LIFE PHASES IN A BACTERIAL CULTURE

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Several important contributions to our knowledge of the numbers of bacteria present in a culture medium at certain stages in the development of the culture have been made recently, but with the exception of the work of Slator (1917), apparently there has been no effort to coordinate these results and develop a complete mathematical theory of such changes in numbers. The present paper is an attempt to analyze the results of these authors and to present certain phases which have apparently been neglected heretofore.

When bacteria, particularly cells from an old culture, are inoculated into a suitable culture medium, as broth, the bacteria will at first remain unchanged in numbers; then multiplication begins, the numbers increase at first slowly, then more rapidly until a certain minimum average generation time is reached; this after a time begins to increase, and there is a negative acceleration in growth, which finally ceases; the numbers remain constant for a time, then the bacteria begin to die off.

Lane-Claypon[1] recognized four periods or phases in the life of a bacterial culture as follows:

1. Initial period of slow growth or even no growth.
2. Period of regular growth.
3. Period during which numbers remain more or less stationary.
4. Period during which the numbers of living bacteria are decreasing.

It would seem, however, that the life phases are somewhat more complex than indicated by the preceding statement. A study of the results secured by various authors indicates that seven relatively distinct periods may be differentiated. These may be recognized easily by plotting the logarithms of the numbers of bacteria against time. Chart 1 is such a plot with the seven phases indicated. It will be noted that points designating the beginning and end of each phase are points where a curve changes to a straight line and vice versa.

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[1] Jour. Hyg., 1909, 9, p. 239.

These various growth phases may be designated as follows:

1. *Initial Stationary Phase.*—During this phase the number of bacteria remains constant, and the plot is a straight line parallel to the x axis indicated by 1 — a.

2. *Lag Phase, or Positive Growth Acceleration Phase.*—During this phase the average rate of increase in numbers per organism increases with the time, giving rise to the curve a — b. This increase in rate of growth per organism does not continue indefinitely but only to a certain point determined by the average minimal generation time per organism under the conditions of the test.

3. *Logarithmic Growth Phase.*—During this phase the rate of increase per organism remains constant, in other words, the minimal average generation time is maintained throughout the period. This gives rise to the straight line b — c.

4. *Phase of Negative Growth Acceleration.*—During this phase the rate of growth per organism decreases, that is, the average generation time is increased.

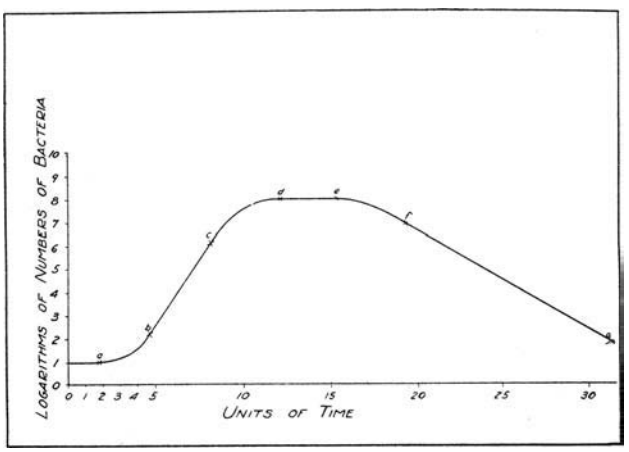

Chart 1.—Diagrammatic plot of logarithms of numbers of bacteria present in a culture.

The bacteria continue to increase in numbers, but less rapidly than during the logarithmic growth phase. This is the curve c — d.

5. *Maximum Stationary Phase.*—During this period there is practically no increase in the numbers of bacteria. The plot gives a straight line d — e parallel to the x axis. The rate of increase per organism is zero and the average generation time infinity.

6. *Phase of Accelerated Death.*—During this phase the numbers of bacteria are decreasing, slowly at first and with increasing rapidity, until the establishment of a logarithmic death rate. In the terminology used in the growth phases, the average "rate of death per organism" is increasing to a certain maximum. This gives the curve e — f.

7. *Logarithmic Death Phase.*—During this phase the "rate of death per organism" remains constant, the plot of the logarithms gives a straight line with a negative slope. This is represented by f — g.

If death and life in the bacterial cell could be regarded as reversible processes, we might expect the appearance of an eighth phase, a negative acceleration of the death rate.

It may be noted that the seven phases previously defined are in a sense arbitrary. The curve, if it could be plotted with data absolutely accurate, would probably be smooth; in other words, the portions designated as straight lines are probably curves, but with curvature so slight that they may be treated mathematically as straight lines without the introduction of any error commensurate in value with the inevitable experimental errors.

These various life phases of the bacterial culture will be discussed in some detail.

I. INITIAL STATIONARY PHASE

Spore-producing bacteria exhibit this growth phase particularly well. If a suspension of bacterial spores be placed in a suitable culture medium microscopic observation will show that growth does not apparently begin immediately. There can be no increase in numbers until the spores have germinated and begun to multiply. Samples of equal volume taken during this period show no increase in numbers. While this stage is most prominent with sporulating organisms it is by no means always absent in nonspore formers, as is shown by results of Lane-Claypon,[1] Penfold,[2] and others. In other words, there is evidence that in old cultures of many bacteria the cells are in a relatively dormant stage, the physiologic equivalent of sporulation though without the spore morphology. When such cells are planted in a suitable medium there will be an appreciable interval before a single cell will have resumed growth sufficiently to divide. During this phase the rate of increase per cell would be zero, and the average generation time infinity. The equation of the curve which represents this phase would be

$$b = B$$

where b = Number of bacteria after time t,

B = Initial number of bacteria.

Little work has been done on this phase. The conditions which determine its length are probably those influencing the length of other phases.

It should be noted that some cultures will not show this phase at all. If there are any actively dividing bacteria in the inoculum at the time of inoculation it will be absent or very transitory. It is therefore probable that the phase of the culture from which the transfer is made will affect the length of this phase.

[2] Jour. Hyg., 1914, 14, p. 215.

II. LAG PHASE, OR PHASE OF POSITIVE GROWTH ACCELERATION

This phase apparently has not been differentiated from the preceding by previous writers. This is illustrated by the definition of latent period given by Chesney:[3] "By latent period or lag is meant the interval which elapses between the time of seeding and the time at which maximum rate of growth begins." The necessity for differentiation of the two phases is not urgent except when the first is long continued.

The lag phase may be defined as that period elapsing between the beginning of multiplication and the beginning of the maximum rate of increase per organism.

The phenomenon of bacterial lag was apparently first noted by Müller,[4] and was later studied by Rahn[5] and by Coplans.[6] Penfold[2] gave the first adequate discussion of the various theories which might be suggested to explain the phenomenon. Chesney[3] later made a careful study of the lag phase with special reference to the growth of the pneumococcus. A mathematical analysis of the lag phase was given by Penfold and Ledingham[7] and elaborated by Slator.[8]

Theories of Bacterial Lag.—Penfold[2] has enumerated some nine different theories as to the cause of bacterial lag, all of which he discards as inadequate. Inasmuch as certain of these have been maintained by other writers, and perhaps some discarded hastily, they will be briefly summarized and reasons for discarding given under the following seven heads.

1. The organism must excrete some essential substance into the medium before maximal growth can occur. Experiments show, however, that subcultures taken from cultures showing maximal growth do not show any lag period.

2. Adaptation to a new medium requires time. This must be discarded, inasmuch as transfers to the same medium may show lag.

3. Some of the bacteria transferred are not viable, and die off early. Inasmuch as enumeration is by plating and not by direct counting, the organisms not viable would never be enumerated.

4. Bacteria may agglutinate and plating would then be an enumeration of clumps and not of individual bacteria. While this may be a factor in some cases, it cannot explain the lag which still persists when adequate precautions against confusion from this source have been taken.

[3] Jour. Exper. Med., 1916, 24, p. 387.
[4] Ztschr. f. Hyg. u. Infektionskr., 1895, 20, p. 245.
[5] Centralbl. f. Bakteriol., 1906, Abt. 2, 16.
[6] Jour. of Path. and Bacteriol., 1909, 14, pp. 1-27.
[7] Jour. Hyg., 1914, 14, p. 242.
[8] Ibid., 1917, 16, p. 100.

5. Accumulated products of metabolism may injure the bacterial cell, the length of the lag phase is the time required to recover from the injury. Penfold rejects this explanation as inadequate. Chesney, however, insists that lag is "an expression of injury which the bacterial cell has sustained from its previous environment." This conception may well be an approximation of the truth, though probably not entirely accurate.

6. "The inoculum consists of organisms having individually different powers of growth, and during the lag the selection of the quick growing strain occurs in response to some selecting agent in the peptone." It is possible that this might occur in cultures which were not "pure lines," or which contained several strains, but there is no proof of its occurrence in pure strains. While there are undoubtedly some differences in the rates of multiplication of individual bacteria in the same culture, they are insufficient to account for the great differences characteristic of the lag period.

7. Bacteria must overcome an "inertia" before reaching maximal growth rate. Penfold dismisses this on the basis of certain experiments in which he chilled rapidly growing bacteria, and thus stopped multiplication, which was resumed at its former rate when the optimum temperature was restored. While it is probable that Penfold is correct in discarding inertia in this sense as a factor, nevertheless a modification of this theory is in the opinion of the writer the only adequate one suggested.

The explanation favored by Penfold is in fact a variant of the last. He believes that certain essential constituents of the bacterial protoplasm, probably synthesized in steps, must be present in the bacterial cell in optimum concentration or at least the intermediate bodies of the steps of the synthesis. When the bacteria cease growing these intermediate bodies diffuse from the cell and disappear, and before maximal growth can begin in a new medium these bodies must again be synthesized. This theory in effect holds that the loss of these substances gives rise to inertia. During the lag phase the bacteria are gradually recovering from injury.

It is probable that none of the preceding are wholly satisfactory explanations of the lag phase. An explanation more in accord with observed facts may be found in the assumption by the bacterial cells of a "rest period" comparable to the resting stages so often assumed by higher forms. It is a well known fact that at certain stages in the life history of many plants certain cells or tissues are developed which pass into a resting stage. When these are morphologically well differentiated they are termed spores, sclerotia, etc., in the lower forms, and seeds, bulbs, tubers, etc., among the higher types. In many other cases cells or tissues pass into a similar resting stage as a result of certain environmental influences, without showing marked morphologic differentiation. These resting cells are usually aroused to renewed growth and activity only as the result of certain stimuli. The cold of winter followed by the warmth of spring may be the stimulus which causes buds to develop. Some seeds will germinate only after the seed coat has decayed or has been scratched or corroded by acid. Bacterial spores form at certain stages in the life history of the bacteria, but do

not usually germinate in the parent culture in spite of abundant moisture, food and optimum temperature. Germination takes place under the stimulus of change to some new medium. It is altogether probable that most bacteria, whether spore producers or not, enter into such a resting stage. When not morphologically differentiated as a spore this resting period is probably more transitory than in a spore, but it is nevertheless just as real.

What happens, then, when a considerable number of bacteria in the resting stage are transferred to a medium suitable for development? If we were to examine the culture microscopically we would find that the bacteria would not all begin development at once, probably for the same reason that seeds placed under uniform favorable conditions for growth do not all germinate at the same instant. Cell division will occur in a few cells first, followed by larger and larger numbers at succeeding intervals of time until a maximum has been reached and passed, and at last all the cells have "germinated." As soon as a cell has actually germinated, there would seem to be no a priori reason why the cells should not thereafter multiply rapidly, showing practically at once a minimum generation time. There is no more reason to suppose that the length of time it takes a bacterial cell to germinate will affect its subsequent rate of growth than to assume that plants derived from seeds slow in sprouting grow more slowly than those from seeds soonest sprouted. After any cell had once "germinated" then, it would proceed to increase in numbers in geometrical progression. Theoretically the lag period would continue until the last viable cell had started to multiply; practically, however, it ceases before this as the rapid increase in the bacteria which have germinated soon makes the ungerminated cells such a small fraction of the whole number that their inclusion is within the limits of error of measurement of the numbers present.

MATHEMATICAL ANALYSIS OF THE LAG PERIOD

The lag period has been previously defined as that period during which there is an increase in the average rate of multiplication of the bacteria, an increase from zero to some constant which is maintained during the succeeding period. Another statement is that it includes the period during which there is a decrease in the average generation time. It should be noted that when used in this sense, the term generation time means the time required for the bacteria to double in numbers, if they continued growing at the same rate. At any given instant during this period there will be some cells not multiplying at all, these at that particular instant would have an infinite generation time, and the term average generation time would cease to have any meaning. In other words, during this period there is an acceleration in the rate of growth.

Let us first examine the equation of growth if the rate of increase per organism should remain constant, that is, the average generation time should not vary. Let

b = number of bacteria after time t,
B = initial number of bacteria.

It is evident that the rate of increase in number of bacteria at any instant will vary directly as the number of bacteria, or expressed in terms of the calculus:

$db/dt = kb$ where k is a constant. Now

$$\frac{db/dt}{b} = k$$

Therefore k is the rate of growth per organism, or the velocity coefficient of growth. On integration this becomes,

$$\ln b = kt + \text{constant of integration.}$$

The constant of integration is found to be $\ln B$ by taking $t = 0$. The equation then becomes

$$\ln b/B = kt$$

This may be interpreted as the equation of a straight line, hence when $\ln b$ is plotted against t, a straight line with slope k will be secured.

The curve showing the number of bacteria after any time may be derived from the above equation

$$\ln b/B = kt$$
$$b/B = e^{kt}$$
and $$b = Be^{kt}$$

This equation may be derived without resort to the calculus as follows:

Let n = number of generations in time t
g = generation time.

At the end of time t one organism will have produced 2^n bacteria, then

$$b = B2^n$$
Now $$n = t/g$$
$$b = B\,2^{t/g}$$
Let $$2^{1/g} = e^k$$
then $$b = Be^{kt}$$
and $$k = \ln 2/g$$

Since the rate of growth varies inversely as the generation time, k may be regarded as this rate of growth per organism, or the velocity coefficient.

Inasmuch as rate of growth per organism is a function of time, it is a matter of interest to determine just what the relationship may be existing between them. Penfold and Slator have suggested relationships empirically determined from experimental data. Apparently there has been no attempt to derive the relationship from theoretical considerations.

Assume that the lag phase represents the time required for all the viable bacteria planted to "germinate." Take as the time of "germination" the instant that the cell first divides to form two individuals. It is assumed that as soon as an organism begins dividing its rate of increase is at once constant. Let this be k'.

Let w = number of bacteria that are dividing after time t, = progeny of all bacteria that have germinated within time t

z = number of bacteria that have not germinated.

The rate of increase per organism [f(t)] at any instant is given by the following equation:

$$f(t) = \frac{k'w}{w+z} = k'.\ 1/(1+z/w) \qquad (1)$$

It is apparent that if the numbers of bacteria "germinating" during each unit of time are plotted against time, a curve may be secured resting on the x axis at both ends, one of the forms of a probability curve. The general equation for such a curve has been shown by Karl Pearson to be

$$y = c(1+x/a_1)^{m_1}(1-x/a_2)^{m_2}$$

in which m_1 and m_2 are constants, c equals the maximum ordinate, and $-a_1$ and a_2 are intercepts of curve with x axis.

Let y be the number of bacteria germinating at time t, then

$$y = c(1+t/a_1)^{m_1}(1-t/a_2)^{m_2}$$

and $a_1 + a_2$ is the total length of the lag period.

The total number of bacteria which will germinate in time dt is ydt. Since the number of bacteria developing after time t from one organism is $2^{t/g}$, those which will develop after time t from those beginning growth during time dt is $y2^{t/g}dt$, and the total number of bacteria developed after time t from those starting growth within that time is

$$\int_{-a_1}^{t} y2^{t/g}dt$$

Therefore $$w = \int_{-a_1}^{t} c(1+t/a_1)^{m_1}(1-t/a_2)^{m_2}\ 2^{t/g}dt$$

The total number of bacteria which "germinate" within time t is

$$\int_{-a_1}^{t} ydt$$

The total number not germinated is

$$B - \int_{-a_1}^{t} ydt$$

$$z = B - \int_{-a_1}^{t} ydt$$

The relationship probably existing between rate of growth per organism and t may be shown by substituting the values secured for w and z in the equation (1).

$$f(t) = k' \frac{1}{1 + \dfrac{B - \displaystyle\int_{-a_1}^{t} c(1+t/a_1)^{m_1}(1-t/a_2)^{m_2}dt}{\displaystyle\int_{-a_1}^{t} c(1+t/a_1)^{m_1}(1-t/a_2)^{m_2}\ 2^{t/g}dt}}$$

All efforts to simplify this expression or put it into usable form have thus far failed. The only points where the exact relationship is known are when $t = 0$,

$f(t) = 0$, and when $t = a_1 + a_2$, $f(t) = k'$. It is evident that the relationship existing between rate of growth per organism and t during the lag period is quite complex.

The problem may also be attacked by the empirical derivation of a formula for a plotted curve by a critical examination of the data of the lag phase. This has been done by Ledingham and Penfold. These authors first reduced all figures to a seeding of 1, that is, the numbers of bacteria found at successive stages of the lag phase were divided by the initial number of bacteria. The logarithms of these numbers were plotted against the logarithms of times. This gave a curve which appeared to be logarithmic. The logarithms of the logarithms of the numbers of bacteria were then plotted against the logarithms of the times. These points were found to lie approximately on a straight line. If n is the slope of this line, and c the intercept with the x axis, the equation of the line is

$$n = \frac{\log (\log b)}{(\log t) - c} \qquad (2)$$

From this they derive the equation

$$t^n = k \log b$$

$$\text{Since} \quad \ln b = \ln 10 . \log b$$
$$\ln b = \ln 10 . t^n/k = k't^n$$

$$\text{where} \quad \frac{\ln 10}{k} = k'$$

Therefore $b = e^{k't^n}$ and for a seeding of B bacteria $b = Be^{k't^n}$
It is evident that this equation and the equation for regular growth

$$b = Be^{kt}$$

are special forms of the equation

$$b = Be^{\mu kt}$$

in which $\mu = f(t)$. In the equation for constant rate of growth per organism, $\mu = 1$, and in the Ledingham-Penfold equation $\mu = t^{n-1}$, and in the equation of initial stationary phase $\mu = 0$ and $b = B$. The equation developed,

$$b = Be^{k't^n}$$

has two constants which must be evaluated for each particular experiment. An equation of this general form was tested out by Ledingham and Penfold (1914) on data from eight series of experiments, and was found to give remarkably consistent results. The value of n in these experiments varied from 1.56 to 2.7 six being below 2.0. The value of k in the equation

$$t^n = k \log b/B,$$

varied from 2329 to 1,045,000.

The tables given by Chesney for increase of bacteria during the lag period afford an opportunity for testing independently the validity of the Ledingham-Penfold equation, or its generalized form.

Slator after a study of the data of Penfold (1914) concluded that in every experiment recorded there existed a relationship between the two constants n and k such that an equation could be derived in which there would appear

but one undetermined constant n. He found by examination that the following relationship always held:

$$\frac{\log k/n}{n} = \text{constant} = 2.024$$

$$k = 105.7^n$$

Substituting the value for k in the Ledingham-Penfold equation

$$t^n = k \log b/B$$
$$t^n = n\ 105.7^n \log b/B = 105.7^n \log b^n/B^n$$

Slator uses the general form of equation

$$kt^n = \log b/B$$

This becomes $(.00945)^n t^n = \log b^n/B^n$.

While the equation as developed holds for the work of Penfold, Slator generalizes into the form

$$C^n t^n = \log b^n/B^n$$

in which C might have some value other than .00945. This can be put into the form of the equations

$$b^n = B^n 10^{C^n t^n}$$
$$\text{or} \quad b^n = B^n e^{k^n t^n}$$

The advantage of Slator's generalized equation over that of Ledingham and Penfold, at least for the lag period, is not apparent.

MEASUREMENT OF LAG

A numerical expression indicating the amount of lag may be secured in either of two principal ways: (*a*) An expression may be secured which will involve directly the length of the lag period, this may be termed "period of lag measurement;" (*b*) an expression may be secured which will give a numerical value to the degree of depression of rate of multiplication at any time during the progress of the lag period. This may be termed the "time index of lag."

(*a*) Period of Lag Measurement: Three suggestions have been made as to methods of measuring lag in terms involving the length of the lag period. These have been defined by Penfold.

1. The actual length of the lag period may be measured.

2. Coplans (1909) measured the restraint of growth in terms of minimum generation time. It may be expressed by the formula

$$\frac{t - ng}{g}$$

where t = length of lag period

n = number of generations during lag period.

g = minimum generation time.

3. The average generation time for the first part of the period may be compared with that of any succeeding period.

(*b*) Time Index of Lag: The degree or amount of lag at any instant during the lag period may have a numerical value assigned to it in either of two ways; the ratio of the generation time at any instant to the minimal generation time characteristic of the logarithmic period of increase may be determined, or, the rate of change or increase per organism at any given instant during the lag period may be compared with the similar rate of increase per organism during the logarithmic period. Inasmuch as the rate of growth must vary inversely as the generation time, it is evident that these two methods of expressing results will have a constant ratio.

1. *Measurement of Lag by Comparison of Generation Times.*—The problem is to secure the ratio of the generation time of the bacteria at any time during the lag phase to the minimal generation time. It should be recalled that the term generation time as used here is not a time average, but that length of time required for the bacteria present to double in number if the average rate increase per individual remained constant.

It was earlier developed that the expression

$$b = B\ 2^{t/g}$$

represents the equation of growth if the rate of increase per individual remains constant. Differentiating and solving for g,

$$g = b\ \ln\ 2\ dt/db \qquad (1)$$

The value of dt/db may be determined for the lag phase by differentiation of either of the equations

$$b = Be^{kt^n} \qquad (2)$$

$$\text{or}\quad b^n = B^n e^{k^n t^n} \qquad (3)$$

Differentiating (2)

$$db/b = knt^{n-1}dt$$

$$dt/db = 1/bknt^{n-1} \qquad (4)$$

Substituting the value of dt/db in (1)

$$g = \frac{b\ \ln\ 2}{bknt^{n-1}} = \ln\ 2/knt^{n-1}$$

The ratio between the value of generation time as determined by this formula during the period of lag and the minimum value of g as determined during the logarithmic period gives a numerical index to the degree of lag at any instant.

If the equation

$$b^n = B^n e^{k^n t^n}$$

be chosen as the more general for the lag period (as developed from the work of Slator), the expression for generation time becomes:

$$g = \ln\ 2/k^n t^{n-1}$$

2. Measurement of Lag by Comparisons of Rates of Increase Per Cell.—The work of Slator suggests the possibility of measuring lag at any instant during the lag phase by a comparison of the rates of increase per cell with similar rates for the logarithmic period.

This may be determined from either lag phase equation

$$b = Be^{kt^n}$$
$$\text{or} \quad b^n = B^n e^{k^n t^n}$$

Differentiate

$$db/dt = bknt^{n-1}$$

The rate of increase per organism at any instant is therefore

$$\frac{db/dt}{b} = knt^{n-1}$$

The corresponding rate of increase per organism during the logarithmic period is

$$\frac{db/dt}{b} = k'$$

The ratio knt^{n-1}/k' gives the numerical index desired.

If the second equation of the lag phase be employed the ratio becomes

$$k^n t^{n-1}/k'$$

It may be noted that the so-called "constant of growth" during the lag period, the expression $\frac{db/dt}{b}$, used by Slator and termed z is directly proportional to the μ of the equation

$$b = Be^{\mu kt}$$

III. THE LOGARITHMIC PHASE

The logarithmic phase of bacterial growth in a culture is that time during which there is a maximum rate of growth per organism, that is, the time during which a certain minimum generation time is maintained. The various relationships which define this period have for the most part been developed in the discussion of the lag phase. They are as follows:

If B = number of bacteria at beginning of logarithmic period,
b = number of bacteria after time t,
n = number of generations in time t,
g = generation time,
k = velocity coefficient of growth,

$$b = B\,2^n = B\,2^{t/g} = Be^{kt}$$

$$g = \frac{t \ln 2}{\ln b - \ln B}$$

$$n = \frac{\ln b - \ln B}{\ln 2}$$

$$k = 1/t.\ \ln.\ b/B$$

This phase of bacterial growth has perhaps been more investigated than any other. The mathematical relationships during this period are comparatively simple. It is evident that any effect of change of environmental conditions on the rate of increase of bacteria will be manifested through a change in the generation time. For every variable in the environment there is of course an optimum for each kind of organism, that is, a condition or concentration such that the generation time is minimal.

There is need for careful mathematical study of the effect of temperature changes, changes in concentration of nutrients, of hydrogen ions, of inhibiting substances, etc., on the rate of growth. It will be noted that the equation

$$k = 1/t \cdot \ln \cdot b/B$$

is one form of the expression for the value of the velocity coefficient of a monomolecular reaction. It has been shown that a similar (not identical) expression holds for the logarithmic death period of bacteria. Will the following expression

$$k = 1/tC^n \cdot \ln b/B$$

hold where C is the concentration of some nutrient or inhibiting substance, and n a constant?

The temperature coefficient per degree or per 10 degree rise in temperature is in need of study, particularly near the minimum and maximum growth temperatures. This temperature coefficient over certain ranges has been determined for some bacteria. Lane-Claypon gives the value per 10° and 35° as 2 to 3 with B. coli. Similar results were secured from 20° to 30° by Hehewerth (1901) and Barber (1908).

IV. PHASE OF NEGATIVE ACCELERATION OF GROWTH

It is a matter of common laboratory observation that bacteria do not long maintain their maximum rate of growth, the logarithmic phase does not usually persist more than a few hours in quick growing types of bacteria. The average generation time apparently lengthens until at the close of the period the bacteria are no longer dividing.

The general equation of this portion can be written, as for the preceding phases

$$b = Be^{\mu_k t}$$

During this phase the μ varies as some function of the time, from the ^{l} of the logarithmic period to 0. Apparently the exact relationship between μ and t during this phase has not been studied. It is apparent that as t increases μ must decrease, but a mathematical characterization has not been successful. The reasons for the decreased rate of growth per organism are complex. Among them may be enumerated the following:

1. The average rate of growth per cell will decrease with the increase in concentration of the injurious products of metabolism.

2. The average rate of growth per organism will decrease with decrease in the available food supply, or with some single limiting factor of this food supply.

3. As the period progresses a larger and larger proportion of the cells go into the "resting stage" and are withdrawn from those dividing or multiplying.

4. It is probable that before this period is completed some cells die.

Slator has suggested that the curve might be described by

$$b^n = Bne^{akn^t n}$$

where n, k and a are constants suitably adjusted. Until further data are accumulated an attempt to evaluate these constants will prove difficult. From analogy with preceding and succeeding equations, it is possible that the growth equation of this phase might be

$$b = Bek^{t-n} \text{ and } \mu = t^{-n-1}$$

V. The Maximum Stationary Phase

During this period there is theoretically no change in the total number of bacteria present. If we still employ the useful general expression

$$b = Bc^{\mu kt}$$

μ during this time remains zero, and the number of bacteria is constant.

Persistence of this phase must mean the balancing of increase and death. The rate of increase of bacteria must be such as to quite exactly make good the loss from death.

Investigations as to the length of this phase, and the influence of environment upon it are needed. With some organisms the phase is very transitory if it can be said truly to occur at all, with other forms apparently it persists for some time before there is marked any tendency to decrease in numbers.

VI. Phase of Accelerated Death Rate

Sooner or later the number of bacteria which die in a unit time will exceed the increase. In other words, as soon as bacteria reach the "resting stage" we may assume that they begin to die off, but they do not all reach this stage at the same instant. For some time there is an acceleration in the rate of death. The μk of the equation

$$b = Be^{\mu kt}$$

varies from zero to the velocity coefficient (constant) of the logarithmic death period. It also becomes negative in sign. It increases numerically in value during this period as time increases. During this stage the curve apparently is just the reverse of that of the lag period.

It is not improbable that the equation of the curve during this period will be found to be

$$b = Be^{-k't^n}$$

When $t = 0$, $b = B$. As t increases, b will be found to decrease more and more rapidly. Data are not at hand to prove the reliability of this equation. This stage probably does not persist long in most cultures, the velocity coefficient of death soon reaching a certain maximum.

VII. LOGARITHMIC DEATH PHASE

It was first shown by Madsen and Nyman and later by Chick that when bacteria are subjected to the action of unfavorable environment such as the presence of disinfectants they die off in accordance with the law which governs monomolecular reactions. If the logarithms of the numbers of surviving bacteria after various lengths of time are plotted against time, they will be found to lie on a straight line. The slope of this line is negative. This slope is the velocity coefficient of the reaction.

$$-k = 1/t \cdot \ln b/B$$
$$\text{or} \quad k = 1/t \cdot \ln B/b$$

The equation of the curve of the surviving bacteria is

$$b = Be^{-kt}$$
$$\text{or} \quad B = be^{kt}$$

This behavior of the bacteria has been abundantly verified by experimentation. It has been found to be of great service in the evaluation of disinfectants.

The effect of concentration of disinfectants has been developed principally by the work of Paul, Bierstein and Reuss, and of Chick[9] and the results generalized by Phelps. It is found that a change in the concentration of a particular disinfectant will change the velocity coefficient of the death rate in accordance with the following relationship:

$$k = k'C^n$$

where k' is the velocity coefficient of the original, and k the velocity coefficient with new concentration C, and n is a constant. For a different concentration the equation then becomes,

$$k' = \frac{1}{C^n t} \ln \frac{B}{b}$$

and the equation of the curve of surviving bacteria becomes

$$b = Be^{-kC^n t}$$

[9] Jour. Hyg., 1912, 12, p. 414.

Determination of the values of k and n for a disinfectant and a comparison of these values with those determined for some standard, as phenol, constitute efficient characterization of the disinfectant.

The Rideal-Walker and the Hygienic Laboratory phenol coefficients of disinfectants are determined by the use of facts inherent in these formulae. If the same concentration of two disinfectants are to be compared, we may place the same number, B of bacteria per unit of solution in each, and determine the length of time it takes to reduce the number of living bacteria to less than one per loop. Under these conditions the time required to change b to a certain number b′ is determined. The only undefined quantities left are t and k in the equations

$$b' = Be^{-k't'}$$
$$b' = Be^{-k''t''}$$
$$\therefore \quad k't' = k''t''$$
$$\text{or} \quad k'/k'' = t''/t'$$

that is, the velocity coefficients are inversely proportional to the times required for "disinfection." By determining variations in the values of these ratios with different concentrations one may approximate the values of n in the equation

$$b = Be^{-kC^n t}$$

If, in addition, the effect of heat be determined in accelerating the death rate of the bacteria, a relatively complete diagnosis of the characteristics of the disinfectant is at hand.

SUMMARY

1. There are at least seven life phases during the development of a culture of bacteria.

2. The general equation which represents the curve of the plot of numbers of bacteria against time is $b = Be^{\mu kt}$.

3. During the first or initial stationary stage μ is equal to zero, b is equal to B and there is no change in the numbers of bacteria.

4. During the second or lag phase μ is a function of time, increasing with time from 0 to 1. The relationship between μ and time is complex, but it is approximated by the equation

$$\mu = t^{n-1}$$

and the growth curve equation becomes

$$b = Bekt^n$$

5. During the third or logarithmic growth phase $\mu = 1$ and the equation becomes

$$b = Be^{kt}$$

where k is related to the minimum generation time as follows:

$$k = \frac{\ln 2}{g}$$

and the equation of the growth curve is

$$b = Be^{kt} = Be^{(t \ln 2)/g}$$

6. During the fourth period or phase of negative growth acceleration μ decreases from the 1 of the logarithmic period of growth to 0. It is a function of time, decreasing with time. The relationship is complex, and no satisfactory evaluation of μ in terms of constants and time has been secured. It is possible the equation of growth may assume the form

$$b = Bekt^{-n} \text{ and } \mu = t^{-n-1}$$

7. During the fifth period or maximum stationary phase μ remains equal to 0 and b equals B.

8. During the sixth period or phase of accelerated death rate μ varies from 0 to — 1. From analogy with the lag phase, the equation of growth during this phase may be

$$b = Be^{-kt^n}, \text{ and } \mu = -t^{n-1}$$

9. During the seventh period or phase of logarithmic decrease μ remains constant at -1, the growth curve having the equation

$$b = Be^{-kt}$$

10. The lag phase is interpreted as the time during which bacteria are gradually emerging from a resting stage. It is not improbable that the numbers of bacteria which emerge at various successive periods of time are distributed in accordance with some probability curve.