Statistics and Dynamics of Procaryotic Cell Populations*

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ABSTRACT

The formulation of a mathematical theory of a cell population requires the ability to specify quantitatively the physiological state of individual cells of the population. In procaryotic cells (bacteria and blue-green algae), the intracellular structure is of a relatively simple nature, and it is postulated that the physiological state of such a cell is specified by its biochemical composition. If we postulate further that the growth rate of a cell and its fission probability depend only on the cell's current physiological state and on the current state of the cell's environment, then an equation of change for the distribution of physiological states in a population can be derived. In addition, an equation of change for the state of the cellular environment can be obtained. These equations allow us to predict the statistical and dynamical behavior of a cell population from information obtained by analysis of cellular and subcellular structure and function.

INTRODUCTION

The problem of describing quantitatively the phenomena occurring during the proliferation of a population of organisms has been considered for a long time. The name of Malthus is associated with the law of exponential growth, and attempts to develop mathematical models of population growth have appeared frequently since his time.

Populations of unicellular organisms, which reproduce essentially asexually by binary fission, have been favorite subjects for such attempts,

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since many of the complications inherent in the multicellular or coenocytic type of organization or in sexual reproduction are not present therein. It is, in fact, only with such unicellular populations that the present paper is concerned. Moreover, mutation will not be considered.

The early investigators of microbial growth perceived regularities in the growth and death of microbial populations that led them to seek the so-called equation of the growth curve; that is, the quantitative description of the increase of cell number in a population inhabiting a (partially) closed living space. It soon became apparent, however, that there was no *unique* equation of the growth curve. Rather, the experimental growth curve for a given organism could be altered at will by changing environmental factors or even by changing the history or number of cells used to start the population. Thus, the growth curve obtained when a population was started with a given number of cells from an "old" culture was markedly different from that obtained when the population was started with the same number of cells from a "young" culture; see, e.g., Henrici [19]. Undoubtedly, the nonuniqueness of the growth curve has been a strong factor contributing to the "indifferent attitude" (Lamanna and Mallette [26], page 379) of biologists toward theoretical attempts to "explain" the growth curve.

The advance of experimental microbiology beyond simple counting techniques introduced a further complication for theories of population dynamics: not all individuals in a population are equivalent, even in a genetically homogeneous population. For instance, there is a distribution of cell sizes and a distribution of cell life-spans in any population. (The life-span of a cell is the duration of time between the instant the cell was formed by fission of its parent and the instant the cell itself divides. That various authors use other terms, e.g., generation time, to denote what is here called the life-span should be remembered.) Moreover, the nature of such distributions changes as the population develops (see, e.g., Henrici [19]). A description of distributions of characters in pure-culture microbial populations is little considered in current microbiology, in spite of the fact (for instance) that understanding of many selectional mechanisms must be based on a knowledge of distributions of characters. It is only in comparatively recent years that the problem of describing distributions of characters throughout a microbial population has been considered from the theoretical point of view, and the models used for this purpose are so simple as not to reflect current experimental knowledge concerning subcellular structure and dynamics.

A further complication for theories of population dynamics arises from the interaction of a population with its environment. Obviously, growth must alter the cellular environment, both by the removal of chemical substances necessary for formation of new protoplasmic material and metabolism and by release of by-products of synthesis and metabolism. It is also well known that the nature of the cellular environment affects both the nature and rate of population growth. In constructing a theory of population dynamics, these interactions are usually ignored, but to do so places severe restrictions on the theory.

The problem considered in this paper is that suggested by the foregoing discussion. We try to formulate a theory that recognizes that the growth curve is not unique because growth itself is not unique, but rather depends on factors, both internal and external, that must themselves be described by the theory. The theory must acknowledge that cells are not all equivalent and that in any population there is a distribution of cellular characters. The theory should establish, in principle, what that distribution is and how it changes in dynamic situations. Finally, the theory must account for the effect of growth on the cellular environment and the reciprocal effect of the cellular environment on growth.

A closed set (i.e., closed in the sense that the number of independent equations of the theory equals the number of unknown variables) of equations constituting a theory meeting the foregoing requirements is presented herein. The equations are no more than one *possible framework* for a theory of microbial population dynamics. Possible is emphasized because it is not known to what extent the concepts developed herein will be adequate for the task at hand; framework is emphasized because the theory will yield quantitative predictions about a specific experimental situation only if hypotheses more specific than those made here are advanced. In other words, the equations contain unknown *functions* (as opposed to unknown *constants*) that must be determined by experiment or made the subjects of appropriate models of cellular behavior that can be tested by experiment.

Despite these disclaimers, the theory developed has value for a number of reasons: First, it provides a yardstick for the assessment of other, less general theories. That is, the theory may make it possible to discard a contemplated model or to state the conditions under which it could be valid. Examples will be given. Second, the theory provides a different perspective from which to view the general problem of cellular growth and fission. As such, it ought to suggest new experiments to be done.

Third, the theory provides a possible framework for a quantitative description of microbial population dynamics. In this regard, the theory could also be extended to cover the interaction of two or more populations inhabiting the same living space (i.e., be extended to provide the basis for a quantitative microecology). Finally, the theory provides a new set of equations having application in many other, nonbiological situations. Mathematicians might possibly find the study of these equations rewarding and interesting.

THE PHYSIOLOGICAL STATE VECTOR

Although this paper is concerned with cell populations, it is first necessary to discuss cellular and subcellular structure and function, since the behavior of single cells determines, together with the environment, the behavior of the population.

By the term state of a cell is meant the chemical composition of structures of the various levels of organization within the cell, and the geometrical arrangement of such structures with respect to each other. According to current mechanistic views of life, knowledge of a cell's state and of conditions in the cell's environment are sufficient to determine the rates and kinds of activities carried on by the cell.

Obviously, a *quantitative* specification of state is a prerequisite for any theory of cellular behavior that depicts or suggests the *modus operandi* of cellular phenomena.* Clearly, complete specification of the state of a cell, as just defined, would be impossible to obtain. Hence, plausible simplifying hypotheses must be made.

The view of cellular state taken here is almost exclusively a biochemical one, and one that is more or less indifferent to the existence of levels of organization within the cell. Thus, let $Z_i(t)$ represent the amount of the ith biochemical entity (nucleic acid, protein, lipid, etc.) in a single cell selected at random** from a population of interest at time t. Specifica-

* Thus, consider the phenomenon mentioned earlier, in which inoculum taken from a young culture leads to a shorter lag phase than does an equal amount of viable inoculum from an old culture. The terms young and old are simply convenient ways to summarize experimental observations; they do not tell us what mechanism is responsible for the altered lag phase duration. Hence, terms such as young and old do not give a quantitative specification of cell state.

** Thus, $Z_i(t)$ is a random variable. We adhere to the usual convention of denoting random variables by capital letters; particular values of a random variable will be denoted by the corresponding lowercase letter.

tion of all of the Z_i is then nothing more than specification of the biochemical composition of the cell at time t. Notice that Z_i is taken without regard to level of organization or location within the cell.

Denote the ordered set of numbers $Z_1(t), Z_2(t), \ldots$, by $\mathbf{Z}(t)$, as,

$$\mathbf{Z}(t) = \begin{bmatrix} Z_1(t) \\ Z_2(t) \\ \vdots \end{bmatrix}. \tag{2.1}$$

(Vectors will be denoted throughout by boldface letters, as is customary. No distinction with regard to notation will be made for vectors belonging to different vector spaces.) The fundamental simplifying hypothesis made here is that knowledge of the set of quantities embodied in $\mathbf{Z}(t)$ is sufficient to specify, in a restricted sense, the current rates and kinds of biological activities occurring within the cell, if similar knowledge of conditions in the environment currently surrounding the cell is available.* Hence, we call $\mathbf{Z}(t)$ the *physiological state vector*** because, by hypothesis, it defines the physiological activity of the cell at time t.

The theory based on the fundamental simplifying hypothesis (fsh) just made will clearly be inadequate to handle situations in which there is appreciable "compartmentalization" (Moses and Lonberg-Holm [32]) of subcellular activities, with transport resistances between compartments. Presumably, the state in this case would be specified by a *matrix* if only two levels of subcellular organization—compartments (organelles) and molecules—are considered. That is, the rows of the matrix would identify the various molecules of interest and the columns of the matrix would identify the various compartments of the cell; see, e.g., Horovitz and Nooney [21].

We do not pursue the matrix description of state further in this paper for several reasons. First, it introduces difficulties such as the question whether organelles arise *de novo* or from the fission of existing organelles. Second, it does not do away with the problem of describing levels of organization but merely transfers it to lower (subcellular) levels.

* The fundamental simplifying hypothesis comes close to, if indeed it is not identical with, that which Dean and Hinshelwood ([7], page 9) call the "principle of total integration."

** State vectors have been used by various authors in other ways; see, e.g., Hahn [16]. Obviously, the various meanings should not be confused.

Thus, may compartments themselves be compartmented? Third, it seems important to work out the consequences of the simpler, i.e., vector, description of state before proceeding to the more complex, i.e., matrix, description of state. Finally, it is possible that in bacteria and the blue-green algae, the so-called *procaryotic cells* (Stanier and van Niel [47] state that E. Chatton first used the terms procaryotic and eucaryotic in 1937), the compartmentalization is so slight as to render the vector description of state adequate. This is certainly suggested by the remark of Stanier and van Niel that "*No unit of structure smaller than the cell in its entirety is recognizable as the site of either metabolic unit process*" (i.e., respiration and photosynthesis; emphasis in the original [47], page 25). Hence, we postulate that the vector description of physiological state is adequate for procaryotic cells and leave the development of a matrix description of physiological state for future work on eucaryotic cells.

In spite of the stated limitations of the vector description of state, it is not so lame and halt that it cannot carry the burden of accommodating many of the phenomena of population growth. For instance, Dean and Hinshelwood ([8], page 546) note that the cell seems to partake of the nature of a chemical compound, a complex macromolecule, a colloidal particle, a "bag of enzymes," of a "system containing various substances engaged in an integrated set of chemical reactions within a suitably permeable wall." The vector description of state is not inconsistent with any of these interpretations. Again, the vector description of state can accommodate such phenomena as feedback control of enzyme induction (protein synthesis or removal of repression), diauxie phenomena, altered metabolic pathways, the occurrence of a lag phase, and the dependence of the lag phase duration on past history of the inoculum. It can even accommodate a transport resistance (or assistance) across the cell membrane. Of course, whether it can do these things with quantitative correctness is a question for experiments to decide.

DEFINITIONS AND HYPOTHESES

In order to develop a set of equations for the description of growth of a population, it is necessary to introduce certain definitions and to make hypotheses of a less general character than the fsh. This section deals with such definitions and hypotheses.

As a first hypothesis, we assume that the population and the population environment under consideration are spatially homogeneous and disregard

the inhomogeneities found by ecologists. It is not necessary to make this hypothesis, since we could consider events occurring in an infinitesimal amount of living space. However, this complicates the mathematics somewhat, and since in practice essentially spatially homogeneous conditions can be obtained in the laboratory, the first hypothesis is advanced.

Consider a cell selected at random from a spatially homogeneous population at time t. The physiological state $\mathbf{Z}(t)$ of this cell will be a random vector. The totality of all possible physiological state vectors defines *physiological state space*, which we denote by $\mathfrak{B}$. The boundary of $\mathfrak{B}$ is denoted by $\mathfrak{S}$.

We are interested in the probability that $\mathbf{Z}(t)$ will be contained in a certain small "volume" $d\mathfrak{v}$ of physiological state space; hence, we define a density function* $f_{\mathbf{Z}}(\mathbf{z}, t)$ such that

$$P[\mathbf{Z}(t) \in d\mathfrak{v} | H] = f_{\mathbf{Z}}(\mathbf{z}, t)\, d\mathfrak{v} \qquad (3.1)^{**}$$

where the density function satisfies the normalizing condition

$$\int_{\mathfrak{B}} f_{\mathbf{Z}}(\mathbf{z}, t)\, d\mathfrak{v} = 1. \qquad (3.2)$$

Of course, integration is a multiple integration, carried out over all of physiological state space. In Eq. (3.1), the probability of occurrence of a state is indicated as being conditional on a set of hypotheses H. These are the totality of hypotheses that constitute the theory of growth developed in this paper.

Let $n(t)$ be the population density at time t; that is, $n(t)$ is the number of individuals present, per unit amount of living space, at time t. (Questions may arise as to whether a cell is one individual or two individuals; i.e., when does fission occur? Presumably, this difficulty can be overcome by a definition; a definition, that fortunately, need not be made at this stage of development.) As a second hypothesis, we assume that $n(t)$ is an ordinary (i.e., nonrandom) function of time. Possibly, this hypothesis would not have to be made either, since Moyal [33] has given theorems from which we could develop a theory even if $n(t)$ were taken to be a random variable.

* A density function will always be denoted by f. The random variable whose density is given thereby will be identified by a subscript.

** Herein, $d\mathfrak{v}$ is the element of volume of physiological state space $dz_1\, dz_2 \ldots$. The expression $\mathbf{Z}(t) \in d\mathfrak{v}$ indicates that $z_1 < Z_1(t) < z_1 + dz_1, z_2 < Z_2(t) < z_2 + dz_2, \ldots$

If $n(t)$ is known, then the number of cells in an amount ΔV of living space having physiological state in the volume Δv of physiological state space has *expected* value

$$n(t) f_{\mathbf{z}}(\mathbf{z}, t)\, \Delta v\, \Delta V.$$

The *actual* number in $\Delta v\, \Delta V$ would vary about expected value, with a standard deviation probably of order $n^{1/2}$.

Hence, as a third hypothesis, we assume that the population density is always sufficiently large so that the actual number of cells in $\Delta v\, \Delta V$ is given, to a sufficient degree of approximation, by the expected value. In many propagation situations, the population density is quite large, so we expect the third hypothesis to be justified therein. There are, however, important situations, such as the emergence of a mutant strain, the development of a clone, or the infection of a host by a small number of parasites, where the hypothesis would not be justified.

Let $c_1, c_2, \ldots$ be the concentrations of the various chemical substances present in the cellular environment. The c_j are to be interpreted in the usual macroscopic sense; that is, they represent time-smoothed quantities so that local concentration fluctuations on the molecular level are averaged out. (Ordinarily, this need not be mentioned, but such fluctuations may be important in that they contribute to the randomness of cellular growth rates—see the following section; we are indebted to Prof. H. O. Halvorson for this suggestion.) We then define a vector $\mathbf{c}(t)$ such that

$$\mathbf{c}(t) = \begin{bmatrix} c_1(t) \\ c_2(t) \\ \vdots \end{bmatrix}. \tag{3.3}$$

Of course, the physical factors of temperature and pressure also are important in defining the nature of the cellular environment. However, we assume that these factors are fixed in what follows, so that the vector $\mathbf{c}(t)$ defines the *state of the cellular environment.*

As a fourth hypothesis, we assume that there is no direct communication between individuals of the population. That is, there are no protoplasmic connections between cells through which influences can be transmitted. Of course, indirect communication between individuals falls within the scope of the theory, because, according to the fsh, the physiological activity of cells is determined by the physiological state

of the cell and the state of the environment. In turn, the state of the environment is influenced by the presence of cells, since these are extracting substrates from, and releasing metabolic products to, the environment.

As a final hypothesis, we specialize the fsh by assuming that the current rates of all cellular processes depend only on the current physiological state of the cell and the current state of the cell's environment (hence, the fifth hypothesis is a Markov hypothesis; see, e.g., Bharucha-Reid [4]); that is, on the two vectors $\mathbf{Z}(t)$ and $\mathbf{e}(t)$. Thus, rates of cellular processes do not depend explicitly on time or on past cell history; they do depend implicitly on time and on past cell history, since $\mathbf{Z}$ and $\mathbf{e}$ depend on time and are determined by past history. It follows from the fourth hypothesis, of course, that rates in cell A depend on $\mathbf{e}$ and the vector $\mathbf{Z}$ for cell A, but not on the vector $\mathbf{Z}$ for cell B or for any other cell.

GROWTH AND FISSION OF SINGLE CELLS

At this point, it is necessary to consider the two major phenomena of cell growth and cell division. (Maintenance and death are also discussed.) The hypotheses stated in the preceding section are to be placed in mathematical form by defining three functions related to the rates of growth and fission.

Consider cell growth first. Let $\dot{Z}_i(t)$ be the rate at which the amount of the ith biochemical entity is changing in a single cell selected at random from a population. In general, we have to assume that $\dot{Z}_i(t)$ is a random variable, not only because we select the cell at random but also because we are considering very small-scale processes, where fluctuations in rates due to molecular chaos may be important.

The *growth rate vector* $\dot{\mathbf{Z}}(t)$ of a cell is the ordered set of numbers

$$\dot{\mathbf{Z}}(t) = \begin{bmatrix} \dot{Z}_1(t) \\ \dot{Z}_2(t) \\ \vdots \end{bmatrix}. \tag{4.1}$$

Growth of a cell is thus regarded as a motion through physiological state space, the velocity of motion being defined by the growth rate vector.

According to the fsh and hypotheses four and five of the foregoing section, the growth rate vector $\dot{\mathbf{Z}}(t)$ of a cell is a function (as opposed to a functional of the history of the vectors $\mathbf{Z}$ and $\mathbf{e}$) only of the vectors $\mathbf{Z}(t)$

and $\mathbf{c}(t)$, and is not an explicit function of time. The nature of the functionality is random, however; that is, $\dot{\mathbf{Z}}(t)$ is a random function of $\mathbf{Z}(t)$ and $\mathbf{c}(t)$. Hence, we define a conditional density function $f_{\dot{\mathbf{Z}}|\mathbf{Z}}(\dot{\mathbf{z}}, \mathbf{z}, \mathbf{c})$ such that

$$P[\dot{\mathbf{Z}}(t) \in d\dot{\mathbf{v}} \,|\, \mathbf{Z} = \mathbf{z}, H] = f_{\dot{\mathbf{Z}}|\mathbf{Z}}(\dot{\mathbf{z}}, \mathbf{z}, \mathbf{c})\, d\dot{\mathbf{v}} \tag{4.2}$$

where $d\dot{\mathbf{v}}$ is the element of volume $d\dot{z}_1\, d\dot{z}_2 \ldots$ in growth rate space. The conditional density is normalized

$$\int_{\dot{\mathfrak{V}}} f_{\dot{\mathbf{Z}}|\mathbf{Z}}(\dot{\mathbf{z}}, \mathbf{z}, \mathbf{c})\, d\dot{\mathbf{v}} = 1 \tag{4.3}$$

where $\dot{\mathfrak{V}}$ is the multidimensional space defined by all possible growth rate vectors. The mean growth rate of a single cell of physiological state $\mathbf{z}$ in environment of state $\mathbf{c}$ is then the vector

$$\bar{\dot{\mathbf{Z}}}(\mathbf{z}, \mathbf{c}) = \int_{\dot{\mathfrak{V}}} \dot{\mathbf{z}} f_{\dot{\mathbf{Z}}|\mathbf{Z}}(\dot{\mathbf{z}}, \mathbf{z}, \mathbf{c})\, d\dot{\mathbf{v}}. \tag{4.4}$$

The conditional density function $f_{\dot{\mathbf{Z}}|\mathbf{Z}}$ is the first of the unknown functions that arise in the development of the theory. Either $f_{\dot{\mathbf{Z}}|\mathbf{Z}}$ is found by suitable experiments or made the subject of a growth model which in turn would have to be tested experimentally. It will turn out that not $f_{\dot{\mathbf{Z}}|\mathbf{Z}}$ but only $\bar{\dot{\mathbf{Z}}}$ is needed for our purposes.

The second major phenomenon to consider is that of cell fission. The view taken here is that fission represents the culmination of a system of rate processes that, by an appropriate definition, can be recognized as occurring at an instant in time.

By the fish and hypotheses four and five of the preceding section, the instant of fission depends on the vectors $\mathbf{Z}(t)$ and $\mathbf{c}(t)$, but not explicitly on time. Hence, we define a time-specific probability of fission $\sigma(\mathbf{z}, \mathbf{c})$ such that the probability that a cell of physiological state $\mathbf{z}$ at time t in environment of state $\mathbf{c}$ will undergo fission in the time interval t to $t + dt$ is

$$\sigma(\mathbf{z}, \mathbf{c})\, dt.$$

The time-specific probability of fission is the second of the unknown functions that arise in the development of the theory.

In connection with $\sigma(\mathbf{z}, \mathbf{c})$ it should be noted, first, that it is not subject to any normalizing condition, such as Eq. (4.3), since it is not a density function. For purposes of the theory, all that is required of $\sigma(\mathbf{z}, \mathbf{c})$ is that it be nonnegative. (It may even be unbounded if a given physiological state is certain to produce fission.)

Second, the concept of death, as used in microbiology (see, e.g., Rahn [41]), can be introduced into the theory through the function $\sigma(\mathbf{z}, \mathbf{c})$. In microbiological usage, a cell is dead if it does not divide under optimal environmental conditions. Hence death would be that condition (or subregion of physiological state space) for which $\sigma(\mathbf{z}, \mathbf{c})$ is vanishingly small, for a given $\mathbf{c}$. If $\mathbf{c}$ is changed, $\sigma(\mathbf{z}, \mathbf{c})$ may no longer be vanishingly small; this accommodates the observation that cells may sometimes be revived by placing them in a more favorable environment. However, if death is defined as lysis of the cell, then a new time-specific probability would have to be introduced into the theory.

Finally, the view taken here assigns no specific cause for cell division. Rather, it postulates that there are conditions—internal and external—that make fission more probable or less probable. Of course, if we knew the sequence of events that culminates in fission, it would be possible, in principle, to arrive at the expression for the time-specific probability of fission.

The last of the unknown functions required in the development of the theory concerns the partioning of biochemical components between daughter cells at fission. In general, it cannot be assumed that the split is "even"; rather, a distribution of partitions is assumed.

Hence, we define a partitioning function $p(\mathbf{z}, \mathbf{z}', \mathbf{c})$ such that the probability that fission of a mother cell of physiological state $\mathbf{z}'$ in an environment of state $\mathbf{c}$ will produce a daughter of physiological state in $d\mathbf{v}$ is

$$p(\mathbf{z}, \mathbf{z}', \mathbf{c})\, d\mathbf{v}.$$

Clearly, $p(\mathbf{z}, \mathbf{z}', \mathbf{c})$ is normalized

$$\int_{\mathcal{V}} p(\mathbf{z}, \mathbf{z}', \mathbf{c})\, d\mathbf{v} = 1. \tag{4.5}$$

(It also has the property that it is zero for all $z_i > z_i'$, since the amount of any biochemical entity does not increase at the instant of fission.) If

we assume that all biochemical species are conserved at fission, then $p(\mathbf{z}, \mathbf{z}', \mathbf{c})$ must be such that

$$p(\mathbf{z}, \mathbf{z}', \mathbf{c}) = p(\mathbf{z}' - \mathbf{z}, \mathbf{z}', \mathbf{c}), \tag{4.6}$$

since if the physiological state of one of the daughter cells is $\mathbf{z}$, then the physiological state of the other daughter cell must be $\mathbf{z}' - \mathbf{z}$. Finally, it is easy to show from Eqs. (4.5) and (4.6) that

$$\int_{\mathfrak{B}} \mathbf{z} p(\mathbf{z}, \mathbf{z}', \mathbf{c})\, d\mathbf{v} = \tfrac{1}{2}\mathbf{z}'. \tag{4.7}$$

That is, the expected amount of a biochemical component in a daughter cell is one half the amount of that biochemical entity in its mother cell. Equation (4.7) does not, however, imply that an "even split" is necessarily the most probable result of fission.

THE EQUATION OF CHANGE FOR THE DISTRIBUTION OF STATES

The integro-differential equation describing the distribution of states and its change in time can be obtained by making a "number balance" on cells of a given region (infinitesimal) of physiological state space and on a specified region of physical (i.e., actual) space. A number balance is simply an accounting for all cells that leave, enter, or remain in the given regions of physiological state and physical spaces. In words, the balance for the time interval between times t and $t + \Delta t$ reads

① ②
No. of cells that enter by growth + No. of cells that enter by fission

③
— No. of cells that leave by growth, fission, or washout

④
+ No. of cells present at time t that do not leave

⑤
= No. of cells present at time $t + \Delta t$. (5.1)

(There is also a term for loss of cells that have entered in time t to $t + \Delta t$, but this is a higher-order differential and can be neglected.) Formulation of Eq. (5.1) in terms of growth rate and fission probabilities will lead to

the required integro-differential equation for $f_{\mathbf{Z}}$, the density of the distribution of physiological states.

The region of physiological state space over which the balance of Eq. (5.1) is taken is chosen to be $\Delta v = \Delta z_1 \Delta z_2, \ldots$, where the Δz_i are arbitrary. The region of physical space over which the balance is taken is the total volume V of the growth vessel. It is presumed that the growth vessel may be of the chemostat (Novick and Szilard [35]), bactogen (Monod [31]), or a related type. In these devices, fresh nutrient medium containing no organisms is fed continuously to the vessel, and culture is removed continuously, and at the same volumetric rate. Thus, the volume of the culture remains constant. Provision is made for agitating the liquid in the vessel. It is assumed that agitation is sufficient to provide "perfect mixing" (see, e.g., Denbigh [9]). Perfect mixing implies that the probability that a cell in the vessel at time t will be washed out of the vessel in time interval t to $t + \Delta t$ is independent of the position or past history of the cell. The transition probability for washout must then be $(1/\theta)\,\Delta t$, where $\theta = V/q$, and q is the volumetric flow rate through the vessel. (Although it may not be entirely evident, the perfect mixing hypothesis follows from the first hypothesis of Section 3. Most discussions of mixing in the engineering literature are in terms of deterministic material balance equations; see, e.g., Aris [2]. It is possible, however, to consider mixing from the viewpoint of stochastic processes, and so obtain the expressions for washout probability, age distribution, and so on.) θ is called the *nominal holding time*; the reciprocal of θ is the *dilution rate*. In *batch* culture (i.e., no flow through the vessel), the dilution rate is zero.

Consider term 1 of Eq. (5.1) first. The following propositions* concerning a single cell chosen at random from the population are defined.

A_i: $z_i - \dot{z}_i \Delta t < Z_i(t) < z_i - \dot{z}_i \Delta t + \Delta z_i$.

A: $A_1 A_2 A_3 \cdots$.

A_i': $z_i < Z_i(t + \Delta t) < z_i + \Delta z_i$.

A': $A_1' A_2' A_3' \cdots$.

C: The cell washes out in time t to $t + \Delta t$.

* The notation $A_1 A_2 A_3 \cdots$ means that proposition A_1 *and* proposition A_2 *and* proposition A_3, etc., are true. The symbol $P(A|H)$ denotes the probability of proposition A, given that the set of hypotheses H is true.

D: The cell divides in time t to $t + \Delta t$.

E: The cell does not divide or wash out in time t to $t + \Delta t$.

By the rules of probability theory, we have

$$P(AE|H) = P(A|H)P(E|AH). \tag{5.2}$$

But by definition of the density function

$$P(A|H) \approx f_{\mathbf{Z}}(\mathbf{z} - \dot{\mathbf{z}}\,\Delta t, t)\,\Delta \mathfrak{v}.$$

Moreover, since C, D, and E are mutually exclusive and exhaustive propositions,

$$P(E|AH) = 1 - P(C|AH) - P(D|AH).$$

By the hypothesis of perfect mixing, the washout probability is

$$P(C|AH) \approx \frac{1}{\theta}\,\Delta t,$$

whereas by the definition of the time-specific probability of fission, the fission probability is

$$P(D|AH) \approx \sigma(\mathbf{z} - \dot{\mathbf{z}}\,\Delta t, \mathbf{c})\,\Delta t.$$

Hence, we have

$$P(AE|H) \approx f_{\mathbf{Z}}(\mathbf{z} - \dot{\mathbf{z}}\,\Delta t, t)\,\Delta \mathfrak{v}\left\{1 - \left[\frac{1}{\theta} + \sigma(\mathbf{z} - \dot{\mathbf{z}}\,\Delta t, \mathbf{c})\right]\Delta t\right\}. \tag{5.3}$$

Now

$$P(A'A|H) \approx P(AE|H) f_{\dot{\mathbf{Z}}|\mathbf{Z}}(\dot{\mathbf{z}}, \mathbf{z} - \dot{\mathbf{z}}\,\Delta t, \mathbf{c})\,\Delta \dot{\mathfrak{v}}$$

where $\Delta \dot{\mathfrak{v}} = \Delta \dot{z}_1 \Delta \dot{z}_2 \ldots$. Hence, by the total probability theorem (see, e.g., Papoulis [36])

$$P(A'|H) \approx f_{\mathbf{Z}}(\mathbf{z}, t + \Delta t) \tag{5.4}$$

$$\approx \Delta \mathfrak{v} \int_{\dot{\mathfrak{v}}} f_{\mathbf{Z}}(\mathbf{z} - \dot{\mathbf{z}}\,\Delta t, t)\left\{1 - \left[\frac{1}{\theta} + \sigma(\mathbf{z} - \dot{\mathbf{z}}\,\Delta t, \mathbf{c})\right]\Delta t\right\} f_{\dot{\mathbf{Z}}|\mathbf{Z}}(\dot{\mathbf{z}}, \mathbf{z} - \dot{\mathbf{z}}\,\Delta t, \mathbf{c})\,d\dot{\mathfrak{v}}.$$

Therefore, by the third hypothesis of Section 3, the number of cells in term 1 of Eq. (5.1) is $Vn(t)P(A'|H)$, where $P(A'|H)$ is given by Eq. (5.4).

The integral in Eq. (5.4) may be simplified considerably by the use of Taylor's theorem. In fact, we find thereby that

$$Vn(t)P(A'|H) \approx VW_{\mathbf{Z}}(\mathbf{z}, t)\,\Delta\mathfrak{v} - VW_{\mathbf{Z}}(\mathbf{z}, t)\sigma(\mathbf{z}, \mathbf{c})\,\Delta t\,\Delta\mathfrak{v} - V\nabla_{\mathbf{Z}} \cdot [\bar{\dot{\mathbf{Z}}}(\mathbf{z}, \mathbf{c})W_{\mathbf{Z}}(\mathbf{z}, t)]\,\Delta t\,\Delta\mathfrak{v} \tag{5.5}$$

in which $\nabla_{\mathbf{Z}} \cdot (\ )$ denotes the operation

$$\nabla_{\mathbf{Z}} \cdot \mathbf{V} = \sum_i \frac{\partial}{\partial z_i} V_i \tag{5.6}$$

where $\mathbf{V}$ is any vector-valued function of $\mathbf{z}$ and

$$W_{\mathbf{Z}}(\mathbf{z}, t) \equiv n(t) f_{\mathbf{Z}}(\mathbf{z}, t) \tag{5.7}$$

is the nonnormalized density* of the distribution of states; i.e., the number of cells, per unit volume of culture, with $\mathbf{Z}(t) \in \Delta\mathfrak{v}$. In deriving Eq. (5.5) from Eq. (5.4), terms of degree two and higher in Δt have been neglected.

The number of cells in term 2 of Eq. (5.1) can be written down at once, in terms of the time-specific probability of fission and the function $p(\mathbf{z}, \mathbf{z}', \mathbf{c})$; the number is

$$2V\,\Delta\mathfrak{v}\,\Delta t \int_{\mathfrak{Z}} \sigma(\mathbf{z}', \mathbf{c})p(\mathbf{z}, \mathbf{z}', \mathbf{c})W_{\mathbf{Z}}(\mathbf{z}', t)\,d\mathfrak{v}',$$

in which the total probability theorem and the third hypothesis of Section 3 have again been used. The factor of two arises because fissions are binary.

The number of cells in terms 3 and 4 of Eq. (5.1) is, except for terms of second degree in $\Delta\mathfrak{v}$, the number of cells present at time t, namely,

$$VW_{\mathbf{Z}}(\mathbf{z}, t)\,\Delta\mathfrak{v}.$$

Finally, the number of cells in term 5 of Eq. (5.1) is

$$VW_{\mathbf{Z}}(\mathbf{z}, t + \Delta t)\,\Delta\mathfrak{v}.$$

Substitution of all of these results into Eq. (5.1) and simplification yields

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* We adopt the convention that $W_{\mathbf{X}}$ is the nonnormalized density of any random quantity $\mathbf{X}$; that is, $W_{\mathbf{X}} \equiv nf_{\mathbf{X}}$.

$$\frac{\partial}{\partial t} W_{\mathbf{Z}}(\mathbf{z}, t) + \nabla_{\mathbf{Z}} \cdot [\bar{\dot{\mathbf{Z}}}(\mathbf{z}, \mathbf{c}) W_{\mathbf{Z}}(\mathbf{z}, t)]$$

$$= 2 \int_{\mathfrak{B}} \sigma(\mathbf{z}', \mathbf{c}) p(\mathbf{z}, \mathbf{z}', \mathbf{c}) W_{\mathbf{Z}}(\mathbf{z}', t)\, d\mathfrak{v}' - \left(\frac{1}{\theta} + \sigma(\mathbf{z}, \mathbf{c}) \right) W_{\mathbf{Z}}(\mathbf{z}, t). \quad (5.8)$$

Equation (5.8) is the *equation of change for the distribution of states*; as such it represents the first of the fundamental equations of theory.

We note that $\bar{\dot{\mathbf{Z}}}$ rather than $f_{\dot{\mathbf{Z}}|\mathbf{Z}}$ appears in the final equation. Hence, the same result would have been obtained if growth had been assumed to be deterministic, with growth rate $\bar{\dot{\mathbf{Z}}}$.

Solutions of Eq. (5.8) must satisfy an initial condition, of course. That is, the distribution density $W_{\mathbf{Z}}(\mathbf{z}, t)$ must be known at some initial time if predictions are to be made. In addition, we require that solutions of Eq. (5.8) satisfy a regularity condition. The regularity condition is that $\bar{\dot{\mathbf{Z}}}(\mathbf{z}, \mathbf{c}) W_{\mathbf{Z}}(\mathbf{z}, t)$ shall vanish everywhere on the boundary $\mathfrak{S}$ of physiological state space:

$$\bar{\dot{\mathbf{Z}}}(\mathbf{z}, \mathbf{c}) W_{\mathbf{Z}}(\mathbf{z}, t) = \mathbf{0}, \qquad \mathbf{z} \in \mathfrak{S}. \quad (5.9)$$

Biologically speaking, the regularity condition may be taken as defining the boundary of physiological state space. That is to say, biological considerations determine what physiological states are impossible or meaningless (e.g., states with any Z_i infinite are impossible, and states with any Z_i negative are meaningless), and the location of such states fixes the boundary of $\mathfrak{B}$. Equation (5.9) simply states that no cells cross over into impossible or meaningless states. Equation (5.9) is not a boundary condition; rather, it may be regarded as a constraint upon the functions $\bar{\dot{\mathbf{Z}}}$ and σ.

In a certain case, a less general form of Eq. (5.8) might be adequate. This case would arise if the instant of fission were determined exactly (i.e., without random variations) by the state of the cell. If such were the case, there would be some hypersurface in physiological state space representing a set of critical conditions for fission. When the trajectory of a cell in physiological state space brought it up to this surface, fission would occur at the instant the surface is reached. This means, in effect, that the time-specific probability of fission σ is zero everywhere in $\mathfrak{B}$ except on the critical surface for fission, where it is infinite.

The assumption that there exists in physiological state space a critical surface for fission is by no means tantamount to the assumption that

fission occurs at a critical mass, a critical size, or a critical value of any other *single* parameter of the cell. To be sure, under some controlled conditions, the mass (or size) may be closely grouped around some mean fission mass (size), and Koch and Schaechter [24] emphasize this fact and use it as a basis for a model of the statistics of cell division. However, their observation that "... the critical size of bacteria at division changes monotonically, and without increase in its coefficient of variation, throughout the reorganization that accompanies a shift of a growing culture from one medium to another medium in which the growth rate and bacterial size are different" suggests that attainment of a certain size is not the *cause* of fission but is only *correlated* with the occurrence of fission. The observation suggests that the cause of fission is to be sought instead in the attainment of a set of intracellular conditions.

Let us divide the boundary $\mathfrak{S}$ of physiological state space into two parts. On one part, $\mathfrak{S}_n$, no fission occurs and the regularity condition, Eq. (5.9), holds. The remaining part, $\mathfrak{S}_d$, of the boundary is the critical surface for fission; a cell divides as soon as it reaches $\mathfrak{S}_d$. Let $\boldsymbol{\lambda}$ be a vector of unit magnitude normal to $\mathfrak{S}_d$, and choose its direction such that it points out of $\mathfrak{V}$.

Application of the same principles used to derive Eq. (5.8) then yields the conservation equation for the degenerate case as

$$\frac{\partial}{\partial t} W_{\mathbf{Z}}(\mathbf{z}, t) + \nabla_{\mathbf{Z}} \cdot [\dot{\bar{\mathbf{Z}}}(\mathbf{z}, \mathbf{e}) W_{\mathbf{Z}}(\mathbf{z}, t)] = -\frac{1}{\theta} W_{\mathbf{Z}}(\mathbf{z}, t)$$

$$+ 2 \int_{\mathfrak{S}_d} p(\mathbf{z}, \mathbf{z}', \mathbf{e}) [\dot{\bar{\mathbf{Z}}}(\mathbf{z}', \mathbf{e}) \cdot \boldsymbol{\lambda}(\mathbf{z}')] W_{\mathbf{Z}}(\mathbf{z}', t)\, d\mathfrak{s}' \qquad (5.10)$$

where $d\mathfrak{s}'$ is an element of surface in $\mathfrak{S}_d$. Equation (5.10) is given for the sake of completeness; in the remainder of the paper, we shall assume that it is necessary to use the general form, Eq. (5.8).

It should be mentioned that the foregoing equations are similar to those that have been used recently for the description of a number of nonbiological problems. These problems include crystallization from a supersaturated solution [45], change of catalyst activity in a fluidized bed reactor [3], and coalescence and droplet breakage in a two-phase system [51, 52]. Indeed, Randolph [44] and Hulburt and Katz [22] have derived "population balance" equations that are more general than Eq. (5.8) in the sense that spatial variations of the density $W_{\mathbf{Z}}$ are per-

mitted. Since equations such as Eq. (5.8) describe a wide class of problems, it seems that a thorough mathematical study of the equations would be profitable.

THE EQUATIONS OF CHANGE FOR THE CELLULAR ENVIRONMENT; STOICHIOMETRY

The second fundamental equation of the theory must be a description of the effects of the factors that change conditions in the cellular environment. As emphasized earlier, cellular chemical processes play a prime role in determining the changes that occur in the cellular environment. Hitherto, we have considered such processes in an abstract manner, and have contented ourselves with the postulate that there exist functions $\bar{\mathbf{Z}}$ that describe them. It is now desirable to become more specific, and to take the view of Dean and Hinshelwood [8], cited earlier, that the cell is a "system containing various substances engaged in an integrated set of chemical reactions within a suitably permeable wall."

The task will be much simplified if we adopt a convenient notation from chemical reactor analysis (see, e.g., Aris [2], Section 2.2). Let A_i represent the ith kind of biochemical making up the cell and its environment. It is convenient to regard a chemical substance inside the cell as different from the same chemical substance outside the cell. Thus, A_I might represent, say, glucose inside the cell, whereas A_{I+1} might represent glucose outside the cell. We arbitrarily adopt the convention that $A_1, A_2, \ldots, A_I$ represent "cellular" substances whereas $A_{I+1}, A_{I+2}, \ldots$ represent "environmental" substances. If A_i is a cellular substance, its amount in a single cell is Z_i. Similarly, if A_i is an environmental substance, its concentration in the environment is c_i.

The *stoichiometry* of the reactions that occur within the cell and on its boundaries can then be summarized neatly by the algebraic formula

$$\sum_i \alpha_{ij} A_i = 0, \qquad j = 1, 2, \ldots, \tag{6.1}$$

where the summation extends over all chemical substances of concern, and the index j runs over all independent reactions that occur. [The concept of independence of reactions is discussed by Aris ([2], Section 2.3).] The quantity α_{ij} is the *stoichiometric coefficient* of the ith chemical substance in the jth reaction. The following conventions regarding the sign of α_{ij} are adopted.

(i) $\alpha_{ij} > 0$ if A_i is produced by the jth reaction.
(ii) $\alpha_{ij} = 0$ if A_i does not participate in the jth reaction.
(iii) $\alpha_{ij} < 0$ if A_i is consumed by the jth reaction.

It is emphasized that Eq. (6.1) represents true chemical reactions and that as such the α_{ij} are not variables but are strictly constant.

Let R_j be the rate of the jth reaction in a single cell. The reaction rates R_j are random variables, of course, for the same reasons that the $\dot{Z}_i$ are random variables. The reaction rate R_j is defined such that the rate of production (consumption) of A_i by the jth reaction in the cell is $\alpha_{ij}R_j$ if α_{ij} is positive (negative). This definition has the advantages of not being dependent on a specific choice of chemical species for the expression of the rate, and of always yielding a nonnegative reaction rate.

The total rate of production (consumption) of A_i in the cell, denoted previously by $\dot{Z}_i$, is then just

$$\dot{Z}_i = \sum_j \alpha_{ij} R_j, \qquad i = 1, 2, \ldots, I, \tag{6.2}$$

where the summation extends over all reactions occurring.

We can define a *reaction rate vector* $\mathbf{R}$ as the ordered set of numbers

$$\mathbf{R} = \begin{bmatrix} R_1 \\ R_2 \\ \vdots \end{bmatrix}. \tag{6.3}$$

(Of course, the dimensionality of the vector $\mathbf{R}$ is in general not the same as the dimensionality of the vectors $\mathbf{Z}$ and $\dot{\mathbf{Z}}$.) By the hypotheses of Section 3, $\mathbf{R}$ is a random, vector-valued function of the two vectors $\mathbf{Z}$ and $\mathbf{c}$.

Let $\bar{\mathbf{R}}(\mathbf{z}, \mathbf{c})$ represent the expected value of $\mathbf{R}$, given that $\mathbf{Z}(t) = \mathbf{z}$. Further, define two matrices $\boldsymbol{\beta}$ and $\boldsymbol{\gamma}$ by

$$\boldsymbol{\beta} = [\beta_{ij}] = \begin{bmatrix} \alpha_{11} & \alpha_{12} & \cdots \\ \vdots & \vdots & \\ \alpha_{I1} & \alpha_{I2} & \cdots \end{bmatrix}, \tag{6.4a}$$

$$\boldsymbol{\gamma} = [\gamma_{ij}] = \begin{bmatrix} \alpha_{I+1,1} & \alpha_{I+1,2} & \cdots \\ \alpha_{I+2,1} & \alpha_{I+2,2} & \cdots \\ \vdots & \vdots & \end{bmatrix}. \tag{6.4b}$$

That is, $\boldsymbol{\beta}$ is the matrix of stoichiometric coefficients for cellular substances, whereas $\boldsymbol{\gamma}$ is the matrix of stoichiometric coefficients for environmental substances.

Then by Eq. (6.2), it follows that the expected growth rate vector of a cell may be written in terms of the expected reaction rate vector by

$$\bar{\dot{\mathbf{Z}}}(\mathbf{z}, \mathbf{c}) = \boldsymbol{\beta} \cdot \bar{\mathbf{R}}(\mathbf{z}, \mathbf{c}) \tag{6.5}$$

where the dot indicates matrix multiplication of the matrix and the vector. Similarly, the expected rate of *consumption* of substances from the environment by a single cell of physiological state $\mathbf{z}$ is given by the vector

$$-\boldsymbol{\gamma} \cdot \bar{\mathbf{R}}(\mathbf{z}, \mathbf{c}).$$

We are now in a position to write the equation that governs changes in the cellular environment. This can be done by making a mass balance on the various chemical substances present in the environment.

If the balance is taken over the environmental medium in the volume V of the growth vessel, the balance for any environmental species reads, in words:

$$\begin{aligned} &\textcircled{1} \\ &\text{Rate of accumulation in the environment} \\ &\qquad \textcircled{2} \qquad\qquad\qquad\qquad \textcircled{3} \\ &= \text{Rate of input with the feed} - \text{Rate of output with the washout} \\ &\qquad\qquad\qquad\qquad \textcircled{4} \\ &- \text{Rate of consumption by the population.} \end{aligned} \tag{6.5}$$

(This balance is written on the liquid phase in the growth vessel. If a gas phase is also present, a further term, involving a so-called mass transfer coefficient, must be introduced into the balance. The equations for this case are given by Fredrickson and Tsuchiya [15].)

By the total probability theorem, the rate of consumption of environmental substances due to cells of state $\mathbf{Z}(t) \in d\mathbf{v}$ is

$$-V[\boldsymbol{\gamma} \cdot \bar{\mathbf{R}}(\mathbf{z}, \mathbf{c})] W_{\mathbf{Z}}(\mathbf{z}, t)\, d\mathbf{v};$$

thus the rate of consumption due to all cells is the vector

$$- V\boldsymbol{\gamma} \cdot \int_{\mathfrak{B}} \bar{\mathbf{R}}(\mathbf{z}, \mathbf{c}) W_{\mathbf{Z}}(\mathbf{z}, t)\, d\mathfrak{v}$$

where again the total probability theorem is used. Hence, Eq. (6.5) becomes simply

$$\underset{①}{} \quad \underset{②}{} \quad \underset{③}{} \qquad\qquad \underset{④}{}$$

$$V \frac{d\mathbf{c}}{dt} = q\mathbf{c}_f - q\mathbf{c} + V\boldsymbol{\gamma} \cdot \int_{\mathfrak{B}} \bar{\mathbf{R}}(\mathbf{z}, \mathbf{c}) W_{\mathbf{Z}}(\mathbf{z}, t)\, d\mathfrak{v} \tag{6.6}$$

where $\mathbf{c}_f$ is the state vector of the feed stream. The equation is based on hypotheses stated earlier, of course; in particular, the first hypothesis of Section 3 must be valid if the vector $\mathbf{c}$ is even to have meaning. Also, the various terms in Eq. (6.6) correspond to the similarly numbered terms in Eq. (6.5).

To summarize this and the preceding section, we state that the distribution of states is governed by the equation

$$\frac{\partial}{\partial t} W_{\mathbf{Z}}(\mathbf{z}, t) + \nabla_{\mathbf{Z}} \cdot [(\boldsymbol{\beta} \cdot \bar{\mathbf{R}}(\mathbf{z}, \mathbf{c})) W_{\mathbf{Z}}(\mathbf{z}, t)]$$

$$= 2 \int_{\mathfrak{B}} \sigma(\mathbf{z}', \mathbf{c}) p(\mathbf{z}, \mathbf{z}', \mathbf{c}) W_{\mathbf{Z}}(\mathbf{z}', t)\, d\mathfrak{v}' - \left(\frac{1}{\theta} + \sigma(\mathbf{z}, \mathbf{c}) \right) W_{\mathbf{Z}}(\mathbf{z}, t). \tag{6.7}$$

Solutions of Eq. (6.7) must satisfy an appropriate initial condition and the regularity condition, Eq. (5.9). The state of the environment is governed by the set of equations (6.6), which, when rewritten slightly, are

$$\frac{d\mathbf{c}}{dt} = \frac{1}{\theta} (\mathbf{c}_f - \mathbf{c}) + \boldsymbol{\gamma} \cdot \int_{\mathfrak{B}} \bar{\mathbf{R}}(\mathbf{z}, \mathbf{c}) W_{\mathbf{Z}}(\mathbf{z}, t)\, d\mathfrak{v}. \tag{6.8}$$

Solutions of Eq. (6.8) must also satisfy appropriate initial conditions. We call Eqs. (6.7) and (6.8) the *master equations of change.*

The *data* needed in any given problem are knowledge of:

(i) the two stoichiometric matrices $\boldsymbol{\beta}$ and $\boldsymbol{\gamma}$;

(ii) the dependence of the expected reaction rate vector $\bar{\mathbf{R}}$ on the physiological state vector $\mathbf{z}$ and the state of the environment $\mathbf{c}$;

(iii) the dependence of the time-specific probability of fission σ on $\mathbf{z}$ and $\mathbf{c}$;

(iv) the dependence of the partioning function p on the physiological states of mother and daughter cells ($\mathbf{z}'$ and $\mathbf{z}$, respectively) and on $\mathbf{c}$;

(v) initial conditions for $W_{\mathbf{Z}}$ and $\mathbf{c}$.

MOMENTS OF THE DISTRIBUTION OF STATES; "NONSEGREGATED" GROWTH MODELS

As a first application of the theory, we show how it can be used to derive a number of growth models that have appeared in the literature. We then show that, according to the theory, these models have a more restricted range of validity than had been supposed earlier.

Equations equivalent to those of a large number of proposed growth models can be obtained by taking moments—the zeroth and the first—of Eq. (6.7). So far as is known, second and higher moments of Eq. (6.7) do not lead to equations that have appeared in the literature; hence, we do not consider moments higher than the first here. The set of equations (6.8) also appears in some models, of course (i.e., those that do not neglect the effect of growth on the environment).

The zeroth moment of Eq. (6.7) is obtained by integrating each term of that equation over all of physiological state space. The regularity condition, Eq. (5.9), and the properties of the partitioning function $p(\mathbf{z}, \mathbf{z}', \mathbf{c})$ as expressed by Eqs. (4.5)–(4.7) then show that

$$\frac{dn}{dt} = -\frac{1}{\theta} n + kn \tag{7.1}$$

in which

$$k = \int_{\mathfrak{Z}} \sigma(\mathbf{z}, \mathbf{c}) f_{\mathbf{Z}}(\mathbf{z}, t)\, d\mathfrak{v} \tag{7.2}$$

is called the specific multiplication rate. Equation (7.1) represents a number balance on cells in the culture; the rate of accumulation is composed of a multiplication term and a washout term, as expected. If there is no washout (batch culture), if $f_{\mathbf{Z}}$ is independent of time, and if σ is independent of $\mathbf{c}$ (or if $\mathbf{c}$ is not changing with time), then the equation shows that the culture density will increase exponentially (Malthus's law). In continuous culture, the equation shows that a necessary condition for steady state is that $k = (1/\theta)$.

The *Verhulst-Pearl model* (the so-called logistic law) is obtained from Eq. (7.1) if

$$k = a_1 - b_1 n \tag{7.3}$$

where a_1 and b_1 are constants. (Obviously, they would have to depend on initial medium composition for batch cultures, or on composition of the feed stream for continuous cultures.) Equation (7.2) shows that k is the average over the population of the time-specific probability of fission; as such, Eq. (7.3) may provide a reasonable fit of data, but it is obvious that if conditions (c) are changed appreciably, Eq. (7.3) must fail.

Davis [6] attributes to Volterra a model that is obtained from Eq. (7.1) by supposing that

$$k = a_2 - b_2 n - \int_0^t K(t, t')n(t')\,dt'. \tag{7.4}$$

The introduction of a weighted time integral ($K(t, t')$ is the weight function) of the population density is an attempt to account for the past history of the population on current growth.

More flexible models can be obtained by taking the first moment of Eq. (6.7). Multiplication of Eq. (6.7) by $\mathbf{z}$ and integration of each term of the resulting equation over all of physiological state space will yield the desired moment equations. The first moment of the distribution of states is denoted by the vector $\mathbf{x}$ and is of course

$$\mathbf{x} = \int_{\mathfrak{B}} \mathbf{z} W_{\mathbf{Z}}(\mathbf{z}, t)\,d\mathfrak{v}. \tag{7.5}$$

The first moment of Eq. (6.7) then yields

$$\frac{d}{dt}\mathbf{x} = -\frac{1}{\vartheta}\mathbf{x} + \boldsymbol{\beta} \cdot \int_{\mathfrak{B}} \bar{\mathbf{R}}(\mathbf{z}, \mathbf{c}) W_{\mathbf{Z}}(\mathbf{z}, t)\,d\mathfrak{v}. \tag{7.6}$$

The following formulas are needed to arrive at Eq. (7.6).

$$2\int_{\mathfrak{B}} \mathbf{z}\,d\mathfrak{v} \int_{\mathfrak{B}} \sigma(\mathbf{z}', \mathbf{c}) p(\mathbf{z}, \mathbf{z}', \mathbf{c}) W_{\mathbf{Z}}(\mathbf{z}', t)\,d\mathfrak{v}' = \int_{\mathfrak{B}} \mathbf{z}\sigma(\mathbf{z}, \mathbf{c}) W_{\mathbf{Z}}(\mathbf{z}, t)\,d\mathfrak{v}. \tag{7.7}$$

$$\int_{\mathfrak{B}} \mathbf{z}\{\nabla_{\mathbf{Z}} \cdot [(\boldsymbol{\beta} \cdot \bar{\mathbf{R}}(\mathbf{z}, \mathbf{c})) W_{\mathbf{Z}}(\mathbf{z}, t)]\}\,d\mathfrak{v} = -\boldsymbol{\beta} \cdot \int_{\mathfrak{B}} \bar{\mathbf{R}}(\mathbf{z}, \mathbf{c}) W_{\mathbf{Z}}(\mathbf{z}, t)\,d\mathfrak{v}. \tag{7.8}$$

Equation (7.7) may be established by interchange of the order of integra-

tion and application of Eq. (4.7). Equation (7.8) may be established by integration by parts and application of the regularity condition, Eq. (5.9).

The elements of the vector $\mathbf{x}$ are the total amounts of the various kinds of cellular biochemical components present in unit volume of culture; hence, we call $\mathbf{x}$ the *concentration of biochemicals vector*. Equation (7.6) is then seen to represent material balances on the various kinds of biochemicals present in the cells. The parallelism of Eq. (7.6) with the equations of change for the cellular environment, Eq. (6.8), is to be noted.

A class of models with several representatives in the literature may be obtained from Eqs. (7.6) and (6.8) by supposing that the average reaction rate vector is expressible as a function *only* of $\mathbf{x}$ and of $\mathbf{c}$ (here we depart from the convention that a lowercase letter represents a specific value of the random variable represented by the corresponding capital letter):

$$\int_{\mathfrak{X}} \bar{\mathbf{R}}(\mathbf{z}, \mathbf{c}) W_{\mathbf{Z}}(\mathbf{z}, t)\, d\mathfrak{v} = \mathbf{r}(\mathbf{x}, \mathbf{c}) \tag{7.9}$$

so that the equations of the class of models can be written as

$$\frac{d\mathbf{x}}{dt} = -\frac{1}{\theta}\mathbf{x} + \boldsymbol{\beta} \cdot \mathbf{r}(\mathbf{x}, \mathbf{c}), \tag{7.10a}$$

$$\frac{d\mathbf{c}}{dt} = \frac{1}{\theta}(\mathbf{c}_f - \mathbf{c}) + \boldsymbol{\gamma} \cdot \mathbf{r}(\mathbf{x}, \mathbf{c}). \tag{7.10b}$$

Tsuchiya, Fredrickson, and Aris [50] have proposed that models leading to Eqs. (7.10) be called structured distributed models. That is, they are structured in the sense that cells are recognized as possessing at least a biochemical structure (composition) subject to change, and they are distributed in the sense that the models do not recognize that life is segregated into structural and functional units—cells—but rather regard life to be uniformly distributed throughout the culture. Non-segregated would probably be a better term than distributed, and we adopt the former designation here.

Specific examples of structured, nonsegregated models are given by Hinshelwood [20],* Perret [37], Ramkrishna *et al.* [43], and Swanson

* It might appear that Hinshelwood's equations are not part of a nonsegregated model, since they do contain the population density as a variable. However, it does not appear as an independent variable since n is a linear scalar function of the vector $\mathbf{x}$ according to the hypothesis concerning cell division that Hinshelwood advances.

et al. [48]. One of the principal reasons for developing this kind of model is that it leads to a mathematical problem that is much simpler than that posed by the more general theory outlined herein. Thus, nonsegregated models lead to a system of ordinary differential equations which, though generally nonlinear, are much easier to solve than the integro-differential equation of change of the distribution of states.

In using nonsegregated models, it is tacitly assumed that the model is complete; that is, that there is no variable in the model for which there is not an independent equation. This assumption can be tested in the light of the present theory.

Thus, suppose that intracellular kinetics is a set of first-order branching reactions of the type considered by Perret [37]:

$$\begin{array}{l} \rightarrow A_i \rightarrow A_{i+1} \rightarrow \\ \quad \downarrow \\ \quad A_k \rightarrow \end{array}$$

For such a case, the expected growth rate vector $\bar{\mathbf{R}}$ for a cell is a *linear* function of the physiological state vector $\mathbf{z}$:

$$\bar{R}_j = \sum_l k_{jl} z_l \tag{7.11}$$

where k_{jl} are the elements of a matrix of rate constants. [Equation (7.11) is written out in component form since for *transfer reactions* (i.e., those involving environmental chemical substances) $\bar{R}_j$ depends on $\mathbf{c}$. For all intracellular reactions, however, Eq. (7.11) is sufficient by the hypothesis of first-order kinetics.] Hence, Eq. (7.9) shows that for this case

$$r_j = \sum_l k_{jl} x_l. \tag{7.12}$$

Clearly, no variables other than $\mathbf{x}$ (and $\mathbf{c}$) appear, and the assumption that the model is complete is justified. In particular, the higher moments of the distribution of states do not appear in taking the averages called for by Eq. (7.9).

However, if intracellular kinetics become somewhat more complicated, and so lead to a *nonlinear* dependence of $\bar{\mathbf{R}}$ on $\mathbf{z}$, then the foregoing convenient circumstance does not obtain, and the vector $\mathbf{r}$ depends not only on $\mathbf{c}$ and $\mathbf{x}$, but also on the higher moments of the distribution of states. In particular, if some of the important reactions in the cell exhibit second-order or Michaelis-Menten kinetics, then the two equations (7.10a) and (7.10b) will not form a complete model.

This conclusion is apparently discouraging, since we could have reasonably expected to learn a good deal about intracellular processes by study and experimental testing of nonsegregated models. Some hope is provided, however, by the reflection that neglect of the dependence of the reaction rate vector on the higher moments of the distribution of states may not be a very bad assumption. The actual degree of approximation involved appears to be a purely mathematical problem, and one on which we do not comment here.

To close this section, we consider a frequently used special case of the class of nonsegregated models. This special case was motivated, no doubt, by the fact that experimental population studies do not provide complete data on the time course of change of each of the x_i, or even on the time course of change of very many of the x_i. In fact, what is usually measured in such studies is some single quantity that serves as a measure of the concentration of the culture. Among such quantities are (see, e.g., Yamanna and Mallette [26]) dry weight per unit volume, fresh volume per unit volume, nitrogen per unit volume, and optical density. A little reflection will show that any such measure of concentration is a linear combination of the concentrations of the various biochemicals present in the cells. Hence, if we let y be the measure of concentration, and call it the *concentration of biomaterial*, then y is related to the concentrations of the various biochemical substances by

$$y = \mathbf{m} \cdot \mathbf{x} \tag{7.13}$$

in which $\mathbf{m}$ is a constant (row) vector. This definition includes any of the quantities just listed as measures of concentration; appropriate definition of $\mathbf{m}$ must be made for each quantity, of course.

Taking the matrix product of $\mathbf{m}$ with Eq. (7.6) shows that y satisfies

$$\frac{dy}{dt} = -\frac{1}{\theta} y + (\mathbf{m} \cdot \boldsymbol{\beta}) \cdot \int_{\mathfrak{V}} \bar{\mathbf{R}}(\mathbf{z}, \mathbf{c}) W_{\mathbf{Z}}(\mathbf{z}, t)\, d\mathfrak{v}. \tag{7.14}$$

This equation is, of course, generally true, but the model under consideration is obtained if we suppose that the integral appearing in Eq. (7.14) depends only on y and $\mathbf{c}$. Hence, the model becomes

$$\frac{dy}{dt} = -\frac{1}{\theta} y + (\mathbf{m} \cdot \boldsymbol{\beta}) \cdot \mathbf{r}(y, \mathbf{c}), \tag{7.15a}$$

$$\frac{d\mathbf{c}}{dt} = \frac{1}{\theta} (\mathbf{c}_f - \mathbf{c}) + \boldsymbol{\gamma} \cdot \mathbf{r}(y, \mathbf{c}). \tag{7.15b}$$

Tsuchiya, Fredrickson, and Aris [50] call models in which a single parameter describes the biomaterial *unstructured.*

A specific example of an unstructured model is that of Monod [31]. In Monod's model, only one reaction is considered, and this is assumed to obey Michaelis-Menten kinetics. Monod's model is usually the starting point for discussions of continuous microbial culture.

Clearly, unstructured models are subject to all of the restrictions on nonsegregated models cited previously. But they are subject to the even more stringent restriction that the results of a whole series of chemical reactions be representable as the result of a single chemical reaction; i.e., that there is a single *rate-limiting* step. Moreover, Eqs. (7.15) written with the time derivatives are somewhat misleading, since as Perret [37] points out, such models can only be valid in the steady state.

REPETITIVE GROWTH

The considerations of the preceding section show that, in general, it is not possible to treat the dynamics of procaryotic cell populations without bringing the concept of a distribution of states into the treatment. In general, therefore, one must attempt to apply Eqs. (6.7) and (6.8), together with regularity and initial conditions, to each specific problem of concern.

The mathematical problem posed by these equations appears to be extremely difficult, however, and numerical solutions for the simplest case of unidimensional physiological state and environmental state vectors require considerable time, even on a large digital computer (Eakman [11]). The experimental problem of determining the unknown functions and constants appearing in the equations is, no doubt, even more formidable. Hence, we seek a special kind of growth situation, in which the mathematical and experimental problems are reduced to a minimum of complexity. Such a situation is suggested by what Campbell [5] has called *balanced growth.*

In order to place the concept of balanced growth in quantitative terms, it is necessary first to rewrite the moment equation (7.6) in somewhat different terms. This is because Campbell's definition of balanced growth is in terms of the rate of synthesis of the various cellular biochemical substances, whereas Eq. (7.6) is given in terms of the average rates of the cellular reactions.

Define a set of quantities μ_i by

$$\mu_i = \sum_j \frac{\beta_{ij} \int_{\mathfrak{V}} \bar{R}_j(\mathbf{z}, \mathbf{c}) f_{\mathbf{Z}}(\mathbf{z}, t)\, d\mathfrak{v}}{\int_{\mathfrak{V}} z_i f_{\mathbf{Z}}(\mathbf{z}, t)\, d\mathfrak{v}} \tag{8.1}$$

where the summation extends over all cellular reactions occurring. Then Eq. (7.6) can be rewritten in component form as

$$\frac{d}{dt} x_i = -\frac{1}{\theta} x_i + \mu_i x_i. \tag{8.2}$$

The significance of the quantities μ_i may be deduced from either Eq. (8.1) or Eq. (8.2). These equations show that μ_i is the rate of synthesis of the ith kind of cellular biochemical per unit volume of culture and per unit concentration of the ith kind of cellular biochemical component. Hence, the μ_i are called the *specific synthesis rates* of biochemical entities. In general, the μ_i are not constants, but depend on n, $\mathbf{c}$, $\mathbf{x}$, and the higher moments of the distribution of physiological states.

Campbell [5] says that "... growth is *balanced* over a time interval if, during that interval, every extensive property of the growing system increases by the same factor." [The concept of the balanced growth *medium* is somewhat older than the concept of balanced growth. A medium is said to be balanced for a particular growth situation if growth changes the concentration of all chemicals present in the medium by the same factor (Lilly [27]). Unfortunately, no medium can be truly balanced, since if growth *decreases* the concentration of some chemicals in the environment, it will *increase* the concentration of others; also, the demand for the various chemicals may change as the physiological state changes.] The extensive properties of a growing system of procaryotic cells are the number of cells in the system, the amounts of the various kinds of cellular biochemical constituents present in the system, and all linear combinations of the latter. In other words, the quantities n, $\mathbf{x}$, and y are the extensive properties of the growing system.

Suppose the foregoing quantities change from n, $\mathbf{x}$, and y at time t to $n + dn$, $\mathbf{x} + d\mathbf{x}$, and $y + dy$ at time $t + dt$. Then growth is balanced in time interval t to $t + dt$ if

$$\frac{dn}{n} = \frac{dx_1}{x_1} = \frac{dx_2}{x_2} = \ldots = \frac{dy}{y}, \tag{8.3}$$

or, because of Eqs. (7.1), (7.14), and (8.2), if

$$k = \mu_1 = \mu_2 = \ldots = \nu, \tag{8.4}$$

in which

$$\nu \equiv \frac{(\mathbf{m} \cdot \boldsymbol{\beta}) \cdot \int_{\mathfrak{V}} \bar{\mathbf{R}}(\mathbf{z}, \mathbf{c}) W_{\mathbf{Z}}(\mathbf{z}, t)\, d\mathfrak{v}}{\mathbf{m} \cdot \int_{\mathfrak{V}} \mathbf{z} W_{\mathbf{Z}}(\mathbf{z}, t)\, d\mathfrak{v}} \tag{8.5}$$

is the specific rate of increase of the concentration of biomaterial y. One sees from Eq. (8.3) that

$$\frac{d}{dt}\left[\frac{x_i}{\sum_{k=1}^{I} x_k}\right] = 0, \tag{8.6}$$

i.e., that the relative composition of the total biomaterial present is unchanging in balanced growth. It also follows that the average amounts per cell of the various biochemicals do not change with time.

One of the most significant features of balanced growth is that it is a type of steady-state situation; this is indicated, for instance, by Eq. (8.6). Equation (8.6) shows that in balanced growth, the biological material in a culture has reached a state of dynamic equilibrium with its environment, as Dean and Hinshelwood [8] emphasize. In general, the composition of the cellular environment is changing with time, since growth processes do alter the environment. Hence, if balanced growth occurs, it must be a situation in which either (i) the environmental conditions are not changing with time, because the whole culture is growing in a steady-state (as in continuous steady-state propagation), or (ii) the rates of cellular processes, as expressed by the functions σ, $\bar{\mathbf{R}}$, and p, are independent of the environmental conditions, which are changing. That is, σ, $\bar{\mathbf{R}}$, and p do not depend on $\mathbf{c}$. If growth is balanced because the first of the foregoing alternatives is true, we say that growth is balanced and restricted. If growth is balanced because the second alternative is true, we say that growth is balanced and *unrestricted*. The terms restricted

balanced growth and unrestricted balanced growth are used by Lamanna and Mallette [26]. The rationale for the terminology is that if steady state has been established in the environment as well as in the cell population, but the steady-state growth and multiplication rates are dependent on the environmental conditions prevailing, then some factor* or factors in the environment must be limiting (restricting) the rates of growth and multiplication. The term unrestricted growth implies that no environmental factors limit the rates of cellular processes. It is theoretically possible to operate a continuous propagator such that both of the foregoing alternatives are true; this situation is also called unrestricted balanced growth. (Unrestricted balanced growth is achieved by controlling the rate of washout through some measure of population concentration. The possibility of obtaining unrestricted balanced growth by this means was suggested and explored by Novick [34] and by Fox and Szilard [12]; Fredrickson and Tsuchiya [15] discuss some dynamical consequences of introducing an external feedback circuit.)

In his discussion, Campbell [5] states that steady-state continuous propagation is balanced growth,** and that exponential batch growth may approximate balanced growth.† He says, however, that synchronous batch growth is not balanced, nor is initial development of a single clone of the many in a batch culture in balanced growth.

The biological situation in the last two cases mentioned possesses many features in common with that in the first two cases mentioned. The difference is that in the former cases we are concerned with whole populations of cells, whereas in the latter cases we are concerned with single cells. Since Campbell's definition of balanced growth is in terms of averages over a distribution of states (the "extensive properties of a growing system"), it is clear why the latter two cases cannot be classified as balanced growth. Indeed, it is relatively meaningless to speak of balanced growth of a single cell. Hence, a more general concept than Campbell's balanced growth, one that will allow us to speak in terms of individual cells, is needed to exploit those features that are common in all of the foregoing cases. Since the present theory is in terms of distribu-

* The limiting factor may act either as a substrate or as an inhibitor.

** This is true only if the feed to the growth vessel contains no organisms. If the feed contains organisms, as in a cascade of vessels, growth cannot in general be balanced, except in the first vessel. This will be shown later.

† But Campbell remarks that there is no logical reason that growth need be exponential to be balanced.

tions of states, a concept based on growth of single cells certainly falls within the scope of the theory.

The common feature in all of the cases mentioned in the foregoing is that, apart from statistical fluctuations, *the same sequence of cellular events (the "life cycle" of the cell) repeats itself over and over again, and at the same rate, in all cells of the population.* When growth is of this nature, we say that it is *repetitive*—repetitive, that is, from the point of view of single cells. (Harris [18] used the term regenerative to describe a situation that is a special case of the one called repetitive growth here; the latter term seems preferable, since regenerative growth has a specialized, and different, meaning in developmental biology.) Clearly, the cases called balanced growth by Campbell are examples of repetitive growth, but synchronous batch growth (here we wish to preserve the distinction between synchron*ous* growth and synchron*ized* growth that was made by Abbo and Pardee [1]) and growth of a clone may also be cases of repetitive growth.

It seems important to establish rigorous criteria for repetitive growth, since an experimentalist must be sure that cellular events are in fact cyclic when he seeks to determine the sequence of events in the life cycle of a cell. The criticism by Dean and Hinshelwood [7] of conclusions based on synchronous culture techniques shows that the necessity for proof of a cyclic sequence of events is not generally recognized.

The verbal definition of repetitive growth suggests that when such a situation prevails, the *age* of a single cell, that is, the time elapsed since it was formed by fission, is an index or measure of the degree of progression of the cell in its life cycle. That is, two cells of the same age should be, on the average, just as far along the path leading to fission, since in repetitive growth the sequence of cellular events repeats itself over and over and at the same rate. In particular, these two cells should have, on the average, the same physiological state. Therefore, in repetitive growth, if one is given the age of a cell, he should be able to predict the physiological state of the cell in the statistical sense.

To formulate the concept mathematically, let A be the age of a cell selected at random from a culture. Let $f_{\mathbf{Z}A}(\mathbf{z}, a, t)\, d\mathbf{v}\, da$ be the probability that the cell has state $\mathbf{Z}(t) \in d\mathbf{v}$ and age $A(t)$ such that $a < A(t) < a + da$. By the laws of probability theory, one can then write

$$\begin{aligned} f_{\mathbf{Z}A}(\mathbf{z}, a, t) &= f_{\mathbf{Z}|A}(\mathbf{z}, a, t) f_A(a, t), \\ &= f_{A|\mathbf{Z}}(a, \mathbf{z}, t) f_{\mathbf{Z}}(\mathbf{z}, t), \end{aligned} \tag{8.7}$$

where f_A is the density of the distribution of ages, and the conditional densities $f_{\mathbf{Z}|A}$ and $f_{A|\mathbf{Z}}$ satisfy the normalizing conditions

$$\int_{\mathfrak{B}} f_{\mathbf{Z}|A}(\mathbf{z}, a, t)\, d\mathfrak{v} = 1, \tag{8.8a}$$

$$\int_0^\infty f_{A|\mathbf{Z}}(a, \mathbf{z}, t)\, da = 1. \tag{8.8b}$$

Hence, it also follows that

$$f_{\mathbf{Z}}(\mathbf{z}, t) = \int_0^\infty f_{\mathbf{Z}|A}(\mathbf{z}, a, t) f_A(a, t)\, da \tag{8.9a}$$

and

$$f_A(a, t) = \int_{\mathfrak{B}} f_{A|\mathbf{Z}}(a, \mathbf{z}, t) f_{\mathbf{Z}}(\mathbf{z}, t)\, d\mathfrak{v}. \tag{8.9b}$$

Equations (8.7)–(8.9) are generally true, of course, but *in repetitive growth, the conditional density $f_{\mathbf{Z}|A}$ does not depend, either explicitly or implicitly, on the time t.* For if the conditional density $f_{\mathbf{Z}|A}$ were changing with time, then rates of cellular processes would also be changing with time.

The lack of dependence of $f_{\mathbf{Z}|A}$ on time is the mathematical definition of repetitive growth. In the next section, it will be shown that repetitive growth is possible only if the functions $\sigma(\mathbf{z}, \mathbf{c})$, $\bar{\mathbf{Z}}(\mathbf{z}, \mathbf{c})$, and $p(\mathbf{z}, \mathbf{z}', \mathbf{c})$ do not introduce dependence on time, either explicit or implicit, into Eq. (6.7). Thus, necessary conditions for repetitive growth are either that σ, $\bar{\mathbf{Z}}$, and p be independent of $\mathbf{c}$, or that $\mathbf{c}$ be independent of time. It has already been suggested that such are necessary conditions for balanced growth. In the following paragraphs, when deductions based on the hypothesis that growth is repetitive are made, it will also be assumed that σ, $\bar{\mathbf{Z}}$, and p are independent of time, since these are necessary conditions for repetitive growth.

It is convenient to define two special cases of repetitive growth. These are based on the time dependence or independence of the density of the age distribution, f_A. If f_A as well as $f_{\mathbf{Z}|A}$ is independent of time, then

we say that repetitive growth is also *balanced* growth. Here the term balanced growth is used in exactly the same sense as that used by Campbell [5]. For if both f_A and $f_{\mathbf{Z}|A}$ are independent of t, then Eq. (8.9a) shows that $f_{\mathbf{Z}}$ is also independent of t. Any time dependence of the nonnormalized density $W_{\mathbf{Z}}$ is then confined to the population density n. Inspection of Eq. (6.7) shows that $d \ln n/dt$ must be a constant in balanced repetitive growth. This conclusion requires cognizance of the observation, made earlier, that σ, $\bar{\mathbf{Z}}$, and p either do not depend on $\mathbf{c}$, or else $\mathbf{c}$ is independent of t, in repetitive growth. Hence, in repetitive balanced growth, proliferation is either exponential* or exactly balanced by washout (steady-state continuous propagation). For the ith kind of biomaterial, Eq. (7.5) shows that

$$\frac{1}{n}\frac{dn}{dt} = \frac{1}{x_i}\frac{dx_i}{dt}$$

in the case where $f_{\mathbf{Z}}$ is independent of t. But this is, of course, just the condition that growth be balanced.

If f_A does depend on t, but growth is repetitive, we say that repetitive growth is *synchronous*. (Again we distinguish synchronous from synchronized growth, as in [1]; we also assert that synchronous growth as defined earlier is identical to the situation called synchronous growth in [1].) The justification for this terminology is that if f_A depends on t but growth is repetitive, n does not increase exponentially. But this implies that the probability that a cell of unspecified physiological state will divide in a time interval dt is a function of time. (In the so-called pure birth process, in which cell state is unspecified, this probability is independent of time and proliferation is exponential; see, e.g., Bharucha-Reid [4]. Thus, the pure birth process applies only to balanced growth situations.) Fission is therefore in partial synchrony, since it is more probable at some times that at others. This can also be seen from Eqs. (7.1) and (7.2).

In synchronous growth, $f_{\mathbf{Z}|A}$ is independent of time, but $f_{A|\mathbf{Z}}$ is not. This is because different ages must be associated with a given physiological state as cells go through their life cycle. It was for this reason that

* Therefore, Campbell's statement that "the concepts of logarithmic [exponential] growth and balanced growth are thus independent" is incorrect. Batch balanced growth must be exponential growth, though the converse is not true, as Perret [37] emphasizes.

repetitive growth was defined in terms of $f_{\mathbf{Z}|A}$ rather than in terms of $f_{A|\mathbf{Z}}$.

Since repetitive growth is defined with reference to a conditional density function, we must know how to calculate that function from the equations of change of the population and the definition of cell age. In order to make such a calculation, it is necessary to have the joint density function $f_{\mathbf{Z}A}$ and the density f_A of the age distribution.

The differential equation describing the joint density $f_{\mathbf{Z}A}$ (actually, the nonnormalized density $W_{\mathbf{Z}A}$) can be derived by induction from Eq. (5.8). Thus, suppose the dimensionality of the space considered is increased by one, with the added dimension being the age A of the cell. The equation for the joint density $W_{\mathbf{Z}A}$ can then be written down at once, if we recognize that

$$\dot{\bar{A}} = 1; \tag{8.10}$$

that is, that the rate of ageing is not random. Hence, we have

$$\frac{\partial}{\partial t} W_{\mathbf{Z}A}(\mathbf{z}, a, t) + \frac{\partial}{\partial a} W_{\mathbf{Z}A}(\mathbf{z}, a, t) + \nabla_{\mathbf{Z}} \cdot [\bar{\dot{\mathbf{Z}}}(\mathbf{z}, \mathbf{c}) W_{\mathbf{Z}A}(\mathbf{z}, a, t)]$$

$$= -\left(\frac{1}{\theta} + \sigma(\mathbf{z}, c)\right) W_{\mathbf{Z}A}(\mathbf{z}, a, t). \tag{8.11}$$

Equation (8.11) is valid for $a > 0$ (*not* for $a \geqslant 0$). The integral on the right-hand side of Eq. (5.8) does not have a corresponding term in Eq. (8.11) since cells just formed by fission have age zero, by definition. In order to account for this, a number balance is made on cells of age 0 to da and state $\mathbf{Z}(t) \in d\mathbf{v}$. If Eq. (5.1) is used for this purpose, we obtain the result

$$W_{\mathbf{Z}A}(\mathbf{z}, 0, t) = 2 \int_0^\infty da \int_{\mathfrak{R}} \sigma(\mathbf{z}', \mathbf{c}) p(\mathbf{z}, \mathbf{z}', \mathbf{c}) W_{\mathbf{Z}A}(\mathbf{z}', a, t)\, d\mathbf{v}' \tag{8.12}$$

where the factor of two results because fissions are binary, and where infinitesimals in the balance equation have been neglected.

Equation (8.12) is a boundary condition that solutions of Eq. (8.11) must satisfy. Thus, the joint density $W_{\mathbf{Z}A}$ may be found, in principle, by solution of Eqs. (8.11) and (8.12), once we have solved the master equations (6.8) and (6.7) for $W_{\mathbf{Z}}$ (and $\mathbf{c}$).

The differential equation for W_A can be deduced by integrating each term of Eq. (8.11) over all of physiological state space; this yields

$$\frac{\partial}{\partial t} W_A(a,t) + \frac{\partial}{\partial a} W_A(a,t) + \int_{\mathfrak{B}} \nabla_{\mathbf{z}} \cdot [\dot{\bar{\mathbf{z}}}(\mathbf{z},\mathbf{c}) W_{\mathbf{z}A}(\mathbf{z},a,t)]\, d\mathfrak{v}$$

$$= -\frac{1}{\theta} W_A(a,t) + \int_{\mathfrak{B}} \sigma(\mathbf{z},\mathbf{c}) W_{\mathbf{z}A}(\mathbf{z},a,t)\, d\mathfrak{v}. \tag{8.13}$$

Application of Green's theorem to the multiple integral on the left-hand side of Eq. (8.13) gives the surface integral of $\bar{\mathbf{z}} W_{\mathbf{z}A}$ over $\mathfrak{S}$. The regularity condition Eq. (5.9) then shows that this integral vanishes. If we put

$$\Gamma(a,\mathbf{c},t) = \int_{\mathfrak{B}} \sigma(\mathbf{z},\mathbf{c}) f_{\mathbf{z}|A}(\mathbf{z},a,t)\, d\mathfrak{v}, \tag{8.14}$$

then Eq. (8.13) becomes

$$\left(\frac{\partial}{\partial t} + \frac{\partial}{\partial a} + \frac{1}{\theta} + \Gamma(a,\mathbf{c},t)\right) W_A(a,t) = 0. \tag{8.15}$$

This is Von Foerster's [53] equation. The significance of the quantity Γ may be deduced from the total probability theorem; this shows that $\Gamma\, dt$ is the probability that a cell of age a at time t will divide in the time interval t to $t + dt$. Harris [18] calls Γ the age-specific death rate, because fission can be looked on as the "death" of the mother cell. We do not adopt this terminology here, however.

As is true of Eq. (8.11), Eq. (8.15) is not valid for $a = 0$. The boundary condition on Eq. (8.15) for $a = 0$ may be found by integrating both terms of Eq. (8.12) over all of physiological state space. This yields

$$W_A(0,t) = 2\int_0^{\infty} \Gamma(a,\mathbf{c},t) W_A(a,t)\, da, \tag{8.16}$$

in which Eq. (4.5) has been used. The boundary condition Eq. (8.16) is an essential part of the solution of the binary fission problem. It was given by Von Foerster [53] and by Fredrickson and Tsuchiya [14].

The density of the age distribution function can be found by solution of Eqs. (8.15) and (8.16). Since $W_{\mathbf{z}}$, $W_{\mathbf{z}A}$, and W_A are then known, we can calculate the conditional densities $f_{\mathbf{z}|A}$ and $f_{A|\mathbf{z}}$.

Equation (8.15) and its attendant boundary condition Eq. (8.16) are valid, of course, whether or not growth is repetitive. However, if growth is repetitive, Eq. (8.14) shows that the fission probability function Γ will not depend on time t, and so we can regard the age of a cell as the single parameter that determines (statistically) when a cell will divide. If growth is not repetitive, cell age does not have this useful property.

Solutions of Eqs. (8.16) and (8.16) for balanced repetitive growth have been given by Powell [39], Harris [18],* and Fredrickson and Tsuchiya [14]. Powell and Harris did not derive their solutions from Eqs. (8.15) and (8.16), however. A partial solution for synchronous repetitive growth has been given by Tsuchiya, Fredrickson, and Aris [50]; this solution is of interest since it suggests the theorem that synchronous repetitive growth tends to degenerate to balanced repetitive growth. Trucco [49] has discussed the general nature of solutions of Von Foerster's equation.

Von Foerster's equation and its attendant boundary condition permit the calculation of the age distribution for repetitive growth. We consider next the calculation of the distribution of any given cellular character. We define a cellular character $S(t)$ to be any nonnegative single-valued scalar function of the physiological state vector of a cell:

$$S(t) = g(\mathbf{Z}(t)). \tag{8.17}$$

For instance, if $g(\mathbf{Z}(t))$ is $\mathbf{m} \cdot \mathbf{Z}(t)$, where $\mathbf{m}$ is the same vector that appears in Eq. (7.13), then $S(t)$ is the amount per cell of the character whose concentration in the culture is $y(t)$. Hence, $S(t)$ could be the dry weight of the cell, its fresh (wet) volume, its nitrogen content, etc.

In order to find the distribution of $S(t)$, we first find the joint density $f_{\mathbf{Z}S}$ by the same procedure used to find $f_{\mathbf{Z}A}$. This shows that $f_{\mathbf{Z}S}$ must satisfy the equation

$$\frac{\partial}{\partial t} W_{\mathbf{Z}S}(\mathbf{z}, s, t) + \frac{\partial}{\partial s} [\dot{\bar{S}}(\mathbf{z}, c) W_{\mathbf{Z}S}(\mathbf{z}, s, t)] + \nabla_{\mathbf{Z}} \cdot [\dot{\bar{\mathbf{Z}}}(\mathbf{z}, c) W_{\mathbf{Z}S}(\mathbf{z}, s, t)]$$

$$= -\left(\frac{1}{\theta} + \sigma(\mathbf{z}, c)\right) W_{\mathbf{Z}S}(\mathbf{z}, s, t)$$

* Harris [18] and Tsuchiya, Fredrickson, and Aris [50] show that the fission probability function Γ is related to the density of the distribution of life spans L by $\Gamma(a) = f_L(a)/(1 - \int_0^a f_L(l)\, dl)$. The present discussion shows clearly that this relation is valid only when growth is repetitive. Rahn [42], Kendall [23], and others have advanced models for $f_L(l)$; because of the relation between f_L and Γ, these models can be regarded as solutions of Eqs. (8.15) and (8.16).

$$+ 2\int_0^\infty ds' \int_{\mathfrak{B}} \sigma(\mathbf{z}', \mathbf{c}) q(\mathbf{z}, \mathbf{z}', s, s', \mathbf{c}) W_{\mathbf{Z}S}(\mathbf{z}', s', t)\, d\mathbf{v}' \tag{8.18}$$

in which

$$\bar{\dot{S}}(\mathbf{z}, \mathbf{c}) = \nabla_{\mathbf{z}} g(\mathbf{z}(t)) \cdot \bar{\dot{\mathbf{Z}}}(\mathbf{z}, \mathbf{c}) \tag{8.19}$$

and $q(\mathbf{z}, \mathbf{z}', s, s', \mathbf{c})\, d\mathbf{v}\, ds$ is the probability that fission of a mother cell of state $\mathbf{z}'$ (and so character s') will produce a daughter cell of state $\mathbf{Z}(t) \in d\mathbf{v}$ and character $s < S(t) < s + ds$. Clearly,

$$q(\mathbf{z}, \mathbf{z}', s, s', \mathbf{c}) = p(\mathbf{z}, \mathbf{z}', \mathbf{c})\, \delta(s - g(\mathbf{z})) \tag{8.20}$$

where δ is Dirac's delta function. The partitioning function for the cellular character is

$$r(s, s', \mathbf{c}) = \int_{\mathfrak{B}} \int_{\mathfrak{B}} q(\mathbf{z}, \mathbf{z}', s, s', \mathbf{c})\, d\mathbf{v}\, d\mathbf{v}', \tag{8.21}$$

and this is normalized

$$\int_0^\infty r(s, s', \mathbf{c})\, ds = 1 \tag{8.22a}$$

and obeys the "conservation principle"

$$r(s, s', \mathbf{c}) = r(s' - s, s', \mathbf{c}). \tag{8.22b}$$

Integration of each term of Eq. (8.18) over all of physiological state space, with application of Green's theorem and the regularity condition, then gives the following equation for the density W_S:

$$\frac{\partial}{\partial t} W_S(s, t) + \frac{\partial}{\partial s} [\bar{\dot{S}}(s, \mathbf{c}, t) W_S(s, t)]$$

$$= -\left(\frac{1}{\theta} + \Gamma'(s, \mathbf{c}, t)\right) W_S(s, t) + 2\int_0^\infty \Gamma'(s', \mathbf{c}, t) r(s, s', \mathbf{c}) W_S(s', t)\, ds' \tag{8.23}$$

in which

$$\bar{\dot{S}}(s, \mathbf{c}, t) = \int_{\mathfrak{B}} \bar{\dot{S}}(\mathbf{z}, \mathbf{c}) f_{\mathbf{Z}|S}(\mathbf{z}, s, t)\, d\mathbf{v} \tag{8.24}$$

and

$$\Gamma'(s, \mathbf{c}, t) = \int_{\mathfrak{X}} \sigma(\mathbf{z}, \mathbf{c}) f_{\mathbf{Z}|S}(\mathbf{z}, s, t)\, d\mathbf{v}; \tag{8.25}$$

$\Gamma'\, dt$ is of course the probability that a cell having $S(t) = s$ will divide in time interval t to $t + dt$.

Equation (8.23) is valid for any kind of growth situation if $\bar{S}$, Γ', and r are functions of time t. However, the equation is not very useful if these functions do depend on t. In balanced repetitive growth, but not in synchronous repetitive growth, the conditional density $f_{\mathbf{Z}|S}$ is independent of time, so that the three functions $\bar{S}$, Γ', and r are independent of t. In balanced repetitive growth, $f_{\mathbf{Z}|S}$ is independent of time because *all* densities are independent of time therein. But $f_{\mathbf{Z}|S}$ is not independent of time in synchronous repetitive growth. This is because $S(t)$ specifies only a hypersurface—not a unique point—in physiological state space. In repetitive growth, the age $A(t)$ specifies (statistically) a unique point in physiological state space so that $f_{\mathbf{Z}|A}$ is independent of time, whether growth is synchronous or balanced.

Equation (8.23) was derived by Eakman, Fredrickson, and Tsuchiya [11] by a very different procedure from that used here. Following Koch and Schaechter [24], they assumed that "cell mass" [which is one possible cellular character that can be defined by Eq. (8.17)] is an index of physiological state and by mass balance established the equation. This procedure is analogous to that commonly used to derive Von Foerster's equation; for that purpose, cell age is assumed to be an index of physiological state. The considerations of this section show that age is a *useful* index of physiological state only in repetitive growth. Nevertheless, Von Foerster's equation and, to a lesser extent, Eq. (8.23), do provide tractable mathematical apparatus for the quantitative study of repetitive growth.

Models of cellular growth and division based on cell age as an index of physiological state are sometimes criticized on the basis that they assume no correlation between the life-spans of sister cells, whereas in fact a strong positive correlation is observed (Schaechter *et al.* [46]; Powell [39, 40]). For instance, Kubitschek [25] dismisses the previously mentioned models of Rahn and Kendall on this basis. Hence, it might be inferred that Von Foerster's equation must also be dismissed, since therein cell age is regarded as an index of physiological state. Such an inference is not justified, however, because the age distribution in Von

Foerster's equation refers to a so-called total or absolute probability: the probability that a cell has a given age without regard to the age of its parent cell at fission. Hence, Von Foerster's equation can say nothing about the correlation of life-spans of sister cells because to do so requires a conditional probability.

Eakman, Fredrickson, and Tsuchiya [11] have shown that Eq. (8.23) does predict correlations between the life-spans of sister cells when appropriate conditional probabilities are introduced and related to the density f_S. However, predicted correlations would probably be good only if "cell mass" (or any other cellular character selected) were a good predictor of fission.

The master equations of change given in Section 6 are capable of predicting correlations between sister cells or between mother and daughter cells. The signs and magnitudes of such correlations cannot be established without becoming a good deal more specific about the functions σ, p, and $\bar{\mathbf{Z}}$, however. Hence, the subject of correlations must be dropped at this point.

To close this section, we consider an example that shows that the definition of repetitive growth is too general, and must be qualified. Suppose the feed stream to a propagation vessel contains organisms. This might be the case, for instance, if we consider the second, third, and subsequent vessels in a cascade (series) arrangement of propagation vessels. The moment equations, Eqs. (7.1) and (8.2), must be generalized for this case; clearly, the generalizations will be

$$\frac{dn}{dt} = \frac{1}{\theta}(n_f - n) + kn \tag{8.26}$$

$$\frac{dx_i}{dt} = \frac{1}{\theta}(x_{if} - x_i) + \mu_i x_i \tag{8.27}$$

where the subscript f denotes conditions in the feed stream.

There is no a priori reason why a steady state cannot be achieved in a cascade of vessels. If in fact a steady state is achieved, then of course $f_{\mathbf{Z}|A}$ (and all other density functions as well) will be independent of time, and by definition, growth will be repetitive.

However, we are faced here with the paradox that growth is not now balanced. For the moment equations, with $dx_i/dt = dn/dt = 0$ yield

$$k = \frac{1}{\theta}\left(1 - \frac{n_f}{n}\right),$$

$$\mu_i = \frac{1}{\theta}\left(1 - \frac{x_{if}}{x_i}\right).$$

Inasmuch as there is no reason why the ratios x_{if}/x_i must be the same for all i, and the same as the ratio n_f/n, the conditions for balanced growth are not satisfied.

The biological reason why this situation is not balanced growth is clear. When cells are washed from one vessel to another, they suddenly "see" a different environment than that from which they came. That is, the environment changes from a concentration (vector) $\mathbf{c}_f$ to a concentration $\mathbf{c}$. This evokes a response ("cell modulation"—Weiss [54]) such that changes in cellular composition and properties are brought about.

In order to resolve, or rather to avoid, the paradox, it is necessary to restrict the definition of repetitive growth to cell populations in which all clones of the population have "seen" the same history of environmental conditions for an indefinite time in the past. It is not necessary that this be a constant history, of course. Since the ancestors of some of the cells in a vessel of a cascade may have been in that vessel for many generations, whereas other cells in the vessel may have just entered, the definition of repetitive growth does not apply, and in fact, such growth is not repetitive.

THE ATTAINMENT OF REPETITIVE GROWTH

Repetitive growth is not attained immediately upon starting growth of a cell population; indeed, repetitive growth may never be attained in some cases. Hence, two questions arise: What experimental observations will be sufficient to establish the existence of repetitive growth? What are the necessary and sufficient conditions on the functions σ, $\bar{\mathbf{R}}$, and p associated with a cell, the applied external conditions $\mathbf{c}_f$ and θ, the initial distribution of states, and the initial environmental conditions, that will guarantee that the master equations of change will predict repetitive growth during some time interval? We are at present unable to give satisfactory answers to these questions, but some suggestions can be made.

Consider first the question of establishing experimental criteria by which balanced repetitive growth can be recognized. Sufficient conditions for balanced repetitive growth are that all the specific synthesis rates (μ_i) and the specific multiplication rate (k) be equal and time independent, and that all (normalized) density functions and conditional density functions be time independent. Obviously, it is not possible to measure

all of these quantities and density functions, and such sample measurements as are obtained will reflect the occurrence of experimental errors. Hence, proof of balanced repetitive growth must be by statistical inference. An attack on this problem, which is by no means trivial, has been made by Hanson [17] for the continuous cultivation of unicellular algae.

Perret ([37], page 614) remarks that "the exponential state [i.e., balanced repetitive growth] could ... be nearly attained during logarithmic growth in batch culture, under suitable conditions; but a mass of experimental evidence throughout the literature suggests that such conditions are rarely realized in practice." If Perret's estimate is accurate, it would explain, for instance, why it is often so difficult to reconcile quantitatively the data from batch growth with data from continuous propagation. It would also imply that Von Foerster's equation, with Γ independent of t, does not apply to batch growth. Hence, further work along the lines suggested by Hanson would appear to be fruitful.

We are unable at present to give any useful experimental criteria by which synchronous repetitive growth can be recognized unequivocally. However, it is possible to state conditions under which growth exhibiting synchrony of fission is *not* synchronous repetitive growth. Thus, one possible way to obtain a synchronous repetitive growth situation is to start with a population in balanced repetitive growth, and remove from the population—without altering the environment—cells of a selected range of physiological states. (This would produce perfect synchrony, for one generation, if a single cell were isolated.) Such selection can be done mechanically; the filtration technique of Maruyama and Yanagita [28] may represent a suitable approach. We are not sure that the resulting population will be in synchronous repetitive growth, but it certainly will not be if the starting population was not in balanced growth.

With regard to the second question raised in the foregoing, that of the necessary and sufficient conditions for growth to be repetitive, it is possible to show that a necessary condition is that the functions σ, $\bar{\mathbf{Z}}$, and p do not depend, either explicitly or implicitly, on time. The equation of change for the conditional density $f_{\mathbf{Z}|A}$ can be derived from Eq. (8.11). We introduce the relation $W_{\mathbf{Z}A} = W_A f_{\mathbf{Z}|A}$ into Eq. (8.11) and apply Von Foerster's equation to the result, thereby obtaining the following nonlinear equation of change for $f_{\mathbf{Z}|A}$:

$$\frac{\partial f_{\mathbf{Z}|A}}{\partial t} + \frac{\partial f_{\mathbf{Z}|A}}{\partial a} + \nabla_{\mathbf{Z}} \cdot [\bar{\dot{\mathbf{Z}}} f_{\mathbf{Z}|A}] = -\left[\sigma - \int_{\mathfrak{V}} \sigma f_{\mathbf{Z}|A}\, d\mathfrak{v}\right] f_{\mathbf{Z}|A}. \qquad (9.1)$$

The boundary condition for this equation may be derived from Eqs. (8.12) and (8.16); it is

$$f_{\mathbf{z}|A}(\mathbf{z}, 0, t) = \frac{\int_{\mathfrak{A}} \sigma(\mathbf{z}', \mathbf{c}) p(\mathbf{z}, \mathbf{z}', \mathbf{c}) f_{\mathbf{z}}(\mathbf{z}', t)\, d\mathbf{v}'}{\int_{\mathfrak{A}} \sigma(\mathbf{z}', \mathbf{c}) f_{\mathbf{z}}(\mathbf{z}', t)\, d\mathbf{v}'} \tag{9.2}$$

Inspection of these equations shows that time independence of σ, $\dot{\mathbf{z}}$, and p is a necessary condition for the vanishing of the time derivative of $f_{\mathbf{z}|A}$; i.e., for repetitive growth.

The question of sufficient conditions for repetitive growth is related to the question of necessary conditions on applied external conditions ($\mathbf{c}_f$), initial distribution of physiological states, and initial state of the environment for repetitive growth. When a culture is started, or when an established culture is "shocked" by a sudden change of environmental conditions (as in certain synchronization techniques), growth is not immediately balanced thereafter, and there is a period of time in which the culture adjusts to the new environmental conditions. If and when this adjustment is completed, we say that growth is balanced. In order to establish sufficient conditions for balanced growth, therefore, the stability of the equations governing the culture must be investigated. No progress has been made along these lines, so we turn to other matters.

MODELS FOR NONREPETITIVE GROWTH

Repetitive growth is the situation in which, as Dean and Hinshelwood [7] put it, the cell population is in a state of dynamic equilibrium with its environment. Hence, if we wish to study the sequence of events in the "normal" cell cycle, then clearly a repetitive growth situation must be established.

On the other hand, all those situations in which a population is *responding to changes* in its environment are *not* repetitive growth. Some of these situations, such as the occurrence of a lag phase following inoculation into a new medium, the induction of enzyme synthesis, and the growth of synchron*ized* cultures, have provided insight into the workings of the cell, and may be expected to provide even more insight in the future.

Mathematical study of nonrepetitive growth appears to be an extremely formidable task. The convenient simplifications of repetitive growth no longer can be made, and we must go back to the full set of equations, Eqs. (6.7) and (6.8). From the computational point of view, the principal obstacle is the indefinitely large dimensionality of physiological state space. If any computations are to be made, this dimensionality must be reduced drastically. The fastest computers presently available could possibly solve problems in a reasonable time (minutes or hours at most) if the physiological state vector had no more than three or four elements; this reduction in dimensionality must be made if nonrepetitive growth is to be attacked mathematically.

One possibility for reduction of dimensionality is suggested by the work of Ramkrishna *et al.* [43] on nonsegregated models. They showed that some of the salient features of the lag phase of growth could be modeled by dividing the biomaterial of a population into two categories, one category being "nucleic acids," the other, everything else in the biomass. To model the induction and repression of enzyme synthesis, this would probably be too crude, and one or two further categories of biomass would have to be introduced.

At any rate, the work cited suggests that in *specific situations*, it may be possible to "lump" various biochemical entities together and so reduce the dimensionality of state space without sacrificing the ability of the equations to predict essential phenomena. Thus, define a contracted physiological state vector $\mathbf{Z}^c$ by

$$\mathbf{Z}^c = \mathbf{h}^s \cdot \mathbf{Z} \tag{10.1}$$

where $\mathbf{h}^s$ is a constant matrix with number of rows equal to the dimensionality of $\mathbf{Z}^c$, and number of columns equal to the dimensionality of $\mathbf{Z}$. There would be no point to the procedure if the dimensionality of $\mathbf{Z}^c$ were not less (and much less) than that of $\mathbf{Z}$.

The number of biochemical reactions that are to be considered must also be reduced. Hence, define a contracted vector of reaction rates $\mathbf{R}^c$ by

$$\mathbf{R}^c = \mathbf{h}^r \cdot \mathbf{R} \tag{10.2}$$

where again $\mathbf{h}^r$ is a constant matrix.

Finally, the dimensionality of the space of environmental conditions should also be reduced, and this can be done by introducing the contracted vector $\mathbf{e}^c$:

$$\mathbf{e}^c = \mathbf{h}^e \cdot \mathbf{e} \tag{10.3}$$

where $\mathbf{h}^e$ is a third constant matrix. A choice of the three matrices $\mathbf{h}^s$, $\mathbf{h}^r$, and $\mathbf{h}^e$ defines a specific model for growth.

The mean growth rate vector of a cell in the contracted physiological state space can be written in terms of the contracted mean reaction rate vector if a contracted stoichiometric matrix for cellular substances is defined:

$$\overline{\dot{\mathbf{Z}}}^c = \boldsymbol{\beta}^c \cdot \overline{\mathbf{R}}^c. \tag{10.4}$$

Here, $\boldsymbol{\beta}^c$ is the contracted stoichiometric matrix for cellular substances. From Eqs. (10.1) and (10.2) it follows that $\boldsymbol{\beta}^c$ is to be found by solving the matrix equation

$$\mathbf{h}^s \cdot \boldsymbol{\beta} = \boldsymbol{\beta}^c \cdot \mathbf{h}^r. \tag{10.5}$$

Similarly, the contracted stoichiometric matrix for environmental substances $\boldsymbol{\gamma}^c$ is to be found by solving

$$\mathbf{h}^e \cdot \boldsymbol{\gamma} = \boldsymbol{\gamma}^c \cdot \mathbf{h}^r. \tag{10.6}$$

The equation of change for $W_{\mathbf{Z}^c}$, the density of the distribution of $\mathbf{Z}^c$ among cells, can be derived from the master equations of change by the same kind of procedure used to derive Eq. (8.23). The result will be an equation of the same form as Eq. (5.8), but with $\mathbf{Z}^c$ replacing $\mathbf{Z}$; thus, the dimensionality of the problem is reduced. The mean growth rate vector in contracted physiological state space will be

$$\int_{\mathfrak{V}} (\mathbf{h}^s \cdot \bar{\mathbf{Z}}) f_{\mathbf{Z}|\mathbf{Z}^c} \, d\mathfrak{v},$$

and the time-specific fission probability will be

$$\int_{\mathfrak{V}} \sigma f_{\mathbf{Z}|\mathbf{Z}^c} \, d\mathfrak{v}.$$

In general, the mean growth rate vector and the time-specific fission probability for the contracted equation of change will depend on $\mathbf{z}^c$ (the location in contracted physiological state space), $\mathbf{c}$, *and* time t.

Obviously, the procedure will be useful only if the time dependence of both the growth rate vector and the time-specific fission probability in contracted physiological state space is eliminated or rendered negligible.

Whether or not any transformations such as Eqs. (10.1)–(10.3) can accomplish this aim is a matter to be settled by experiment. However, the success of the nonsegregated models of Ramkrishna *et al.* [43] in giving at least a qualitative description of lag phenomena suggests that the contraction procedure may be useful. It must be emphasized that a model that is useful in the analysis of one nonrepetitive growth situation may not be useful in the analysis of a different one.

The contraction procedure just outlined is in common use in chemical kinetics. Therein, if "rate-controlling steps" or "Bodenstein intermediates" (Frank-Kamenetsky [13]) can be identified, the dimensionality of the kinetics problem can be reduced. Hence, construction of growth models (specification of the matrices $\mathbf{h}^s$, $\mathbf{h}^r$, and $\mathbf{h}^e$) is, at least in part, the identification of rate-controlling steps and Bodenstein intermediates in a specific growth situation.

CONCLUDING REMARKS

Much of current biological research is concerned with *analysis* of biological phenomena. The recent spectacular successes of molecular biology make it the most obvious example of the trend toward analysis, but examples from other approaches to biology are not hard to find. For instance, most current biological work utilizing the mathematical and deductive methods of physical science deals with analysis of specific phenomena, such as transport across biological membranes or catalysis of biological reactions by enzymes.

We have attempted to take a *synthetic* viewpoint here. That is, we have tried to show how information gleaned from analysis of subcellular phenomena can be used to describe and predict the behavior of cells and cell populations. The foregoing statement does not imply that such information will be sufficient to effect a synthesis; clearly, it is necessary for synthesis.

We have tried to point out that a tendency of some makers of theories of population growth to focus attention on the organisms while ignoring the organisms' environment is unfortunate. Henrici's remark [19] regarding this is still pertinent: "Where these [i.e., mathematical analyses of the growth curves of bacteria] are not interpreted in terms of organisms, substrate, products of metabolism or other definite factors, they do not seem to be very helpful to an understanding of the phenomena . . . ." The concepts necessary for bringing the environment into a quantitative

description of growth—stoichiometric relationships and conservation principles—were stated by M'Kendrick and Pai [29], and were emphasized by Monod [30, 31]. The concepts are implicit in the work of Hinshelwood [20], and Fredrickson and Tsuchiya [14] pointed out that they apply also to theories in which cells are endowed with a degree of individuality. It is hoped that introduction of stoichiometry and conservation principles into the mathematics of population growth will prove to be a stimulus rather than just an added burden of nonlinearity.

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