THE GROWTH OF BACTERIAL CULTURES

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INTRODUCTION

The study of the growth of bacterial cultures does not constitute a specialized subject or branch of research: it is the basic method of Microbiology. It would be a foolish enterprise, and doomed to failure, to attempt reviewing briefly a "subject" which covers actually our whole discipline. Unless, of course, we considered the formal laws of growth for their own sake, an approach which has repeatedly proved sterile. In the present review we shall consider bacterial growth as a method for the study of bacterial physiology and biochemistry. More precisely, we shall concern ourselves with the quantitative aspects of the method, with the interpretation of quantitative data referring to bacterial growth. Furthermore, we shall consider exclusively the positive phases of growth, since the study of bacterial "death," i.e., of the negative phases of growth, involves distinct problems and methods. The discussion will be limited to populations considered genetically homogeneous. The problems of mutation and selection in growing cultures have been excellently dealt with in recent review articles by Delbrück (1) and Luria (2).

No attempt is made at reviewing the literature on a subject which, as we have just seen, is not really a subject at all. The papers and results quoted have been selected as illustrations of the points discussed.

DEFINITION OF GROWTH PHASES AND GROWTH CONSTANTS

DIVISION RATE AND GROWTH RATE

In all that follows, we shall define "cell concentration" as the number of individual cells per unit volume of a culture and "bacterial density" as the dry weight of cells per unit volume of a culture.

Consider a unit volume of a growing culture containing at time t_1 a certain number x_1 of cells. After a certain time has elapsed,

all the cells have divided once. The number of cells per unit volume (cell concentration) is then

$$x = x_1 \cdot 2;$$

after n divisions it will be

$$x = x_1 \cdot 2^n.$$

If r is the number of divisions per unit time, we have at time t_2:

$$x_2 = x_1 \cdot 2^{r(t_2 - t_1)}$$

or using logarithms to the base 2.

$$r = \frac{\log_2 x_2 - \log_2 x_1}{t_2 - t_1} \quad \cdots\cdots\cdots\cdots [1]$$

where r is the mean division rate in the time interval $t_2 - t_1$. In defining it we have considered the increase in cell concentration. When the average size of the cells does not change in the time interval considered, the increase in "bacterial density" is proportional to the increase in cell concentration. Whether growth is estimated in terms of one or the other variable, the growth rate is the same[1].

However, as established in particular by the classical studies of Henrici (3), the average size of the cells may vary considerably from one phase to another of a growth cycle. It follows that the two variables, cell concentration and bacterial density, are not equivalent. Much confusion has been created because this important distinction has been frequently overlooked. Actually, one or the other variable may be more significant, depending on the type of problem considered. In most of the experimental problems of bacterial chemistry, metabolism, and nutrition, the significant variable is bacterial density. Cell concentration is essential only in problems where division is actually concerned, or where a knowledge of the elementary composition of the populations is important (mutation, selection, etc.).

[1] The use of log base 2 in place of log base 10 simplifies all the calculations connected with growth rates. It is especially convenient for the graphical representation of growth curves. If $\log_2$ of the bacterial density ($\log_2 = 3.322 \log_{10}$) is plotted against time, an increase of one unit in ordinates corresponds to one division (or doubling). The number of divisions that have occurred during any time interval is given by the difference of the ordinates of the corresponding points. It is desirable that this practice should become generalized.

Although the two variables are not equivalent, it is convenient to express growth rates in the same units (i.e., number of doublings per hour) in both cases. When cell concentrations have been estimated, it is equivalent to the true division rate. When bacterial density is considered, it expresses the number of doublings of bacterial density per unit time, or the division rate of cells postulated to be of constant average size. In all that follows, unless specified, we shall consider growth and growth rates in terms of bacterial density.

These definitions involve the implicit assumption that in a growing culture all the bacteria are viable, i.e., capable of division or at least that only an insignificant fraction of the cells are not capable of giving rise to a clone. This appears to be a fairly good assumption, provided homogeneous populations only are considered. It has been challenged however [Wilson (4)] on the basis of comparisons of total and viable counts. But the cultures examined by Wilson were probably not homogeneous (see p. 378), and the value of the viable count in determining the "absolute" number of cells which should be considered viable under the conditions of the culture is necessarily doubtful (see p. 378). Direct observations by Kelly & Rahn (5) contradict these findings and justify the assumption. [See also Lemon (42) and Topley & Wilson (43).]

Growth Phases

In the growth of a bacterial culture, a succession of phases, characterized by variations of the growth rate, may be conveniently distinguished. This is a classical conception, but the different phases have not always been defined in the same way. The following definitions illustrated in Fig. 1 will be adopted here:

1. lag phase: growth rate null;
2. acceleration phase: growth rate increases;
3. exponential phase: growth rate constant;
4. retardation phase: growth rate decreases;
5. stationary phase: growth rate null;
6. phase of decline: growth rate negative.

This is a generalized and rather composite picture of the growth of a bacterial culture. Actually, any one or several of these phases may be absent. Under suitable conditions, the lag and acceleration

phases may often be suppressed (see p. 388). The retardation phase is frequently so short as to be imperceptible. The same is sometimes true of the stationary phase. Conversely, more complex growth cycles are not infrequently observed (see p. 389).
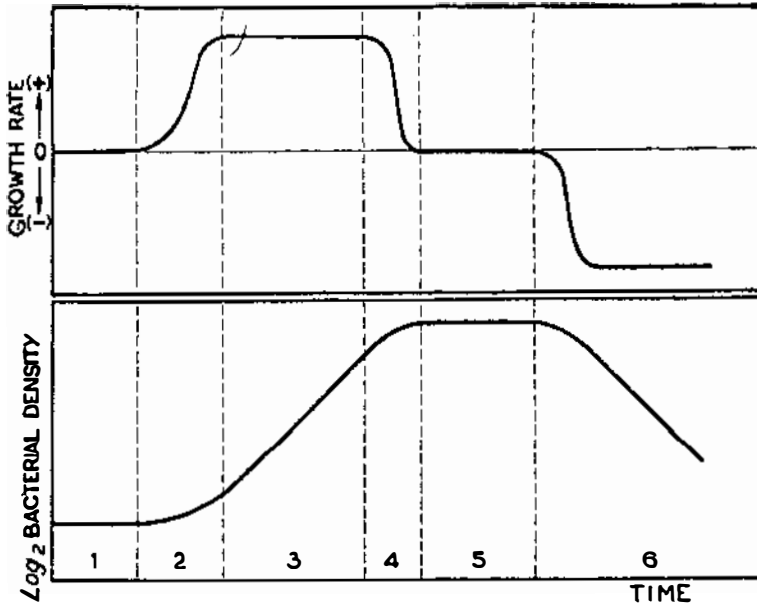

FIG. 1.—Phases of growth. Lower curve: log bacterial density. Upper curve: variations of growth rate. Vertical dotted lines mark the limits of phases. Figures refer to phases as defined in text (see p. 373).

GROWTH CONSTANTS

The growth of a bacterial culture can be largely, if not completely, characterized by three fundamental growth constants which we shall define as follows:

Total growth:[2] difference between initial (x_0) and maximum (x_{max}) bacterial density:

$$G = x_{max} - x_0.$$

Exponential growth rate: growth rate during the exponential phase (R). It is given by the expression

$$R = \frac{\log_2 x_2 - \log_2 x_2}{t_2 - t_1}$$

[2] "Croissance totale," Monod 1941.

when $t_2 - t_1$ is any time interval within the exponential phase.

Lag time and growth lag.—The lag is often defined as the duration of the lag phase proper. This definition is unsatisfactory for two reasons: (*a*) it does not take into account the duration of the acceleration phase; (*b*) due to the shape of the growth curve, it is difficult to determine the end of the lag phase with any precision.

As proposed by Lodge & Hinshelwood (6), a convenient lag constant which we shall call lag time (T_l) may be defined as the difference between the observed time (t_r) when the culture reaches a certain density (x_r) chosen within the exponential phase, and the "ideal" time at which the same density would have been reached (t_i) had the exponential growth rate prevailed from the start, i.e., had the culture grown without any lag $T_l = t_r - t_i$, or

$$T_l = t_r - \frac{\log_2 x_r - \log_2 x_0}{R}.$$

The constant thus defined is significant only when cultures having the same exponential growth rate are compared. A more general definition of a lag constant should be based on physiological rather than on absolute times. For this purpose, another constant which may be called growth lag (L) can be defined as

$$L = T_l \cdot R.$$

L is the difference in number of divisions between observed and ideal growth during the exponential phase. T_l and L values are conveniently determined graphically (Fig. 2).

ON TECHNIQUES

ESTIMATION OF GROWTH

Bearing these definitions in mind, a few general remarks may be made about the techniques employed for the estimation of bacterial density and cell concentrations.

Bacterial density.—For the estimation of bacterial density, the basic method is, by definition, the determination of the dry weights. However, as it is much too cumbersome (and accurate only if relatively large amounts of cells can be used) it is employed mainly as a check of other indirect methods.

Various indirect chemical methods have been used. Nitrogen determinations are generally found to check satisfactorily with

dry weights. When cultures are grown on media containing an ammonium salt as sole source of nitrogen, estimations of the decrease of free ammonia in the medium appear to give adequate results (7). Estimations of metabolic activity (oxygen consumption, acid production) may be convenient (8), but their use is obviously
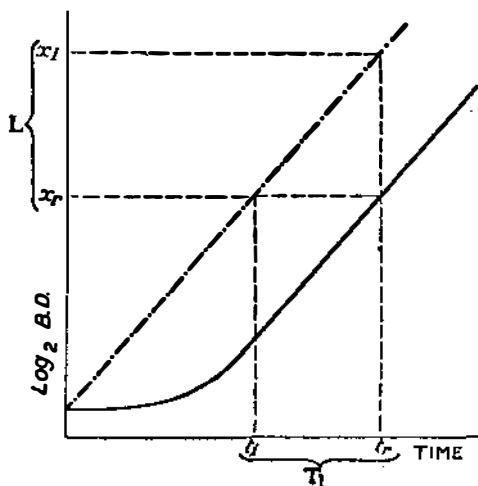

FIG. 2.—Lag time and growth lag. Solid line = observed growth. Dotted line = "ideal growth" (without lag). T_l = lag time. L = growth lag. (See text p. 375.)

very limited. Centrifugal techniques have been found of value (9).

The most widely used methods, by far, are based on determinations of transmitted or scattered light. (Actually, the introduction around 1935 of instruments fitted with photoelectric cells has contributed to a very large degree to the development of quantitative studies of bacterial growth.) We cannot go here into the physical aspects of this problem [for a discussion of these see (10)]. What should be noted is that in spite of the widespread use of the optical techniques, not enough efforts have been made to check them against direct estimations of cell concentrations or bacterial densities. Furthermore a variety of instruments, based on different principles, are in use. The readings of these instruments are often quoted without reference to direct estimations as arbitrary units of turbidity, the word being used in an undefined

sense, or as "galvanometer deflections" which is worse. This practice introduces no little confusion and indeterminacy in the interpretation and comparison of data. It should be avoided.

Whatever instruments are used, the readings should be checked against bacterial density or cell concentration determinations, and the checks should be performed not only on different dilutions of a bacterial suspension, but at various times during the growth of a control culture. Only thus will the effects of variations of size of the cells be controlled. Without such controls it is impossible to decide whether the readings can be interpreted in terms of bacterial density or cell concentration, or both, or neither.

Actually, the instruments best fitted for the purpose appear to be those which give readings in terms of optical density ($\log I_0/I$). With cultures well dispersed, it is generally found that optical density remains proportional to bacterial density throughout the positive phases of growth of the cultures (11). When this requirement is fulfilled, optical density determinations provide an adequate and extremely convenient method of estimating bacterial density.

It is often convenient to express optical density measurements in terms of cell concentrations. For this purpose, the two estimations should be compared during the exponential phase. The data, expressed as cell concentrations, may then be considered as referring to "standard cells," equal in size to the real bacteria observed during the exponential phase, larger than bacteria in the stationary phase and probably smaller than those in the acceleration phase.

Cell concentration.—Cell concentration determinations are performed either by direct counts (total counts) or by indirect (viable) counts. The value of the first method depends very much on technical details which cannot be discussed here. Its interpretation depends on the properties of the strains (and media) and is unequivocal only with organisms which do not tend to remain associated in chains or clumps. Total counts are evidently meaningless when there is even a slight tendency to clumping.

The same remarks apply to the indirect, so called viable, counts made by plating out suitable dilutions of the culture on solid media. The method has an additional difficulty, as it gives only the number of cells capable of giving rise to a colony on agar under conditions widely different from those prevailing in the culture. Many organisms, such as pneumococci (12), are extremely sensitive to

sudden changes in the composition of the medium. The mere absence of a carbon source will induce "flash lysis" of *Bacillus subtilis* (13). Such effects may be, in part at least, responsible for the discrepancies often found between total and viable counts.

In spite of these difficulties viable counts retain the undisputed privilege of being by far the most sensitive method and of alone permitting differential counting in the analysis of complex populations. In the latter case, relative numbers are generally the significant variable, and whether or not the counts give a reasonably accurate estimation of absolute cell concentrations is unimportant.

METHODS OF CULTURE

Although the methods of culture will vary according to the problems investigated, certain general requirements must be met in any case. The most important one is that the cultures should be constantly mixed, homogenized, and in equilibrium with the gas phase. This is achieved either by shaking or by bubbling air (or other gas mixtures) or both. Bubbling is often found inefficient unless very vigorous, when it may provoke foaming which should be avoided. Slow rocking of a thin layer of liquid is the simplest and probably the best procedure. [For detailed descriptions of techniques, see (14).]

Various techniques for the continuous renewal of the medium have been described (15) and should be found useful for certain types of experiments (see p. 385).

The composition of the medium is largely determined by the nature of the experiment, and the properties of the strains. One general rule should however, so far as possible, be followed in the planning of quantitative growth experiments. As a culture grows, the conditions in the medium alter in a largely uncontrollable and unknown way. Therefore, the observations should be performed while the departure from initial conditions may still be considered insignificant. The more dilute the cultures, the closer will this requirement be met. The sensitivity of optical density measurements makes it practicable to restrict most experiments to a range of bacterial densities not exceeding 0.25 mg. dry weight per ml.

THE PHYSIOLOGICAL SIGNIFICANCE OF THE GROWTH CONSTANT

TOTAL GROWTH

Limiting factors.—The metabolic activity of bacterial cells

modifies the composition of the medium in which they grow. Depending on the initial conditions, and on the properties of the strains, one or another, or several, of these changes will eventually result in a decrease of the growth rate, bringing the exponential phase to an end, and leading more or less rapidly to the complete cessation of growth.

The factors most commonly found to be limiting can, as a rule, be classified in one of the following groups: (a) exhaustion of nutrients; (b) accumulation of toxic metabolic products; and (c) changes in ion equilibrium, especially pH.

The physiological significance of the constant G (total growth) depends on the nature of the limiting factor. It is uninterpretable when the limiting factor is unknown, or when several factors cooperate in limiting growth. The conditions of an experiment where G is to be estimated must therefore be such that a single limiting factor is at work. This may be considered to be the case only where it can be shown that no change, other than the one considered, plays a significant part both in breaking the exponential phase and in stopping the growth. Provided these requirements are met, the utilization of G as a measure of the effect of a limiting factor is warranted.

Actually, the estimation of G is especially useful when the limiting factor is a single, known, essential nutrient. Under such conditions, it can be a most convenient tool for the study of many aspects of nutritional problems. The principles of this technique and some general results will be considered in the next paragraphs.

Nutrients as limiting factors.—The bacteria most commonly studied are chemoorganotrophs[3] requiring an organic compound as carbon and energy source, a hydrogen acceptor, inorganic ions, and carbon dioxide. Most of the parasitic (and many saprophytic) bacteria are chemoorganoheterotrophs requiring, in addition to the above diet, certain specific organic molecules (growth factors).

Any one of the essential nutritional requirements of an organism is, by definition, a potential limiting factor. With organisms able to grow on simple defined media (whether they are organoautotrophs or organoheterotrophs), the composition of a medium is easily adjusted so that the concentrations of all essential nutrients are in large excess compared to one of them, which then becomes the sole limiting factor, provided its concentration is kept

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[3] The Cold Spring Harbor Nomenclature (16) is adopted here.

low enough to eliminate interference from other potential limiting factors (pH changes, accumulation of metabolic products, etc.). Within the limits thus defined, the relation between G and the initial concentration (C) of the nutrient is, as a very general rule, found to be the simplest possible, namely, linear and to conform to the equation:

$$G = KC.$$

This relation implies that the amount of limiting nutrient used up in the formation of a unit quantity of cell substance is independent of the concentration of the nutrient. It implies also that growth stops only when the limiting nutrient is completely exhausted, or, in other words, that there is no threshold concentration below which growth is impossible (11).

Neither of these conclusions can be considered strictly true of course, and the linear relation cannot be taken for granted a priori. But it does seem to be a general approximation, and even a remarkably accurate one in many cases (Fig. 3 and Table I). Where
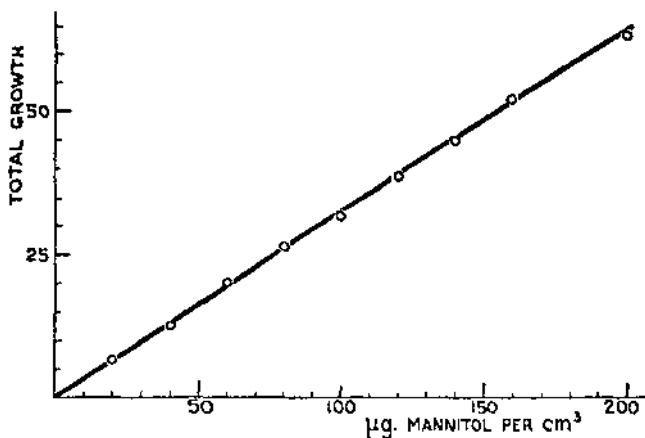

FIG. 3.—Total growth of *E. coli* in synthetic medium with organic source (mannitol) as limiting factor. Ordinates: arbitrary units. One unit is equivalent to 0.8 μg. dry weight per ml. (11).

it holds, the estimation of G affords a simple and direct measure of the growth yield (K) on the limiting nutrient, or

$$\frac{G}{C} = K = \frac{\text{amount of bacterial substance formed}}{\text{amount of limiting nutrient utilized}}.$$

When the proportion of the dry weight representing substance derived from the limiting nutrient is known, it is a measure of the fraction assimilated. If G is expressed as "standard" cell concentration, $1/K$ represents the amount of limiting nutrient used up in the formation of a "standard" cell. Thus, when determined under proper conditions, G is a constant of perfectly clear and fundamental significance; it is a measure of the efficiency of assimilatory processes.

TABLE I

Total growth of purple bacteria with acetate as
limiting factor [after Van Niel (9)]

Acetate (mg/ml.)	0.5	1.0	2.0	3.0
Total growth (mg/ml.)	0.18	0.36	0.70	1.12
K	0.36	0.36	0.35	0.37

Extensive data on G and K values are available only with respect to the organic source (9, 11). Little is known of K values in the case of inorganic sources. Owing to the development of microbiological assay methods, abundant data are available on the quantitative relations between growth of many bacteria and concentration of a variety of growth factors. But the major part of these data do not bear any known relation to G or any other definable growth constant, which is most unfortunate. It does seem at least probable that in many instances, the measurement of total growth, under conditions insuring homogeneity and limitation of growth by a single factor, could with advantage replace estimations of "turbidity at 16 hours," or "galvanometer deflections at 24 hours." It can be predicted with confidence that in most cases linear relations would be found [see e.g. (44)], permitting the estimation of K, and on which simpler and more reproducible methods of assay could be based. Furthermore, an intelligible and very valuable body of quantitative data on nutritional requirements of bacteria would thus become accumulated.

The remarkable degree of stability and reproducibility of K values, for a given strain and a given compound under similar

conditions, should be emphasized. Contrary to the other growth constants, it seems to be very little affected by hereditary variability (45).

In general, of the three main growth constants, total growth is the easiest to measure with accuracy and the most stable. Its interpretation is simple and straightforward, provided certain experimental requirements are met. These are remarkable properties, which could, it seems, be put to much wider use than has hitherto been done, especially with the focussing of attention on problems of assimilatory and synthetic metabolism.

EXPONENTIAL GROWTH RATE

The exponential phase as a steady state: rate determining steps.— The rate of growth of a bacterial culture represents the over-all velocity of the series of reactions by virtue of which cell substance is synthesized. Most, if not all, of these reactions are enzymatic, the majority probably are reversible, at least potentially. The rate of each, considered alone, depends on the concentrations of the reactants (metabolites) and on the amount of the catalyst (enzyme).

During the exponential phase, the growth rate is constant. It is reasonable to consider that a steady state is established, where the relative concentrations of all the metabolites and all the enzymes are constant. It is in fact the only phase of the growth cycle when the properties of the cells may be considered constant and can be described by a numeric value, the exponential growth rate, corresponding to the over-all velocity of the steady state system.

It has often been assumed that the over-all rate of a system of linked reactions may be governed by the slowest, or master, reaction. That this conception should be used, if at all, with extreme caution, has also been emphasized (17, 18). On theoretical grounds, it can be shown that the over-all rate of a system of several consecutive reversible enzymatic reactions depends on the rate and equilibrium constant of each. The reasons for this are obvious, and we need not go into the mathematics of the problem. A master reaction could take control only if its rate were very much slower than that of all the other reactions. Where hundreds, perhaps thousands, of reactions linked in a network rather than as a chain are concerned, as in the growth of bacterial cells, such a

situation is very improbable and, in general, the maximum growth rate should be expected to be controlled by a large number of different rate-determining steps. This makes it clear why exponential growth rate measurements constitute a general and sensitive physiologic test which can be used for the study of a wide variety of effects, while, on the other hand, quantitative interpretations are subject to severe limitations. Even where the condition or agent studied may reasonably be assumed to act primarily on a single rate determining step, the over-all effect (i.e., the growth rate) will generally remain an unknown function of the primary effect.

Although very improbable, it is of course not impossible that the exponential growth rate could in certain specific cases actually
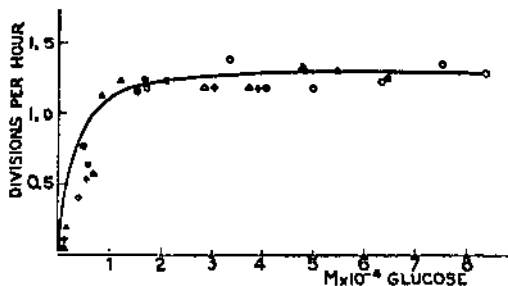

FIG. 4.—Growth rate of *E. coli* in synthetic medium as a function of glucose concentration. Solid line is drawn to equation (2) with $R_K = 1.35$ divisions per hour, and $C_1 = 0.22$ M $\times 10^{-4}$ (11). Temperature 37° C.

be determined by a single master reaction. But such a situation could hardly be assumed to prevail, in any one case, without direct experimental evidence. Some recent attempts at making use of the master reaction concept in the interpretation of bacterial growth rates are quite unconvincing in that respect (19).

Rate-concentration relations.—Notwithstanding these difficulties, relatively simple empirical laws are found to express conveniently the relation between exponential growth rate and concentration of an essential nutrient. Examples are provided in Figs. 4 and 5. Several mathematically different formulations could be made to fit the data. But it is both convenient and logical to adopt a hyperbolic equation:

$$R = R_K \frac{C}{C_1 + C} \quad \dots \dots \dots \dots \dots \dots \dots [2]$$

similar to an adsorption isotherm or to the Michaelis equation. In the above equation C stands for the concentration of the nutrient. R_K is the rate limit for increasing concentrations of· C. C_1 is the concentration of nutrient at which the rate is half the maximum.

The constant R_K is useful in comparing efficiency in a series of related compounds as the source of an essential nutrient. So far extensive data are available only with respect to the organic source (11). The value of R_K may vary widely when different
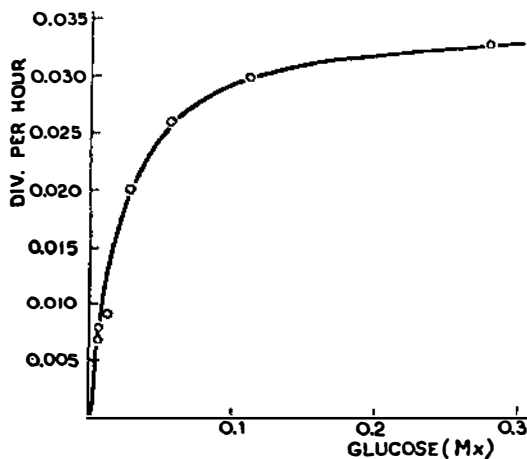

FIG. 5.—Growth rate of *M. tuberculosis* in Dubos' medium, as a function of glucose concentration. Solid line drawn to equation (2) with $R_K = 0.037$ and $C_1 = M/45$ (20).

organic sources are compared under otherwise identical conditions. There is no doubt that it is related to the activity of the specific enzyme systems involved in the breakdown of the different compounds, and it can be used with advantage for the detection of specific changes (e.g., hereditary variation) affecting one or another of these systems (30).

The value of C_1 should similarly be expected to bear some more or less distant relation to the apparent dissociation constant of the enzyme involved in the first step of the breakdown of a given compound. Furthermore, since a change of conditions affecting primarily the velocity of only one rate-determining step will, in general (but not necessarily), be only partially reflected in the

over-all rate, one might expect C_1 values to be lower than the corresponding values of the Michaelis constant of the enzyme catalysing the reaction. This may explain why C_1 is often so small, compared to the concentrations required for visible growth, that its value may be difficult to determine, and the exponential growth rate appears practically independent of C (19).

It may be of interest to note that in a few instances exceptionally large values of C_1 have been obtained. For instance for *Mycobacterium tuberculosis*, on Dubos' medium, the value of C_1 for glucose is $M/45$, i.e., some 1,000 times its value for *Escherichia coli*. Whether this is due to a very low affinity of an enzyme or whether it reflects a peculiar permeability property of the membrane of this organism is not known (20).

Growth rate determinations as a null point method.—Although the growth rate is an unknown function of a large number of variables, quantitative comparisons of the effects of conditions or agents affecting it through the same rate-determining reaction (or system of reactions) are possible (at least in principle) by using growth rate measurements as a null point method, that is to say by determining the equivalent conditions at which a certain, conveniently chosen, value of R obtains. This general method is susceptible of many applications, especially in the study of antagonistic effects. Here reliable and sensitive methods for distinguishing between various types of antagonistic effects, and determining the relative activities of different antagonists, are needed. Theoretically the most sensitive comparisons should be afforded by determining, at various absolute concentrations, the ratios of inhibitor and antagonist at which a given per cent decrease of R (over uninhibited controls) occurs.

Although this may not always prove practicable, there is little doubt that growth rate measurements do yield data, not only more accurate, but essentially more informative, than "turbidity at 16 hours" or "galvanometer deflections at 72 hours." The studies of McIllwain on the pantoyl taurine-pantothenate antagonism (8) adequately illustrate this point. They clearly show, in particular, the importance of distinguishing between effects on growth rate and on total growth [see also (21 to 24)].

Linear growth.—Since we are discussing the interpretation of exponential growth rates, it may be worthwhile to consider the case when growth is linear with time, although, to the reviewer's

knowledge, this has been clearly observed only once (25), actually during the residual growth of a streptomycin-requiring *B. subtilis* in a medium containing no streptomycin (Fig. 6). The interpretation is obvious, albeit surprising. Growth must be limited by one enzyme or system of enzymes, the activity of which is constant. In other words, in the absence of streptomycin, one rate-determining enzyme ceases to be formed, so that by being outgrown by the
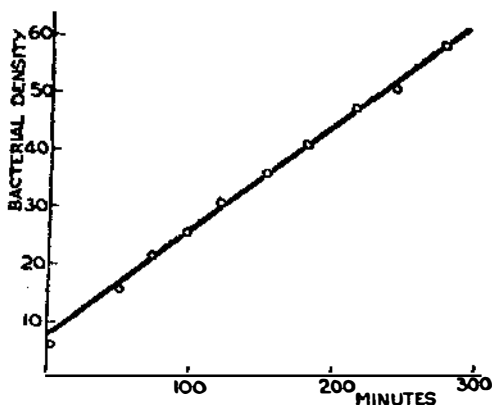

FIG. 6.—Residual growth of a streptomycin requiring strain of *Bacillus subtilis* in the absence of streptomycin. Growth is linear for over 4 hr. (25).

other enzymes, it eventually achieves true mastery and sets the system at its own constant pace, disregarding the most fundamental law of growth.

Similar systems could be artificially set up by establishing a constant, limited supply of an essential metabolite (using an organism incapable of synthesizing it), while all other nutrients would be in excess. Such a technique should prove useful for certain studies of metabolism (see p. 378).

LAG TIME

Types of lag.—The lag and acceleration phases correspond to the gradual building up of a steady state. The growth lag (L) may be considered a measure of the physiological distance between the initial and the steady state. Depending on the specific conditions and properties of the organism, one or several or a large number of reactions may determine the rate of this building up

process. Furthermore each rate-determining reaction may be affected in either or both of two ways: (a) change in the amount and activity of the catalyst; (b) change in the concentration of the reactants (metabolites).

When the phenomenon is associated with the previous ageing of the cells of the inoculum, the chances are that it involves at once a large number of reactions, and specific interpretations are impossible. Furthermore an apparent lag may be caused if a large fraction of the incoulated cells are not viable (18). When, however, the lag can be shown to be controlled primarily by only one reaction, or system of reactions, the measurement of lag times becomes a useful tool for the study of this reaction. This may often be achieved by a careful preconditioning of the inoculated cells, and appropriate choice of media [see e.g. (26)]. In point of fact this technique amounts to artificially creating conditions where one or a few rate limiting steps become true master reactions, at least during the early stages of the lag.

Theoretically, the lagging of a reaction may be due either to insufficient supply of a metabolite or to the state of inactivity of the enzyme. In the first case, the technique may be used for the study of certain essential metabolites synthesized by the cell itself during growth, and consequently difficult to detect and identify otherwise. Few examples of this sort are available besides the glutamine effects studied by McIlwain et al. (27) and the detection of metabolites able to replace carbon dioxide (26), but it is probable that the method could be developed.

In the second case, the technique may be useful in the study of enzyme activation or formation. The magnesium effects described by Lodge & Hinshelwood (28) and the sulfhydryl effects described by Morel (29) should probably be attributed to the reactivation of certain enzymes or group of enzymes. However, lag effects are especially interesting in connection with the study of enzymatic adaptation.

Lag and enzymatic adaptation.—Enzymatic adaptation is defined as the formation of a specific enzyme under the influence of its substrate (30). If cells are transferred into a medium containing, as sole source of an essential nutrient, a compound which was not present in the previous medium, growth will be impossible unless and until an enzyme system capable of handling the new substrate is developed. If other potential factors of lag are elimi-

nated, the determination of lag times becomes a means of studying
the adaptive properties of the enzyme system involved (Fig. 7).

The technique has proved especially useful for the study of
adaptive enzymes attacking organic compounds serving as sole
organic source (11, 31). The work of Pollock (32) shows that it
can also be applied in the case of adaptive systems specific for
certain hydrogen acceptors (nitrate and tetrathionate). A further
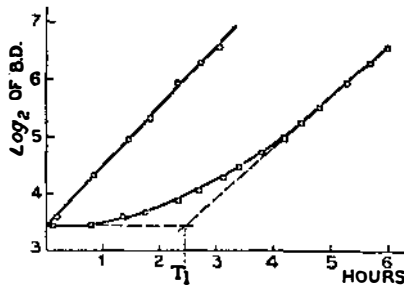development of the technique is suggested by the work of Stanier

FIG. 7.—Growth of *E. coli* in synthetic medium with glucose (circles) and
xylose (squares) as organic source. Culture previously maintained on arabinose
medium, temperature 37° C. Growth on glucose proceeds without any lag. Lag
time (T_l) on xylose is approximatively 2.5 hours (46).

(33) and Cohen (34) on the possibility of identifying metabolic
pathways through a systematic study of cross adaptation.

In general, lag-time measurements may be especially useful in
the detection and preliminary identification of adaptive effects,
but they could not, of course, replace more direct methods of
estimating enzymatic activities.

A broader approach to the problem of relations between lag
and enzymatic adaptation should also be considered. As empha-
sized by Hinshelwood (18), the lag and acceleration phases repre-
sent essentially a process of equilibration, the functioning of a
regulatory mechanism, by virtue of which a certain enzyme
balance inside the cells is attained. That such a mechanism must
exist is obvious, since in its absence, the cells could not survive
even slight variations of the external environment. However, the
nature of the postulated mechanisms is still completely obscure.
The kinetic speculations of Hinshelwood, although interesting as
empirical formulations of the problem, do not throw any light on,

the nature of the basic mechanisms involved in the regulation of enzyme formation by the cells.

The most promising hypothesis for the time being appears to be that this regulation is insured through the same mechanism as the formation of adaptive enzymes, which implies the assumption that all the enzymes in a cell are more or less adaptive. The competitive effects observed in enzymatic adaptation (11, 35, 36) agree with the view that the regulation may be the result of a continuous process of selection of mutually interacting enzymes or enzyme-forming systems (30, 37). The kinetics of bacterial growth and, in particular, the lag and acceleration phases certainly constitute the best available material for the study of this fundamental problem.

Division lag.—The largest discrepancies between increase in bacterial density and increase in cell concentration are generally observed during the lag and acceleration phases. This phenomenon has been the subject of much confused discussion (38). Actually, it has been demonstrated by Hershey (39, 40) that a definite lag in cell concentration may occur even when there is no detectable lag in bacterial density. This must mean that cell division mechanisms may be partially inhibited under conditions which do not affect the growth rate and general metabolism of the cell. A number of interesting observations by Hinshelwood *et al.* (18) point to the same conclusion. Further studies on the phenomenon are desirable, as they should throw some light on the factors of cell division in bacteria.

THE INTERPRETATION OF COMPLEX GROWTH CYCLES

Multiple exponential phases.—In many cases, the growth cycle does not conform to the conventional scheme represented in Fig. 1. The interpretation of these complex growth cycles will be briefly discussed here.

One of the most frequently encountered exceptions is the presence of several successive exponential phases, characterized by different values of R and separated by angular transition points. This should in general be interpreted as indicating the addition or removal of one or more rate-determining steps in the steady state system. This type of effect may result from a change in the composition of the medium, for instance from the exhaustion of a compound partially covering an essential nutritional requirement

(34), or from the transitory accumulation of a metabolite, which will eventually serve as a secondary nutritional source (41).

Interpretations are more delicate, and more interesting, when the cause is a change in the composition of the cells themselves. Such effects are frequently encountered with various bacteriostatic agents and have been discussed at length by Hinshelwood (18). But the deliberate confusion entertained by this author between selective and adaptive mechanisms has obscured, rather than clarified, the interpretation of these effects.
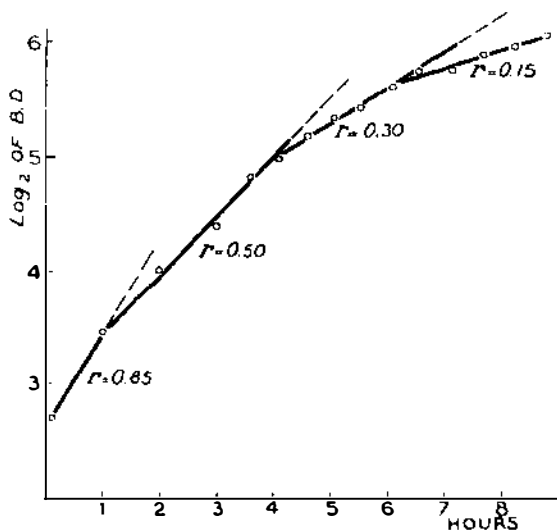

FIG. 8.—Growth of *E. coli* in synthetic medium under suboptimal partial pressure of carbon dioxide (3×10^{-5}). r = growth rate.

In some cases, the phenomenon can be reasonably ascribed to the exhaustion of a reserve metabolite in the cells. An interesting example is afforded by the growth of coli under suboptimal partial pressures of carbon dioxide (26). As seen in Fig. 8 as much as three or four exponential phases can be clearly distinguished suggesting the successive exhaustion of several reserve metabolites, each independently synthesized with the participation of carbon dioxide, a conclusion which is borne out by other lines of evidence.

Diauxie.—This phenomenon is characterized by a double growth cycle consisting of two exponential phases separated by a phase during which the growth rate passes through a minimum,

even becoming negative in some cases. It is found to occur in media where the organic source is the limiting factor and is constituted of certain mixtures of two carbohydrates. The evidence indicates that each cycle corresponds to the exclusive utilization of one of the constituents of the mixture, due to an inhibitory effect of one of the compounds on the formation of the enzyme attacking the other (Fig. 9). This striking phenomenon thus reveals the existence
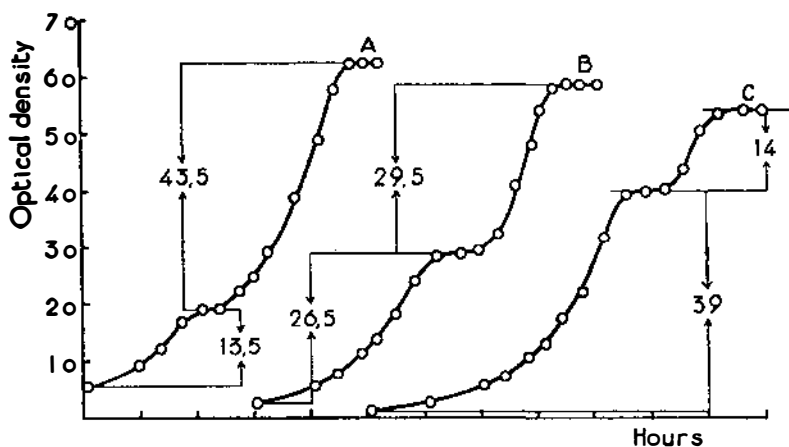
FIG. 9.—Diauxie. Growth of *E. coli* in synthetic medium with glucose+sorbitol as carbon source.

The figures between arrows indicate total growth corresponding to each cycle.

    (a) Glucose 50 μg. per ml.; sorbitol 150 μg. per ml.

    (b) Glucose 100 μg. per ml.; sorbitol 100 μg. per ml.

    (c) Glucose 150 μg. per ml.; sorbitol 50 μg. per ml.

Total growth corresponding to first cycle is proportional to glucose concentration. Total growth of second cycle is proportional to sorbitol concentration (11).

of interactions between closely related compounds in the formation of specific enzymes and has proved valuable in the study of certain aspects of enzymatic adaptations (11, 30, 35). It may perhaps be susceptible of certain technical applications, e.g., for the quantitative analysis of certain mixtures of carbohydrates.

CONCLUDING REMARKS

The time-honored method of looking at a tube, shaking it, and looking again before writing down a + or a 0 in the lab-book has led to many a great discovery. Its gradual replacement by

determinations of "turbidity at 16 hours" testifies to technical progress, primarily in the manufacturing and advertising of photelectric instruments. This technique however is not, properly speaking, quantitative, since the quantity measured is not defined. It might be a rate, or a yield, or a combination of both.

In any case, this technique does not take advantage of the fact that the growth of bacterial cultures, despite the immense complexity of the phenomena to which it testifies, generally obeys relatively simple laws, which make it possible to define certain quantitative characteristics of the growth cycle, essentially the three growth constants: total growth (G), exponential growth rate (R), and growth lag (L). That these definitions are not purely arbitrary and do correspond to physiologically distinct elements of the growth cycle is shown by the fact that, under appropriately chosen conditions, the value of any one of the three constants may change widely without the other two being significantly altered. The accuracy, the ease, the reproducibility of bacterial growth constant determinations is remarkable and probably unparallelled, so far as biological quantitative characteristics are concerned.

The general physiological significance of each of the growth constants is clear, provided certain experimental requirements are met. Under certain specific conditions, quantitative interpretations in terms of the primary effect of the agent studied may even be possible. The fallacy of considering certain naive mechanistic schemes, however, as appropriate interpretations of unknown, complex phenomena should be avoided.

There is little doubt that, as further advances are made towards a more integrated picture of cell physiology, the determination of growth constants should and will have a much greater place in the experimental arsenal of microbiology.

LITERATURE CITED

1. DELBRÜCK, M., *Ann. Missouri Botan. Garden*, **32**, 223–33 (1945)
2. LURIA, S. E., *Bact. Revs.*, **11**, 1–40 (1947)
3. HENRICI, A. T., *Morphologic Variation and the Rate of Growth of Bacteria*, 194 pp. (C. C Thomas, Springfield, Ill., 1928)
4. WILSON, G. S., *J. Bact.*, **7**, 405 (1922)
5. KELLY, C. D., AND RAHN, O., *J. Bact.*, **23**, 147 (1932)
6. LODGE, R. M., AND HINSHELWOOD, C. N., *J. Chem. Soc.*, 213–219 (1943)
7. FISHER, K. C., AND ARMSTRONG, F. H., *J. Gen. Physiol.*, **30**, 263 (1947)
8. MCILWAIN, H., *Biochem. J.*, **38**, 97–105 (1944)
9. VAN NIEL, C. B., *Bact. Revs.*, **8**, 1–118 (1944)
10. DOGNON, A., in *Techniques de laboratoire*, 197–210 (Masson & Cie, Paris, 1947)
11. MONOD, J., *Recherches sur la croissance des cultures bactériennes*, 211 pp. (Hermann & Cie, Paris, 1942)
12. DUBOS, R. J., *J. Exptl. Med.*, **65**, 873–83 (1937)
13. MONOD, J., *Ann. inst. Pasteur*, **68**, 444 (1942)
14. MONOD, J., *Ann. inst. Pasteur* (In press)
15. JORDAN, R. C., AND JACOBS, S. E., *J. Bact.*, **48**, 579 (1944)
16. LWOFF, A., VAN NIEL, C. B., RYAN, F. J., AND TATUM, E. L., *Cold Spring Harbor Symposia Quant. Biol.*, **11**, 302–3 (1946)
17. BURTON, A. C., *J. Cellular Comp. Physiol.*, **9**, 1 (1936)
18. HINSHELWOOD, C. N., *The Chemical Kinetics of the Bacterial Cell*, 284 pp. (Clarendon Press, Oxford, 1946)
19. JOHNSON, F. H., AND LEWIN, I., *J. Cellular Comp. Physiol.*, **28**, 47 (1946)
20. SCHAEFER, W., *Ann. inst. Pasteur*, **74**, 458–63 (1948)
21. MCILWAIN, H., *Biol. Revs.*, **19**, 135 (1944)
22. MCILWAIN, H., *Advances in Enzymol.*, **7**, 409–60 (1947)
23. WYSS, O., *Proc. Soc. Exptl. Biol. Med.*, **48**, 122 (1941)
24. KOHN, H. I., AND HARRIS, J. S., *J. Pharmacol. Exptl. Therap.*, **73**, 343 (1941)
25. SCHAEFFER, P., *Compt. rend.*, **228**, 277–79 (1949)
26. LWOFF, A., AND MONOD, J., *Ann. inst. Pasteur*, **73**, 323 (1947)
27. MCILWAIN, H., FILDES, P., GLADSTONE, G. P., AND KNIGHT, B. C. J. G., *Biochem. J.*, **33**, 223 (1939)
28. LODGE, R. M., AND HINSHELWOOD, C. N., *J. Chem. Soc.*, 1692–97 (1939)
29. MOREL, M., *Ann. inst. Pasteur*, **67**, 449 (1941)
30. MONOD, J., *Growth*, **11**, 223–89 (1947)
31. MONOD, J., *Ann. inst. Pasteur*, **69**, 179 (1943)
32. POLLOCK, M. R., AND WAINWRIGHT, S. D., *Brit. J. Exptl. Path.*, **29**, 223–40 (1948)
33. STANIER, R. Y., *J. Bact.*, **54**, 339 (1947)
34. COHEN, S. S., *J. Biol. Chem.*, **177**, 607–19 (1949)
35. MONOD, J., *Ann. inst. Pasteur*, **71**, 37 (1945)
36. SPIEGELMAN, S., AND DUNN, R., *J. Gen. Physiol.*, **31**, 153–73 (1947)
37. SPIEGELMAN, S., *Cold Spring Harbor Symposia Quant. Biol.*, **11**, 256–77 (1946)
38. WINSLOW, C. E., AND WALKER, H. H., *Bact. Revs.*, **3**, 147–86 (1939)
39. HERSHEY, A. D., *J. Bact.*, **37**, 290 (1939)

40. HERSHEY, A. D., *Proc. Soc. Exptl. Biol. Med.*, **38,** 127–28 (1938)
41. LWOFF, A., *Cold Spring Harbor Symposia Quant. Biol.*, **11,** 139–55 (1946)
42. LEMON, C. G., *J. Hyg.*, **33,** 495 (1937)
43. TOPLEY, W. W. C., AND WILSON, G. S., *Principles of Bacteriology and Immunity*, 3rd Ed., 2054 pp. (Williams & Wilkins, 1946)
44. LWOFF, A., QUERIDO, A., AND LATASTE, C., *Compt. rend. soc. biol.*, **130,** 1580 (1939)
45. MONOD, J. (Unpublished data)
46. MONOD, J. (Unpublished data)