of generation time measurements of a given population, and their relationship to the functions $f(\tau)$ and $g(\tau)$.

The long-time solution is discussed in section 4. We generalize some previous results which relate the steady state age distribution of a growing cell population to the generation-time distribution function $g(\tau)$. In section 5, we discuss how the transition kernel $K(\tau, \tau')$ can be inferred from experiment. The generation-time frequency function $f(\tau)$ and the generation-time distribution function $g(\tau)$ are found in section 6 for a special assumed form of the kernel function K.

2. Quantitative Features of Microbial Populations

A microbial population growing in an ideal constant environment will, after some transient period achieve a *steady state of exponential growth*. By "ideal" we mean that not only are environemental conditions such as nutritional requirements, pH, and temperature kept constant, but the bacterial spatial density is not permitted to get too great so that effects of "crowding" are negligible. In this state the number of organisms in the total population, $N(t)$, will increase asymptotically as $e^{\gamma t}$, where γ is a positive constant. At the same time, the fraction of the population whose age is in a given age interval will approach a steady state value independent of the time. The growth constant γ, and the steady state age distribution $\bar{n}(a)$, where a is the age of a given cell, are intrinsic properties of the system which depend on the environment but are independent of the initial conditions, i.e. on the way the culture was initiated.

Another intrinsic property of a microbial population growing in a steady state is the generation-time distribution function, determined as follows. Select from a steady state population, at some specified time, a cohort of cells all of which are of age 0, i.e., they are newly formed. There exist experimental procedures for doing this [18]. Determine the fraction of these cells which undergo cell division in the subsequent time interval τ to $\tau + d\tau$. This number is set equal to $g(\tau)\, d\tau$, and the function $g(\tau)$ so determined is called the steady state *generation-time distribution function.*

In addition to the steady state of "exponential growth" described above, usually achieved by the batch culture method of growth, there is also the "continuous culture" method for growing cells. Here bacteria are grown in a chemostat or turbidostat which is continuously diluted so as to maintain a fixed total number of cells in the apparatus. We shall refer to cells grown in such a manner as being in the *steady state of the chemostat*, or simply the chemostat steady state. The generation time distribution, defined as above, of cells grown in the chemostat steady state was shown by Powell [16] to be intimately connected to that obtained in the batch culture steady state. Both of these steady states of growth correspond to special cases of the general state of growth which we consider herein.

The *generation-time frequency function* $f(\tau)$ mentioned earlier may be defined operationally as follows. In a batch culture select for observation some cells, in an arbitrary manner. Wait until these selected cells divide. Of course, the cells

do not all divide at the same time. Measure the times elapsed until the daughter cells divide again. Call $f_2(\tau)\,d\tau$ the fraction of the second generation or daughter cells for which the time elapsed between birth and subsequent division is between τ and $\tau+d\tau$. Repeat this procedure for the newborn or third generation cells, and call $f_3(\tau)\,d\tau$ the fraction of these cells with a generation time between τ and $\tau+d\tau$. Continue this process for N generations. Since for each cell in the first generation there are two cells in the second generation, four cells in the third, etc., define formally the average function $f(\tau;N)=\sum_{j=2}^{N} 2^{j-1} f_j(\tau) / \sum_{j=2}^{N} 2^{j-1}$. For N large, $f(\tau;N)$ will presumably approach a limit, $f(\tau)$. Equivalently, the $f_j(\tau)$ will approach a limiting form $f(\tau)=\lim_{j\to\infty} f_j(\tau)$. As pointed out by Powell, $f(\tau)$ will be the same as $g(\tau)$ only if there are no correlations between the generation times of mother and daughter cells. If such correlations do exist, $f(\tau)$ and $g(\tau)$ will be different. Powell also noted the $f(\tau)$ would coincide with $g(\tau)$ in a population in which one of the daughters of each dividing cell was removed from the population at birth. We shall later give explicit examples of $g(\tau)$ and $f(\tau)$ for some simple illustrative forms of the kernel $K(\tau,\tau')$.

We shall investigate the intimate connections among $K(\tau,\tau')$, the growth constant γ, the steady state age distribution $\bar{n}(a)$, and the generation-time distribution function $g(\tau)$. Given the latter three quantities from observation, these relations constitute consistency requirements which restrict but do not uniquely determine the transition probability $K(\tau,\tau')$. In the absence of a direct experimental determination of $K(\tau,\tau')$ for a given cell population, the functional form of $K(\tau,\tau')$ may be prescribed somewhat arbitrarily. For certain special assumptions regarding this functional form, the available relations among the known observables and $K(\tau,\tau')$ can serve to uniquely determine K. Thus, if K is assumed to be a function of τ only, which implies that there is no correlation between mother and daughter generation times, then K is uniquely determined by $g(\tau)$, and the theory may be shown to be equivalent to the age-time formalism. We shall investigate in some detail a particular functional form of K in section 6. This form of K can represent a birth law which is compatible both with the short-time behavior inferred from Prescott's experiments, and with the existence at large times of a generation-time distribution function.

3. Mathematical Model of Cell Populations

The basic equation governing the cell density function $n=n(a,t;\tau)$ is assumed to be the following,

$$\frac{\partial n}{\partial t}(a,t;\tau)+\frac{\partial n}{\partial a}(a,t;\tau)=-\lambda n(a,t;\tau)\quad 0<a\leqq\tau, \tag{1}$$

where λ is the fractional loss of cells per unit time, due to death or disappearance, but not cell division. This meaning is not the same as that for the similar term appearing in the age-time formalism where λ also represents "losses" due to cell

division. We assume here that λ may depend on a but is independent of τ. We could in principle consider λ also to be a function of time, in order to represent special circumstances, but we shall not do so here. The function n satisfies a boundary or birth condition, given as

$$n(0, t; \tau) = 2 \int_0^\infty K(\tau, \tau')\, n(\tau', t; \tau')\, d\tau'. \tag{2}$$

Because mother cells give birth to daughter cells which have associated with them some generation time τ, the corresponding probability of such birth must be unity. Therefore, the transition probability K satisfies the normalization condition

$$\int_0^\infty K(\tau, \tau')\, d\tau = 1. \tag{3}$$

Equation (1) may be viewed as a flux equation which states that, in the absence of cell death, the age of a cell increases uniformly with time. Equation (2) expresses the contribution to new cells of age zero and generation time τ from mothers of all different generations. The factor 2 on the right hand side of (2) reflects the assumption that, when a cell divides, it produces 2 daughter cells. The density function n must also satisfy an initial condition,

$$n(a, 0; \tau) = \varphi(a; \tau), \tag{4}$$

where φ is a prescribed function of a and τ.

Before going on to find the solution of (1) we note that by choosing λ appropriately, (1) represents different experimental conditions. Thus, $\lambda = 0$ corresponds to the growth of cells in a batch culture in which there are no cell deaths. In this case the system will approach, as t increases, a steady state of exponential growth for which $n(a, t; \tau) \to e^{\gamma_0 t}\, n(a; \tau)$, where γ_0 and $n(a; \tau)$ are determined by K. If, on the other hand, we set λ equal to a constant independent of a, and this constant equal to γ_0, we represent a continuous culture grown in a chemostat in which the dilution is such as to maintain a constant number of cells. Then, as t approaches infinity, $n(a, t; \tau)$ approaches a time independent function we denote by $n_c(a; \tau)$, where the subscript c indicates chemostat conditions. In general, any constant λ leads to a population growth proportional to $\exp[(\gamma_0 - \lambda)t]$. Making $\lambda > \gamma_0$ corresponds to extinction of the population, a situation we shall not consider here.

We shall now give the solution to equation (1) by means of the generation expansion, given by Rubinow [8]. Thus, let

$$n(a, t; \tau) = \sum_{j=1}^{\infty} n_j(a, t; \tau), \tag{5}$$

where $n_j(a, t; \tau)$ is the age-time generation density function associated with the j-th generation. The function n_1 representing the initial cohort of cells or first generation, chosen to satisfy the initial condition $n_1(a, 0; \tau) = \varphi(a; \tau)$, has the form

$$n_1(a,t;\tau)=\varphi(a-t;\tau)\exp\left[-\int_{a-t}^{a}\lambda(\xi)\,d\xi\right],\ 0\leqq t\leqq a\leqq\tau. \tag{6}$$

For $j\geqq 2$ we have

$$\begin{aligned} n_j(0,t;\tau)&=2\int_0^\infty K(\tau,\tau')\,n_{j-1}(\tau',t;\tau')\,d\tau',\\ n_j(a,t;\tau)&=n_j(0,t-a;\tau)\exp\left[-\int_0^a\lambda(\xi)\,d\xi\right],\ t>a. \end{aligned} \tag{7}$$

Using (6) in (7), we obtain the more explicit result

$$n_2(a,t;\tau)=2\int_{t-a}^{\infty}K(\tau,\tau')\,\varphi(\tau'+a-t;\tau')\exp\left[-\int_{\tau'+a-t}^{\tau'}\lambda(\eta)\,d\eta-\int_0^a\lambda(\xi)\,d\xi\right]d\tau', \tag{8}$$

$$\begin{aligned} n_j(a,t;\tau)&=2\int_0^{t-a}K(\tau,\tau')\,n_{j-1}(\tau',t-a;\tau')\exp\left[-\int_0^a\lambda(\xi)\,d\xi\right]d\tau',\ t>a,\\ &\qquad j=3,4,5,\ldots. \end{aligned} \tag{9}$$

The solution (5—9) expressed in terms of generations may be expected to be useful if the behavior of the population is desired for a period of several generations or so following the initial time, i. e. the short-time behavior.

We introduce here the function $\psi_j(\tau)$ defined as

$$\psi_j(\tau)\equiv\int_0^\infty n_j(0,t;\tau)\,dt,\ j\geqq 2, \tag{10}$$

which represents the number of cells of the j-th generation, born at any time which have a generation time τ. When $\lambda\neq 0$, some of these cells will of course not survive until age τ and thus will not produce any offspring. Using (7) and (8) in (10), we find that

$$\psi_j(\tau)=2\int_0^\infty K(\tau,\tau')\exp\left[-\int_0^{\tau'}\lambda(\xi)\,d\xi\right]\psi_{j-1}(\tau')\,d\tau',\ j>2, \tag{11}$$

with $\psi_1(\tau)$ defined by the expression

$$\psi_1(\tau)=\int_0^\tau\varphi(x;\tau)\exp\left[-\int_x^\tau\lambda(\xi)\,d\xi\right]dx. \tag{12}$$

Now when $\lambda=0$, the population doubles with each generation, so that the total number of cells in the j-th generation is $N_0\,2^{j-1}$, where $N_0=\int_0^\infty d\tau\int_0^\tau\varphi(a;\tau)\,da$ is the number of cells present at $t=0$. Hence, for $\lambda=0$, $\psi_j(\tau)/N_0\,2^{j-1}$ is the fraction of cells in the j-th generation with generation time τ, and is denoted as $f_j(\tau)$. From (11), $f_j(\tau)$ satisfies the equation

$$f_j(\tau)=\int_0^\infty K(\tau,\tau')\,f_{j-1}(\tau')\,d\tau,\ j\geqq 2, \tag{13}$$

where $f_1(\tau)\equiv\psi_1(\tau)/N_0$.

As $j\to\infty$, $f_j(\tau)\to f(\tau)$, with $f(\tau)$ determined by the equation

$$f(\tau)=\int_0^\infty K(\tau,\tau')f(\tau')\,d\tau'. \tag{14}$$

$f(\tau)$ is the generation-time frequency function discussed in section 2.

Hence, if K is known, then $f(\tau)$ is in principle determined by this equation. For example if $K(\tau,\tau')=k(\tau)$, which implies there is no correlation between the generation times of mother and daughter cells, cells, then $f_j(\tau)=k(\tau)=f(\tau)$ for all $j\geqq 2$, because, by definition, $\int_0^\infty f_j(\tau)\,d\tau=\int_0^\infty f(\tau)\,d\tau=1$. If however $K(\tau,\tau')$ does depend on τ', then $f_j(\tau)$ for small j is not equal to $f(\tau)$ even when the initial sample $\varphi(a;\tau)$ is taken from a population in a steady state of exponential growth. The relationship between $f(\tau)$, $g(\tau)$ and $k(\tau)$ is further clarified by the specific example discussed in section 6.

4. The Long-Time Solution

A solution to equation (1) which is useful for investigating the long-time behavior of the population will now be presented. First, we note that if we go over from $n(a,t;\tau)$ to $y(a,t;\tau)$ by means of the transformation

$$n(a,t;\tau)=y(a,t;\tau)\exp\left[-\int_0^a \lambda(\xi)\,d\xi\right], \tag{15}$$

then $y(a,t;\tau)$ satisfies the equation

$$\frac{\partial y}{\partial t}+\frac{\partial y}{\partial a}=0, \tag{16}$$

subject to the boundary condition

$$y(0,t;\tau)=2\int_0^\infty K(\tau,\tau')\,y(\tau',t;\tau')\exp\left[-\int_0^{\tau'}\lambda(\xi)\,d\xi\right]d\tau', \tag{17}$$

and the initial condition

$$y(a,0;\tau)=\varphi(a;\tau)\exp\left[\int_0^a \lambda(\xi)\,d\xi\right]. \tag{18}$$

Because of the simple form of equation (16), it is obvious than an arbitrary function of $(t-a)$ formally satisfies the equation, and that

$$y(a,t;\tau)=y(0,t-a;\tau),\quad t>a. \tag{19}$$

Hence, from equation (18), a solution which satisfies the initial condition and which is valid for $a>t$ is

$$y(a,t;\tau)=\varphi(a-t;\tau)\exp\left[\int_0^{a-t}\lambda(\xi)\,d\xi\right],\quad a>t. \tag{20}$$

We rewrite equation (17) as

$$y(0,t;\tau)=2\int_0^t K(\tau,\tau')\,y(\tau',t;\tau')\exp\left[-\int_0^{\tau'}\lambda(\xi)\,d\xi\right]d\tau' \\ +2\int_t^{\infty} K(\tau,\tau')\,y(\tau',t;\tau')\exp\left[-\int_0^{\tau'}\lambda(\xi)\,d\xi\right]d\tau'. \tag{21}$$

In the first integral on the right, we replace $y(\tau', t; \tau')$ by the expression (19), valid for $t>\tau'$,

$$y(\tau',t;\tau')=y(0,t-\tau';\tau'),\quad t>\tau'. \tag{22}$$

In the second integral on the right we replace $y(\tau', t; \tau')$ by the expression (20) which is valid for $\tau'>t$,

$$y(\tau',t;\tau')=\varphi(\tau'-t;\tau')\exp\left[\int_0^{\tau'-t}\lambda(\xi)\,d\xi\right],\quad \tau'>t. \tag{23}$$

Combining (19) and (21)–(23), we obtain the result

$$y(a,t;\tau)=2\int_0^{t-a} K(\tau,\tau')\,y(0,t-a-\tau;\tau')\exp\left[-\int_0^{\tau'}\lambda(\xi)\,d\xi\right]d\tau' \\ +2\int_{t-a}^{\infty} K(\tau,\tau')\,\varphi(\tau'+a-t;\tau')\exp\left[\int_0^{\tau'+a-t}\lambda(\xi)\,d\xi\right]d\tau',\quad t>a. \tag{24}$$

Combining (20) and (24) with (15) leads to the formal solution, alternative to (5)—(9),

$$n(a,t;\tau)=\begin{cases}\varphi(a-t;\tau)\exp\left[-\int_{a-t}^{a}\lambda(\xi)\,d\xi\right], & t<a,\\ 2\exp\left[-\int_0^a\lambda(\xi)\,d\xi\right]\left\{\int_0^{t-a}K(\tau,\tau')\,n(0,t-a-\tau';\tau')\exp\left[-\int_0^{\tau'}\lambda(\xi)\,d\xi\right]d\tau'\right. \\ \qquad\left.+\int_{t-a}^{\infty}K(\tau,\tau')\,\varphi(\tau'+a-t;\tau')\exp\left[\int_0^{\tau'+a-t}\lambda(\xi)\,d\xi\right]d\tau'\right\} & t>a.\end{cases} \tag{25}$$

Equation (25) is useful for examining the behavior of the solution for large times. Thus, we expect that, for a population which is growing or at least maintaining itself, the density function $n(a, t; \tau)$ will have the asymptotic form

$$n(a,t;\tau)\sim e^{\gamma t}\,n(a;\tau),\quad t\to\infty, \tag{26}$$

with $n(a;\tau)$ independent of the initial distribution $\varphi(a;\tau)$ for $\gamma\geqq 0$. Here $n(a;\tau)\,da d\tau$ is the fraction of cells in the steady state whose generation times lie between τ and $\tau+d\tau$, and whose ages are between a and $a+da$. Substituting (26) into (25), dividing by $e^{\gamma t}$, and letting $t\to\infty$, we obtain for the asymptotic steady state distribution,

$$n(a;\tau)=\begin{cases}C\exp\left[-\gamma a-\int_0^a\lambda(\xi)\,d\xi\right]g(\tau), & a\leqq\tau,\\ 0, & \tau<a,\end{cases} \tag{27}$$

$$g(\tau)=2\int_0^\infty K(\tau,\tau')\exp\left[-\gamma\tau'-\int_0^{\tau'}\lambda(\xi)\,d\xi\right]g(\tau')\,d\tau'. \tag{28}$$

Here

$$g(\tau)\equiv n(0;\tau)/\int_0^\infty n(0;\tau)\,d\tau \tag{29}$$

is defined as the generation-time distribution of newly formed cells in the steady state, and C is a normalization constant chosen so as to make $\int_0^\infty da\int_0^\infty n(a;\tau)\,d\tau=1$. This function is the generation-time distribution function discussed in section 2.

The growth rate γ and the generation time distribution function $g(\tau)$ are determined by $K(\tau,\tau')$ through (28). By integrating (28) with respect to τ, we obtain the result

$$1=2\int_0^\infty \exp\left[-\gamma\tau'-\int_0^{\tau'}\lambda(\xi)\,d\xi\right]g(\tau')\,d\tau'. \tag{30}$$

Essentially this same result is attributable to early investigators in population biology such as Lotka and Euler [19].

The normalized age distribution in the steady state, $\bar{n}(a)$ is the age distribution of cells in the steady state without regard to their generation time. From (27), we find that

$$\bar{n}(a)\equiv\int_a^\infty n(a;\tau)\,d\tau=C\exp\left[-\gamma a-\int_0^a\lambda(\xi)\,d\xi\right]\int_a^\infty g(\tau)\,d\tau. \tag{31}$$

The above expressions simplify when $\lambda(a)=\lambda_0$, a constant independent of a. Setting $\gamma+\lambda_0=\gamma_0$, the growth rate when there are no losses, (28), (30) and (31) now assume the forms

$$g(\tau)=2\int_0^\infty K(\tau,\tau')\,e^{-\gamma_0\tau'}\,g(\tau')\,d\tau', \tag{32}$$

$$1=2\int_0^\infty e^{-\gamma_0\tau}\,g(\tau)\,d\tau, \tag{33}$$

$$\bar{n}(a)=2\gamma_0\,e^{-\gamma_0 a}\int_a^\infty g(\tau)\,d\tau. \tag{34}$$

These results reduce to those of Powell and others [15, 17] for the case when $\lambda=0$. Note that the results can be interpreted as representing either the case of batch culture without losses for which $\gamma=\gamma_0$ and $\lambda=0$, or to the case of continuous culture in the chemostat, for which $\lambda=\gamma_0$ and $\gamma=0$.

We see that the cell population density function $n(a,t;\tau)$ contains all the information of interest regarding the kinetics of a cell population, whether it relates to the observable distribution functions $\bar{n}(a)$, $f(\tau)$, or $g(\tau)$. Many useful theoretical results relating to the steady state were derived by Powell. However,

because these were not related to or presented in the context of a cell density function as in the present work, Powell found it necessary to introduce new distribution functions to discuss particular aspects of his investigations. The relationship among these distribution functions is not always apparent. Here, we show how some of these functions are easily obtained from the cell density function.

For example, $h(\tau)$, the fraction of cells in the steady state (of whatever age) with generation times between τ and $\tau+d\tau$, is defined by the expression

$$h(\tau)\equiv\int_0^\tau n(a;\tau)\,da. \tag{35}$$

This function is not the same as the generation function $g(\tau)$. In fact, the relation between them is readily obtained for the case when λ is a constant by substituting (27) and (34) into (35). The result is

$$h(\tau)=2\left[1-e^{-\gamma_0\tau}\right]g(\tau), \tag{36}$$

in agreement with Powell [17].

The fraction of cells in the steady state undergoing mitosis per unit time with generation times between τ and $\tau+d\tau$ is denoted the "carrier distribution" by Powell [16]. It is easily seen from (27) and (34) that $c(\tau)$ is defined in terms of n as

$$c(\tau)\equiv\frac{n(\tau;\tau)}{\int_0^\infty n(\tau;\tau)\,d\tau}. \tag{37}$$

When λ is constant, it follows immediately with the aid of (27) that

$$c(\tau)=2\,e^{-\gamma_0\tau}\,g(\tau). \tag{38}$$

The rate of appearance of cells in the steady state with generation time τ is given as $h(\tau)\frac{dN}{dt}$. This must equal $[2\,g(\tau)-c(\tau)]\frac{dN}{dt}$, where $2\,g(\tau)\frac{dN}{dt}$ represents the rate of appearance of cells of generation time τ, and $c(\tau)\frac{dN}{dt}$ represents the rate of disappearance of cells of age τ. Hence,

$$h(\tau)=2\,g(\tau)-c(\tau). \tag{39}$$

This relation may be directly verified when λ is constant with the aid of equations (36) and (38).

We note here that when λ is constant, $h(\tau)/g(\tau)=2\left(1-e^{-\gamma_0\tau}\right)$ is less than one for $\tau<(ln\,2)/\gamma_0$, the doubling time, and greater than one for larger values of τ. This relation corresponds to the fact that a cell picked at random is less likely to have a short generation time than a newborn one. It is this difference between $g(\tau)$ and $h(\tau)$ which is responsible for the fact that when cells are selected from the population at random, irrespective of age, the generation time distribution of their progeny (which we called $f_2(\tau)$ previously) will not be equal, when $K(\tau,\tau')$ depends on τ',

to $g(\tau)$, which represents the fraction of cells newly born at some specified time with generation time τ. This difference will be illustrated by a particular example in section 6.

5. The Transition Kernel K (τ, τ')

We have tacitly assumed here that, starting from an arbitrary initial distribution $n(a, 0; \tau)$: (1) $\lim_{t\to\infty} [e^{-\gamma t} n(a, t; \tau)] = n(a; \tau)$ exists, and (2) $n(a; \tau)$ is independent of the form of $n(a, 0; \tau)$. The validity of these assumptions depends on the transition probability $K(\tau, \tau')$ having certain "smoothing" properties, e.g. for each τ', $K(\tau, \tau')$ should be a "smooth" function of τ. We expect K to have such properties in all realistic cases and shall not investigate here in any detail the precise necessary and sufficient conditions on $K(\tau, \tau')$ for this to be true. (The interested reader is referred to the paper of Harris [20] for a discussion of these questions in a somewhat different context.) We note however that a necessary condition for a stationary asymptotic state to exist in accordance with assumption (2), is clearly that (28) have a unique solution $g(\tau)$ which is non-negative and normalizable. If the solution of (28) is not unique, then it is certainly possible for $n(a, t; \tau)$ to remember the initial state for all times.

For example, if $K(\tau, \tau') = \delta(\tau - \tau')$, which corresponds to one of the possibilities of cell growth considered by Rubinow [8], namely that each daughter cell has exactly the same generation time as its mother, then with λ constant, (28) has the family of solutions $g(\tau) = \delta(\tau - \hat{\tau})$ with $2e^{-\gamma\hat{\tau}} = 1$, and $\hat{\tau}$ arbitrary. In this case it is intuitively clear that the progeny of the cells with the shortest generation time in the initial colony will always become dominant after a long time, as has previously been pointed out [8].

An example for $K(\tau, \tau')$ for which our assumptions are valid is the following,

$$K(\tau, \tau') = k(\tau), \tag{40}$$

a function independent of τ' (and not containing any delta functions). This assumption corresponds to the other hypothesis considered by Rubinow of no correlations between mothers and daughters.

We shall indicate that with this assumption, the theory can be reduced to the ordinary age-time formalism. Thus, consider the age-density function which is independent of generation times, defined as

$$\tilde{n}(a, t) \equiv \int_a^\infty n(a, t; \tau)\, d\tau, \quad t \geqq 0. \tag{41}$$

Then, if the initial distribution $n(a, 0; \tau) = \varphi(a, \tau)$ has the special form

$$\varphi(a, \tau) = \tilde{n}(a, 0) \frac{k(\tau)}{\int_a^\infty k(\tau)\, d\tau}, \tag{42}$$

where $\tilde{n}(a, 0)$ is given by (41), then it is readily shown by integrating equation (1) over τ from a to ∞ that $\tilde{n}(a, t)$ is the solution of the age-time equation [7]

$$\frac{\partial \tilde{n}}{\partial t}+\frac{\partial \tilde{n}}{\partial a}=-\tilde{\lambda}\tilde{n},$$
$$\tilde{\lambda}=\lambda(a)+\lambda_m(a), \tag{43}$$
$$\lambda_m(a)=\frac{k(a)}{\int_a^\infty k(\tau)\,d\tau}.$$

Here $\lambda_m(a)$ represents the disappearance rate due to cell division. In addition, $\tilde{n}(a, t)$ satisfies the boundary condition

$$\tilde{n}(0, t)=2\int_0^\infty \tilde{n}(a, t)\,\lambda_m(a)\,da, \tag{44}$$

and an initial condition in accordance with (41), namely

$$\tilde{n}(a, 0)=\int_a^\infty \varphi(a;\tau)\,d\tau. \tag{45}$$

With this correspondence, the short-time solution (5—9) becomes

$$\tilde{n}(a, t)=\sum_{j=1}^{\infty}\tilde{n}_j(a, t),$$

$$\tilde{n}_1(a, t)=\tilde{n}(a-t, 0)\exp\left[-\int_{a-t}^{a}\lambda(\xi)\,d\xi\right]\frac{\int_a^\infty k(\tau)\,d\tau}{\int_{a-t}^\infty k(\tau)\,d\tau},$$

$$=\tilde{n}(a-t, 0)\exp\left[-\int_{a-t}^{a}\tilde{\lambda}(\xi)\,d\xi\right],\quad t<a, \tag{46}$$

$$\tilde{n}_2(a, t)=2\int_{t-a}^{\infty}\lambda_m(\tau')\,\tilde{n}_1(\tau', t-a)\,d\tau'\exp\left[-\int_0^a\tilde{\lambda}(\xi)\,d\xi\right],\quad t>a,$$

$$\tilde{n}_j(a, t)=2\int_0^{t-a}\lambda_m(\tau')\,\tilde{n}_{j-1}(\tau', t-a)\,d\tau'\exp\left[-\int_0^a\tilde{\lambda}(\xi)\,d\xi\right],\quad j\geqq 3,\ t>a.$$

In deriving these expressions, we have made use of the relations

$$n(a, t; a)=\tilde{n}(a, t)\,\frac{k(a)}{\int_a^\infty k(\tau)\,d\tau}$$

and (47)

$$\int_a^\infty k(\tau)\,d\tau=\exp\left[-\int_0^a\lambda_m(\xi)\,d\xi\right].$$

These results generalize some formulas of Rubinow [8] for the special case $\tilde{n}(a, 0) = N_0 \delta(a)$. The solution (46) could of course be derived quite readily directly from equations (43—45).

We shall turn now to the question posed in the introduction, namely, can a $K(\tau, \tau')$ be found which has the feature that over a period of several generations or so, it tends to make the newborn population remember the generation time of its ancestors, while over long times, it diffuses that memory so that its generation distribution function is as shown in Fig. 1. In principle, $K(\tau, \tau')$ could be experimentally determined, although the task for a given bacterial species would undoubtedly be very arduous. It would require the selection of a subpopulation of cells of a given generation time τ', and the determination of the generation times of their offspring. This procedure would then have to be repeated for many different values of τ'.

Rather less arduous is the determination of the generation-time distribution function $g(\tau)$. It can be accomplished directly by selecting a cohort N_0 of newborn cells from a population of cells growing in a steady state of exponential growth, and then observing the times at which these cells divide. An alternative procedure is to measure the total population of this cohort as a function of time, for a period which is greater than the minimum generation time τ_0, and less than the minimum time for the appearance of granddaughter cells, $2\tau_0$. Then the total population of the cohort $N(t)$ is given by the expression $N(t) = N_0 \left[1 + \int_0^t g(\tau)\, d\tau\right]$, so that $g(t) = N_0^{-1}\, dN(t)/dt$, for $t < 2\tau_0$. Although this procedure does not determine $g(\tau)$ for all values of τ, in practice it may be expected that $g(\tau)$ is negligible for $\tau \geqq 2\tau_0$. Such a procedure was utilized by Harvey [21] utilizing some data of Helmstetter [22]. The method, however, is beset by the difficulty of finding the derivative to a curve which must be fitted to data points having associated experimental errors.

We assume that such an experimental determination of $g(\tau)$ has been made. A typical skewed distribution curve is shown in Fig. 1, taken from the work of Rubinow [8], and based on data of Prescott [2] for the *HS* strain of *Tetrahymena geleii* cells. The manner of obtaining the data was not sufficiently described by Prescott for us to determine whether $f_j(\tau)$, $f(\tau)$, $g(\tau)$, or some other distribution function was actually measured. But no matter, these distribution functions are all similar, and for our present purposes, it is sufficient to assume that it represents $g(\tau)$. The solid line in the Fig. is given by the following expression [8], a gamma distribution with a time delay τ_0,

$$g(\tau) = \frac{\alpha^{\nu+1}}{\Gamma(\nu+1)} (\tau - \tau_0)^{\nu} e^{-\alpha(\tau - \tau_0)}, \quad \tau \geqq \tau_0, \tag{48}$$

where $\tau_0 = 85$ min., $\nu = 5.318$, $\alpha = (\nu+1)/(\bar{\tau} - \tau_0)$, and $\bar{\tau} = 111$ min., the mean generation time.

Given $g(\tau)$, the following theoretical question arises: to what extent is $K(\tau, \tau')$ prescribed. Obviously, since $g(\tau)$ is a function of only one variable, we cannot expect that it will, by itself, uniquely determine $K(\tau, \tau')$ which is a function of two variables.

We shall therefore assume for illustrative purposes a certain form for $K(\tau, \tau')$, such that it is essentially a function of only a single variable. Taking into account mathematical convenience and biological reasonableness, we choose K to be of the form

$$K(\tau, \tau') = \beta\, \delta(\eta - \eta') + (1-\beta)\, k(\eta - \rho\eta'). \tag{49}$$

Here $\eta = \tau - \tau_0$, $\eta' = \tau' - \tau_0$, so that $\eta, \eta' \geqq 0$, β and γ are constants, $0 \leqq \beta < 1$, and $k(x)$ is a non-negative normalized function defined for $x \geqq 0$, so that $\int_0^\infty k(x)\, dx = 1$. Equation (49) states, essentially, that for cells with a given generation time τ', a certain fraction β of them give rise to progeny with the same generation time τ', while the remaining fraction give rise to progeny with generation times which are "shifted" in accordance with the function $k(\eta - \rho\eta')$.

Equation (32) is a constraint that the function k must satisfy. Setting $g(\tau) = \varphi(\eta)$, which is the form of equation (48), equation (32) with the substitution of equation (49) for K becomes the following,

$$\varphi(\eta) = 2\, e^{-\gamma_0 \tau_0} \left[\beta\, e^{-\gamma_0 \eta}\, \varphi(\eta) + (1-\beta) \int_0^{\eta/\rho} k(\eta - \rho\eta')\, e^{-\gamma_0 \eta'}\, \varphi(\eta')\, d\eta' \right] \tag{50}$$

Taking the laplace transform of (50) yields

$$\tilde{\varphi}(s) = 2\, e^{-\gamma_0 \tau_0} \left[\beta \tilde{\varphi}(s + \gamma_0) + (1-\beta)\, \tilde{k}(s)\, \tilde{\varphi}(\rho s + \gamma_0) \right] \tag{51}$$

where $\tilde{\varphi}(s) = \int_0^\infty e^{-s\eta}\, \varphi(\eta)\, d\eta$ and $\tilde{k}(s) = \int_0^\infty e^{-s\eta}\, k(\eta)\, d\eta$ are the laplace transforms of $\varphi(\eta)$ and $k(\eta)$, respectively. Solving equation (51) for $\tilde{k}(s)$ yields

$$\tilde{k}(s) = \left[\tilde{\varphi}(s) - 2\beta\, e^{-\gamma_0 \tau_0}\, \tilde{\varphi}(s + \gamma_0) \right] / \left[(1-\beta)\, 2\, e^{-\gamma_0 \tau_0}\, \tilde{\varphi}(\rho s + \gamma_0) \right]. \tag{52}$$

We note that, for a given $\varphi(\eta)$, γ_0 is determined by (33) via the relation

$$\tilde{\varphi}(\gamma_0) = \tfrac{1}{2}\, e^{\gamma_0 \tau_0} \tag{53}$$

This equation will always have a unique solution γ_0, because $\tilde{\varphi}(s)$ is a monotone decreasing function of s with $\tilde{\varphi}(0) = 1$ and $\tilde{\varphi}(\infty) = 0$ provided $\varphi(\eta)$ does not behave like $\delta(\eta)$ as $\eta \to 0$.

Equation (52) gives the laplace transform of $k(x)$, for a given $\varphi(\eta)$, β and ρ. In order that the $k(x)$ so obtained be "acceptable", we must be sure that $k(x) \geqq 0$ for $x \geqq 0$. This condition imposes some constraints on β and ρ. It is always possible to choose $\beta = 0$, $\rho = 0$, and $k(\eta) = \varphi(\eta)$, corresponding to there being no correlation between the generation times of a cell and that of its offspring. As previously indicated, such a choice does not seem biologically reasonable, and also does not fit the data of Prescott if $N(t)$ is followed beyond the second generation [8]. Indeed if we require that $k(x)$ should not behave like $\delta(x)$ as $x \to 0$, then we must have $\tilde{k}(s) \to 0$ as $s \to \infty$. Hence, whenever $\tilde{\varphi}(s)$ behaves as Cs^{-b} when $s \to \infty$, with C and b some positive constants, which indeed is the case for $g(\tau)$ given in (48), and $\rho \neq 0$, we must choose $\beta = \frac{1}{2}\, e^{\gamma_0 \tau_0} = \tilde{\varphi}(\gamma_0)$. We shall consider the case $\rho = 0$ in the next section.

For $g(\tau)$ given by (48), $\tilde{\varphi}(s)$ is readily calculated to be

$$\tilde{\varphi}(s)=\frac{\alpha^{\nu+1}}{(\alpha+s)^{\nu+1}}. \tag{54}$$

Substituting the above equation into (52) yields for $\tilde{k}(s)$ the following explicit form,

$$\tilde{k}(s)=\frac{\alpha^{\nu+1}[(\alpha+s+\gamma_0)^{\nu+1}-(\alpha+s)^{\nu+1}][\alpha+\gamma_0+\rho s]^{\nu+1}}{[(\alpha+\gamma_0)^{\nu+1}-\alpha^{\nu+1}](\alpha+s+\gamma_0)^{\nu+1}(\alpha+s)^{\nu+1}}, \tag{55}$$

where γ_0 is determined by the relation $2e^{-\gamma_0\tau_0}=(1+\gamma_0/\alpha)^{\nu+1}$ and ρ is an undetermined parameter which can be chosen to fit other experimental data, such as the behavior of $N(t)$ at later times.

The inverse laplace transform of (55) can be found simply whenever ν is an integer. For $\nu=0$ we find

$$k(x)=\frac{\alpha[\gamma_0+(1-\rho)\alpha]}{\alpha+\gamma_0}e^{-x}\left[1-\frac{(1-\rho)(\gamma_0+\alpha)}{\gamma_0+(1-\rho)\alpha}e^{-\gamma_0 x}\right], \tag{56}$$

with $x=\eta-\rho\eta'\geqq 0$. We see that $k(x)$ will be non-negative as long as $\gamma_0+(1-\rho)\alpha>0$. In this case, $K(\tau,\tau')$ is given by (49) and (56) as

$$K(\tau,\tau')=\beta\delta(\tau-\tau')+(1-\beta)k[\tau-\tau_0-\rho(\tau'-\tau_0)], \tag{57}$$

with $k(x)$ given by (56).

6. The Relation between g (τ) and f (τ)

To illustrate the relation between the functions $g(\tau)$ and $f(\tau)$ explicitly in a particular context, we assume a particularly simple form of $K(\tau,\tau')$ obtained by setting $\rho=0$ in (49), namely,

$$K(\tau,\tau')=\beta\delta(\tau-\tau')+(1-\beta)k(\tau),\quad 0\leqq\beta<1, \tag{58}$$

Here, for the sake of generality, we have not necessarily restricted k to be zero for $\tau<\tau_0$. Substituting (58) into (13), we find immediately that

$$f_j(\tau)=\beta^{j-1}f_1(\tau)+[1-\beta^{j-1}]k(\tau),\quad j\geqq 1. \tag{59}$$

Letting $j\to\infty$, we see that $f(\tau)=k(\tau)$, independent of β, for $\beta<1$. $g(\tau)$ is determined by (50), and with $\rho=0$, it becomes

$$g(\tau)=\frac{(1-\beta)k(\tau)}{1-2\beta e^{-\gamma_0\tau}}. \tag{60}$$

Here γ_0 is determined by the normalization condition

$$(1-\beta)\int_0^\infty \frac{k(\tau)\,d\tau}{1-2\beta e^{-\gamma_0\tau}}=1, \tag{61}$$

equivalent to (33).

We note that (60) is a valid solution only if $\beta < 1/2$. Otherwise, $g(\tau)$ becomes negative for some small value of τ. This restriction is relaxed partially or completely if a minimum generation time τ_0 is introduced. We see from (60) that $g(\tau)$ is smaller than $f(\tau)$ especially for cells with large generation times.

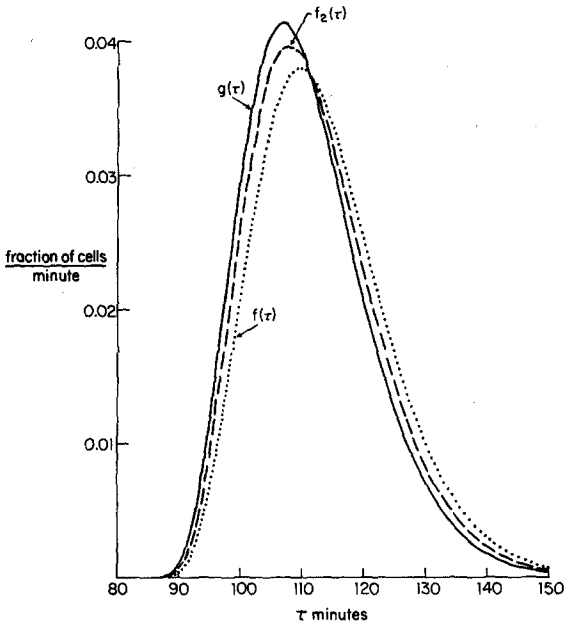

Fig. 2. The functions $g(\tau)$ (solid line), $f(\tau)$ (dotted line), and $f_2(\tau)$ (dashed line) are illustrated for the same population in steady exponential growth. These curves are based on equations (48), (60), and (64), respectively, with β arbitrarily set equal to 0.8

Consider now an experiment in which N_0 cells are selected at random from a population in steady exponential growth, for which λ is constant, and with K given by (58). Call the time of selection $t=0$. The cell density function at $t=0$ is then $n(a, 0; \tau) = \varphi(a, t) = n(a; \tau)$ where, according to (27), the generation distribution of the selected cells is

$$n(a; \tau) = C e^{-\gamma_0 a} g(\tau), \quad a \leqq \tau, \tag{62}$$

and $g(\tau)$ is given by (60). From (12), (27), (30), and the definition of $f_1(\tau)$, it follows that

$$f_1(\tau) = 2(1 - e^{-\gamma_0 \tau}) g(\tau) = 2(1 - e^{-\gamma_0 \tau}) \frac{(1-\beta) k(\tau)}{1 - 2\beta e^{\gamma_0 \tau}}. \tag{63}$$

The $f_j(\tau)$ for $j \geqq 2$ can now be computed from (59), thus,

$$f_2(\tau) = (1-\beta)[1+2\beta-4\beta e^{-\gamma_0 \tau}] k(\tau)/(1-2\beta e^{-\gamma_0 \tau}) = [1+2\beta-4\beta e^{-\gamma_0 \tau}] g(\tau), \quad (64)$$

$$\begin{aligned} f_3(\tau) &= (1-\beta)[1+\beta+2\beta^2-2\beta(1+2\beta) e^{-\gamma_0 \tau}] k(\tau)/(1-2\beta e^{-\gamma_0 \tau}) \\ &= [1+\beta+2\beta^2-2\beta(1+2\beta) e^{-\gamma_0 \tau}] g(\tau), \end{aligned} \quad (65)$$

and so forth. These equations show explicitly that when cells are selected at random from a population even in steady exponential growth, the distribution functions $f_j(\tau)$, $g(\tau)$, and $f(\tau)$ are distinct. This aspect is illustrated in Fig. 2 where the curves for $g(\tau)$, $f(\tau)$, and $f_2(\tau)$ are shown, with $g(\tau)$ given by equation (48). The curves for $f(\tau) = k(\tau)$ and $f_2(\tau)$ are based on equations (60) and (64), respectively, with the value of β arbitrarily chosen to be 0.8.

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