DYNAMICS OF MICROBIAL CELL POPULATIONS

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I. Introduction

Biological systems in general, and their interaction with their environment, abound with an overwhelming complexity of detail through which general concepts and relationships cannot readily be perceived. Because of this complexity, many biologists are concerned with details, and have tended to neglect problems associated with integrated systems. On the other hand, engineers who have not yet encountered such complexity in manufacturing facilities are concerned with integrated systems which are just beginning to approach the complexity of biological systems. The limited work discussed below offers quantitative analyses of some simpler biological phenomena, and suggests experimental work that may profitably be carried out.

Standard biology texts list the related phenomena of growth and reproduction as minimal criteria for all biological systems. Hence this review is principally a summary of attempts to apply engineering analysis to elucidate growth and replication phenomena in very simple systems: cultures of unicellular organisms reproducing by binary fission. Parenthetically we suggest that other biological phenomena may also be examined with profit by chemical engineers, in particular the field of intracellular transport and transformation. If we assume that biological systems and processes have been optimized during the course of their evolution, such studies may lead to improved methods of treating industrial systems. The study of biological systems so as to apply the knowledge gained ("bionics") to nonbiological systems is familiar to electrical engineers.

Numerous investigations are being made of continuous propagation of microorganisms with a view to potential industrial applications, without much attention to the relations between batch and continuous processing. *All* the aspects observed in batch propagation must somehow manifest themselves in continuous propagation. Moreover, the dynamics of nonassociated cell populations warrants closer scrutiny *per se* because of basic scientific interest in the growth and reproduction of any organism with associated cells, including man. For instance, study of "aging" in microbial cultures may yield information useful to the medical subbranch of geriatrics. Again, the dynamics of microbial growth and reproduction may be of interest to demographers in today's study of the "population explosion."

II. Possible Approaches to the Problem

A fundamental generalization of modern biology is the cell theory: that life is segregated into structural and functional units—cells—and that new cells arise only from pre-existing cells, at least on earth at the present time (see O1). In any complete theory of cellular population dynamics, the *number* of cells occupying a given region will be the fundamental variable. However, in many practical cases the model that one constructs omits certain variables, or admits them in a modified form. Thus the experimental number of microbial organisms may be replaced by the volume or dry weight of cells, the optical density of a cell suspension, or another measure.[1] A model for correlating such data would use the empirical measure of "concentration" of living substance, and would treat "life" as something distributed throughout the whole physical region in which cells occur. Hence one can classify a model of population dynamics as representing either a *distributed* or a *segregated* population. The "distributed" model is the simpler, since the

[1] Methods for counting cell numbers or measuring cell volume, etc., are discussed by Buchanan and Fulmer (B10).

process of *reproduction* is not (and cannot be) treated in this model. The process of *growth* can, of course, be treated in both models.

Another basis for classification of models of population dynamics is provided by the observation that cells may be assumed to be either *structured* or *unstructured*. In a segregated model, we assume a cell to be structured if we specify some means of *distinguishing* it from its fellows. This means may be visual, by comparison of morphology and size under the microscope, or it may be indirect, by comparison of the mass and chemical composition of cells. When we deal with a distributed model, the population will be "structured" if the composition of the population varies with the conditions of propagation. In other words, specification of the *state* of the population requires more than specification of a single quantity.

Finally, one may use either *stochastic* (probabilistic) or deterministic models. In fact, a population of microbial cells is always segregated and structured, and its growth and reproduction should be treated stochastically. On the other hand, the biological knowledge and mathematical tools necessary for the formulation and study of a completely general model do not exist, and a less general approach gives useful results.

III. Unstructured Models

A. Segregated, Stochastic Models

We consider a population of indistinguishable individuals contained in some region V of space, that is, indistinguishable by any technique available to us. The number of individuals present at time t is a random variable $X(t)$, the "population size," that can assume only the discrete values $0, 1, 2, \ldots, x_m$; here x_m is some positive integer (which may be very large, depending on the size of V, the availability of nutrients in V, etc.). At this point, the extensive random variable $X(t)$ is to be sharply distinguished from the intensive population density (the number of organisms per unit volume of V); the population density need not be an integer.

The question to answer is: given an initial population size $X(0) = x_0$, and certain information about the probability of cell fission, what is the probability, $P_x(t)$, that $X(t) = x$ for $t > 0$? Clearly, the question can be answered by experiment, using the frequency interpretation of probability. One measures the population sizes in a large number (ensemble) of "identical" systems V as functions of time; the fraction of systems in which $X(t) = x$ is then an approximation to the required probability.

We desire a *model* that will reproduce and/or predict the experimental observations. Such models have been constructed by statisticians and mathematicians. The model selected here is "the pure birth process," discussed in detail in many places [cf. Bharucha-Reid (B7) or Bailey (B1)].

Consider the following propositions:

A: $X(t + \Delta t) = x$

B_k: $X(t) = x - k$; $\quad k = 0, 1, \ldots, x - x_0$

C: $X(0) = x_0$; cells reproduce by binary fission, cells are indistinguishable, etc.

That is, A is a proposition concerning the *state* (x) of the system at time $t + \Delta t$, whereas the B_k are propositions concerning the state of the system at time t.

By definition of $P_x(t)$, we have[2]

$$P_x(t + \Delta t) = P(AB_0 + AB_1 + \cdots + AB_{x-x_0} \mid C)$$

Hence, by Rule 2 of the theory of probability,

$$P_x(t + \Delta t) = P(AB_0 \mid C) + P(AB_1 \mid C) + \cdots + P(AB_{x-x_0} \mid C) \tag{1}$$

since terms such as $P(AB_0AB_1 \mid C)$ must vanish (propositions B_0 and B_1 are mutually exclusive). By Rule 1 of the theory of probability,

$$\begin{aligned} P(AB_k \mid C) &= P(B_k \mid C)\, P(A \mid B_kC) \\ &= P_{x-k}(t)\, P(A \mid B_kC) \end{aligned} \tag{2}$$

This is as far as the theory of probability can carry us. We must now introduce a model from which the conditional probabilities $P(A \mid B_kC)$ can be calculated.

We assume that proliferation of cells is a *Markov process* (B7). With this assumption, the probabilities of transitions $X(t) = x - k$ to $X(t + \Delta t) = x$ will depend only on the state of the system at the beginning of the time interval t to $t + \Delta t$. In particular, the probabilities of transition will be independent of the history of the system prior to time t. Hence the model is the set of equations

$$\begin{aligned} P(A \mid B_0C) &= 1 - f(x)\, \Delta t - O[(\Delta t)^2] \\ P(A \mid B_1C) &= f(x - 1)\, \Delta t + O[(\Delta t)^2] \\ P(A \mid B_2C) &= O[(\Delta t)^2] \end{aligned} \tag{3}$$

etc., where O means "of the order of," and f is an arbitrary but nonnegative function of its argument. Equations (3) say that the probability of one fission in a very short time interval is very much less than the probability of no fissions in that time interval, and similarly for two fissions, etc. The transitions for which probabilities are assumed in Eqs. (3) refer to increases in population size; this is tantamount to specifying the number of fissions, since in binary fission, each fission increases the population size by one unit.

[2] The appendix gives a brief review of probability theory, applicable here.

From Eqs. (1–3) we get

$$P_x(t+\Delta t) = P_x(t)\cdot\{1 - f(x)\,\Delta t - O[(\Delta t)^2]\} + P_{x-1}(t)\cdot\{f(x-1)\,\Delta t + O[(\Delta t)^2]\} + O[(\Delta t)^2]$$

or, if this is rearranged and Δt allowed to approach zero, then

$$dP_x(t)/dt = -f(x)\,P_x(t) + f(x-1)\,P_{x-1}(t) \tag{4}$$

which is the difference-differential equation describing the model. Equation (4) describes only the *probability* of occurrence of various states (x) as a function of time and a property ($f(x)$) of the system and its surroundings; we make no attempt to derive a functional equation for the state itself.

A set of initial conditions is needed for the solution of the above problem. If the initial state is $X(0) = x_0$ ($x_0 = 1, 2, \ldots, x_m$), then the required initial conditions are

$$P_x(0) = \begin{cases} 1, & x = x_0 \\ 0, & x \neq x_0 \end{cases} \tag{5}$$

As an example of a stochastic growth process we consider the special case where

$$f(x) = \lambda x \tag{6}^3$$

where λ is a constant. Since the probability of a single fission in the time interval Δt is directly proportional to the population size, the population size should increase without limit as $t \to \infty$, and we must let x_m be infinite.

The problem to be solved is now

$$dP_x(t)/dt = -\lambda x\,P_x(t) + \lambda(x-1)\,P_{x-1}(t) \tag{4a}$$

subject to the initial conditions given by Eq. (5). The solution is easily found by Laplace transform methods and the process of induction; it is

$$P_x(t) = \binom{x-1}{x-x_0} e^{-x_0\lambda t}(1 - e^{-\lambda t})^{x-x_0} \tag{7}$$

where the $\binom{n}{m}$ are the binomial coefficients. The moments $M_i(t)$ of the distribution could in principle be calculated from Eq. (7), but it is simpler to use the method of generating functions (cf. F1).

[3] If we had taken $f(x) = \gamma$ (constant), $x_0 = 0$, the problem would describe the *Poisson process*, for which

$$P_x(t) = e^{-\gamma t}\frac{(\gamma t)^x}{x!}$$

This cannot be the model of a growth process, of course, since it violates the basic principle that cells arise only from pre-existing cells.

The generating function $G(s, t)$ is defined by

$$G(s, t) = \sum_{x=x_0}^{\infty} P_x(t)s^x \tag{8}$$

The rth moment of the distribution is of course

$$M_r(t) = E[X^r(t)] = \sum_{x=x_0}^{\infty} x^r P_x(t) \tag{9}$$

Hence one sees that the moments can be found from the generating function by the formulas

$$M_1(t) = \frac{\partial G(1, t)}{\partial s} \tag{10}$$

$$M_2(t) = \frac{\partial G(1, t)}{\partial s} + \frac{\partial^2 G(1, t)}{\partial s^2} \tag{11}$$

etc. In addition, since $X(t)$ must have *some* value equal to or greater than x_0, we require

$$\sum_{x=x_0}^{\infty} P_x(t) = 1 \tag{12}$$

whence $G(1, t)$ must equal unity.

From Eq. (4a) and the definition of the generating function, we find that G must satisfy

$$\frac{\partial G(s, t)}{\partial t} = \lambda s(s - 1)\frac{\partial G(s, t)}{\partial s} \tag{13}$$

To obtain this result, we must remember that $P_x(t) = 0$ if $x < x_0$. The general solution of Eq. (13) may be found; the required special solution is obtained by using the initial condition, Eq. (5), which shows that

$$G(s, 0) = s^{x_0} \tag{14}$$

The generating function is then

$$G(s, t) = \left[1 - \left(\frac{s - 1}{s}\right)e^{\lambda t}\right]^{-x_0} \tag{15}$$

Equation (15) is seen to satisfy the condition required by Eq. (12).

From Eqs. (10) and (11), we find the mean and the variance of the distribution to be

$$E[X(t)] = M_1(t) = x_0 e^{\lambda t} \tag{16}$$

$$V[X(t)] = M_2(t) - M_1{}^2(t) = x_0 e^{\lambda t}(e^{\lambda t} - 1) \tag{17}$$

Equation (16) shows that the expected rate of increase of population size is

exponential. Thus, the problem just solved is the stochastic analog of the *Malthusian* growth process developed in the next section.

We note that

$$dM_i/dt = \lambda M_i \tag{18}$$

That is, the expected multiplication rate depends explicitly only on the expected state, and not on time. In other words, Eq. (18) is the differential equation of an *autonomous* system. Most of the models that have been proposed have the property of being autonomous.

One further conclusion of importance can be gleaned from these results. Consider the "spread" of observed population sizes about the mean value. A measure of the spread is the standard deviation or square root of the variance. The relative spread of observations is then the ratio of the standard deviation to the mean (often called the coefficient of variation), which for this case is

$$\left(\frac{1 - e^{-\lambda t}}{x_0}\right)^{1/2}$$

Hence, if x_0 is small, we see that the degree of unpredictability in the results is large; that is, if many identical systems are observed, their population sizes will be widely scattered about the mean.

On the other hand, chemical engineers and most microbiologists rarely deal with growth situations in which x_0 is small.[4] A lower bound for the size of systems used in these disciplines is something like one cubic centimeter. Ordinarily, there will be 10^4–10^{12} organisms in this volume, and the relative spread will range from 0.0001 up to 1%. By the central limit theorem, this means that only about 31% of identical systems observed would have population sizes differing from the expected size by more than the relative spread calculated above. Since an uncertainty of even 1 percent is usually well within the precision of conventional methods for determining population size, we can regard the growth process as deterministic for the practical purposes of engineering or microbiology. This re-expresses the well-known fact that we *can* predict with fair precision what a population size undergoing logarithmic growth will be at subsequent times.

Note, however, that if we choose to include cellular structure in our model we must then consider the *distribution* of structures in the cells of the population, and elements of randomness again enter the picture.

B. Deterministic Models: Segregated or Distributed

We now define with some precision the physical arrangement of systems of concern. Let the population of cells be contained in a vessel ("reactor" or

[4] A notable exception occurs in counting experiments [cf. Halvorson and Ziegler (H2) and Ziegler and Halvorson (Z2)].

"propagator") of effective volume V. Cells are suspended in a liquid phase that contains nutrients including gases essential for their growth. Byproducts of cellular metabolism accumulate in the liquid. The contents of the propagation vessel are so well stirred that the distribution of cells and of chemical species is uniform throughout.

In biological parlance, the liquid phase is called the *medium*; the liquid phase with suspended cells is called the *culture*. The word *growth* refers to the totality of metabolic processes (physical and chemical) that results in production of protoplasmic material, production of metabolic byproducts, consumption of *substrates* (reactants, nutrients), etc. Later we shall refer to the growth of a single cell; here growth refers to the culture as a whole.

The case where the propagation vessel is charged with a growth medium and *inoculated* (seeded) with cells at some datum of time, and the culture is left to grow without subsequent addition or removal of material from the vessel (with the possible exception of gases), is called *batch growth*. This is the most common mode of propagation in current practice; the scale of batch propagation varies from test tube size in laboratories to 100,000-gallon fermentors or larger industrial installations. Batch growth was the case just treated in the last section on stochastic growth models. It is in connection with batch growth that the terms "lag phase," "log growth phase,"[5] "stationary phase," and "phase of decline" have arisen.

An alternate mode of propagation is provided by the so-called *chemostat* or continuous propagator.[6] In the continuous propagator, fresh medium containing no cells is fed to the vessel at a constant rate of Q volumes per unit time. Culture is removed from the vessel at the same rate, so that the effective volume of the vessel is constant. Variations on this scheme are possible; one might feed fresh medium intermittently, or one might place the feed rate under control of some output variable (such as the pH or optical density of the culture). In other cases a cascade of vessels might be used. For simplicity, we consider here only a single vessel where the feed rate is constant.

Evidentally a steady-state situation may be possible in the continuous propagator. In this situation, cells grow in an unchanging physical and chemical environment (hence the name "chemostat"). This is one of the reasons that continuous propagation has excited the interest of microbiologists [cf. the proceedings of the Czech symposium (M2) and the S. C. I. symposium (S4)].

The mathematics of continuous propagation, for populations of unstructured cells, is easily written down: Let N be the population density of cells in the propagator, and let C be the concentration of "protoplasm"

[5] This is a misnomer; the kind of growth referred to is exponential, rather than logarithmic. In this review, the term "exponential growth" will be used.

[6] In engineering practice, such a system is often called a continuous stirred-tank reactor (C*).

therein. The latter term is used roughly; it may refer to volume of wet cells per unit volume of culture (determined, e.g., by centrifugation), to the concentration of cellular nitrogen (as determined by chemical analysis), etc. *These two methods of expressing cell quantity are not always proportional.* The population density is not used in distributed models.

The *growth rate* may be expressed as either

R_c = rate of production of *cells* (number) per unit volume of culture (strictly, this is a *multiplication rate*)

or

R_p = rate of production of "protoplasm" per unit volume of culture

There is not necessarily a linear relation between R_c and R_p.

Number or material balance on V then yields

$$dN/dt = -(1/\theta)N + R_c \tag{19}$$

$$dC/dt = -(1/\theta)C + R_p \tag{20}$$

where $\theta = V/Q$ is the nominal holding time.[7] In batch propagation, $1/\theta = 0$ (infinite holding time), so that the growth rate becomes the time derivative of population density or concentration of protoplasm.

If a steady state is obtained in a continuous propagator, then clearly

$$r_c \equiv R_c/N = 1/\theta \tag{21}$$

$$r_p \equiv R_p/C = 1/\theta \tag{22}$$

that is, the specific growth rates (r_c or r_p) must equal the reciprocal holding time. Cells and protoplasm are washed out as rapidly as they are formed.

Expressions for Growth Rate. At this stage, a model must be introduced if further progress is to be made, and we choose a deterministic model. All deterministic models which have been introduced (with two exceptions) have been autonomous; they assume that the growth rates are explicit functions only of the *state* of the system, and not of time. But state in an unstructured model can only refer to population density or to concentration of protoplasm, since, by definition, a model is unstructured if only one state variable appears. Thus, the model is

$$R_c = R_c(N) \tag{23}$$

$$R_p = R_p(C) \tag{24}$$

[7] Microbiologists prefer to use the reciprocal of θ, which they call the "dilution rate." Herein, we adhere to the usual chemical engineering practice of using the holding time. Both usages have their advantages, of course.

By a Maclaurin series expansion of Eq. (23), for instance, we get

$$R_c = R_c(0) + \frac{dR_c(0)}{dN} N + \frac{d^2R_c(0)\, N^2}{dN^2\ 2!} + \cdots \tag{25}$$

But $R_c(0)$ must be zero, since cells arise only from pre-existing cells.

If we neglect all but the second term of Eq. (25), we have Malthus' law[8]:

$$R_c = \mu N \tag{26}$$

where μ is a constant. Thus, for a batch system with $N = N_0$ when $t = 0$, Eqs. (19) and (26) show that growth is exponential. On the other hand, we have for a continuous propagator

$$dN/dt = -(1/\theta)N + \mu N$$

whence the condition for steady state is

$$\mu = 1/\theta \tag{27}$$

The population density at steady state is indeterminate, and no steady state is possible unless Eq. (27) is fulfilled exactly. As a matter of fact Eq. (27) grossly contradicts experience, since in continuous propagation there is a *range* of holding times in which steady state can be achieved. Hence something is wrong with the Malthus model.

If all but the second and third terms in Eq. (25) are neglected, we get the law of Verhulst and Pearl (W2, P1), also called the "logistic law":

$$R_c = \mu N(1 - \beta N) \tag{28}$$

where μ and β are positive constants. In batch growth Eq. (28) yields the *logistic curve*:

$$N = \frac{N_0 e^{\mu t}}{1 - \beta N_0(1 - e^{\mu t})} \tag{29}$$

One sees that N approaches $1/\beta$ as $t \to \infty$. Equation (29) is a more realistic form than Malthus' law, since there is now a stationary phase following exponential growth.

Application of Eq. (28) to continuous propagation yields the steady-state condition

$$1/\theta = \mu(1 - \beta N) \tag{30}$$

According to this equation, all holding times greater than $1/\mu$ will yield steady states with nonzero population density. If $\theta = 1/\mu$, a steady state will also

[8] According to Malthus, populations tend to increase exponentially, unless checked by some factor, such as a shortage of nutrients. A survey of some of the early literature on population growth is given by Whittaker (W2) and by Pearl (P1).

be obtained, but N will be zero therein. In contrast to Eq. (27), Eq. (30) yields the steady-state population density uniquely.

Clearly the foregoing result is "better" than that obtained from Malthus' law. However, any model such as Eq. (23) or (24) is suspect because it does not account for the effect of environment on growth, or the reciprocal effect of growth on environment; this will be considered shortly. Also experimental data exist which cannot be reconciled with such models. Finn and Wilson (F2), in a study of the continuous propagation of yeast, found that under certain conditions there did not appear to be any steady state; rather, the population density oscillated about some mean value, with a well-defined frequency. This brings the question of *stability* into the discussion; we will show that Eq. (23) is incompatible with the existence of the phenomenon observed by Finn and Wilson.

The question of stable operation of ordinary chemical reactors apparently originated with van Heerden (H6). Analyses of the stability question for such systems were given by Bilous and Amundson (B8) and Aris and Amundson (A2). Analysis depends on a purely mathematical result obtained by Liapunov (L3). This result is known as Liapunov's theorem, or the stability theorem, and a proof may be found in Davis' book (D1). A more advanced treatment of the stability problem is given by Hahn (H1). The analysis given below is essentially that of Spicer (S5).

If we do not specialize Eq. (23), the equation describing the dynamics of the continuous propagator is

$$dN/dt = -(1/\theta)N + R_c(N) \tag{31}$$

We denote possible steady-state population densities by $\tilde{N}$; these are given by

$$R_c(\tilde{N})/\tilde{N} = 1/\theta \tag{32}$$

There may be multiple steady states if Eq. (32) has more than one real positive root.

Application of the stability theorem shows that the steady-state $\tilde{N}$ will be a *stable node* if

$$R_c(\tilde{N})/\tilde{N} > dR_c(\tilde{N})/dN \tag{33}$$

The steady state $\tilde{N}$ is an *unstable node* if the inequality is reversed. As shown by Spicer (S5), the inequality (33) will be fulfilled if and only if the derivative of the *specific* growth rate [Eq. (21)] is negative: $dr_c(\tilde{N})/dN < 0$.

On biological grounds, we have to assume that $dr_c(N)/dN$ is *never* positive. This derivative is zero in the Malthusian case: in other cases, if the derivative were positive, growth in batch cultures would be faster than exponential (a circumstance which is never observed). It was for this reason that β was restricted to positive values in the Verhulst–Pearl law.

It is easy to show that the restriction $dr_c(N)/dN < 0$ allows only one real positive root for Eq. (32). Thus there are not multiple steady states. The one steady state must be a node rather than a focus, so that no oscillations will be observed in approach to the steady state, and Eq. (23) cannot accommodate the Finn and Wilson phenomenon.

Finn and Wilson tried to circumvent this difficulty by introducing the *ad hoc* hypothesis that R_c was also an explicit function of time; this does away with the autonomous nature of the model. By so doing, they were able to make use of earlier work by Baron (B2) to obtain predictions of oscillation in population density.

Volterra (V2) postulated an unstructured growth model involving a "hereditary factor."[9] His equation for batch growth is

$$dN/dt = \mu N\left[1 - \beta N - \int_0^t N(t')\, K(t - t')\, dt'\right] \tag{34}$$

It is difficult even to obtain numerical solutions of this equation, in the general case. However, if the kernel K is a constant (say K_0), the equation may be solved numerically. Plots of the solution may be found in Davis' book (D1) on p. 418. If $K_0 = 0$, one has the logistic equation. For $K_0 > 0$, a set of curves exhibiting exponential growth, a stationary phase, and a phase of decline are predicted for batch growth.

Volterra's equation and the modification of Eq. (23) proposed by Finn and Wilson are the only unstructured growth models that are not autonomous. As we have seen, the justification for models in which growth rates are explicit functions of time lies in the failure of simple autonomous growth models to predict certain observed phenomena. There are, however, other ways in which one can accommodate the phenomena without doing away with the convenient character of autonomous models.

One method was first suggested by M'Kendrick and Pai (M11), and completed by Monod (M12). Applications of it have been made by Monod (M13), Novick and Szilard (N2), Novick (N1), Maxon (M7), Herbert *et al.* (H10), and others. M'Kendrick and Pai assumed that growth would proceed at maximum rate (exponential growth in batch cultures) only if an "unlimited supply of nutriment" were available. When substrates for growth have been consumed, growth must stop. Hence they postulated that the growth rate is

$$R_c = vNC_s \tag{35}$$

where C_s is the concentration of the substance in the medium limiting growth (it is tacitly assumed that there is only one such substance). This in turn is influenced by the amount of growth which has occurred; i.e., M'Kendrick

[9] "Heredity" in Volterra's sense is not to be confused with the geneticist's use of the word.

and Pai assume a *stoichiometric relation* of the form

$$-R_s = aR_c \tag{36}$$

where $-R_s$ is the rate of consumption of substrate, and a is a stoichiometric coefficient.

In batch growth, Eqs. (35) and (36) lead to Eq. (29), the logistic equation with $\mu = \nu(C_{s0} + aN_0)$ and $\beta = a/(C_{s0} + aN_0)$, where C_{s0} is the initial substrate concentration. The constants μ and β are not now things simply to be determined by experiment, but must depend on initial conditions (C_{s0} and N_0) in a specific manner.

Monod (M12) made two improvements on the theory of M'Kendrick and Pai. In the view of the latter authors, the essential process involved in growth may be represented as a chemical reaction

$$V + aS \rightarrow 2V + \text{byproducts}$$

Here, V represents organisms and S is substrate. M'Kendrick and Pai took the measure of V to be the population density N. Inasmuch as a stoichiometric relation is assumed, however, the measure of V ought not to be population density, since the amount of substrate necessary to form one organism does not remain constant under different circumstances of propagation. Monod recommended instead that one use "*la masse de la substance vivante*," which fits the description of the quantity we called C earlier. In other words, a distributed model, rather than a segregated model, is to be used if stoichiometric relations appear. Monod's stoichiometric postulate is then

$$R_s = -aR_p \tag{37}$$

where a has different units than the a in Eq. (36).

Next, Monod recognized that the growth rate could have a maximum value,[10] and he postulated that the substrate dependence of growth rate followed the Michaelis–Menten (M8) form:

$$R_p = \frac{\mu C C_s}{K + C_s} \tag{38}$$

where μ is now the maximum specific growth rate obtained when C_s is much greater than the Michaelis constant K. Equations (37) and (38) together are a complete statement of Monod's model, and they may now be applied to various special cases.

For instance, in *batch* growth with

$$\left.\begin{aligned} C &= C_0 \\ C_s &= C_{s0} \end{aligned}\right\} \text{at } t = 0$$

[10] M'Kendrick and Pai (M11) saw this also, but did not incorporate it into their theory.

we find

$$\frac{dC}{dt} = -\frac{1}{a}\frac{dC_s}{dt} = \frac{\mu C C_s}{K + C_s} \tag{39}$$

The first equation of the foregoing gives

$$C_{s0} - C_s = a(C - C_0)$$

whence the second equation may be integrated to yield

$$\frac{K}{aC_0 + C_{s0}} \ln \frac{C_{s0}}{C_{s0} - a(C - C_0)} + \frac{K + aC_0 + C_{s0}}{aC_0 - C_{s0}} \ln \frac{C}{C_0} = \mu t \tag{40}$$

Provided that $C_{s0} \gg K$ and $C_{s0} \gg aC_0$, C will increase exponentially at first; however, growth will eventually stop, and the maximum concentration of protoplasm reached will be

$$\lim_{t \to \infty} C = C_0 + \frac{C_{s0}}{a} \approx \frac{C_{s0}}{a}$$

No phase of decline is predicted.

The postulated cause of the stationary phase is that all substrate is consumed. The existence of exponential growth in the initial stages of the batch is a consequence of the hyperbolic rate equation, Eq. (38), Thus, for $C_s \gg K$, it becomes approximately

$$R_p \approx \mu C \tag{38a}$$

and the rate is zero order in substrate.

For the continuous propagator, Monod's model requires

$$\frac{dC}{dt} = -\frac{1}{\theta} C + \frac{\mu C C_s}{K + C_s} \tag{41}$$

$$\frac{dC_s}{dt} = \frac{1}{\theta}(C_{sf} - C_s) - \frac{a\mu C C_s}{K + C_s} \tag{42}$$

where C_{sf} is the concentration of limiting substrate in the feed. There are two possible steady states:

$$\text{(A)} \begin{cases} C = 0 \\ C_s = C_{sf} \end{cases}$$

or

$$\text{(B)} \begin{cases} C = \dfrac{1}{a}\left[C_{sf} - \dfrac{K}{\mu\theta - 1}\right] \\ C_s = \dfrac{K}{\mu\theta - 1}, \qquad (\mu\theta > 1) \end{cases}$$

Stability analysis by Liapunov's theorem shows that[11]:

(i) Steady-state (A) is stable if

$$\frac{\mu C_{sf}}{K + C_{sf}} < \frac{1}{\theta}$$

Steady-state (A) is unstable if the inequality is reversed.

(ii) Steady-state (B) is stable if

$$\frac{\mu C_{sf}}{K + C_{sf}} > \frac{1}{\theta}$$

Steady-state (B) is unstable if the inequality is reversed. Moreover, if steady-state (B) is stable, it must be a node; it cannot be a vortex point or a focus.

Steady-state (B) is the interesting one, of course, since steady-state (A) corresponds to complete washout of cells. Stability analysis has shown that the two cannot coexist at the same holding time; either (A) is stable and (B) is unstable, or (B) is stable and (A) is unstable. Moreover, since (B) is a node if it is stable, we see that Monod's model will not predict oscillations—even damped ones—about a steady state of nonzero cell concentration. Hence, in this sense, there has been no improvement over the Verhulst–Pearl model.

However, the ideas involved in Monod's model can be extended somewhat so that predictions of oscillations can be obtained under certain conditions. This idea has been developed by Ramkrishna (R3). He recognized that several processes may be involved in growth, and that combinations of these processes may lead to oscillations of the type found by Finn and Wilson. In his simplest extension of the Monod model, Ramkrishna proposed that growth produces a *staling factor* which inhibits growth. This model, in which structure has not yet been introduced, is an improvement over Monod's model, since it predicts a phase of decline in batch growth, and damped oscillations in certain cases of continuous propagation. Since a generalization of this model to include structure is treated in the next section, we make no further mention of it here.

The problem of growth of mixed cultures (two or more interacting species occupying the same living space) has generated interest among biologists and mathematicians ever since the time of Volterra (V2) and Lotka (L4). Whittaker (W2) states that oscillations in the number of fish of various species present in the Adriatic Sea have been found to be in agreement with some of Volterra's equations; other cases of population interactions are discussed from a purely mathematical point of view by Davis (D1).

Moser (M14) applied extensions of Monod's model to growth of mixed

[11] For details, see Ramkrishna (R3).

cultures in the chemostat. Principal interest here was in the emergence of mutant populations. Very few experimental data exist with which to check the models.

IV. Structured Models: Distributed

A. First Model

The models just discussed have a number of serious deficiencies, despite their being able to provide reasonable descriptions of certain phenomena in a limited number of cases. A most notable deficiency is their inability to predict a lag phase in batch growth. Consideration of this led Ramkrishna (R3) to develop new models in which microbial cultures are endowed with a certain amount of biochemical structure; although these models are distributed rather than segregated, so that phenomena associated with reproduction cannot be treated, the models do predict qualitatively a number of phenomena that cannot be touched by unstructured models.

The basic supposition is that protoplasm is composed of two structural components, whose interaction with each other and with the surrounding medium produces growth. This idea arose in a paper of Weiss and Kavanau (W1). For convenience, we call the two structural components of protoplasm the G mass and the D mass. (In fact, protoplasm is composed of a large number of structural components, whose *geometrical* arrangement is also highly important to life processes.) Tentatively the G mass may be thought of as nucleic acids, and the D mass may be thought of as proteins.

In one of Ramkrishna's models, the processes of growth are assumed to be represented by the following set of "chemical reactions"[12]:

$$G + a_s S \xrightarrow{D} 2G \tag{43a}$$

$$G + a_s' S \xrightarrow{D} G + D + bT \tag{43b}$$

$$G + T \longrightarrow N_G + (1 + b')T \tag{43c}$$

$$D + T \longrightarrow N_D \tag{43d}$$

where T represents an inhibitor produced by growth, and N_G and N_D are "dead" forms of G and D, respectively. The rates of the first two (growth) reactions are assumed to be given by double substrate Michaelis–Menten kinetics (see, e.g., L1); thus, both G mass and D mass must be present for

[12] Ramkrishna's other models are modifications of the scheme proposed above. For instance, inhibitor might be produced by reactions (43a) and (43d), rather than by (43b) and (43c), as above.

growth to occur. The deactivating reactions are assumed to be second order, as written.

For batch growth, the kinetic equations are

$$dC_G/dt = R_G - KC_GC_T \tag{44a}$$

$$dC_D/dt = R_D - K'C_DC_T \tag{44b}$$

$$dC_S/dt = -a_sR_G - a_s'R_D \tag{44c}$$

$$dC_T/dt = bR_D + b'KC_GC_T \tag{44d}$$

in which the C's denote the concentrations of the substances identified by the subscripts. In these equations, we have put

$$R_D = \frac{\mu C_S C_G C_D}{(K_s + C_S)(K_g + C_G)} \tag{45a}$$

$$R_D = \frac{\mu' C_S C_G C_D}{(K_s' + C_S)(K_g' + C_G)} \tag{45b}$$

Although these equations are coupled and nonlinear, numerical solutions can be obtained on the digital computer. Figure 1 shows the results of such a numerical integration procedure applied to the set of Eqs. (44). We see that all phases of the batch growth curve, including a lag phase, are exhibited by

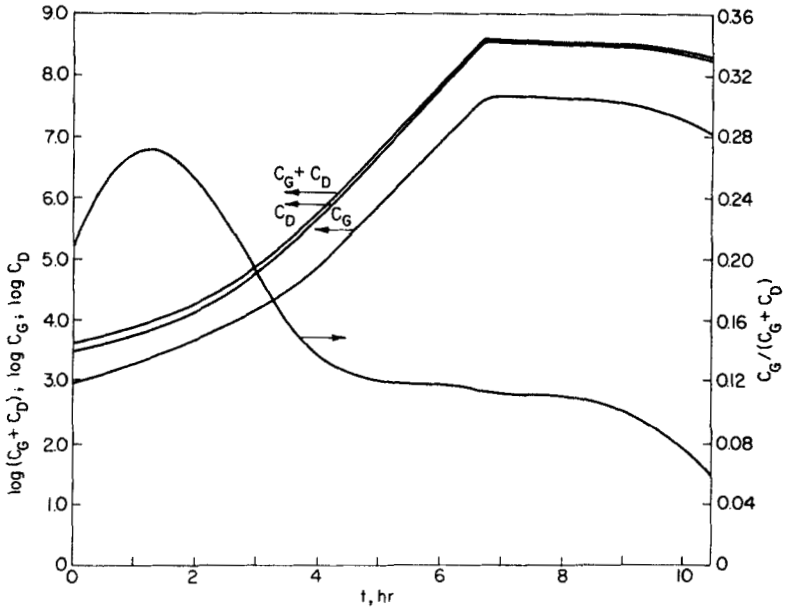


FIG. 1. Computer solution of Eqs. (44) and (45) for batch growth.

the curve of $(C_G + C_D)$—that is, the total concentration of viable protoplasm in the culture—vs time. Moreover, the plot of the fraction of biomass present as G follows the course of fraction of nucleic acids present found experimentally by Malmgren and Hedén in batch growth of *E. coli* (M3).

Other phenomena found in batch growth are predicted by the foregoing model. For instance, the model yields batch growth curves dependent on (i) the relative proportion of G and D in the inoculum, with its principal effect the length of the lag phase; and (ii) the size of the inoculum, with increases in size generally reducing the lag-phase duration. The model shows that the duration of the lag phase is increased for decreased initial amounts of G mass; a culture inoculated with cells from the phase of decline would have a longer lag phase than one from the exponential growth phase (cf. B10). The time course of growth is affected by the "physiological state" or "biological age" of the inoculum, for which a quantitative definition is the fraction of D present.

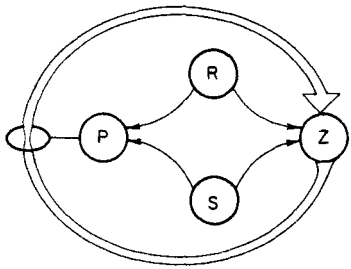
For point (ii) the predictions given above agree with the results of Henrici (H7) on growth of *E. coli*, and with those of Rabotnova and Mineeva (R1) on growth of *Torulopsis utilis* and *Pseudomonas fluorescens*. The maximum concentration of organism attained becomes roughly independent of inoculum size and dependent on initial substrate concentration, as known since Henrici's experiments (H7).

Ramkrishna (R3) has applied this and similar models to continuous propagation, where these structured models display rather complicated stability characteristics; numerical computations indicate multiple steady states, steady states which are foci, and perhaps the existence of limit cycles. Thus the Finn and Wilson phenomenon can be accommodated in principle by a structured model.

B. The Bottleneck Model of the Lag Phase

A distinctive feature of biological growth is that the period of exponential growth (growth phase) is preceded by an induction period (lag phase) during which no growth is observed. In the simpler growth models, in which the growth rate is initially proportional to the population size, the origin of time must be taken at the end of the lag phase. The object of this section is to explore a model which will include both the lag phase and the growth phase. This analysis is related to that of certain production processes in economic theory, known as bottleneck problems (B5), and since it may be a little unfamiliar we give first a verbal description of the method and results of the analysis, which we hope will enable the reader to see what it is all about. Even though this should give a useful overview, it scarcely needs to be added that the detailed mathematical analysis is needed if this approach is to be understood.

A simple model of a biological system that will exhibit a natural lag phase is the following. An organism Z lives on two nutrients R and S. Without loss of generality we can take R to be the nutrient that would be exhausted first, but both are required to maintain the viability of Z; they might, for example, be a "nitrogen" source and an energy source. The growth or reproduction of Z is controlled by a critical product P (such as ATP or a polymerase) and R and S are also required for the formation of P. Schema A shows the system.



In the bottleneck problems of economic theory there is a critical intermediate product and the policy that maximizes the total production starts with a lag phase in which the stock of this critical product is built up; in the metaphor of the problem the "bottleneck" is enlarged. Similarly in our present formulation an objective function must be set up and must be justified in two ways: firstly, by showing that it is biologically meaningful, and secondly, by showing that it gives a behavior that corresponds, at least qualitatively, with observation. In the above model we will assume that the system behaves in such a way as to maximize the population of Z at the time of exhaustion of R. To justify this on the first count we may suppose that a large population of Z confers some advantage on the system in its environment. A larger project is to study both the system and its environment and this would require us to assume some larger objective. On the second count we have to show that the model exhibits a lag phase as an essential part of its optimal policy. We should also like it to show the following features: (*a*) the duration of the lag phase decreases with the decrease of available nutrient, and (*b*) this duration should also decrease with increase in the level of P, so that if growth has started and the organism is transferred into fresh nutrient the lag phase will be shorter. Ultimately we would like to show quantitative agreement between the relations of lag duration and growth rate to nutrient and critical product levels in the model and in a specific biological system or class of systems. This last must await a more detailed confrontation with experiment, but the correct qualitative features are shown. We will now give a verbal description of the equations and solution, referring, where necessary, to the equations of Sections IV,B,1–6 by their number and to Figs. 6, 10, 11(a), 11(b), and 14 to give some impression of the results.

The rate of production of P is assumed to be proportional to the concentration of Z, with a proportionality (or rate) constant k_5 which is the control variable of the system [Eq. (46)]. Thus if $k_5 = 0$, the concentration level of P remains constant. Z reproduces itself at a rate proportional to its own concentration, the rate constant being $(k_1 - k_5)$ [Eq. (47)]. Thus, if $k_5 = 0$, no P is being produced and the growth rate of Z is the greatest that it can be, while if $k_5 = k_1$, P is being produced but the population of Z is constant. We do not allow the production of P at the expense of Z so that k_5 is never greater than k_1 (Eq. 48). The nutrients are used at a rate proportional to the amount of Z present to maintain the viability of the organism and also at an additional rate proportional to the rate of production of P [Eqs. (49) and (50)].

Since the organism cannot be expected to have an innate sense of time, but could have a control mechanism based on concentration levels, we take the concentration of R as the independent variable in place of time by dividing each of the differential equations by the equation for the concentration c_r. The independent variable thus decreases from its value ρ at the beginning of the process to zero at the end. It does indeed turn out that the policy that gives the maximum final concentration of Z involves first a lag phase, during which the concentration of P increases but not that of Z, and then a growth phase, in which the concentration of P remains constant and growth of Z takes place. This only happens if the concentration of R is sufficiently large; if it is small, there is no lag phase and very little growth, but as ρ increases so does the duration of the lag phase and the subsequent rate of growth. There is a definite relation between the concentrations of P, R, and S at the transition from the lag to the growth phase.

To get any further we have to give a definite form to the maximum rate of growth of Z, the constant k_1. In the dimensionless form into which these constants are put [Eq. (51)], this growth rate is γ and is taken to be $a\pi^b\rho\sigma$, where a and b are constants and π, ρ, and σ the concentrations of P, R, and S. By varying a and b we can simulate a number of conditions. If $b = 1$, the effect of the concentration of critical product P on the growth rate is linear. If $b > 1$, it is less than linear at low concentrations of P but rather more than linear at high concentrations: the reverse is true if $b < 1$. This switching surface, as it is called, between the lag and the growth phases has a form such as is shown in Fig. 6. ξ and η are dimensionless forms of the concentrations of R and S [Eq. (86)] and the figure shows the intersection of the switching surface with various planes of constant π. The region below the line $\xi = \eta$ is not of interest since it is not consistent with the assumption that R is the nutrient first exhausted. If, for any level of concentration of P, π, the available nutrient concentrations put the point (ξ, η) to the right of the curve corresponding to the value of π, then the system is in the lag phase and P is produced but Z remains constant. As P is produced the nutrients are exhausted and the point (ξ, η, π) representing the state of the system moves downwards and to the left in Fig. 6. If we think of the axis of π as coming vertically out

of the paper, then this point is moving upwards as well and will soon intersect the switching surface. Suppose that it intersects at such a point as "a" on the curve $\pi = 1.9$ of Fig. 6. In Fig. 11(a) the attempt is made to represent this lag phase in three dimensions. The curve "*abcde*" is the curve $\pi = 1.9$ of Fig. 6 and now lies in the horizontal plane $\pi_s = 1.9$ (the suffix "s" refers to the value at the moment of switching). If switching from the lag to the growth phase takes place at "a," the value of π must have earlier been less than 1.9; the curve representing the state of the system therefore comes up from behind the switching surface as shown. In Fig. 11(b) the growth phase is shown, the vertical axis now being the dimensionless concentration of Z, namely, χ. Since χ has been constant during the lag phase, it has the same value χ_s that it has at the switching surface. Thus $\chi - \chi_s$ is the increase and it is convenient to put the zero of this at the point such as "a" where the switch between phases occurs; this means that the vertical ordinate is really $\chi - \chi_s + \pi_s$. Thus the curve through the point "a" in Fig. 11(b) represents the continuation of the history of the system in which the concentration of Z increases as the nutrients become used up. χ_f is the final value of χ and hence gives the level of Z when R is exhausted. Figure 10 shows the growth of Z as a function of the dimensionless time after switching, $\tau - \tau_s$. This depends on χ_s, the size of the innoculum, for with a larger initial concentration the nutrient is more rapidly exhausted.

Finally, Fig. 14 shows the effect of taking the system in the growth phase and reinoculating it into fresh nutrient. Thus in the first lag phase, which is rather long, the concentration of Z, χ is constant while the concentration of P, π rises. This passes naturally into a growth phase with π remaining constant and χ increasing. The nutrient concentrations ξ and η fall continuously during this period, but before they have been exhausted they are restored to their initial levels. The second lag phase that ensues is very much shorter and is followed by a growth period during which the growth rate is much greater. Thus some of the principal features of biological growth processes are reproduced and the model merits further investigation and incorporation into a larger structure.

1. *Equations for the Simplest Model*

The following equations are the simplest that embody the features of the model we have just described. C will denote concentration with the subscript indicating the species in question.

Production of P. We assume the rate of production of P is proportional to C_z and that the constant of proportionality is the control variable of the system,

$$\frac{dC_p}{dt} = k_5 C_z \tag{46}$$

To say that k_5 is the control variable of the system is to imply that it is some function of the state (C_r, C_s, C_p, C_z), and in looking for the optimal growth policy we are asking just what function k_5 should be to achieve the maximum increase in Z.

Reproduction of Z. We assume that Z is either engaged in reproduction or in formation of P and that the total rate $d(C_z + C_p)/dt$ is proportional to C_z. The constant of proportionality k_1 may be a function of C_r, C_s, and C_p. Thus

$$\frac{dC_z}{dt} = k_1 C_z - \frac{dC_p}{dt} = (k_1 - k_5)C_z \tag{47}$$

We do not allow Z to cannibalize; i.e., P cannot be formed at the expense of Z, so that

$$0 \leqslant k_5 \leqslant k_1 \tag{48}$$

If $k_5 = 0$, then no P is being formed and the growth rate of Z is maximal. If $k_5 = k_1$, Z is not growing but P is increasing.

Use of Nutrients. We assume that the rate of consumption of the two nutrients R and S is proportional to the amount of Z present and that when P is being formed they are consumed at an additional rate proportional to dC_p/dt. Thus

$$\frac{dC_s}{dt} = -k_2 C_z - k_3 \frac{dC_p}{dt} = -(k_2 + k_3 k_5)C_z \tag{49}$$

$$\frac{dC_r}{dt} = -k_4 C_z - k_6 \frac{dC_p}{dt} = -(k_4 + k_5 k_6)C_z \tag{50}$$

Before going further in setting up the problem let us introduce a change of variables. It is unlikely that the organism will have an innate sense of time and know at what future instant the nutrient R will be exhausted. However, it may well be able to detect the level of remaining nutrient and be controlled accordingly. Thus C_r, the concentration of the least-abundant nutrient, is the natural independent variable, rather than the time t. We can obtain equations with C_r as independent variable by dividing each equation by Eq. (50). Let us also introduce the abbreviations

$$\alpha = k_3, \qquad \beta = k_6, \qquad \gamma = k_1/k_4, \qquad \lambda = k_5/k_4, \qquad \omega = k_2/k_4 \tag{51}$$

Then Eqs. (46)–(49) become

$$\frac{dC_p}{dC_r} = \frac{-\lambda}{1 + \beta\lambda} \tag{52}$$

$$\frac{dC_z}{dC_r} = \frac{-\gamma + \lambda}{1 + \beta\lambda} \tag{53}$$

$$0 \leqslant \lambda \leqslant \gamma \tag{54}$$

and

$$\frac{dC_s}{dC_r} = \frac{\omega + \alpha\lambda}{1 + \beta\lambda} \tag{55}$$

At the beginning of the growth process, $t = 0$, we have

$$C_r = \rho, \qquad C_s(\rho) = \sigma, \qquad C_p(\rho) = \pi, \qquad C_z(\rho) = \zeta \tag{56}$$

Thus $C_z(0) - \zeta(\rho)$ is the increase in the amount of Z and is the quantity to be maximized. The optimal policy is the choice of $\lambda(C_r)$, $\rho \geqslant C_r \geqslant 0$, that achieves this maximum and satisfies the restriction of Eq. (54).

2. *Equations for the Optional Growth Policy*

If the optimal choice of $\lambda(C_r)$ were known it could be inserted in Eqs. (51)–(55), and these could be integrated to give the maximum increase

$$C_z(0) - \zeta = \int_{\rho}^{0} \frac{dC_z}{dC_r}\, dC_r = \int_0^{\rho} \frac{\gamma - \lambda}{1 + \beta\lambda}\, dC_r \tag{57}$$

The resulting maximum would of course depend on the initial values used for the integration of the equations, namely, ρ, σ, π, and ζ. Let us therefore write

$$f(\rho, \sigma, \pi, \zeta) = \max \int_0^{\rho} \frac{\gamma - \lambda}{1 + \beta\lambda}\, dC_r \tag{58}$$

where the maximum is by the optimal choice of $\lambda(C_r)$, $0 \leqslant \lambda \leqslant \gamma$, for the whole interval $0 \leqslant C_r \leqslant \rho$. We thus have a problem in the calculus of variations which may be tackled in a variety of ways. We choose here to make use of the notions of dynamic programing, though the methods of classical calculus of variations or the maximum principle of Pontryagin will lead to the same result.

Let us break the interval $(0, \rho)$ into two parts, $(\rho - \delta, \rho)$ and $(0, \rho - \delta)$, where $0 \leqslant \delta \leqslant \rho$. Then

$$f(\rho,\sigma,\pi,\zeta) \equiv \max \left[\int_{\rho-\delta}^{\rho} \frac{\gamma - \lambda}{1 + \zeta}\, dC_r + \int_0^{\rho-\delta} \frac{\gamma - \lambda}{1 + \beta\lambda}\, dC_r \right] \tag{59}$$

The maximization still requires the choice of λ over both intervals, but we should pause to see what the second integral really means. It is just such an integral as in Eq. (58), save that it is over the interval $(0, \rho - \delta)$. It will have a maximum value which is the same function f of the state $(\rho - \delta)$, $C_s(\rho - \delta)$, $C_p(\rho - \delta)$, $C_z(\rho - \delta)$ as the maximum of the integral over the whole interval $(0, \rho)$ is of the state ρ, $C_s(\rho) = \sigma$, $C_p(\rho) = \pi$, $C_z(\rho) = \zeta$. Moreover we shall clearly not get the maximum for the integral over the whole interval if we do

not have the maximum over the subinterval $(0, \rho - \delta)$, for we should have thrown away some advantage we might have had by using a suboptimal policy over the latter part of the interval. Thus

$$f(\rho, \sigma, \pi, \zeta) = \max\left[\int_{\rho-\delta}^{\rho} \frac{\gamma - \lambda}{1 + \beta\lambda} dC_r \right.$$
$$\left. + f(\rho - \delta, \quad C_s(\rho - \delta), \quad C_p(\rho - \delta), \quad C_z(\rho - \delta))\right] \qquad (60)$$

and now the maximization requires only the optimal choice of $\lambda(C_r)$ on the subinterval $\rho \geqslant C_r \geqslant \rho - \delta$, for the optimal choice on $\rho - \delta \geqslant C_r \geqslant 0$ is implied by our using f for the second integral in Eq. (59). Equation (60) is true for all δ, $0 \leqslant \delta \leqslant \rho$, and is an application of Bellman's principle of optimality which states that "an optimal policy has the property that whatever the initial state and decision may be, the remaining decisions constitute an optimal policy with respect to the state resulting from the initial decision."

However, to get a useful equation out of (60) we need to let δ become small and later tend to zero. From Taylor expansions, with the use of Eqs. (52), (53), and (55), we have

$$C_s(\rho - \delta) = C_s(\rho) - \delta C_s'(\rho) + O(\delta^2)$$
$$= \sigma - \delta\left(\frac{\omega + \alpha\lambda}{1 + \beta\lambda}\right) + O(\delta^2)$$
$$C_p(\rho - \delta) = \pi + \delta\left(\frac{\lambda}{1 + \beta\lambda}\right) + O(\delta^2)$$
$$C_z(\rho - \delta) = \zeta + \delta\left(\frac{\gamma - \lambda}{1 + \beta\lambda}\right) + O(\delta^2)$$

Combining these with a Taylor expansion of f about the point ρ, σ, π, ζ, we have

$$f(\rho - \delta, C_s(\rho - \delta), C_p(\rho - \delta), C_z(\rho - \delta)) = f(\rho, \sigma, \pi, \zeta)$$
$$-\delta\left[f_\rho + f_\sigma \frac{\omega + \alpha\lambda}{1 + \beta\lambda} - f_\pi \frac{\lambda}{1 + \beta\lambda} - f_\zeta \frac{\gamma - \lambda}{1 + \beta\lambda}\right] + O(\delta^2) \qquad (61)$$

where f_ρ, etc., denote the partial derivatives $\partial f/\partial\rho$, etc., evaluated at $(\rho, \sigma, \pi, \zeta)$, and $O(\delta^2)$ denotes terms of order δ^2 and smaller. Moreover if δ is small

$$\int_{\rho-\delta}^{\rho} \frac{\gamma - \lambda}{1 + \beta\lambda} dC_r = \delta \frac{\gamma - \lambda}{1 + \beta\lambda} + O(\delta^2) \qquad (62)$$

and substituting Eqs. (61) and (62) in (60) we have

$$(\rho, \sigma, \pi, \zeta) = \max \left[f(\rho, \sigma, \pi, \zeta) + \frac{\delta}{1+\beta\lambda} \{(1+f_\zeta)(\gamma - \lambda) + \lambda f_\pi - (\omega + \alpha\lambda) f_\sigma - (1+\beta\lambda) f_\rho\} + O(\delta^2) \right]$$

This maximization is by choice of λ over a very short interval, $\rho \geqslant C_r \geqslant \rho - \delta$, and this choice does not affect the first term since no λ appears in it. We may therefore take $f(\rho, \sigma, \pi, \zeta)$ outside the maximization sign and subtract it from both sides. If we then divide through by δ and let $\delta \to 0$ the remainder terms will vanish, leaving

$$\max \frac{1}{1+\beta\lambda} \{(1+f_\zeta)(\gamma - \lambda) + \lambda f_\pi - (\omega + \alpha\lambda) f_\sigma - (1+\beta\lambda) f_\rho\} = 0$$

We notice immediately that $(1+\beta\lambda)$ is always positive and that when the maximum of $A(\lambda)/B(\lambda)$, $B(\lambda) > 0$, is zero then $\max A(\lambda) = 0$. Thus the equation could be rearranged and written

$$\max [\{(1+f_\zeta)\gamma - \omega f_\sigma - f_\rho\} + \lambda\{f_\pi - \beta f_\rho - \alpha f_\sigma - (1+f_\zeta)\}] = 0 \qquad (63)$$

The choice to be made to find the maximum is of λ, the value of the control variable when $C_r = \rho$. We have thus reduced a variational problem requiring the choice of a whole function $\lambda(C_r)$, $\rho \geqslant C_r \geqslant 0$, to the choice of one value, $\lambda(\rho)$.

Furthermore the choice of the optimal $\lambda(\rho)$ is particularly simple. Let us write

$$\begin{aligned} \Gamma &\equiv \gamma(1+f_\zeta) - \omega f_\sigma - f_\rho \\ \Delta &\equiv f_\pi - \beta f_\rho - \alpha f_\sigma - (1+f_\zeta) \end{aligned} \qquad (64)$$

then

$$\max(\Gamma + \lambda\Delta) = 0 \qquad (65)$$

Thus if $\Delta > 0$, λ should have its maximum value, namely, γ; whereas if $\Delta < 0$, λ should have its minimum value, namely, zero. In fine,

$$\begin{aligned} \lambda &= 0 \quad \text{if} \quad \Delta < 0 \\ \lambda &= \gamma \quad \text{if} \quad \Delta > 0 \end{aligned} \qquad (66)$$

This policy is good common sense, for the condition $\Delta > 0$ could be written

$$f_\pi > \beta f_\rho + \alpha f_\sigma + f_\zeta + 1$$

Now f_π is $\partial f/\partial\pi$, i.e., the advantage to be gained by increasing π, whereas the left-hand side is a sum of partial derivatives with respect to ρ, σ, and ζ, i.e.,

the advantages to be gained from increasing ρ, σ, and ζ. Since the formation of P is at the expense of growth of Z and involves the consumption of R and S, the optimal policy will only demand the formation of P if the advantage f_π sufficiently dominates the other alternative of growth ($\lambda = 0$). Thus the criterion $\Delta > 0$ defines the lag phase, whereas $\Delta < 0$ is the growth phase.

3. *Boundary Conditions and Solution*

When there is no nutrient R to begin with, the growth process cannot take place whatever the levels of P, S, and Z may be. Thus

$$f(0, \sigma, \pi, \zeta) \equiv 0 \tag{67}$$

and so $f_\sigma = f_\pi = f_\zeta = 0$ when $\rho = 0$. Also $f_\rho > 0$, for if a little R is present some growth is possible. Hence, at $\rho = 0$,

$$\Delta = -\beta f_\rho - 1 < 0$$

and at the last the optimal policy is certainly to grow. This again is common (if somewhat Epicurean) sense, for when very little nutrient is left there is no point in expending it to enhance capacity for a growth that cannot take place—"let us eat, drink, and be merry, for tomorrow we die." Since $\lambda = 0$ the partial differential equation (65) reduces to

$$\Gamma \equiv \gamma(1 + f_\zeta) - \omega f_\sigma - f_\rho = 0 \tag{68}$$

This is a conventional partial differential equation and may be integrated by the method of characteristics. The characteristic equations are

$$\frac{d\zeta}{ds} = \gamma, \qquad \frac{d\rho}{ds} = -1, \qquad \frac{d\sigma}{ds} = -\omega, \qquad \frac{d\pi}{ds} = 0 \tag{69}$$

$$\frac{df}{ds} = -\gamma \tag{70}$$

$$\frac{df_\zeta}{ds} = 0, \qquad \frac{df_\rho}{ds} = -\left(\frac{\partial\gamma}{\partial\rho}\right)(1 + f_\zeta), \qquad \frac{df_\sigma}{ds} = -\left(\frac{\partial\gamma}{\partial\sigma}\right)(1 + f_\zeta),$$
$$\frac{df_\pi}{ds} = -\left(\frac{\partial\gamma}{\partial\pi}\right)(1 + f_\zeta) \tag{71}$$

We observe that Eqs. (69) are just the growth equations (52), (53), and (55), with $\lambda = 0$. The characteristic equations (71) give

$$\frac{d\Delta}{ds} = \alpha\left(\frac{\partial\gamma}{\partial\sigma}\right) + \beta\left(\frac{\partial\gamma}{\partial\rho}\right) - \left(\frac{\partial\gamma}{\partial\pi}\right) \tag{72}$$

since by Eqs. (67) and (71) $f_\zeta \equiv 0$. From Eq. (68), when $\rho = 0, f_\rho = \gamma$; so the

equation for Δ may be integrated with the condition $\Delta = -1$ when

$$s = \rho = 0 \tag{73}$$

Δ will increase as ρ increases if

$$\frac{\partial \gamma}{\partial \pi} > \alpha \frac{\partial \gamma}{\partial \sigma} + \beta \frac{\partial \gamma}{\partial \rho} \tag{74}$$

When Δ changes sign, the growth phase ends, and we move into the lag phase. (It should be remembered that increasing ρ takes us *back* from the end of the process.) The equation for f is then $\Gamma + \gamma\Delta = 0$; i.e.,

$$\gamma f_\pi - (\alpha\gamma + \omega) f_\sigma - (1 + \beta\gamma) f_\rho = 0 \tag{75}$$

The characteristic equations of this are

$$\frac{d\zeta}{ds} = 0, \qquad \frac{d\rho}{ds} = -(1 + \beta\gamma), \qquad \frac{d\sigma}{ds} = -(\omega + \alpha\gamma), \qquad \frac{d\pi}{ds} = \gamma \tag{76}$$

$$\frac{df}{ds} = 0 \tag{77}$$

$$\frac{df_\zeta}{ds} = 0, \qquad \frac{df_\rho}{ds} = (\beta f_\rho + \alpha f_\sigma - f_\pi)\left(\frac{\partial \gamma}{\partial \rho}\right)$$

$$\frac{df_\sigma}{ds} = (\beta f_\rho + \alpha f_\sigma - f_\pi)\left(\frac{\partial \gamma}{\partial \sigma}\right), \qquad \frac{df_\pi}{ds} = (\beta f_\rho + \alpha f_\sigma - f_\pi)\left(\frac{\partial \gamma}{\partial \pi}\right) \tag{78}$$

Hence

$$\begin{aligned} \frac{d\Delta}{ds} &= (\beta f_\rho + \alpha f_\sigma - f_\pi)\left\{\left(\frac{\partial \gamma}{\partial \pi}\right) - \alpha\left(\frac{\partial \gamma}{\partial \sigma}\right) - \beta\left(\frac{\partial \gamma}{\partial \rho}\right)\right\} \\ &= \left\{\alpha\left(\frac{\partial \gamma}{\partial \sigma}\right) + \beta\left(\frac{\partial \gamma}{\partial \rho}\right) - \left(\frac{\partial \gamma}{\partial \pi}\right)\right\}(\Delta + 1) \end{aligned} \tag{79}$$

This shows that both Δ and its derivative are continuous at the transition from the growth to the lag phases and Δ will continue to increase so long as the criterion (74) obtains. This lays out the whole solution in terms of ordinary differential equations.

4. *A Specific Form for the Growth Rate*

We now take a specific form for $\gamma(\rho, \sigma, \pi)$ in order to obtain definite equations and results. γ must certainly vanish if either of the nutrients is absent, and by our hypotheses it must increase with increasing π. Let us set

$$\gamma = a\pi^b \rho\sigma \tag{80}$$

so that with choice of a and b we can simulate a variety of conditions. If $b = 1$, the growth depends linearly on the critical product; for $b < 1$ the increase of π is ultimately less than linear suggesting an inhibition; $b > 1$ implies a small effect for low values of π but a greatly enhanced effect for $\pi \gg 1$. The constant a puts an absolute magnitude on the growth rate; if we think of it as $a'(\pi_c)^{-b}$, it ascribes a critical value π_c to π above which inhibition ($b < 1$) or enhancement ($b > 1$) begins to take effect. Now Eq. (69) shows that π remains constant during the growth phase. Let π_f be its final value (and hence its value throughout growth) and let $\rho_f = 0$, $\sigma_f > 0$, and ζ_f be the final values of ρ, σ, and ζ. Then Eqs. (69) and (70) give

$$\rho = -s, \qquad \sigma = \sigma_f - \omega s = \sigma_f + \omega\rho, \qquad \pi = \pi_f \tag{81}$$

and

$$f = \zeta_f - \zeta = -a\pi_f{}^b\{\tfrac{1}{3}\omega s^3 - \tfrac{1}{2}\sigma_f s^2\} \tag{82}$$

$$= \tfrac{1}{2}a\pi^b\rho^2\{\sigma - \tfrac{1}{3}\omega\rho\} \tag{83}$$

The form of Eq. (83) is obtained from Eq. (82) by substituting for s from Eq. (81) so as to eliminate final values of everything except ζ. By direct substitution it can be seen to satisfy the partial differential equation $\Gamma = 0$, Eq. (68). Substituting in Δ gives

$$\Delta = -1 + \tfrac{1}{2}ab\pi^{b-1}\rho^2(\sigma - \tfrac{1}{3}\omega\rho) - \tfrac{1}{2}a\pi^b\rho\{(\alpha - \omega\beta)\rho + 2\beta\sigma\} \tag{84}$$

and the vanishing of Δ gives a surface in ρ, σ, π space that corresponds to the transition from the lag to the growth phase. Let us explore this surface a little.

The equation $\Delta = 0$ can be solved for σ in terms of π and ρ which will allow us to plot contours of the surface for constant π in the ρ, σ plane. In fact,

$$\sigma = \frac{1 + \tfrac{1}{2}a\pi^b(\alpha - \omega\beta)\rho^2 + \tfrac{1}{6}ab\pi^{b-1}\omega\rho^3}{a\pi^{b-1}\rho(\tfrac{1}{2}b\rho - \beta\pi)} \tag{85}$$

so that σ is positive if $\rho > 2\beta\pi/b$ and this is an asymptote for the contour of constant π. Since we have assumed that the nutrient S is not exhausted first, we have $\sigma_f \geqslant 0$; we are only interested in the region $\sigma > \omega\rho$. For plotting these contours it is convenient to make a scale change through the definitions

$$\xi = b\rho/2\beta, \qquad \eta = b\sigma/2\beta\omega, \qquad V = b^2/4a\beta^3\omega, \qquad W = 1 - \alpha/\beta\omega \tag{86}$$

Then

$$\eta = \frac{V\pi^{1-b} - \tfrac{1}{2}W\pi\xi^2 + \tfrac{1}{3}\xi^3}{\xi(\xi - \pi)} \tag{87}$$

reduces the equation to the form with fewest disposable parameters. In the ξ, η plane we are only interested in the region $\eta \geqslant \xi$.

We note that ω is the ratio of the rates of usage of the two nutrients S and R during growth phase; and α/β is this ratio during the lag phase, with small C_z. Figures 2, 3, 4, and 5 show sets of contours for $V = 0.125$ [cf.

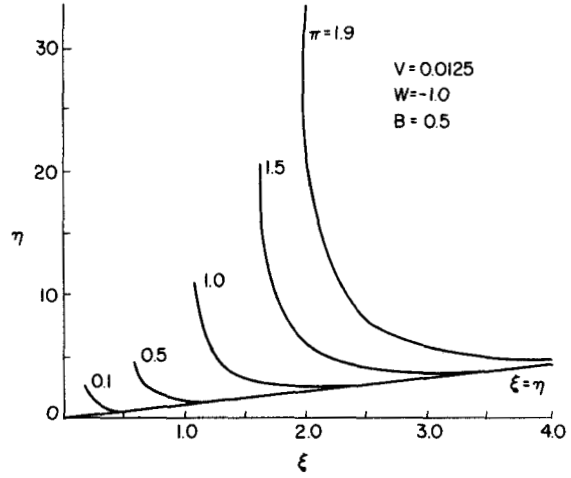


FIG. 2. Contours of the switching surface.

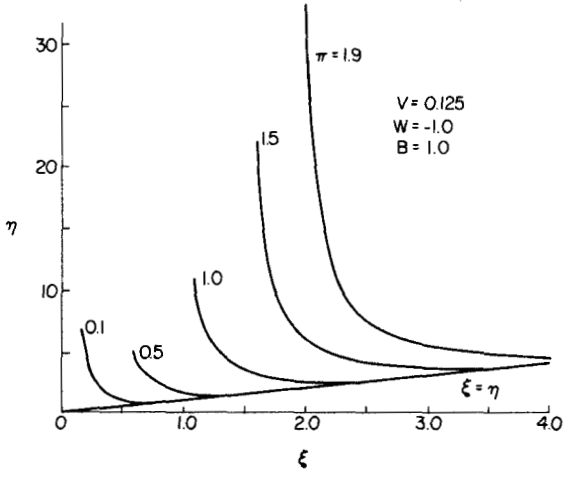


FIG. 3. Contours of the switching surface.

Eq. (86)], $\alpha = \beta$, and $\omega = 0.5$ (i.e., $W = -1$), with an increasing sequence of the exponent b [cf. Eq. (80)]. For $b = 0.5$ and 1.0 the surface is a smooth and fairly uniform ramp. For $b > 1$ the first term dominates when π is small; so

the surface curves back and under, near the plane $\pi = 0$, The sequence of Figs. 6, 3, and 7 shows the effect of increasing V at constant W and b, i.e., of decreasing a or decreasing β (or ω) with $\alpha = \beta$ (or $\alpha = 2\beta\omega$). This evidently

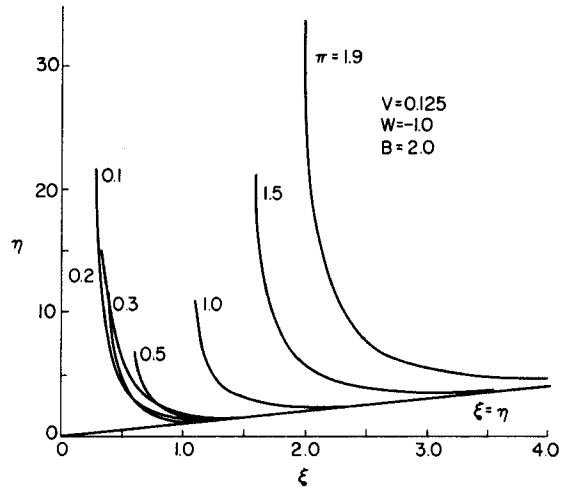


FIG. 4. Contours of the switching surface.

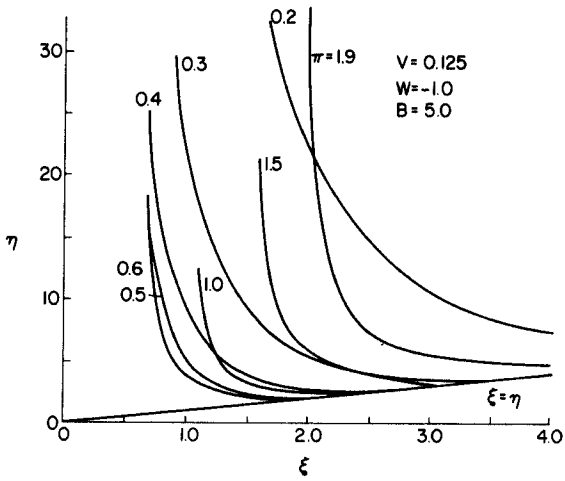


FIG. 5. Contours of the switching surface.

moves the surface away from the η axis but does not change its shape. A comparison of Figs. 3 and 8 shows a much more pronounced effect from an increase of α from $\alpha = 2\beta\omega$ to $\alpha = 8\beta\omega$. Hence the effect of increasing the

ratio α/β is to bring back the switching surface and to give a longer growth period.

We notice that, except for $b > 1$ with small π, the effect of increasing π for given ρ, σ (or ξ, η) is to bring a point beneath the surface nearer to it.

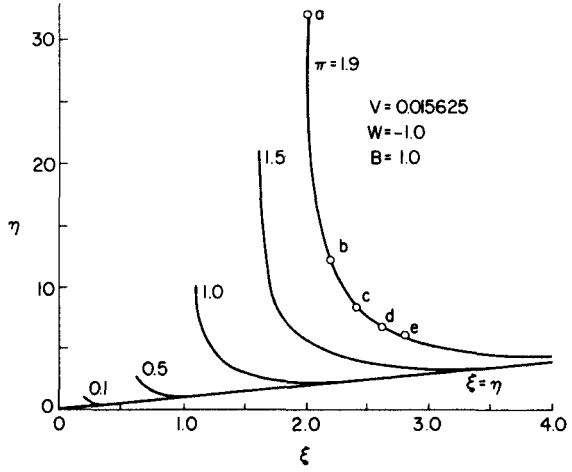


FIG. 6. Contours of the switching surface (see Fig. 3 for $V = \frac{1}{8}$).

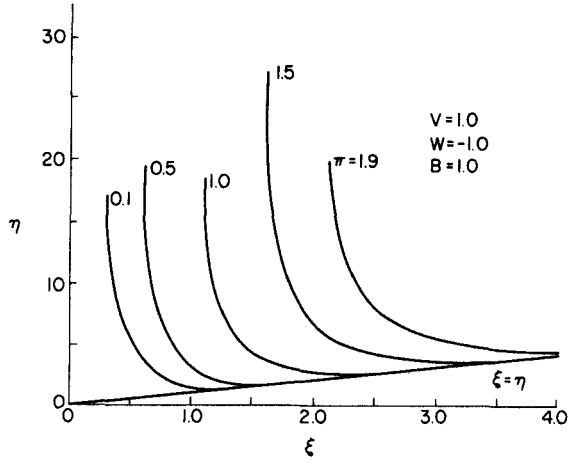


FIG. 7. Contours of the switching surface (see Fig. 3 for $V = \frac{1}{8}$).

In fact, the value of π on the contour passing through a given nutrient point (ξ, η) is the level of critical product for which growth starts immediately. This again is in accordance with a biologically sensible picture.

5. *The Solutions in the Lag and Growth Phases*

With this understanding of the transition from lag to growth, we can set down the equations for the two phases. Let a suffix "s" denote a value at the switching point so that η_s, ξ_s, and π_s satisfy Eq. (87). The value of ζ_s depends actively on the initial value ζ since it has been constant during the

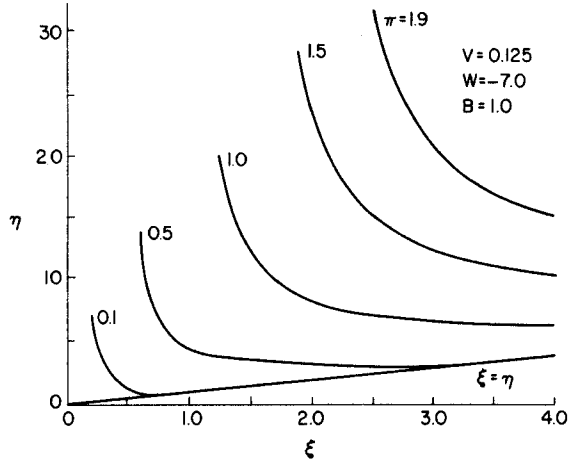


FIG. 8. Contours of the switching surface for $W = -7.0$ (see Fig. 3 for $W = -1.0$).

lag phase; we could normalize it to 1, except that the usage of the two nutrients during the lag phase does depend upon it. Since the change of variables given in Eq. (86) has proven useful for the switching surface when γ is given by (80), we will use it also for the growth equations. In addition, let us set

$$\chi = \tfrac{1}{2}bV\zeta \qquad \text{and} \qquad \tau = k_4 t/\beta V \tag{88}$$

Then during the growth phase

$$\frac{d\eta}{d\xi} = 1, \qquad \frac{d\pi}{d\xi} = 0, \qquad \frac{d\chi}{d\xi} = -\pi^b\xi\eta, \qquad \frac{d\zeta}{d\xi} = \frac{-1}{\chi} \tag{89}$$

These equations may be integrated by quadratures from the switching surface, and give

$$\eta = (\eta_s - \xi s) + \xi, \qquad \pi = \pi_s \tag{90}$$

$$\chi = \chi_s + \pi_s{}^b\{\tfrac{1}{2}(\eta_s - \xi_s)(\xi_s{}^2 - \xi^2) + \tfrac{1}{3}(\xi_s{}^3 - \xi^3)\} \tag{91}$$

$$\tau = \tau_s + \int_{\xi}^{\xi_s} \frac{d\xi}{\chi(\xi)} \tag{92}$$

Thus χ_f (the final value of χ, the amount of the organism present) is given by

$$\chi_f - \chi_s = \tfrac{1}{6}\pi_s{}^b\xi_s{}^2(3\eta_s - \xi s)$$
$$= \tfrac{1}{12}\pi_s{}^b\xi_s \frac{6V + \pi_s\xi_s{}^2(2 - 3W)}{(\xi_s - \pi_s)} \tag{93}$$

and the switching surface might itself be marked with contours of $(\chi_f - \chi_s)$.

Figure 9(a) shows $(\chi - \chi_s)$ as a function of ξ during growth for five switching points, $a, \ldots, e$, located on the contour $\pi = 1.9$ in Fig. 6. These curves can be brought closer together if $(\chi - \chi_s)/\xi_s\eta_s$ is plotted against ξ/ξ_s as shown in Fig. 9(b). From Eqs. (91) and (92) we can determine the actual

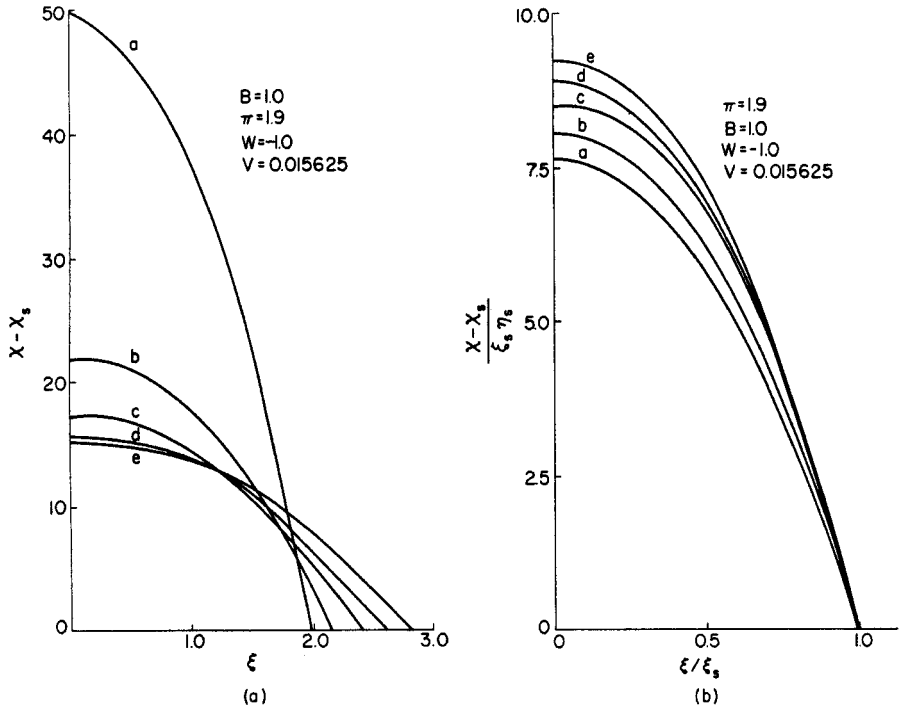


FIG. 9(a, b). Amount of growth as a function of substrate concentration for different initial substrate concentrations.

course of growth $\chi(\tau)$. This will depend on the value of χ_s as the equations clearly show. Figure 10 shows $\chi(\tau)$ for the trajectories from points $a, \ldots, e$ and for $\chi_s = 10/128$, $1/128$, $1/1280$. As $\chi_s \to 0$, the time to achieve the final value χ_f tends to increase without bound. In fact for small χ_s

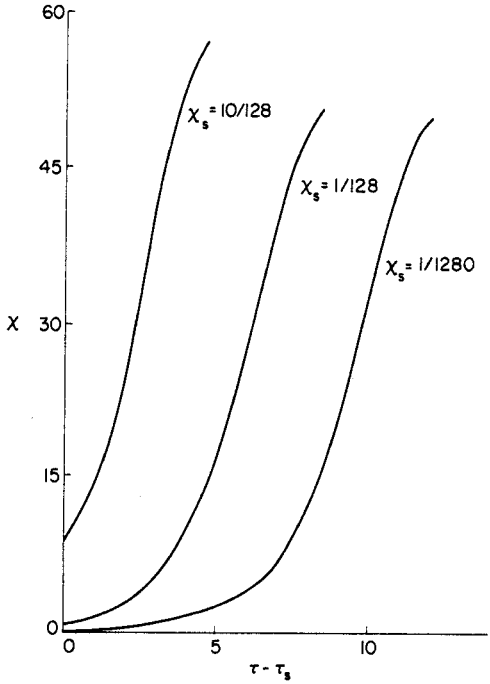


FIG. 10. Time course of growth for different initial inoculum sizes.

$$\tau - \tau_s \sim \frac{1}{\pi_s{}^b \xi_s \eta_s} \ln\left\{1 + \frac{\pi_s{}^b \xi_s \eta_s}{\chi_s}(\xi_s - \xi)\right\} + O(1) \tag{94}$$

where $O(1)$ denotes a term that remains bounded as $\chi_s \to 0$. For large χ_s

$$\tau - \tau_s \sim \frac{\xi_s - \xi}{\chi_s}\left[1 - \frac{\pi_s{}^b}{12\chi_s}\{(4\eta_s - \xi_s)\xi_s{}^2 - 2(\xi_s + \eta_s)\xi(\xi_s + \xi) - \xi^3\}\right] + O(\chi_s{}^{-3}) \tag{95}$$

The switching surface and growth phase are shown in Fig. 11(b), where from the contour of constant π_s on the switching surface several trajectories with ordinate $\chi - \chi_s$ above the π_s plane (i.e., $\chi - \chi_s + \pi_s$ above the base plane) are shown. This may be called a growth surface generated by this particular section of the switching surface.

During the lag phase Eqs. (76) and (77) apply, which in the current notation give

$$\frac{d\chi}{d\xi} = \frac{df}{d\xi} = 0, \qquad \frac{d\eta}{d\xi} = 1 - W\frac{\beta\gamma}{1 + \beta\gamma}, \qquad \frac{d\pi}{d\xi} = -\frac{2}{b}\frac{\beta\gamma}{1 + \beta\gamma} \tag{96}$$

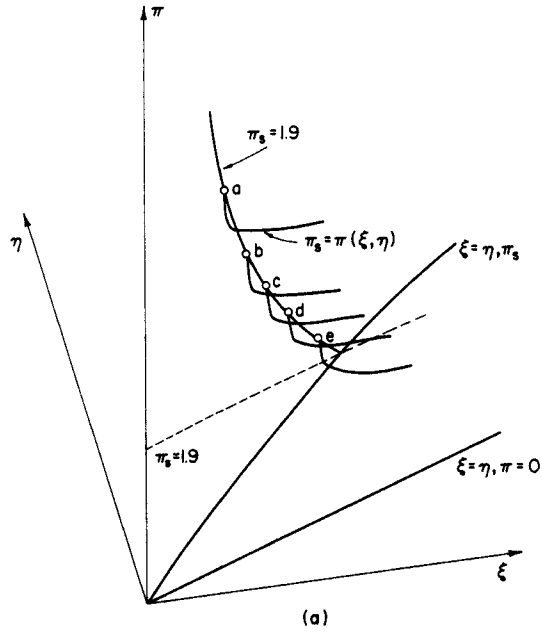


FIG. 11(a). Switching surface and the lag phase as calculated from the model. Three-dimensional plot.

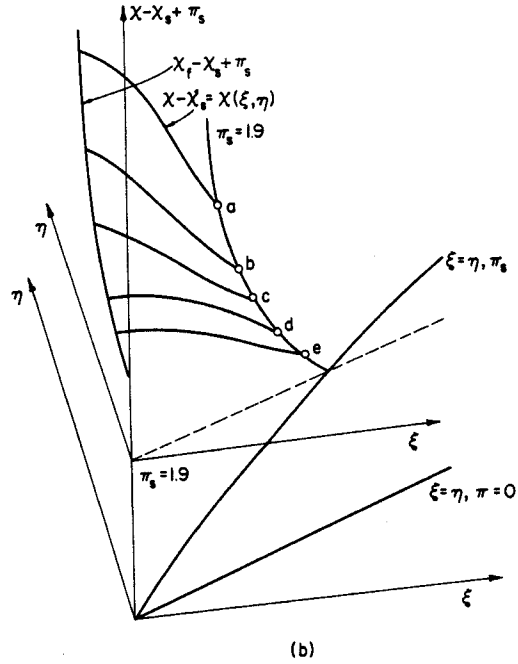


FIG. 11(b). Switching surface and growth phase as calculated from the model. Three-dimensional plot.

where

$$\beta\gamma = \beta a\eta^b \rho\sigma = \pi^b \xi\eta/V \tag{97}$$

These equations are less easy to integrate, apart from the first two which give $\chi = \chi_s, f = f_s$ trivially. We do however notice that

$$\frac{d\pi}{d\xi} = \frac{2}{bW}\left(\frac{d\eta}{d\xi} - 1\right)$$

Hence the trajectory reaching the switching surface at ξ_s, η_s, π_s must lie in the plane

$$\pi = \pi_s + \frac{2}{bW}\{(\eta - \eta_s) - (\xi - \xi_s)\} \tag{98}$$

If $W < 0$ (i.e., $\alpha > \beta\omega$) the projection of the trajectory in the ξ, η plane climbs the plane of Eq. (98) from above the line $\eta = \xi + \eta_s - \xi_s$; if $W > 0$, the trajectory again climbs the plane of Eq. (98), but this time from below the line $\eta = \xi + \eta_s - \xi_s$. In the special case $W = 0$ the projection of the trajectory is the line of unit slope through (ξ_s, η_s). It would be possible to use Eq. (98) to obtain an equation entirely in terms of ξ and η, but it is better to integrate these equations numerically from the point of attachment to the switching surface.

In any event we can learn a lot about the general shape of the solutions of these equations. We observe first that, as we go back in time and ξ increases, π will decrease for $d\pi/d\xi$ is certainly negative. Hence either it will vanish for some ξ or will go asymptotically to zero. By Eq. (98) it must vanish on or be asymptotic to the line

$$\eta = \eta_0 + \xi = (\eta_s - \xi_s + \tfrac{1}{2}bW\pi_s) + \xi \tag{99}$$

This is possible for $b > 1$, for then

$$\pi^{1-b} \sim \left(\frac{b-1}{bV}\right)(\eta_0 + \tfrac{2}{3}\xi)\xi^2 \tag{100}$$

and

$$\beta\gamma \sim \left(\frac{3}{2}\frac{b}{b-1}\right)^{b/(b-1)} V^{1/(b-1)} \xi^{-(b+2)/(b-1)} \tag{101}$$

or for $b = 1$, when

$$\pi \sim \exp[-(1/V)(\eta_\infty + \tfrac{2}{3}\xi)\xi^2] \tag{102}$$

and

$$\beta\gamma \sim V\xi(\eta_\infty + \xi)\exp[-(1/V)(\eta_\infty + \tfrac{2}{3}\xi)\xi^2] \tag{103}$$

However, if $b < 1$, it is possible to reach the plane $\pi = 0$ in a finite time. This

again is reasonable, for $b < 1$ represents an enhancement of the rate at low values of π; hence, it is possible to start from a zero value of π, and reach the switching surface in a finite time. The time is given by the integral

$$\tau_s - \tau = \frac{bV}{2\chi_s} \int_{\pi}^{\pi_s} \frac{d\pi}{\pi^b \xi \eta} \tag{104}$$

where ξ and η have to be evaluated along the path. Again this is dependent on $\chi_s = \chi_0$, the concentration of Z at the start, and it would be possible to get asymptotic expressions for large times.

The curves of Fig. 11(a) for which the ordinate is π show the development of the critical product. Figures 12 and 13 show variation of $(\pi_s - \pi)$ with

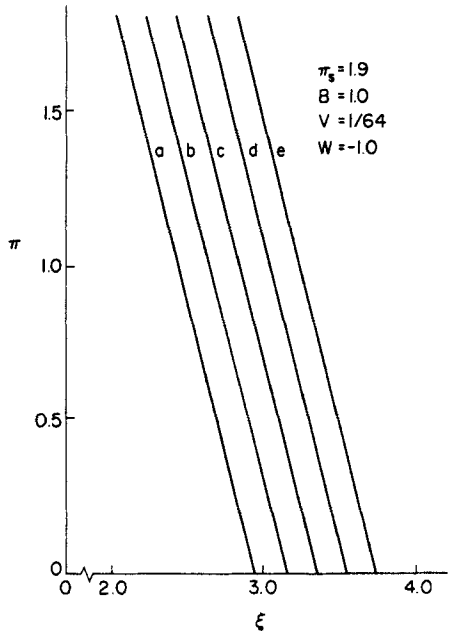


FIG. 12. Concentration of critical product during the lag phase as a function of limiting nutrient concentration.

$(\xi - \xi_s)$ and $(\tau_s - \tau)$, respectively, for the trajectories which join to those of Figs. 9 and 10. Figures 12 and 13 show the lag phase paths π vs ξ and π vs τ.

It may also be shown at the expense of algebraic labor that, when $b > 1$, the path during the lag phase goes asymptotically to the upper side of the underfolded switching surface. Here the lag phase is never preceded by a growth phase; also, growth is not possible if the initial concentration of P puts the point (ξ, η, π) below the switching surface. This also would seem to

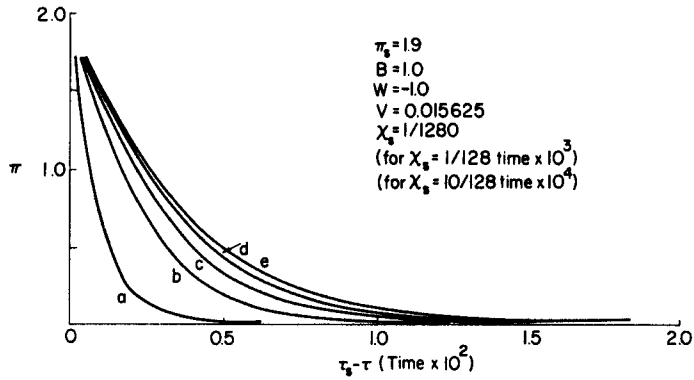


FIG. 13. Concentration of critical product during the lag phase as a function of time, for various initial limiting substrate concentrations.

accord with the fact that $b > 1$ makes the effect of small π most unfavorable.

6. *Transfer into Fresh Nutrient*

As an example of a known bacteriological phenomenon which this model exhibits we may consider a culture during its growth phase transferred into fresh medium; Fig. 14 shows a typical result taken from the calculations with

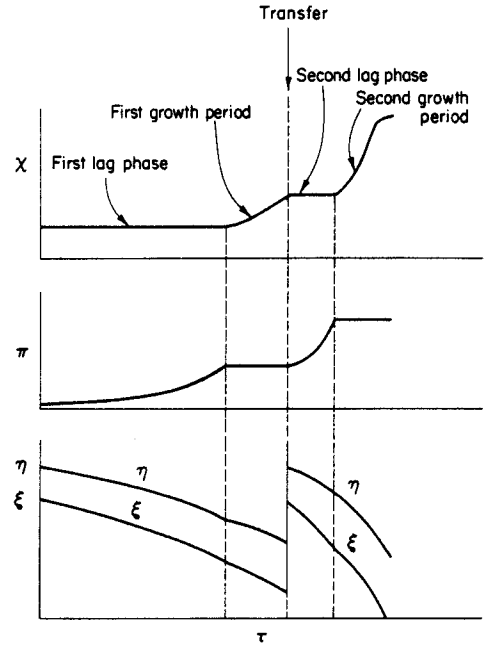


FIG. 14. Lag and growth phenomena upon transfer of cells as predicted from the model.

parameter values $V = 1/64$, $W = -1$, $b = 1$. The first growth phase is preceded by a long lag phase during which the critical product rises to the switching value. After the growth period has started, but before the nutrient has been exhausted, the nutrient concentrations are restored to their original values. There follows a shorter lag phase and a subsequent period of much more rapid growth.

This indeed suggests one of the tests of this model, for if the switching surface has the form we have supposed then there should be values of R and S for which the second lag phase just becomes zero. This would allow the experimental determination of the switching surface and hence of the function $\gamma(\rho, \sigma, \pi)$.

7. *An Improved Model*

The first model has been examined in sufficient detail to show that it has some promise at least, but it is useful to exhibit a slight extension that will embrace the whole life cycle of a culture. The same basic picture is retained, save that the usage of the nutrients is now divided into three parts:

(i) to preserve the viability of Z at rates proportional to C_z;
(ii) in the reproduction of Z at rates proportional to dC_z/dt, provided that $dC_z/dt > 0$ (if Z is actually decreasing we do not allow it to reform the nutrients);
(iii) to form P at rates proportional to dC_p/dt.

Thus we would have

$$\frac{dC_r}{dt} = -k_1 C_z - k_2 \frac{dC_z}{dt} - k_3 \frac{dC_p}{dt} \tag{105}$$

$$\frac{dC_s}{dt} = -k_4 C_z - k_5 \frac{dC_z}{dt} - k_6 \frac{dC_p}{dt} \tag{106}$$

For the growth of Z we have a similar law to that of the previous model with

$$\frac{dC_z}{dt} = K(C_r, C_s, C_p)C_z - k_7 \frac{dC_p}{dt} \tag{107}$$

where this time the form of K is $aC_p{}^b C_r C_s - \Theta$. The new constant Θ that has been introduced here is a constant death rate. If the rate of formation of P is proportional to C_z and is again the biological control variable, we may write

$$\frac{dC_p}{dt} = \frac{k}{k_7} C_z \tag{108}$$

and then

$$\frac{dC_z}{dt} = (K - k)C_z \qquad \text{and} \qquad 0 \leqslant k \leqslant K \tag{109}$$

We shall not treat this model further, but it is clear that it can also include a phase of decline when, the nutrients being exhausted, the population decreases exponentially.

V. Models and Experimental Results

Establishment of the validity of a model of any natural phenomenon rests on the agreement of observations with predictions. Although the term "agreement" is somewhat subjective, we may say that a model is valid when: (i) it correctly predicts trends *in a number of cases*; and (ii) discrepancies between predictions and observations are within the latitude allowed by uncertainties of measurement and uncontrollable experimental variables.

It is important to note that the model should be applied to more than one case; if this is not done, the model is really a "curve fit" and does not command much respect or confidence. A model first becomes plausible if it reproduces observations for two cases quite different from each other. One ought not to rule out a model if it does not yield correct predictions for *all* imaginable experimental situations; the model may be valid for a few special sets of conditions, and it is then the task of inductive research to expand the scope of the model to new conditions.

Consider Monod's model of growth. It is often possible to arrange experimental conditions so that a single substrate does in fact limit growth. One can then proceed to test the more quantitative aspects of the model. Monod's model has been applied to two cases: batch growth and continuous propagation. To fulfill the first requirement for a valid model, it should predict the results of (say) continuous propagation from batch data, if the model is to be accepted.

Figure 15, in terms of dimensionless quantities, shows expected results of Monod's model for continuous propagation; productivity P is the amount of biomass formed per unit time per unit volume of culture:

$$P = QC/V = C/\theta \tag{110}$$

Herbert *et al.* (H10) studied the growth of *Aerobacter cloacae* in both continuous and batch propagators. The limiting substrate was glycerol. Constants μ and a were determined from batch data; the constant K was determined from the holding time at which productivity was a maximum in continuous propagation—not highly accurate, but the best that could be done under the experimental circumstances.

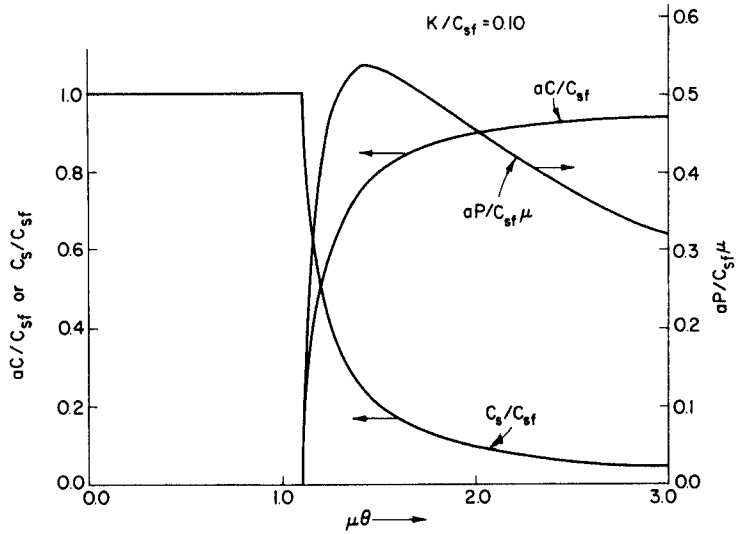


FIG. 15. Continuous propagation—predictions of Monod's model for steady-state values of various quantities as functions of holding time.

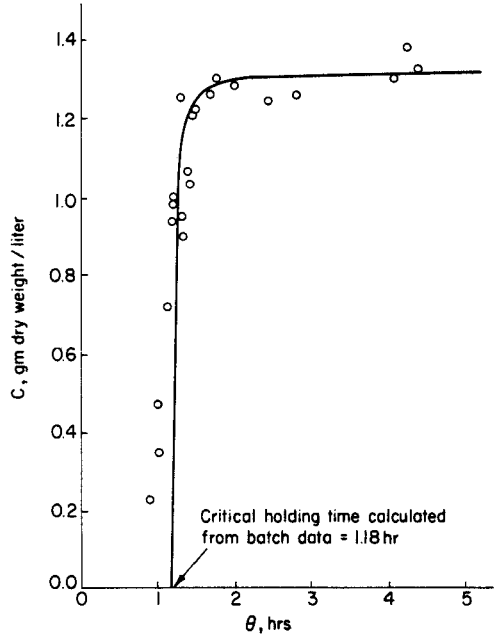


FIG. 16. Comparison of Monod's model with data of Herbert *et al.* (H10) on growth of *Aerobacter cloacae*. Solid line calculated from Monod's model with $\mu = 0.85$ hr^{-1}, $K = 0.0123$ g/liter, $C_{sf} = 2.5$ g/liter, and $a = 1.89$ g/g. Replotted from *J. Gen. Microbiol.* **14**, 601–622 (1956), by permission of Cambridge University Press.

Experimental results of Herbert *et al.* are compared with predictions of Monod's model in Fig. 16. At long holding times, agreement of the model with experiment is good; this is not the case at holding times near the critical, where there is a definite trend not predicted by the model. Thus, it appears that the maximum specific growth rate (μ) is faster than that determined from batch experiments; also the stoichiometric coefficient a changes as θ approaches the critical.

The authors remark that part of the discrepancy may be due to imperfect mixing in the culture vessel. They also imply that the discrepancies may point to inadequacies of the biological side of the model.

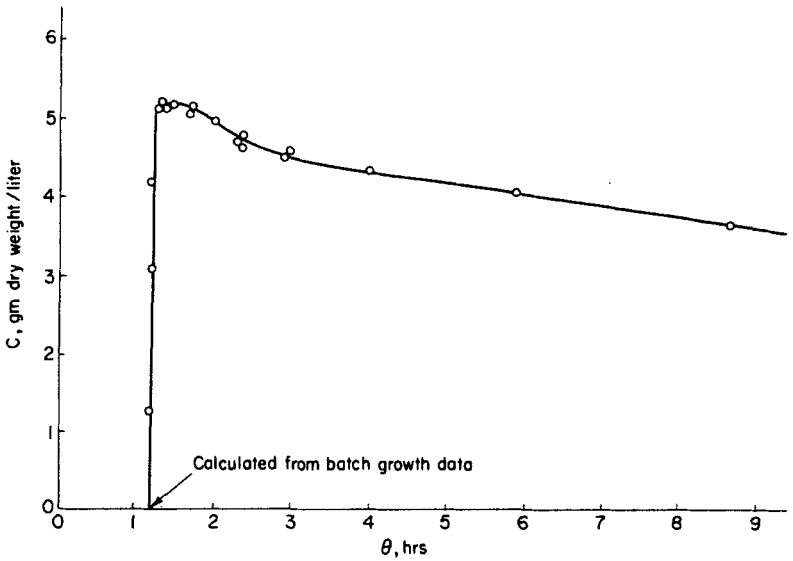


FIG. 17. Herbert's data (H8) on growth in continuous culture: *Aerobacter aerogenes* in glycerol medium. Replotted from p. 48 of "Continuous Culture of Microorganisms: A Symposium," by permission of the Publishing House of the Czechoslovak Academy of Sciences.

Figures 17 and 18 show the results of Herbert (H8) on continuous propagation of *Aerobacter aerogenes* and the yeast *Torula utilis*. Herbert states that, in both cases, the critical holding times agree well with values computed from results of batch experiments. At long holding times, however, there is considerable discrepancy from theory in the declining steady-state cell concentration.

Herbert attributes part of the discrepancy to the occurrence of endogenous metabolism, not accounted for in Monod's model. In other words, cells not only convert substrate into protoplasm, but they also carry on reactions

which consume cell substance. This effect can be incorporated into the model by writing

$$R_p = \frac{\mu C C_s}{K + C_s} - \mu_c C \tag{111}$$

in place of Eq. (38). Here μ_c represents a rate constant for such reactions. Herbert states that incorporation of this term yields a curve having the trend shown in Figs. 17 and 18.

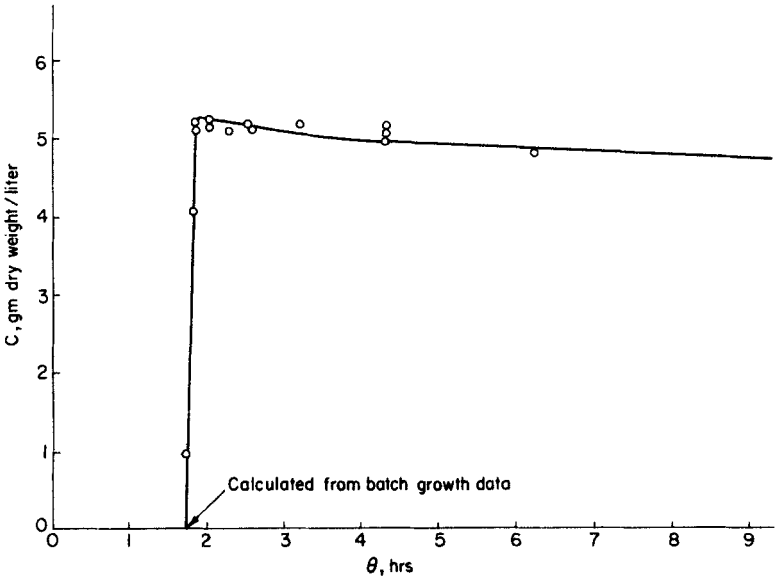


FIG. 18. Herbert's data (H8) on growth in continuous culture: *Torula utilis* in glucose medium. Replotted from p. 48 of "Continuous Culture of Microorganisms: A Symposium," by permission of the Publishing House of the Czechoslovak Academy of Sciences.

In a growing culture the rate of respiration may be assumed proportional to the rate of synthesis; that is,[13]

$$R_{\text{resp}} = \frac{\beta \mu C C_s}{K + C_s} \tag{112}$$

where β is a stoichiometric coefficient. From Eq. (22), the condition for steady

[13] R_{resp} is measured in terms of moles of oxygen consumed per unit volume of culture per unit time. Strictly speaking, a term for endogenous metabolism should also be added to the right-hand side of Eq. (112). In growing cultures, however, this term is usually small; moreover, its inclusion would not alter the conclusions drawn below.

state in the continuous propagator is

$$R_p/C = 1/\theta$$

Hence, from Eqs. (111) and (112), we find that the specific respiration rate should be a linear function of $1/\theta$:

$$\frac{R_{\text{resp}}}{C} = \frac{\beta\mu C_s}{K + C_s} = \beta\left(\frac{1}{\theta} + \mu_c\right)$$

Figure 19 shows Herbert's data on the respiration of *Aerobacter aerogenes.*

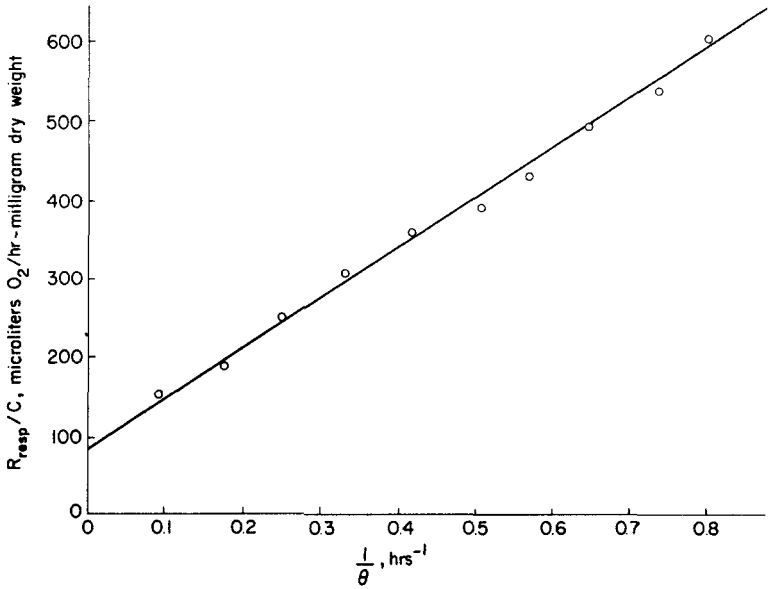


FIG. 19. Herbert's data (H8) on the specific rate of respiration of *Aerobacter aerogenes* in continuous culture. Replotted from p. 49 of "Continuous Culture of Microorganisms: A Symposium," by permission of the Publishing House of the Czechoslovak Academy of Sciences.

These data are in agreement with the foregoing equation, and serve as further confirmation of Herbert's hypothesis.

More recently, Marr and Harvey (M4) have pointed out that occurrence of endogenous metabolism will cause the yield of microbial mass obtained from unit mass of limiting substrate to change with holding time in continuous culture. Thus, if the apparent yield coefficient Y is defined as

$$Y \equiv \frac{R_p}{-R_s}$$

then Eq. (111) and the equation

$$-R_s = a\frac{\mu C C_s}{K + C_s} \tag{37a}$$

yield

$$1/Y = a(1 + \mu_c\theta)$$

where a is the true stoichiometric coefficient. Marr and Harvey were able to verify experimentally the foregoing linear relation between $1/Y$ and θ for a number of organisms.

Growth models of the Monod type have been applied to the microscopic, unicellular algae. Algae are plants (photosynthetic organisms) and as such show some interesting variations from heterotrophic organisms. The driving force for synthetic reactions in algae is not the energy stored in chemical substrates, but is in part, and sometimes exclusively, the energy of light absorbed by the pigment systems of algal cells. Hence, the growth rate of an alga is strongly dependent on the light intensity I seen by the cell.

A new circumstance arises here because the "concentration" of "limiting substrate" is the light intensity, and this can never be uniform in a propagator with an optical path of finite length. In other words, algal propagators cannot be assumed to be "perfectly stirred,"[14] and the macroscopic growth rate must be obtained by some sort of averaging procedure.

Tamiya *el al.* (T1) postulated that the *local* (microscopic) growth rate of an alga follows a kinetic expression similar to that of Michaelis and Menten:

$$R_p = \frac{\mu v C I}{\mu + vI} \tag{113a}$$

At high light intensities, this yields

$$R_p \approx \mu C \tag{113b}$$

and the growth process is said to be *light saturated.* The parameter μ should be strongly temperature dependent. At low light intensities,

$$R_p \approx vCI \tag{113c}$$

The parameter v should be nearly temperature independent.

In an algal culture contained in a rectangular vessel of thickness L and irradiated from one side ($I = I_0$ at $x = 0$), the light intensity may be assumed

[14] However, Rieske *et al.* (R4) found that the rate of the so-called Hill reaction in chloroplast fragments depends on the *average* light intensity in a well-stirred suspension. This finding is consistent with the occurrence of "flashing light effects" (B9, E2, K4), and has been analyzed by Fredrickson *et al.* (F5). Miller *et al.* (M9) report a scheme to utilize the flashing light effect to improve the efficiency of light utilization by algal cultures.

to fall off according to Beer's law:

$$I = I_0 \, e^{-\varepsilon C x} \tag{114}$$

Hence, the average growth rate is expected to depend on the absolute scale of the growth vessel.

The macroscopic rate of growth, assuming a uniform distribution of cells, is then

$$R_p = \frac{1}{L} \int_0^L R_p(x) \, dx$$

$$= \left[\frac{\mu}{\varepsilon C L} \ln \frac{\mu + \nu I_0}{\mu + \nu I_0 \, e^{-\varepsilon C L}} \right] C \tag{115}$$[15]

Tamiya *et al.* (T1) found that Eq. (115) gave a good fit of their data for batch growth of *Chlorella ellipsoidea* in flat glass vessels. The expected temperature dependence of μ and ν was found, and the effect of incident light intensity was as predicted, though there was some inhibition of growth at very high incident intensities.

Equation (115) does not predict a limiting value of C in batch cultures. It must therefore be modified by inclusion of some sort of term for catabolism. The simplest assumption is

$$R_p = \left[\frac{\mu}{\varepsilon C L} \ln \frac{\mu + \nu I_0}{\mu + \nu I_0 \, e^{-\varepsilon C L}} - \mu_c \right] C \tag{116}$$

where μ_c is a constant.

Equation (116) may be substituted into Eq. (20) to yield an equation for continuous culture. The minimum holding time required to maintain a non-zero cell concentration is then given by

$$\frac{1}{\theta} + \mu_c = \frac{\mu \nu \bar{I}_0}{\mu + \nu I_0} \tag{117}$$

whereas in general

$$\frac{1}{\theta} = \frac{R_p}{C} \tag{22}$$

for steady state. Figure 20 shows a plot of steady-state concentration and productivity (C/θ) as a function of holding time for a particular choice of parameters in Eq. (116). The curve of productivity resembles that predicted by Monod's model for growth of nonphotosynthetic organisms. The shape of the concentration-holding time curve is typical of algal propagators (H3).

[15] Equation (115) shows that, at large values of C, R_p becomes independent of C, so that batch growth should be *linear*. This is observed [see, e.g., Myers (M15)].

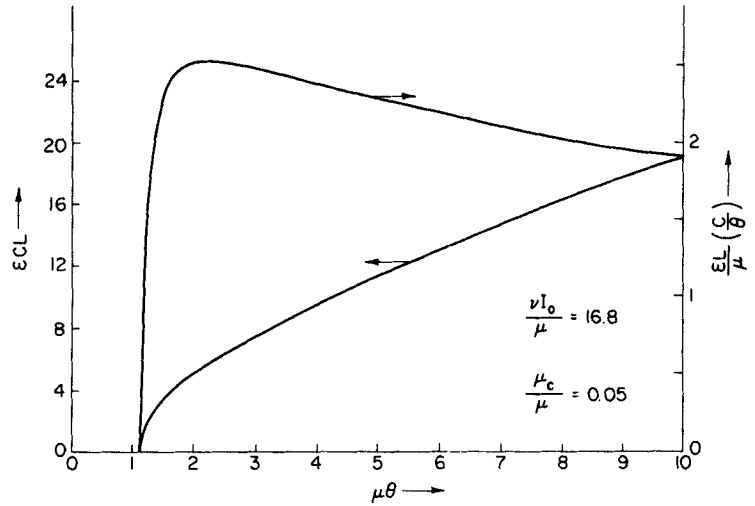


FIG. 20. Continuous propagation of algae as calculated from Eq. (116). Dimensionless cell concentration and productivity as a function of dimensionless holding time. The minimum holding time is a strong function of incident intensity, I_0.

Figure 21 is a plot of productivity vs steady-state cell concentration, calculated from the model with the same parameters as for Fig. 20.

The net rate of production of oxygen by growing algae may be postulated to be proportional to the growth rate R_p. Consumption of oxygen by respiration has already been accounted for in Eq. (116); this is the term involving

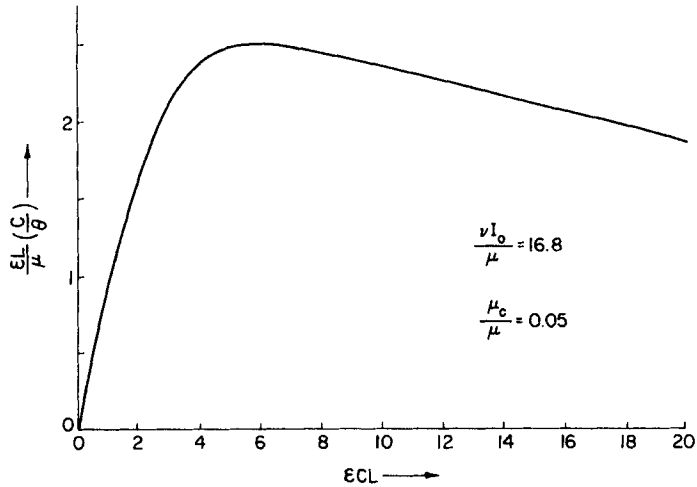


FIG. 21. Continuous propagation of algae as calculated from Eq. (116). Steady-state productivity as a function of steady-state cell concentration.

μ_c. Then R_{photo}, the net rate of oxygen production per unit volume of culture, is

$$R_{\text{photo}} = \beta R_p \tag{118}$$

where β is a stoichiometric coefficient. Hence, from Eq. (22),

$$\frac{R_{\text{photo}}}{C} = \beta \frac{R_p}{C} = \frac{\beta}{\theta} \tag{119}$$

That is, the specific net rate of photosynthesis should be proportional to the reciprocal holding time. Figure 22 shows data collected by Hanson *et al.* (H3) for photosynthesis in continuously propagated cultures of a *Chlorella* species. Equation (119) is seen to give a good "fit" of the experimental data; further corroboration of the theory is the fact that the stoichiometric coefficient β calculated from the overall stoichiometry of the photosynthetic reaction and cell compositions of *Chlorella* taken from the literature (M10) is in rough agreement with the value calculated from the data of Fig. 22.

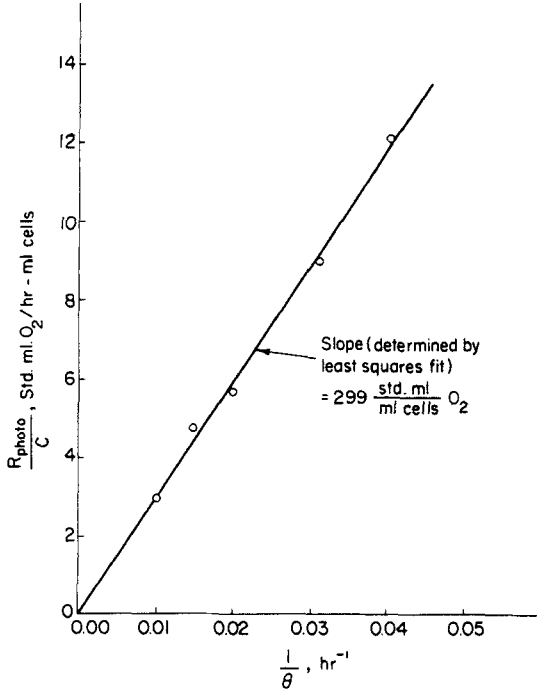


FIG. 22. Data of Hanson *et al.* (H3) on net specific rate of photosynthesis in continuous propagation of algae. This establishes the stoichiometric relation between growth and photosynthesis.

Myers and Graham (M16) studied the growth of *Chlorella ellipsoidea* in continuous culture. Their results are not given in such a way that direct comparison with the batch growth data of Tamiya *et al.* (T1) is possible; however, Myers and Graham found that the productivity at low cell concentrations seemed to be predictable from batch data which they obtained.

Trends of the experimental results of Myers and Graham can be compared with those predicted by Eq. (116). Figure 23 shows the experimental data of

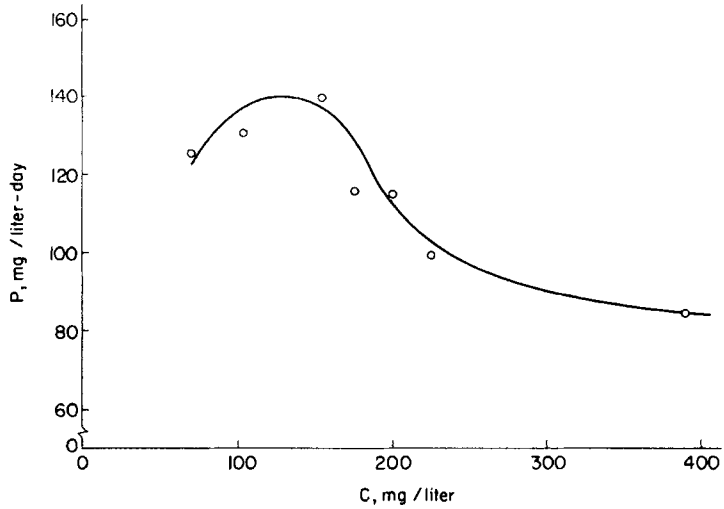


FIG. 23. Productivity of continuous *Chlorella* cultures as determined by Myers and Graham (M16). This should be compared with Fig. 21. Replotted from *Plant Physiol.* **34**, 345–352 (1959), by permission of the American Society of Plant Physiologists.

Myers and Graham. Qualitative agreement of the model with experimental results is indicated in the region of maximal productivity; at high algal concentrations, however, the productivity predicted by the model falls off incorrectly. Hence, the simple model outlined is somewhat inadequate.

Myers and Graham give three additional sets of experimental data which might account for some of the discrepancy. First, they noted that Beer's law is not strictly followed by the algal populations used. Deviations from Beer's law might have been apparent, rather than real, and might be attributed to edge effects. This is a practical, rather than a conceptual difficulty, of course.

Second, manometric experiments on cells taken from steady-state cultures at different holding times showed that the respiration rate of cells per unit mass was not constant; instead, respiration rate fell off with increasing holding time of the steady-state culture.

This result is reminiscent of Herbert's data (H8) on the respiration of

bacteria in continuous culture and of the data of Hanson *et al.* (H3) on photosynthesis in continuous culture. This effect is not necessarily the same, however, and may be indicative of a general slowing down of metabolism in slowly growing cultures.

Finally, Myers and Graham determined irradiance curves[16] for algal cells suspended in buffer solutions; cells were taken from steady-state cultures at different holding times again. At low light intensities, irradiance curves for cells were nearly independent of steady-state holding time. At saturating intensities, however, it was found that photosynthetic rate fell off with increasing holding time. This occurred in spite of the fact that chlorophyll content was highest in cells taken from high population density cultures.

The examples given show that there are marked variations in physiological characteristics of cells taken from continuous cultures at different steady-state conditions. This is also true of cells taken from different phases of batch cultures [see, e.g., Malmgren and Hedén [M3)]. Again, one observes considerable changes in morphology of cells at different conditions in both

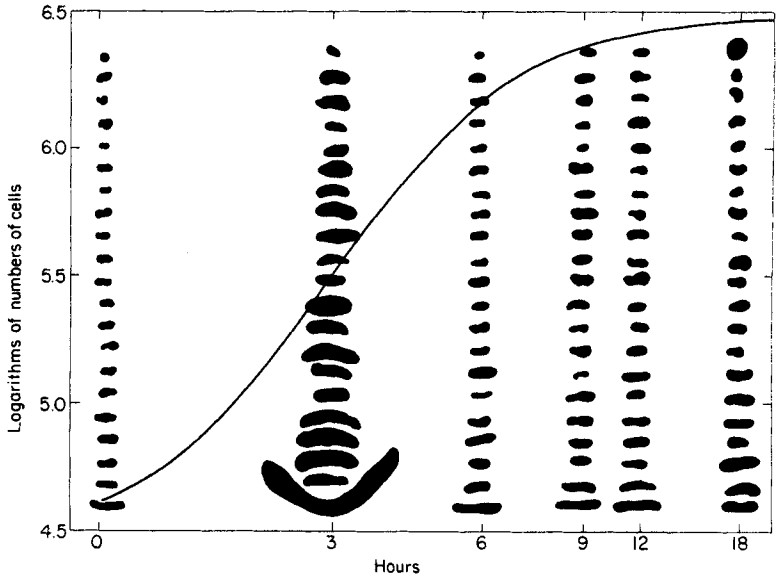


FIG. 24. Henrici's results (H7) showing morphologic variation during batch growth of *Bacillus coli* (*E. coli*). The shapes and sizes of cells selected at random were as suggested by the drawing. Redrawn from "Morphologic Variation and the Rate of Growth of Bacteria," by permission of Charles C. Thomas, Publishers.

[16] An irradiance curve is a plot of photosynthetic rate, as measured by oxygen production per unit cell mass, vs light intensity. Conditions are arranged so that little light is absorbed in passing through the suspension; averaging of rates is thus not necessary.

batch and continuous cultures [see Henrici (H7) and Herbert (H9)]. Figure 24, taken from Henrici's classic work, suggests this.[17]

Clearly, morphologic changes and physiologic changes are correlated in some fashion; Monod's model or extensions of it give no clue as to what this correlation might be. Hence, the basic reason for discrepancies between the model and experiment is probably due to failure of the model to account for cellular *structure*. Models such as those of Ramkrishna (R3) will probably account for some physiologic changes. However, we are also interested in morphologic changes and in reproduction of cells, so that it becomes necessary to consider structured, *segregated* models.

VI. Structured Models: Segregated

The concept of a structured but distributed model can be carried further than in Section IV. However, we turn instead to the more complicated case of structured and segregated models. In other words, we now acknowledge that the culture is made up of *individuals*. [It should be remembered that we are speaking about a culture of cells, not about a population of cells forming a multicellular organism or a tissue; in the latter case there may be "communication" between the cells such that intercellular events achieve a degree of synchrony (cf. G1).]

Cells are continually being formed by fission while others are growing, being washed out, etc., in a continuous culture. Hence, it follows that cells in a culture exhibit a *distribution* of structures. Therefore, the gross metabolic rates of the culture represent averages over the distribution of cell structures. One of the principal problems is then to establish the factors which determine the distribution.

A sophisticated model of the dynamics of cell populations would have to take the distribution of cell structures into account. This presupposes that one has a quantitative *measure* of cell structure. Such a measure is not a single, scalar quantity. Rather, one would have to specify, for instance, how many enzymes of each kind a cell had, the geometrical arrangement of the enzyme molecules, the number and location of RNA and DNA molecules, etc. Undoubtedly the measure for this kind of information would be a matrix (higher order tensor). No one has formulated such a measure, however; probably this is due to lack of basic knowledge concerning the dynamics of subcellular processes.

As a slight digression, we can deduce from the foregoing a principle of

[17] Consideration of varying transport properties of cell walls and membranes may be important in the explanation of why sizes and shapes of microorganisms vary during the different phases of growth.

importance long recognized by those concerned with cultivation of microbial forms. Since structure is described by a set of quantities, rather than by a single quantity, the rate of change of structure—that is, the *growth rate of a cell*—is also described by a set of quantities. Moreover the set of quantities describing the structure follows different paths of development under different circumstances of propagation, so that the set of quantities in the growth-rate measure will bear different relations to one another. Hence a correlation between two simple measures of growth rate, say rate of increase of cell mass and rate of increase of cell numbers, may not be expected to hold when one alters the circumstances of propagation. To put it more precisely, it is not possible to predict the results of continuous propagation from the results of batch propagation when only single measures of growth rate are used. This was, of course, the point of Ramkrishna's work (R3).

A. Steady-State Propagation: the Age Model

To return to the principal line of development, one wonders if there is not some simplified semiempirical way of introducing cell structure (i.e., accounting for differences between cells) which can serve as a basis for a model. Several possibilities exist. The arguments for the first one, at least, are most easily developed by considering the steady state of a continuously propagated culture.

In such a culture, the environmental conditions "seen" by a cell are constant, on the average. By average, we mean the average over periods of time long in comparison to the reciprocal frequencies of random fluctuations of conditions on the molecular scale.[18] Moreover, the distribution of cell structures in this situation is constant.

Hence, if at one time (say, t) we could pick out all cells of a given structure and "follow" this group of cells through its subsequent history, we could establish the distribution of structures in the culture. As a matter of fact, we can do this, in principle, by equating structure with *cell age* (which we hereinafter denote by the symbol τ). We could also equate structure with cell *mass*, and this is done in Part C of this section.

We *define* the age of a cell as the length of time which has elapsed since the cell was formed by fission of its parent. We are arbitrary here, of course, since a definition of the instant of fission is required.[19] Nevertheless, the definition of age for our purpose must be quantitative and operational; it is to be distinguished sharply from qualitative and nonoperational terms such as "biological age," etc., used in the literature.

[18] From the point of view of, say, a bacterial cell, these fluctuations may be rather important.

[19] We are aware that a septum in a potential daughter cell may begin to form before the septum in the parental cell is complete.

In general, the equation of cell age with cell structure can hold rigorously only in the steady state, and then only in the statistical sense. In the unsteady state, environmental conditions are changing with time so that the average pattern of development changes with time. The necessity for a statistical interpretation of our equation is due to two causes: first, random fluctuations of environmental and intracellular conditions on the molecular level mean that two cells having initially the same structure and exposed to the same environment (in the average sense) will develop at the same rate[20] only in the statistical sense; second, two cells just formed by fission (age $\tau = 0$) will have identical structures only in the statistical sense. These factors require a model of populations of structured cells to be statistical in nature. This contrasts with models of populations of unstructured cells, where it was shown that the statistical approach was an unnecessary luxury.

Consider the following propositions concerning the fate of a cell in a steady-state, continuously propagated population:

A. The cell is not washed out nor does it divide in the time interval τ to $\tau + \Delta\tau$.
B. The cell is present in the culture at time τ.
C. The cell was formed by fission of its parent at time zero (so that it has age τ at time τ).

We seek the probability of (B | C), of course; let this be

$$P(\mathrm{B} \mid \mathrm{C}) \equiv P(\tau) \tag{120}$$

so that

$$P(\mathrm{AB} \mid \mathrm{C}) = P(\tau + \Delta\tau)$$

Hence, by Rule 1 of the theory of probability,

$$P(\mathrm{AB} \mid \mathrm{C}) = P(\mathrm{A} \mid \mathrm{BC})\, P(\mathrm{B} \mid \mathrm{C})$$

or

$$P(\tau + \Delta\tau) = P(\tau)\, P(\mathrm{A} \mid \mathrm{BC}) \tag{121}$$

To get $P(\mathrm{A} \mid \mathrm{BC})$, consider the propositions

D. The cell divides in time τ to $\tau + \Delta\tau$.
E. The cell is washed out in time τ to $\tau + \Delta\tau$.

By Rule 2 of the theory of probability,

$$P(\mathrm{A} + \mathrm{D} + \mathrm{E} \mid \mathrm{BC}) = 1 = P(\mathrm{A} \mid \mathrm{BC}) + P(\mathrm{D} \mid \mathrm{BC}) + P(\mathrm{E} \mid \mathrm{BC})$$

or

$$P(\mathrm{A} \mid \mathrm{BC}) = 1 - P(\mathrm{D} \mid \mathrm{BC}) - P(\mathrm{E} \mid \mathrm{BC}) \tag{122}$$

[20] But as Schrödinger (S2) points out, the *results* achieved are deterministic, even though rates are subject to probability considerations.

This follows since A, D, and E are mutually exclusive and exhaustive events.

At this point, models must be introduced for the fission and washout processes. If we assume that the vessel is perfectly mixed, then washout probability ($P(\mathrm{E} \mid \mathrm{BC})$) is independent of cell age, and moreover

$$P(\mathrm{E} \mid \mathrm{BC}) = 1/\theta\, \Delta\tau + O[(\Delta\tau)^2] \tag{123}$$

where $\theta = V/Q$ is the nominal holding time of a particle in the vessel. For the fission probability $P(\mathrm{D} \mid \mathrm{BC})$, consider the most general assumption possible, namely,

$$P(\mathrm{D} \mid \mathrm{BC}) = \Gamma(\tau)\, \Delta\tau + O[(\Delta\tau)^2] \tag{124}$$

where Γ is an arbitrary (but nonnegative and at least piecewise continuous) function of cell age. By postulating the existence of the function $\Gamma(\tau)$, we are saying that the probability of fission of a cell changes with its age; viz., there is not much chance that a young cell will divide but there is a considerable chance that a somewhat older cell will divide in the given time interval when both are in identical environments. For the present, we need not specify the form of $\Gamma(\tau)$; instead, we regard it as a quantity to be determined by experiment. Of course $\Gamma(\tau)$ depends on environmental conditions, but since these are constant in steady-state culture, we need not express the dependence explicitly.

Equation (122) can now be written as

$$P(\mathrm{A} \mid \mathrm{BC}) = 1 - (1/\theta + \Gamma(\tau))\, \Delta\tau - O[(\Delta\tau)^2] \tag{125}$$

so that Eq. (121) yields

$$P(\tau + \Delta\tau) = P(\tau)[1 - (1/\theta + \Gamma(\tau))\, \Delta\tau - O[(\Delta\tau)^2]]$$

whence

$$\frac{dP(\tau)}{d\tau} = -(1/\theta + \Gamma(\tau))\, P(\tau) \tag{126}$$

The initial condition for Eq. (126) is

$$P(O) = 1$$

so that

$$P(\tau) = \exp\left\{-\left(\frac{\tau}{\theta} + \int_0^\tau \Gamma(\tau')\, d\tau'\right)\right\} \tag{127}$$

We may give this result a frequency interpretation. Let $U(\tau)\, d\tau$ be the number of cells, per unit volume of culture, having ages between τ and $\tau + d\tau$. Then

$$U(\tau)/U(0) = P(\tau) \tag{128}$$

since in *steady-state* culture, $U(\tau)/U(0)$ is the fraction of organisms formed at time zero which are still present, undivided, in the vessel at time τ. Then by Eq. (127)

$$U(\tau) = U(0) \exp\left\{-\left(\frac{\tau}{\theta} + \int_0^\tau \Gamma(\tau')\, d\tau'\right)\right\} \tag{129}$$

which is the (nonnormalized) density of the age distribution function. This equation was also derived by Fredrickson and Tsuchiya (F4) by very different methods. Equivalent equations are given by Powell (P4) and Harris (H4).

The function $\Gamma(\tau)$ could be determined indirectly if the ratio $U(\tau)/U(0)$ could be determined. This is not possible, however, and a different but equivalent procedure must be used. In principle, it is possible to determine the distribution of division times in a population by microscopic examination. Let $h(\tau)$ be the density of the distribution of division times; i.e., $h(\tau)\, d\tau$ is the probability that a cell has age between τ and $\tau + d\tau$ when it divides. Then, by Bayes' theorem [Eq. (A.6)], we easily find[21]

$$\Gamma(\tau) = h(\tau)\left(1 - \int_0^\tau h(\tau')\, d\tau'\right)^{-1} \tag{130}$$

Thus, $\Gamma(\tau)$ can be calculated once $h(\tau)$ has been measured experimentally, and the ratio $U(\tau)/U(0)$ can then be calculated from Eq. (129).

The question now arises as to where subcellular kinetics and transport processes enter this picture. A partial answer is that these processes (along with the genetic capabilities of the organism) determine the function $\Gamma(\tau)$. We shall explore below a model of the reproduction process which leads to an explicit equation for $\Gamma(\tau)$.

Beyond the immediate problem of reproduction we are interested in the consumption of substrates by cells, the formation of new cellular material, and the release of metabolic by-products to the environment. One cannot ignore these questions; it is the growth of single cells that determines what the environmental conditions will be (apart from external controls applied), and the environmental conditions determine in turn what the growth and reproductive characteristics of the culture will be.

Suppose that we had either experimental or theoretical evidence concerning the growth rate of single cells in steady-state culture. In the most elementary approach, this information might be given as rate of increase of cell mass. In general, growth rate is not expressible as a single quantity, as stated earlier, but let us ignore this fact here. That is, we assume that the development of cells is independent of the particular circumstances of propagation.

[21] Harris (H4) also gives this equation (Section II, Part 1 of his paper).

Put $r(\tau)$ equal to the average growth rate of a single cell of age τ. The rate is expressed in terms of increase of cell mass and is taken to be an explicit function of cell age. Then the concentration C of biomass present may be established by material balance on the propagation vessel, the result for the steady state is

$$-\frac{C}{\theta} + \int_0^\infty r(\tau)\, U(\tau)\, d\tau = 0 \tag{131}$$

Again, if the addition of one unit of mass to a cell by growth consumes (on the average) α_i mass units of the ith chemical species in the surrounding medium, material balance for the steady state yields

$$\frac{C_{if} - C_i}{\theta} - \int_0^\infty \alpha_i(\tau)\, r(\tau)\, U(\tau)\, d\tau = 0 \tag{132}$$

where C_i is the concentration of ith chemical species in the propagator effluent, and subscript f denotes feed conditions. If the ith species is a product of growth, then α_i is negative, of course. Again, we assume that the stoichiometric coefficients α_i depend on cell age.

The growth rate R_p and the stoichiometric coefficients a_i used in Monod's model represent averages over the distribution of cell ages. Thus, from Eq. (131) and the definition of R_p, we find

$$R_p = \int_0^\infty r(\tau)\, U(\tau)\, d\tau \tag{133}$$

and, from Eqs. (37) and (132),

$$a_i = \int_0^\infty \alpha_i(\tau)\, r(\tau)\, U(\tau)\, d\tau \Big/ \int_0^\infty r(\tau)\, U(\tau)\, d\tau \tag{134}$$

In continuous, steady-state culture, R_p and a_i will in general depend on the holding time, since $U(\tau)$ varies with holding time. Hence the model is able to account for dependence of stoichiometric coefficients and growth rates on the steady-state holding time; in Monod's model, there is no dependence of these quantities on holding time. It is possible, then, for the newer model to rationalize data such as those shown in Fig. 16.

Clearly the procedure just outlined is sufficient to establish a rather flexible model of continuous steady-state propagation. The information necessary for computational purposes is contained in the functions $\Gamma(\tau)$, $r(\tau)$, $\alpha_1(\tau)$, $\alpha_2(\tau)$, ..., and it is these functions that describe intracellular dynamics. Present lack of understanding of these processes indicates that the functions $\Gamma(\tau)$, etc., must be determined experimentally. If, however, we had a model, we could write down what these functions should be.

One such model was proposed by Rahn (R2). In his model, growth of a cell involves replication of a fixed number N of entities (Rahn called these "genes" but we need not do so) within the cell. When all N entities have been replicated, the cell divides, and the process repeats itself.

The rate of replication is random, of course, since we are dealing with very small scale processes. Rahn suggested that his postulated entities were identical, in the sense that the order in which they are replicated is immaterial; indeed, simultaneous replication is not forbidden. Other hypotheses of the same general nature are possible, and have been reported in the literature [see, e.g., Kendall (K2) and Powell (P3)].

Let $X(\tau)$ be the number of entities which have been replicated in a cell of age τ. Of course, $X(\tau)$ is a discrete random variable, which assumes values 0, 1, 2, ..., N. If we put $P_x(\tau)$ equal to $P(X(\tau) = x)$, then it can be shown by a method essentially the same as used for the pure birth process (Section III, A), that $P_x(\tau)$ satisfies the difference-differential equation

$$dP_x(\tau)/d\tau = -\gamma \cdot (N - x)\, P_x(\tau) + \gamma \cdot (N - x + 1)\, P_{x-1}(\tau) \qquad (135)$$

The model from which this is obtained is that the probabilities of the transitions $X(\tau) = x - k$ to $X(\tau + \Delta\tau) = x$ are

$$\{1 - \gamma \cdot (N - x)\, \Delta\tau - O[(\Delta\tau)^2]\} \qquad \text{for} \quad k = 0$$

$$\{\gamma \cdot (N - x + 1)\, \Delta\tau + O[(\Delta\tau)^2]\} \qquad \text{for} \quad k = 1$$

etc. Here the parameter γ must be assumed to be dependent on environmental conditions; in steady-state culture, these are constant, so γ is also constant.

Initial conditions for Eq. (135) are

$$P_x(0) = \begin{cases} 1, & x = 0 \\ 0, & x > 0 \end{cases} \qquad (136)$$

whence it is easily shown that [22]

$$P_N(\tau) = (1 - e^{-\gamma\tau})^N \qquad (137)$$

[22] The solution of Rahn's problem is

$$P_x(\tau) = (-1)^x \binom{N}{x} e^{-N\gamma\tau}(1 - e^{\gamma\tau})^x$$

whence one can easily show that

$$\sum_{x=0}^{N} P_x(\tau) = 1$$

and

$$E[X(\tau)] = N(1 - e^{-\gamma\tau})$$

The latter expression is the result obtained for product accumulation in a first-order batch chemical reaction (deterministic).

Since it is assumed that division follows as soon as N entities are replicated, it follows that $dP_N/d\tau$ must be the density of the distribution of division times. Hence,

$$h(\tau) \equiv \frac{dP_N(\tau)}{d\tau} = \gamma N \, e^{-\gamma\tau}(1 - e^{-\gamma\tau})^{N-1} \tag{138}$$

so that by Eq. (130)

$$\Gamma(\tau) = \frac{\gamma N \, e^{-\gamma\tau}(1 - e^{-\gamma\tau})^{N-1}}{1 - (1 - e^{-\gamma\tau})^N} \tag{139}$$

In this view, the dependence of Γ on environmental conditions results from the dependence of γ on those conditions.

Experimentally, it has been found by Powell (P3), Kubitschek (K5), and others that the function $h(\tau)$ derived from Rahn's hypotheses does reproduce the form of the observed density of division times; values of N reported vary from 25 (F3) to 400 (K5). It so happens, however, that other hypotheses can be made to fit the data as well, and there are observations (such as the correlation of division times of sister cells) which apparently contradict the model.

However, the important point here is the appearance of intracellular dynamics in the functions $\Gamma(\tau)$, etc. In principle these functions can be determined by experiments on *populations* of cells, so that it is not necessary to work with *single cells* to study the mechanism of cellular processes.

B. General Equations for the Age Model

In the preceding section a probabilistic approach was taken to the problem of cell age distribution in continuous steady-state cultures. The density of the age distribution function

$$U(\tau) \Big/ \int_0^\infty U(\tau')\, d\tau'$$

was thereby determined [Eq. (129)], and the effect of environmental conditions on growth was incorporated by letting functions Γ, r, and α_i depend on those conditions, as well as on cell age. The set of equations given is not complete, since it does not determine the steady-state population density at a given holding time. Moreover, one would like to be able to make some statements about the *transient* behavior of populations; this cannot be done from the previous analysis.

We begin this section by developing a differential equation for $U(t, \tau)$, the nonnormalized density of the distribution of cell ages at time t. The population density itself is, of course,

$$N(t) = \int_0^\infty U(t, \tau)\, d\tau \tag{140}$$

In accord with the results of Section III,A, we regard the population density as an ordinary (i.e., nonrandom) variable. On the other hand, the age of randomly selected cells in the culture is a random variable, and we seek the law by which the distribution of ages changes with time.

Consider a cell which at time t has an age between τ and $\tau + d\tau$; for convenience, such a cell is said to be of group τ. The number of cells of group $\tau - d\tau$ at time t, per unit volume, is $U(t, \tau - d\tau)\, d\tau$. If we choose an increment of time $dt = d\tau$, then the fate of cells of group $\tau - d\tau$ during time interval t to $t + dt$ must be one of the following:

A. the cell divides;
B. the cell is washed out;
C. the cell "ages" by an amount $d\tau = dt$.

If (C | D) is true, then the cell will be of group τ at time $t + dt$. In the foregoing, "D" refers to our prior knowledge of the cell; that is, that it was of group $\tau - d\tau$ at time t, etc.

By Rule 2 of the theory of probability

$$P(\mathrm{A} + \mathrm{B} + \mathrm{C} \mid \mathrm{D}) = P(\mathrm{A} \mid \mathrm{D}) + P(\mathrm{B} \mid \mathrm{D}) + P(\mathrm{C} \mid \mathrm{D}) = 1 \tag{141}$$

since A, B, and C are mutually exclusive and exhaustive events. But by definition of $\Gamma(\tau)$ and the hypothesis of perfect mixing

$$P(\mathrm{A} \mid \mathrm{D}) = \Gamma(\tau - d\tau)\, dt$$

$$P(\mathrm{B} \mid \mathrm{D}) = 1/\theta\, dt$$

so that

$$P(\mathrm{C} \mid \mathrm{D}) = 1 - \{1/\theta + \Gamma(\tau - d\tau)\}\, dt \tag{142}$$

Thus Eq. (142) gives the probability $P(\mathrm{C} \mid \mathrm{D})$ that a cell of group $\tau - d\tau$ at time t will be a cell of group τ at time $t + dt$ ($d\tau = dt$).

We now adopt the frequency interpretation of probability. The number of cells of group $\tau - d\tau$ at time t which become cells of group τ at time $t + dt$ is

$$U(t, \tau - d\tau)\, d\tau \cdot \left[1 - \left\{\frac{1}{\theta} + \Gamma(\tau - d\tau)\right\} dt\right]$$

As a matter of fact, this must also be the number of cells (per unit volume) of group τ present at time $t + dt$, since *all* cells of group τ at time t will be washed out, divided, or aged to cells of group $\tau + d\tau$ in time interval t to $t + dt$. The number of such cells, per unit volume, which are "lost" to group τ in t to $t + dt$ is $U(t, \tau)d\tau$. Hence, the *increase* in number of cells, per unit volume, of group τ in time t to $t + dt$ is

$$U(t, \tau - d\tau)\, d\tau\left[1 - \left\{\frac{1}{\theta} + \Gamma(\tau - d\tau)\right\} dt\right] - U(t, \tau)\, d\tau$$

This must be equated to the accumulation of cells of group τ in time t to $t + dt$,

$$\frac{\partial}{\partial t}\left[U(t, \tau)\, d\tau\right] dt$$

Hence,

$$U(t, \tau - d\tau)\, d\tau\left[1 - \left\{\frac{1}{\theta} + \Gamma(\tau - d\tau)\right\} dt\right] - U(t, \tau)\, d\tau = \frac{\partial U(t, \tau)}{\partial t}\, d\tau\, dt$$

This may be rearranged to yield the partial differential equation

$$\left(\frac{\partial}{\partial t} + \frac{\partial}{\partial \tau} + \frac{1}{\theta} + \Gamma(\tau)\right) U(t, \tau) = 0 \tag{143}$$

This equation was derived by Fredrickson and Tsuchiya (F4) by a rather obscure method; a similar equation was found by Behnken *et al.* (B4) in their study of particle growth processes. Note that Eq. (143) for the steady-state case ($\partial U/\partial t = 0$) reduces to that derived earlier, Eq. (126).

Equation (143) as it stands is valid for $\tau > 0$; it does not hold for $\tau = 0$ since there is no term for generation of cells of age zero. A boundary condition for Eq. (143) may be derived by making a number balance on cells of age 0 to $d\tau$.

The number of new cells per unit volume, which are formed in time t to $t + dt$, is

$$2\, dt \int_0^\infty U(t, \tau)\, \Gamma(\tau)\, d\tau$$

The factor 2 arises since each fission produces two new cells. On the other hand, all cells of group 0 at time t will be "lost" to this particular age interval in time t to $t + dt$ ($d\tau = dt$) by fission, washout, or aging. The number so lost is $U(t, 0)\, d\tau$. There is no gain of cells of group 0 by aging of younger cells, since no cells are younger than $\tau = 0$. Hence, the inventory reads

$$2\, dt \int_0^\infty U(t, \tau)\, \Gamma(\tau)\, d\tau - U(t, 0)\, d\tau = \frac{\partial U(t, 0)}{\partial t}\, d\tau\, dt$$

Since the right-hand side is a higher order differential, the required boundary condition is

$$U(t, 0) = 2 \int_0^\infty U(t, \tau)\, \Gamma(\tau)\, d\tau \tag{144}$$

This equation, which must be satisfied in the steady state as well as in the unsteady state, provides the missing piece of information necessary for the complete solution of the steady-state case treated earlier.

In Eqs. (143) and (144), we must take the function Γ to be at least an implicit function of time. Recall that Γ depends on environmental conditions,

and these in turn change with time in the transient case. Hence, if we define the vector **C** by

$$\mathbf{C} = [C_1, C_2, \ldots, C_M]$$

(M = number of chemical species in the environment), then we must write

$$\Gamma = \Gamma(\tau, \mathbf{C})$$

Similar statements may be made about the growth rate per cell r and the stoichiometric coefficients α_i.

The complete set of equations describing the continuous culture in the unsteady state is then

$$\left(\frac{\partial}{\partial t} + \frac{\partial}{\partial \tau} + \frac{1}{\theta} + \Gamma(\tau, \mathbf{C})\right) U(t, \tau) = 0 \tag{145}$$

$$\frac{dC_i}{dt} = \frac{1}{\theta}(C_{if} - C_i) - \int_0^\infty \alpha_i(\tau, \mathbf{C})\, r(\tau, \mathbf{C})\, U(t, \tau)\, d\tau \quad (i = 1, 2, \ldots, M) \tag{146}$$

together with the boundary condition

$$U(t, 0) = 2\int_0^\infty \Gamma(\tau, \mathbf{C})\, U(t, \tau)\, d\tau \tag{147}$$

Of course, a sufficient number of initial conditions must also be specified.

Equation (145) is the number balance (accounting) on cells of group τ; Eq. (147) is the number balance on cells of group 0. Equation (146) is a material balance on the ith chemical species in the medium; Eq. (132) is its steady-state form. In addition a balance on biomass yields the generalization of Eq. (131):

$$\frac{dC}{dt} = -\frac{1}{\theta} C + \int_0^\infty r(\tau, \mathbf{C})\, U(t, \tau)\, d\tau \tag{148}$$

where C is the concentration of biomass. This equation is not independent, since the amount of biomass formed must equal the net amount of mass extracted from the environment.

At this point, a difficulty not present in the steady-state case arises. In the steady state, it seems sufficient to assume that Γ (and also r and the α_i) depends only on cell age τ and environmental conditions **C**. The treatment of Rahn's model given earlier, for example, makes this clear. But in the transient case, it appears that Γ should depend not only on τ and **C**, but also on (laboratory or absolute) time t.

Inspection of Rahn's model can also clarify this point. Consider a cell formed by fission at time t_0. At time $t_0 + \tau$, this cell will have age τ. By Rahn's hypotheses, the probability of replication of one "gene" in time interval $t_0 + \tau$ to $t_0 + \tau + \Delta\tau$, given that x "genes" have been replicated at time $t_0 + \tau$, is

$$\gamma(t_0 + \tau)(N - x + 1)\,\Delta\tau + O[(\Delta\tau)^2]$$

where the parameter γ must now depend on $t_0 + \tau$, since environmental conditions are changing in the transient state. In the steady-state case, γ could be regarded as constant.

Explicit introduction of time (t or t_0) in the function Γ creates a much more difficult problem than that expressed by Eqs. (145)–(147), since the equations are no longer autonomous. Hence, in what follows, we ignore the dependence of Γ on t; assume that Γ has the same form in the unsteady state as in the steady state, and work with the approximate equations already given. This procedure should be adequate, provided that environmental conditions are not changing too fast; that is, not changing much in the mean lifetime of a cell. Of course, if we had an adequate measure of cell structure, we should not have to resort to this kind of subterfuge.

As an example, consider the batchwise growth of a synchronized population of cells. A culture is said to be synchronized when all cells divide at the same time; for our purposes, we say that a culture is synchronized when all cells have the same age.

Various methods of synchronizing cultures have been discovered [see, e.g., Burns (B11) and Maaløe (M1). Some of these involve application of stress—such as sudden temperature changes—but we do not consider these here [since in shock, environmental conditions change rapidly and Eqs. (145)–(147) are not expected to hold]. Rather, we consider that the culture has been synchronized by the filtration technique developed by Maruyama and Yanagita (M6), Abbo and Pardee (A1), and others. Presumably this method does not alter the composition or physical conditions of the growth medium, and the synchronizing action is the selection of cells having narrow ranges of sizes and surface characteristics.

For example, assume that synchronization is perfect, and that it is effected at time $t = 0$ when all cells selected have age $\tau = 0$. The requisite initial condition is then

$$U(0, \tau) = N_0\,\delta(\tau) \tag{149}$$

where N_0 is the initial population density of the synchronized culture, and $\delta(\tau)$ is Dirac's delta function (see E4).

Let us also assume that density of the culture upon synchronization is quite low, so that subsequent growth, at least for some time, does not alter

the medium enough to alter the rates of subcellular processes.[23] If this is the case, Γ will depend only on τ, and will be independent of **C**.

Assume that Γ has the form

$$\Gamma(\tau) = \gamma S(\tau - \tau_0) \tag{150}$$

where

$$S(\tau) = \begin{cases} 0, & \tau < 0 \\ 1, & \tau > 0 \end{cases}$$

is the "unit step function," and γ is the parameter appearing in Rahn's model of cell fission. The form of Eq. (150) is the limit of Rahn's model as the number of entities to be replicated (N) becomes indefinitely large. In actuality, Γ would not be a sharp step as indicated by Eq. (150), but rather would be a smoothly rising step.

The pertinent equation is then

$$\left(\frac{\partial}{\partial t} + \frac{\partial}{\partial \tau} + \gamma S(\tau - \tau_0)\right) U(t, \tau) = 0 \tag{151}$$

and this is to be solved with initial condition Eq. (149) and boundary condition

$$U(t, 0) = 2\gamma \int_{\tau_0}^{\infty} U(t, \tau)\, d\tau \tag{152}$$

The solution may be found by Laplace transform methods; these lead to

$$\begin{aligned} \bar{N}(p) &= \int_0^{\infty} \bar{U}(p, \tau)\, d\tau \\ &= \frac{N_0}{1 - \dfrac{2\gamma}{p + \gamma} e^{-p\tau_0}} \left[\frac{1}{p}(1 - e^{-p\tau_0}) + \frac{e^{-p\tau_0}}{p + \gamma}\right] \end{aligned} \tag{153}$$

where p is the Laplace transform variable, and the bar indicates a transformed quantity. The population density is found by inverting the expression (153) and is

$$\frac{N(t)}{N_0} = 1 + \sum_{m=1}^{P(t)} 2^{m-1} f[\gamma(t - m\tau_0), m] \tag{154}$$

[23] This is not an unreasonable assumption. For instance, growth rate is zero order in substrate concentration as long as that concentration is large enough. Indeed, the very occurrence of exponential growth in batch cultures indicates that metabolic rates are zero order in substrate concentrations therein.

Here $P(t)$ is the largest integer such that $t > P(t)\tau_0$, and f is the function

$$f(y, m) = \frac{1}{(m-1)!} \int_0^y e^{-x} x^{m-1}\, dx \tag{155}$$

The time course of increase of $N(t)$ predicted by Eq. (154) is shown in Fig. 25

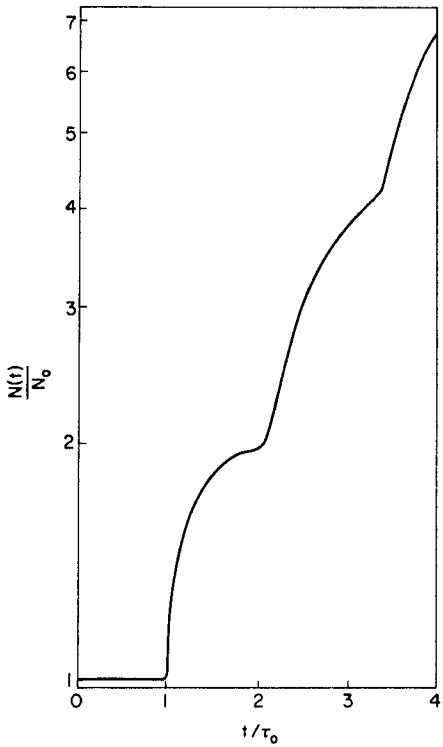


FIG. 25. Time course of population increase in an initially synchronous culture as calculated from Eq. (154).

for $\gamma = 3.91\ \text{h}^{-1}$ and $\tau_0 = 1$ h. This is to be compared, for instance, with the experimental data shown by Abbo and Pardee (A1; their Fig. 1).

Figure 25 shows that synchronization is gradually lost, and growth approximates to exponential increase, as time proceeds.[24] This is not apparent in the data of Abbo and Pardee; perhaps the desynchronization in any one generation of their culture was less than that assumed in the foregoing calculations. The spread in division times shown by their data is then

[24] Asymptotic formulas for the growth-rate constant and the age distribution in exponential growth are given in the example following.

explained by the probability that synchronization was not initially perfect. Of course the form assumed for Γ may be too simple.

Equation (154) does not predict a stationary phase or a phase of decline as time increases. This is so because we neglected the effect of the changing environment on Γ. If we solved the full set of equations[25] (145)–(147), a stationary phase would be predicted.

To close this section, we derive Powell's (P4) asymptotic formula for the density of the age distribution of a batch culture in exponential growth. As before, we assume that the culture is dilute enough so that Γ is not altered by growth,[26] at least for a number of generations. Then the requisite equations are

$$\left(\frac{\partial}{\partial t} + \frac{\partial}{\partial \tau} + \Gamma(\tau)\right) U(t, \tau) = 0 \tag{156}$$

with

$$U(t, 0) = 2 \int_0^\infty \Gamma(\tau)\, U(t, \tau)\, d\tau \tag{157}$$

No initial condition is specified as in the previous example since we seek the asymptotic solution valid after exponential growth has started.

A trial solution is therefore

$$U(t, \tau) = N_0\, e^{\mu t} f(\tau) \tag{158}$$

where $f(\tau)$—the density of the age distribution—must be chosen so that

$$\int_0^\infty f(\tau)\, d\tau = 1 \tag{159}$$

Hence, the population density is

$$N(t) = \int_0^\infty U(t, \tau)\, d\tau = N_0\, e^{\mu t} \tag{160}$$

so that μ is the exponential growth rate constant.

Substitution of (158) into (156) yields

$$df(\tau)/d\tau + (\mu + \Gamma(\tau)) f(\tau) = 0 \tag{161}$$

which by (157) must satisfy

$$f(0) = 2 \int_0^\infty \Gamma(\tau) f(\tau)\, d\tau \tag{162}$$

and also the normalizing condition, Eq. (159).

[25] This can be done numerically on the digital computer [for details, see Eakman (E1)].

[26] Indeed, it appears that exponential growth is possible only if there exists a range of environmental conditions in which Γ is constant.

The solution of (161) is

$$f(\tau) = f(0) \exp\left\{ -\left(\mu\tau + \int_0^\tau \Gamma(\tau')\, d\tau' \right) \right\} \tag{163}$$

so that Eq. (162) requires

$$\tfrac{1}{2} = \int_0^\infty \Gamma(\tau) \exp\left\{ -\left(\mu\tau + \int_0^\tau \Gamma(\tau')\, d\tau' \right) \right\} d\tau \tag{164}$$

Equation (164) gives the growth rate constant μ as a function of Γ. The constant $f(0)$ is determined from

$$f(0) = \left\{ \int_0^\infty e^{-\mu\tau} \exp\left(- \int_0^\tau \Gamma(\tau')\, d\tau' \right) d\tau \right\}^{-1} \tag{165}$$

which is derived from Eq. (159), of course.

Equations (163)–(165) are equivalent to Powell's solution for batch cultures. The analysis here shows that this solution is only valid asymptotically. For a batch culture which has just emerged from the lag phase, there is no reason to expect that the age distribution has the density given by Eq. (163). Therefore, there must be a transient period during which the density changes with time until the form (163) is approached asymptotically. The previous example on a synchronous culture shows that this transient period might last for two or more generations, depending on the initial departure of the age distribution from that given by Eq. (163). Of course the solution given will cease to be valid after sufficient growth has occurred to alter the value of Γ appreciably.

C. A More General Model and Its Possible Extension

Koch and Schaechter (K3) have pointed out a number of unsatisfactory features in use of cell age as an index or measure of cell structure. In particular they note that the model based on this predicts no correlation between the life spans of sister cells, whereas in fact these life spans are found to be correlated. Again, they note that the *mass* of cells at division (bacterial cells, at any rate) shows less "spread" about a mean value than does the age of cells at division. They also report that they have found that the mean size of bacteria, at division, changes in a regular fashion when cells are transferred to a medium in which growth rate and bacterial size are different.

On the basis of these observations and other arguments which we review, a different model for the growth of a population of structured cells was proposed by Koch and Schaechter. The postulates of the model are discussed below.

First: Growth at the cellular level is deterministic. This is essentially the same assumption as made in Parts A and B of this section, where only

the mean or expected growth rate of a cell, r, was found to be necessary for the model. There r was taken to be a function of cell age; in the model of Koch and Schaechter, however, the index of cell structure must be the cell mass m. As mentioned earlier, growth rate depends on environmental conditions, too, so that

$$r = r(m, \mathbf{C}) \tag{166}$$

As a subpostulate, Koch and Schaechter assume that the growth of individual cells of a culture in exponential growth is also exponential, with the same growth rate constant as that of the culture. That is

$$r = \mu(\mathbf{C})m \tag{166a}$$

where (C) is the growth rate "constant" (in terms of cell mass) for the culture as a whole. This postulate is certainly not general,[27] but according to Koch and Schaechter, it is in accord with experimental findings for certain bacteria.

Second: The size (mass) of a cell at division is under genetic and environmental control, but the critical mass for fission shows small random variations about the mean value. The concept that cell division results when cells have attained a critical mass is not new; its history is reviewed by Koch and Schaechter. Random variations of cell size at division may be due to (i) the critical mass varying slightly from cell to cell; or (ii) appearance of visible evidence of fission in individual cells may be premature or delayed. In either case the observed variation is supposed to be due to the accumulation of small effects of a large number of random influences. Hence, as a subpostulate, Koch and Schaechter assume that the distribution of sizes at fission[28] is nearly Gaussian.

Third: Cell division results in an equal or nearly equal division of cytoplasmic mass between the daughter cells. Thus we define a function $g(m', m, \mathbf{C})$ such that $g(m', m, \mathbf{C})\, dm$ is the probability that a daughter cell, formed from a mother of mass m' in a medium of conditions **C**, will have mass m to $m + dm$. Clearly then, g must satisfy the following conditions:

$$g(m', m, \mathbf{C}) = 0 \qquad \text{if} \quad m \geqslant m' \tag{167a}$$

$$\int_0^{m'} g(m', m, \mathbf{C})\, dm = 1 \tag{167b}$$

$$g(m', m, \mathbf{C}) = g(m', m' - m, \mathbf{C}) \tag{167c}$$

[27] This will be discussed in Section VII.

[28] That is, in batch cultures in exponential ("balanced") growth, or in continuous steady-state cultures.

Equation (167c) states that mass is conserved upon division of a cell. Koch and Schaechter assume that fission results in daughter cells of precisely equivalent masses; for this to be true, we must have

$$g(m', m, \mathbf{C}) = \delta(\tfrac{1}{2}m' - m) \tag{168}$$

where δ is Dirac's delta function.

Clearly these postulates suggest why cultures, even though initiated by single cells, do not grow synchronously through many generations.

Koch and Schaechter use these postulates to devise a formula for the distribution of cell masses in a batch culture growing exponentially (they obtained an approximate, asymptotic expression for this case). However, the model is more generally expressed as a set of integrodifferential equations. Let $W(m, t)\, dm$ be the number of cells per unit volume, at time t, having mass between m and $m + dm$. The concentration of biomass C is then

$$C(t) = \int_0^\infty mW(m, t)\, dm \tag{169}$$

then one can show [see, e.g., (E1)] that in a continuous propagator, the pertinent equations for the model just described are

$$\frac{\partial}{\partial t} W(m, t) + \frac{\partial}{\partial m}\left\{r(m, \mathbf{C})\, W(m, t)\right\} + \left\{\frac{1}{\theta} + \Gamma'(m, \mathbf{C})\right\} W(m, t)$$

$$= 2\int_m^\infty W(m', t)\, \Gamma'(m', \mathbf{C})\, g(m', m, \mathbf{C})\, dm' \tag{170}$$

$$\frac{dC_i}{dt} = \frac{1}{\theta}(C_{if} - C_i) - \int_0^\infty \alpha_i(m, \mathbf{C})\, r(m, \mathbf{C})\, W(m, t)\, dm \tag{171}$$

subject to

$$\int_0^\infty \frac{\partial}{\partial m}\{r(m, \mathbf{C})\, W(m, t)\}\, dm = 0 \tag{172}$$

and appropriate initial conditions for $W(m, t)$ and the C_i. Here, the α_i are the stoichiometric coefficients as before, and $\Gamma'(m, \mathbf{C})\, dt$ is the probability that a cell of mass m in environment of conditions $\mathbf{C}$ at time t will divide in time t to $t + dt$. The meaning of Γ' is quite similar to that of the function Γ used in the two previous sections.

Equation (170) may be derived by the method used by Behnken *et al.* (B4) in their study of particle growth processes. The primary difference is that one must account for fission of cells, a possibility not considered in the work cited. Similar equations, but not containing the growth terms, have been considered by Valentas (V1) in his study of droplet breakup and coalescence phenomena in agitated, two-phase systems. Note that if fission always results

in sister cells of exactly the same mass, then the right-hand side of Eq. (170) becomes

$$2\Gamma'(2m, \mathbf{C})\, W(2m, t)$$

Hence, instead of an integrodifferential equation, we now have a functional equation (also a differential equation).

The boundary condition which solutions of Eq. (170) must satisfy could be obtained by making a "number balance" on cells of all sizes present. However, this can be reduced to the boundary condition given, Eq. (172). This is done by integrating Eq. (170) over all m and comparing the result with the number balance on cells of all sizes. In this process, Eq. (167b) must be used.

Equation (172) can also be written as

$$r(0, \mathbf{C})\, W(0, t) = \lim_{m \to \infty} r(m, \mathbf{C})\, W(m, t) \tag{173}$$

and this limit should be zero, since $r(0, \mathbf{C}) = 0$ (cells cannot originate from nothing) and $W(0, t)$ is assumed finite. Equation (173) is the "regularity condition" postulated by Behnken *et al.* (B4) for their equations of the particle growth process.

It is of interest to note that Eqs. (170–172) reduce to Monod's model [Eqs. (41–42)] under certain conditions. These can be established by multiplying Eq. (170) by m and integrating the resulting equation over all m. One finds that Monod's equations are obtained if:

(i) there is a single limiting substrate S so that $\mathbf{C}$ may be replaced by C_s;
(ii) the stoichiometric coefficient α_i (for $i = S$) is constant;
(iii) the function $g(m', m, \mathbf{C})$ is such that

$$\tfrac{1}{2}m' = \int_0^{m'} m g(m', m, C_s)\, dm \tag{174}$$

(iv) the function $r(m, \mathbf{C})$ is such that

$$r(m, C_s) = \frac{\mu C_s}{K + C_s^m} \tag{175}$$

where μ and K are constants.

Equation (174) is always true, as can be shown from Eqs. (167b) and (167c). Equation (175) is seen to be a special case of the postulate of Koch and Schaechter [Eq. (166a)]. The conditions under which Eq. (175) *might* be valid are discussed in Section VII.

As mentioned previously, an important feature of this model is its ability to account for a correlation between the life spans of sister cells. The model which used age as an index of cell structure cannot do this.

With regard to the correlations, there are two extreme cases to be considered:

(1) If the function $g(m', m, \mathbf{C})$ is such that there is a large probability that the daughter cells resulting from a fission will be of nearly the same size whereas the function $\Gamma'(m, \mathbf{C})$ is such that the size of a cell at fission is rather variable, then the life spans of sister cells will have a positive coefficient of correlation.[29] The coefficient of correlation of the life spans of a mother cell and either of its daughters will be slightly negative in this case. Thus the progeny of a cell which was smaller than the average at fission are both likely to have life spans longer than the average, whereas the progeny of a cell which was larger than the average at fission are both likely to have life spans shorter than the average. The negative coefficient of correlation between the life spans of mother and daughter cells obviously follows from the same observations.

(2) If the function $g(m', m, \mathbf{C})$ is such that there is a large probability that the daughter cells resulting from a fission will have quite different sizes whereas the function $\Gamma'(m, \mathbf{C})$ is such that most fissions occur when cell sizes are very close to a certain value, then the life spans of sister cells will have a negative coefficient of correlation. In this case, the coefficient of correlation of the life spans of a mother cell and either of its daughters should be nearly zero. Thus, one daughter cell resulting from a fission is likely to have a life span longer than the average while the other is likely to have a life span shorter than the average. The life span of a daughter is more or less independent of the life span of its mother.

Positive coefficients of correlation between the life spans of sister cells have been observed in the bacteria by Koch and Schaechter (K3) and by Kubitschek (K5). A negative coefficient of correlation between the life spans of mother and daughter cells is reported by Koch and Schaechter (K3). They also find that in the organisms they studied, cells divided into nearly equal parts on fission. Hence, the experimental observations indicate that the first extreme mentioned above is more nearly correct. It is important to note that the theory predicts the correlations described without having to postulate any genetic inhomogeneity of the population; i.e., all cells may be of the same genotype.

It might be possible to extend the model discussed in the foregoing by positing that the mass of cells is of several different kinds; that is, the concept developed by Ramkrishna (R3) might be extended to segregated models. Use of this concept of structure to generalize Eq. (170) would certainly lead to a more flexible model, but the mathematical difficulties inherent in solving the equations of the model are very formidable.

[29] The coefficient of correlation for two discrete random variables is discussed, e.g., by Feller (F1), Section IX,8. For continuous random variables, see pp. 48–53 in Laning and Battin (L2). Estimation of the coefficient of correlation from sampling experiments may be accomplished by the method described, e.g., by Crow *et al.* (C4), p. 158.

VII. Growth of Single Microbial Cells

In the model of growth discussed in Section VI,C, it is necessary to have an expression for the growth rate of a single cell. In this section, we discuss certain papers on growth of single cells which have appeared in the literature.

Von Bertalanffy (B6) hypothesized that growth of a single cell represents the results of competition between two opposing processes: *Aufbau* or assimilation and *Abbau* or endogenous metabolism. He assumed that the rate of *Abbau* is simply proportional to the mass of protoplasm in the cell. On the other hand, *Aufbau* must be assumed to be dependent on environmental conditions (e.g., on concentration of limiting substrate, in particular). Von Bertalanffy also postulated that the rate of assimilation is proportional to the *surface area* of the cell. The intuitive basis for these postulates is given on pp. 240–241 of his book.

The suggestion that the rate of assimilation of substrates by a cell might be proportional to the cell's surface area opens up a new field—biological transport—which this review has not previously considered. Thus, if the assimilation rate is assumed proportional to surface area, this rate could be controlled by the rate of transport of nutrilites from the cell's environment to the cell's interior.

In a steady-state situation, von Bertalanffy writes the growth rate of a single cell as

$$r(m, \mathbf{C}) \equiv dm/d\tau = V_0(\mathbf{C})\sigma - \mu_c m \tag{176}$$

Here σ is the surface area of the cell of mass m and age τ. Some important consequences of Eq. (176) can now be deduced.

Consider the growth of a rod-shaped cell (bacillus). Such a cell grows by elongation along its axis; there is essentially no change in the radius of the rod. Hence, if r is the radius of the cell and l its effective length, then $\sigma = 2\pi rl$ and $m = \pi r^2 l\rho$, where ρ is the mean cell density. Substitution of these equations into Eq. (176) then shows that the length grows as

$$\frac{dl}{d\tau} = \left(\frac{2V_0}{r\rho} - \mu_c\right) l \tag{177}$$

The mass follows the same law for this case of *linear, intercalary* growth:

$$\frac{dm}{d\tau} = \left(\frac{2\,V_0}{r\rho} - \mu_c\right) m \tag{178}$$

Hence, in steady-state propagation, the mass of a bacillus should increase exponentially, up to the time the cell divides.

Equation (178) is exactly the form postulated by Koch and Schaechter (K3) [see Eq. (166a)]. Moreover, Eq. (178) shows that if

(i) $(2V_0/r\rho) \gg \mu_c$

(ii) $V_0 \propto \dfrac{C_s}{K + C_s}$

(C_s = concentration of limiting substrate), then growth of a culture of rod-shaped cells should follow Monod's equations. On the other hand, if Condition (ii) above is valid but Condition (i) is not, then one can show that substitution of Eq. (178) into Eq. (170) leads to Herbert's generalization [Eq. (111)] of Monod's equations.

From this, we conclude that if Monod's equations (or Herbert's generalization thereof) and von Bertalanffy's equation [Eq. (176)] are valid, then the rate of transport of limiting substrate from environment to cell interior is proportional to $C_s/(K + C_s)$. However, if this transport were *Fickian*, the rate should be a linear function of C_s. Hence the simultaneous validity of Eq. (176) and Monod's equations for rod-shaped bacteria would appear to rule out ordinary transport and point instead to some more complicated mechanism.

Cohen and Monod (C2) have summarized experimental evidence which shows indeed that special mechanisms of transport of organic nutrients occur in bacteria. They call such transport systems *permeases*. This term ending in *-ase* implies that the system involves enzymes—an implication not yet proved by available data. At any rate, it is found that permease systems can lead to transport *against* an apparent rise in concentration, as well as other effects not possible with Fickian diffusion. Various hypothetical mechanisms for operation of permease systems yield rates of permeation which exhibit the Michaelis–Menten type of dependence on substrate (including water) concentration. Perhaps it is in the occurrence of one of these mechanisms that the rate equation [Eq. (38)] assumed by Monod and almost all subsequent workers finds its justification. [For further information on biological transport, see, e.g., Christensen (C1).]

For a spherical cell (coccus), one has

$$\sigma = 4\pi r^2$$

$$m = \tfrac{4}{3}\pi r^3 \rho$$

So that according to von Bertalanffy's equation, cell growth should follow the law

$$\frac{dr}{d\tau} = \frac{V_0}{\rho} - \frac{\mu_c}{3} r \tag{179}$$

When integrated, this equation yields a sigmoidal curve. The mass follows

$$\frac{dm}{d\tau} = V_0\left[(4\pi)^{1/3}\left(\frac{3}{\rho}\right)^{2/3}\right]m^{2/3} - \mu_c m \tag{180}$$

which also yields a sigmoidal curve on integration. In this case, there is a maximum possible cell size, given by

$$\lim_{\tau\to\infty} m = \frac{36\pi {V_0}^3}{\rho^2 {\mu_c}^3}$$

Thus, one sees from Eq. (180) that cultures of spherical bacteria should not follow Monod's equations.

Von Bertalanffy cites experimental data in support of his theory. For instance, he shows that the data of Bayne-Jones and Adolph (B3) on growth of single cells of *Bacillus megaterium* (rod-shaped) follow Eqs. (177) and (178), whereas their data (B3) on growth of the yeast *Saccharomyces cerevisiae* (roughly spherical) follow Eqs. (179) and (180). Other data cited are also in agreement with the model, as are more recent data, such as those of Schaechter *et al.* (S1) on growth of three rod-shaped bacteria (strains of *Escherichia coli*, *Proteus vulgaris*, and *Salmonella typhimurium*) and of Prescott (P5) on growth of an organism (*Amoeba proteus*) of irregular but more or less spherical shape. Prescott (P6) states that, with the exception of *Paramecium*, growth of single cells of protozoa is never "autocatalytic" (exponential). His view seems to be that this is a general conclusion, valid for all cells, since he suggests that speculations concerning "autocatalytic" growth of single cells should be stopped.

It must be mentioned that the foregoing treatment of linear growth of rod-shaped cells has assumed growth to be *intercalary*. That is, if we were to observe two points on the axis of the rod, we should observe that the distance between the points increases with time as growth lays down new material between them. (The relatively few experimental observations that have been made do not necessarily support that growth is completely intercalary.)

But a rod-shaped cell or a linear chain of rod-shaped cells can also exhibit another kind of linear growth—*apical*. In this mode of linear growth, cells or filaments of cells increase in length only at their apex; the distance between two adjacent points on the filament axis remains constant.

The experiments of Smith (S3), Stadler (S6), Zalokar (Z1), and Plomley (P2) have shown that the hyphae of various genera of fungi, both septate and nonseptate, exhibit apical linear growth rather than intercalary linear growth.

From these considerations, Emerson (E3) concluded that growth of a fungal culture in liquid medium ought to follow a different law than does growth of bacteria. In particular, he concluded that, for batch growth,

biomass concentration should rise as the cube of the time elapsed, rather than exponentially. Emerson's experimental data show that the third power law fits a larger portion of the growth curve than does the exponential law. The data of Marshall and Alexander (M5) on oxygen consumption by fungal cultures is also better fitted by a cubic law for growth. Neither set of experimental data is convincing, however.

Emerson's prediction of a third power growth law is based on the implicit assumption that the number density of fungal hyphae is spatially uniform and time invariant. Plomley's data (P2) for growth of *Chaetomium globosum* on agar plates are not in agreement with this assumption; they show that the number density of fungal hyphae is neither spatially uniform nor time invariant. Indeed, the *local* situation revealed by Plomley's experiments is strongly suggestive of the usual batch (exponential) growth situation. One still has to explain the data of Emerson (E3) and of Marshall and Alexander (M5), of course. The better "fit" of the third power law as contrasted with the exponential law might be due to oxygen limitation, a possibility supported by scrutiny of Emerson's data.

Again, mycologists have postulated (cf.H5) that the nongrowing central portions of fungal hyphae serve, in part at any rate, as food collectors for the growing apex. Zalokar's observations (Z1) on *cytoplasmic streaming*[30] indicate the essential correctness of this postulate. We suggest that studies of these transport phenomena might yield remarkable results to suitably talented chemical engineers.

VIII. Concluding Remarks

The foregoing review has started from the simplest considerations and proceeded to the analysis of more complex cases in its attempt to describe the dynamics of microbial cell populations. Some of the models discussed in the review are new; others have appeared in the literature but are not generally known by chemical or biochemical engineers. At any rate, treatment of these models is not to be found in any available treatise on biochemical engineering or elsewhere in the chemical engineering literature. Possibly the most important reason for this apparent lack of concern is the feeling that the models advanced are unrealistic and not useful.

The validity of the limited number of models discussed (i.e., whether or not they are realistic) must be tested by experimental work. Hopefully these mathematical descriptions will suggest experiments specifically designed to test their predictions. It is in this process that the principal *present* utility of

[30] Cytoplasmic streaming is the biologists' term for intracellular convection. Its mechanism is not understood. A literature survey is given by Kamiya (K1); see especially pp. 11–13 for streaming in fungal hyphae.

models resides; the experimentalist so engaged will be called upon to display considerable ingenuity and skill.

Appendix. Results from Probability Theory

We give here a résumé of the principal ideas of probability theory. The purpose is not to be comprehensive, but to summarize in a few paragraphs the concepts necessary for following the developments in the text, and to mention a few books in which extensive treatments of the theory are given.

In probability theory, we are concerned with propositions, or assertions about the occurrence of events. Generally speaking, these assertions will be conditional; that is, we assert that such and such is true if this and that are true. Some formal scheme of writing propositions is required, and we adopt the following: (A | B) means that "A is true if B is true." (Another way of putting this is to say that "A is true given B," or "A is true on data B.")

In many cases, we are interested in propositions concerning the occurrence of two or more events. Thus, let (AB | C) mean "both A and B are true, given that C is true," and let (A + B | C) mean "at least one of the events A or B is true if C is true." Finally, we denote the negation of a proposition by a small letter; e.g., (a | B) means "A is not true if B is true."

The probability of propositions is denoted by a capital P; viz., $P(\mathrm{A} \mid \mathrm{B})$ means "the probability that A is true if B is true," with similar meanings for $P(\mathrm{AB} \mid \mathrm{C})$, etc.

Probabilities of several propositions are related by the two fundamental rules given below. These rules may be treated as axioms (J2) or they may be derived from other axioms (C3), (J1). The rules are:

Rule 1:

$$P(\mathrm{AB} \mid \mathrm{C}) = P(\mathrm{A} \mid \mathrm{BC})\, P(\mathrm{B} \mid \mathrm{C}) \tag{A.1}$$
$$= P(\mathrm{A} \mid \mathrm{C})\, P(\mathrm{B} \mid \mathrm{AC})$$

Rule 2:

$$P(\mathrm{A} + \mathrm{B} \mid \mathrm{C}) = P(\mathrm{A} \mid \mathrm{C}) + P(\mathrm{B} \mid \mathrm{C}) - P(\mathrm{AB} \mid \mathrm{C}) \tag{A.2}$$

In addition, we have the *conventions*

Rule 3: Certainty is represented by a unit probability.

Rule 4: Impossibility is represented by a zero probability.

Propositions A and B are said to be *independent* on data C if

Rule 5:

$$P(\mathrm{AB} \mid \mathrm{C}) = P(\mathrm{A} \mid \mathrm{C})\, P(\mathrm{B} \mid \mathrm{C}) \tag{A.3}$$

or, by Rule 1, if

$$P(\mathrm{A}\,|\,\mathrm{BC}) = P(\mathrm{A}\,|\,\mathrm{C}) \qquad \text{and} \qquad P(\mathrm{B}\,|\,\mathrm{AC}) = P(\mathrm{B}\,|\,\mathrm{C})$$

Thus, if A and B are independent on data C, B is irrelevant to A given C, and A is irrelevant to B given C. Rule 5 is not generally true; a *reductio ad absurbdum* of Rule 5 when improperly applied is given by Jeffreys (J2).

Two propositions A and B are said to be *mutually exclusive* on data C if

$$P(\mathrm{AB}\,|\,\mathrm{C}) = 0$$

whence, from Rule 2,

$$P(\mathrm{A} + \mathrm{B}\,|\,\mathrm{C}) = P(\mathrm{A}\,|\,\mathrm{C}) + P(\mathrm{B}\,|\,\mathrm{C}) \tag{A.4}$$

In particular,

$$P(\mathrm{A} + \mathrm{a}\,|\,\mathrm{C}) = 1 = P(\mathrm{A}\,|\,\mathrm{C}) + P(\mathrm{a}\,|\,\mathrm{C}) \tag{A.5}$$

so that a proposition and its negation are mutually exclusive. Mutually exclusive propositions A and B are said to be *exhaustive* if $P(\mathrm{A} + \mathrm{B}\,|\,\mathrm{C}) = 1$; thus, A and a are exhaustive.

Bayes' theorem or the principle of inverse probability is deducible from Rule 1 and is

$$P(\mathrm{A}\,|\,\mathrm{BC}) = P(\mathrm{A}\,|\,\mathrm{C})\,\frac{P(\mathrm{B}\,|\,\mathrm{AC})}{P(\mathrm{B}\,|\,\mathrm{C})} \tag{A.6}$$

According to Jeffreys (J2), Bayes' theorem is the principal rule used in learning from experience. Thus, suppose our belief in the plausibility of A is $P(\mathrm{A}\,|\,\mathrm{C})$; this is called the *prior probability*. If we are given new evidence, B, then Bayes' theorem provides the rule whereby we reassess the plausibility of A; this leads to the *posterior probability*, $P(\mathrm{A}\,|\,\mathrm{BC})$.

Of course, somewhere along the line we have got to assign numerical values to probabilities such as $P(\mathrm{A}\,|\,\mathrm{C})$, $P(\mathrm{B}\,|\,\mathrm{C})$, etc. For this purpose, we need a *model* of the process of interest.

In certain cases, probabilities can be assigned by the so-called "principle of insufficient reason." Thus, suppose we are involved in the vice of coin tossing. Consider the two mutually exclusive propositions

$(\mathrm{A}\,|\,\mathrm{C})$: A head turns up

$(\mathrm{B}\,|\,\mathrm{C})$: A tail turns up

Here, C represents our model of the coin-tossing process; namely, that the coin has two faces only, so that we can get only a head or a tail. These are

mutually exclusive, and we have no reason to choose one over the other. By Rule 2,

$$P(\mathrm{A} + \mathrm{B} \mid \mathrm{C}) = P(\mathrm{A} \mid \mathrm{C}) + P(\mathrm{B} \mid \mathrm{C}) = 1$$

since $P(\mathrm{AB} \mid \mathrm{C}) = 0$ (impossibility is represented by a probability of zero). But we have insufficient reason to choose $(\mathrm{A} \mid \mathrm{C})$ over $(\mathrm{B} \mid \mathrm{C})$ or vice versa; therefore, $P(\mathrm{A} \mid \mathrm{C}) = P(\mathrm{B} \mid \mathrm{C})$, and both probabilities must equal $\frac{1}{2}$, by Rule 2. The same sort of reasoning can be employed for the assignment of probabilities in dice throwing, picking multicolored balls from urns, or in allotting energy levels to systems composed of one mole of an ideal gas. Notice that our model is not necessarily "right"; in the case of coin tossing, the coin might have two heads, or it might be biased in some manner of which we are not aware.

Many cases occur, however, in which the principle of insufficient reason is insufficient for the assignment of probabilities. That is, we are not able to reduce the problem to a statement of mutually exclusive, exhaustive, and equally likely propositions. As an example, consider the disintegration of a radioactive nucleus. We choose this example since it is similar to the processes mentioned in the text.[31]

Suppose we consider the proposition: "The nucleus will undergo fission in the time interval t to $t + \Delta t$." This is conditional, of course, since it presupposes that the nucleus has not undergone fission in earlier time intervals. As a model for calculating the probability of the proposition, adopt the following. The conditional probability will certainly depend on the duration of the time interval, Δt; it should be zero if Δt is zero. The probability should be continuous in Δt, so that the probability does not change by a finite amount in an infinitesimal interval of time. Moreover, the probability should be independent of absolute time t, since we have no reason to expect fission in one time interval to be more likely than fission in any other time interval. Hence, the required probability will be of the form $f(\Delta t)$, and Taylor series expansion about $\Delta t = 0$ subject to hypotheses stated gives

$$f(\Delta t) = k\,\Delta t + O[(\Delta t)^2]$$

where k is a positive constant, and O means "of the order of." The model does not tell us the numerical value of k; that could be estimated by experiment (if the model is "right") or calculated if we understood the processes involved in fission.

[31] As a matter of fact, the problem of radioactive decay is exactly the same as the problem of "washout" of particles from a perfectly mixed, continuous flow vessel. Thus, Eq. (124) of the text can be applied to yield the well-known law of radioactive decay.

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Nomenclature

$a, a_s, a_s', a_t, \ldots$	Stoichiometric coefficients
b, b'	Stoichiometric coefficients
C	Concentration of biomass
$\mathbf{C}$	Concentration of chemical species in environment (vector)
$C_G, C_D, C_s, C_z, C_i, \ldots$	Concentration of species denoted by subscript
D	D mass
E	Expected value
G	Generating function; G mass
h	Density of distribution of division times
I, I_0	Light intensity; incident light intensity
K	Kernel in Volterra's equation [Eq. (34)]
K, K_s, K_s', K_g, K_g'	Michaelis constants
$k_1, k_2, \ldots$	Rate constants in bottleneck model
l	Length of cell
M_r	rth moment of probability distribution
m	Mass of a cell
N	Population density; number of "genes" in Rahn's model
P	Probability; critical product; productivity [Eq. (110)]
Q	Volumetric flow rate
R	Substrate
R_c	Multiplication rate of a culture per unit volume
R_p	Growth rate of a culture per unit volume
R_s	Rate of production of substrate per unit volume
R_{resp}	Rate of respiration per unit volume
R_{photo}	Rate of photosynthesis per unit volume
r	Growth rate of a single cell; radius of a cell
r_c	Specific multiplication rate
r_p	Specific growth rate
S	Substrate
T	Inhibitor
t	Time
U	Nonnormalized density of age distribution
V	Volume of propagator; variance; viable biomass; quantity defined by Eq. (86)
V_0	Growth rate of a single cell, per unit area
W	Quantity defined by Eq. (86); nonnormalized density of mass distribution
X	Population size; random variable
x	Particular value of population size
Y	Apparent yield coefficient
Z	Organism

GREEK LETTERS

α	Defined by Eq. (51)
α_i	Stoichiometric coefficient for ith chemical species
β	Constant in Verhulst–Pearl law; defined by Eq. (51); stoichiometric coefficient
Γ	Defined by Eq. (64); probability of fission (per unit time) of a cell of age τ

Γ' Probability of fission (per unit time) of a cell of mass m
γ Defined by Eq. (51); specific rate of "gene" replication in Rahn's model
Δ Defined by Eq. (64)
δ Dirac's delta function
ε Extinction coefficient in Beer's law
ζ Initial value of C_z
η Defined by Eq. (86)
θ Holding time
λ Constant; defined by Eq. (51)
μ, μ' Maximum specific growth rates
μ_c Specific rate of endogenous metabolism
ν Constant in Eq. (35); specific rate of light reaction in photosynthesis
π Initial value of C_p
ξ Defined by Eq. (86)
ρ Initial value of C_R; density of cell
σ Initial value of C_s; surface area of cell
τ Defined by Eq. (88); age
χ Defined by Eq. (88)

SUBSCRIPTS

f Feed condition
0 Initial condition

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