FACTORS LIMITING BACTERIAL GROWTH

VI. Equations Describing the Early Periods of Increase

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By experiments involving direct measurements of size and growth rate of *Bacterium coli* in nutrient broth (1–3), evidence has been obtained for the following assumptions regarding bacterial growth:

1. Measured as increase of bacterial substance, growth occurs at constant rate under conditions we wish to consider.[1]

2. There is a latent period (L) during which the average cell undergoes no multiplication, but steadily increases in size to a maximum, the maximal average adult size (S_m). This size is characteristic of the species, but is affected by influences similar to those affecting the rate of growth.

3. At the end of the latent period, the average cell abruptly divides into two equal daughter cells, which in turn increase in size to the same maximal value before undergoing fission to produce the second generation. After the latent period is passed, therefore, the size changes $S_m/2 \rightleftarrows S_m$ prevail, and the rate of multiplication is numerically equal to the rate of accretion.

4. The individuals of a culture do not behave exactly as the average cell behaves (chiefly because of differences in initial size), but exhibit properties distributed symmetrically about the mean.[2] Thus the first division of the average cell is preceded and followed by an equal number of first divisions of other cells, fewer in number as they occur further in advance or arrears of the mean. As a result the rhythm of divisions of the majority is soon masked by the divisions of precocious and backward cells. Since the culture now contains cells distributed at random in all stages of fission, the average observed size (Sa) will be three-fourths of the average

[1] For *Bacterium coli*, nutrient broth cultures containing 1 to approximately 10^8 organisms per ml., with carbon dioxide available, and oxygen in excess.

[2] Or, not improbably, skewed. But the accuracy of the data to be considered does not warrant making this distinction.

maximal adult size, until altered by changing environmental circumstances.

Only the first of these assumptions is entirely new. Regarding the third, the evidence is contradictory. Jensen (4) noted that once a given cell had divided, it continued to do so for a time at a constant rate. Others (5, 6), on the contrary, have observed the successive shortening of generation time during early cell divisions which the concept of lag (if applicable to a single cell) implies. However, microscopic observations are almost necessarily limited to cultures on solid mediums, where significant changes in environmental conditions conceivably take place from generation to generation. If only for this reason, it appears justifiable to consider by themselves the results obtained with the present methods.

In what follows we are seeking more rigorous quantitative test of the assumptions outlined above, than was provided by the underlying experiments.

During the latent period, if the number of cells be considered constant (B_0), the increase in average size from S_0 to S_a occurs at constant rate. Then:

$$\frac{dS}{dt} = KS$$

Integrating between the limits S_0 and S_a,

$$t_L = \frac{1}{K} \ln \frac{S_a}{S_0}, \qquad (1)$$

where

t_L = net time in hours occupied by growth in size.
K = fractional increase in size per hour.
S_a = average adult size.
S_0 = average initial size.

After all the cells have divided, the average size S_a remains constant, and the cell numbers increase at constant rate K. Therefore:

$$\frac{dB}{dt} = KB$$

And on integration,

$$t_n = \frac{1}{K} \ln \frac{B}{B_0}, \qquad (2)$$

where

t_n = net time occupied by increase in numbers.
K = fractional increase in numbers per hour.
B = final numbers of cells.
B_0 = initial numbers.

But

$$t_L + t_n = \text{total elapsed time } t.$$

Substituting (1) and (2) into the above:

$$\frac{1}{K}\ln\frac{S_a}{S_0} + \frac{1}{K}\ln\frac{B}{B_0} = t,$$

and

$$\ln B = \ln B_0 + Kt - \ln\frac{S_a}{S_0}.$$

But,

$$K = M \ln 2.^{3}$$

Then

$$\log B = \log B_0 + Mt \log 2 - \log S_a/S_0. \quad (3)$$

Equation (3) predicts bacterial numbers expected at any time after the end of the latent period. Under given conditions of cultivation, M and S_a are constant and B varies only with B_0, t, and S_0.

The value of t_L (equation 1) is not measurable experimentally. But an expression for the latent period (L) representing the time required for growth in size from S_0 to S_m, is derived in a similar way. Thus:

$$L = \frac{1}{K}\ln\frac{S_m}{S_0}$$

Since, from postulate (4), $S_m = 4/3\, S_a$:

$$L = \frac{1}{M \log 2}\log\frac{4S_a}{3S_0} \quad (4)$$

[3] $$M = \frac{K}{2.3 \log 2} = \frac{1}{t}\log\frac{B}{B_0}$$

where M is the reciprocal of the generation time as given by the equation of Buchner (7). M is defined, accordingly, as doublings per hour, or as generations per hour where growth proceeds by binary fission.

Equation (4) refers to the latent period of the average individual cell. For experimental purposes, therefore, L may be defined as the time required for 50 per cent increase in numbers, if it be assumed that the distribution of times of first division of individual cells is normal. Under given conditions of cultivation, L should vary only with S_0.

TABLE I

Observed and Calculated Latent Period

$$\text{Equation (4)}: L = \frac{1}{M \log 2} \log \frac{4S_a}{3S_0}$$

	Source culture	Temperature	Log* bacterial Nos. (log B_0)	Rate of accretion (M)	Initial size (S_0)	Average adult size (S_a)	L† obs.	L‡ obs.	L calc. equation (4)
		°C.	per ml.	doublings/hrs.	$\times 10^{-7}$ mm.³/cell hr.	$\times 10^{-7}$ mm.³/cell hr.	hrs.	hrs.	hrs.
1	*Bact. dysenteriae*§	37.0	3.99	2.5‖	0.85	13.2‖	1.77	1.78	1.76
2	" " ¶	"	3.74	"	2.10	"	1.20	1.37	1.23
3	*Bact. coli***	"	5.92	3.1‖	15.1	16.1‖	0.19	0.19	0.16
4	" " §	"	3.72	"	0.85‖	"	1.50‖	1.51‖	1.51
5	" " ††	"	3.62	"	2.4‖	"	0.99‖	1.02‖	1.02
6	" "	32.5	4.15	2.4	"	15.8	1.32	1.32	1.31
7	" "	28.0	4.54	1.4	"	14.6	2.18	2.15	2.16

* Average of counts made prior to first sustained 10 per cent increase.

† Time required for 50 per cent increase in numbers.

‡ Average of $L = t - nG$ for three or four successive generations; t = median of cell divisions, n = number of generations, $G = 1/M$.

§ Day old aerated broth cultures.

‖ Indicates average values, otherwise single experiments.

¶ 18 hour agar slopes.

** 3 hour broth culture. Equation (4) does not strictly apply to this case. Equation (3) should be used. See definition of "L" above.

†† Day-old stationary broth culture.

S_0 and S_a are expressed in terms of oxygen consumed at zero time in broth. See Methods.

These equations, therefore, describe the significant aspects of the growth and multiplication of any organism growing at constant rate and dividing by binary fission. If growth rate and net change in size are known, the latent period and the course of numerical increase can be predicted. For testing the assumptions outlined above, we have chosen to use the equations (3) and (4), since these are contingent on all the assumptions made,

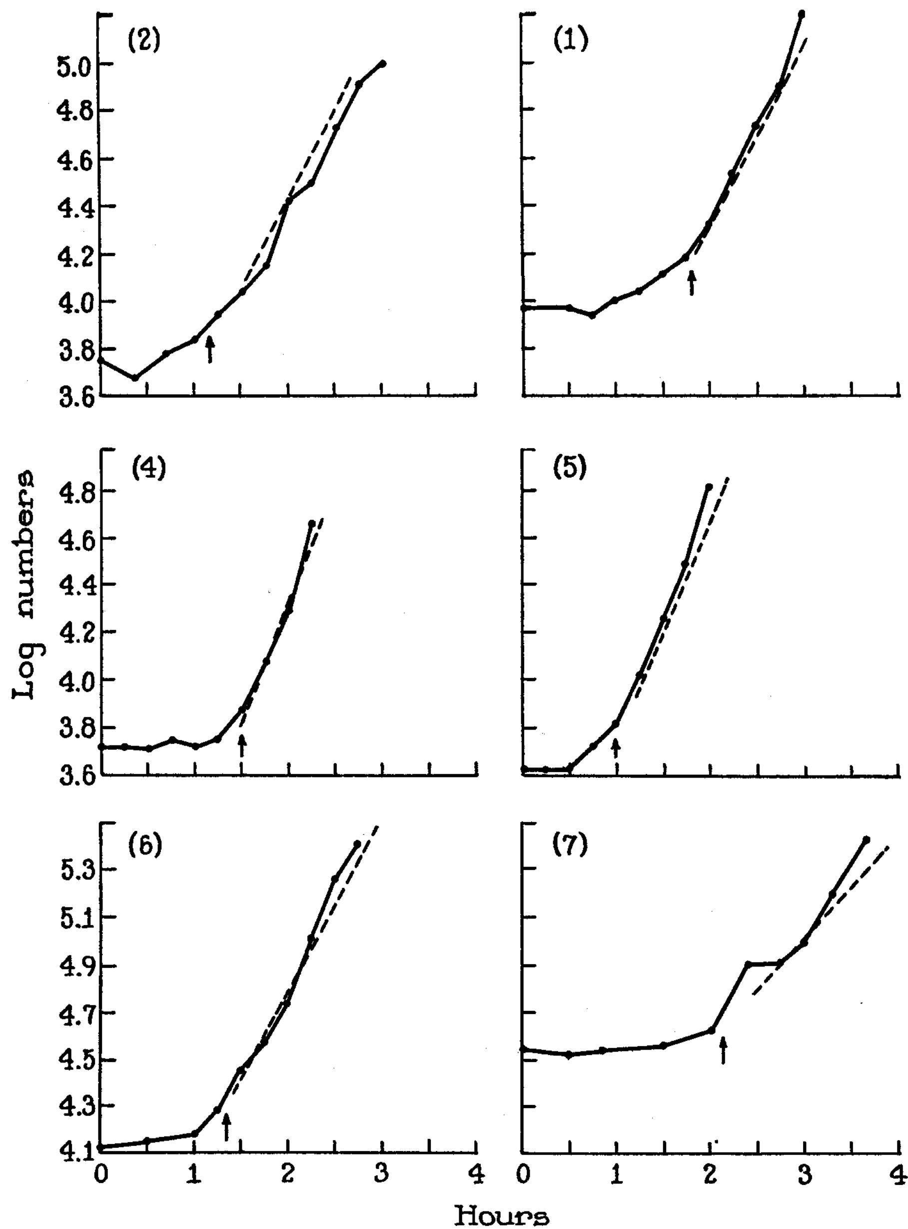

FIG. 1. Calculated and observed bacterial numbers. Equation (3): $\text{Log } B = \text{Log } B_0 + M\, t \log 2 - \log S_a/S_0$.

Lines connecting points are the experimental curves. The broken lines show the course of the calculated numbers. Arrows beneath the curves indicate the end of the observed latent period.

Values substituted in equation (3), and the conditions of the various experiments, are given in Table I.

and since measurements of B and L employ no new methods. All experiments have been carried out with *Bacterium coli,* excepting a few with *Bacterium dysenteriae* (Flexner).

The first problem we have considered is whether the growth of bacteria from young and old cultures, which are known to differ remarkably in size and latent period, is predictable from our hypotheses. That their growth-rates are substantially the same, can be regarded as established by the experiments recently published (2). The data given at that time fit equation (3) by definition, since the same measurements were used to compute size and growth rate. Nothing is to be accomplished, therefore, by re-examination of those data, which leave in question only the validity of the methods used.

This question is best answered by extending the observations to a considerable portion of the population curve, and assigning fixed average values to the postulated constants. New observations have accordingly been made, using conditions under which the rate of accretion and the initial size varied independently over a considerable range. Briefly, it was found that the latent period could be accurately and reproducibly measured, and that equation (4) satisfactorily predicted the times observed (Table 1). In the same experiments, the course of the numbers curve was accurately predicted by equation (3) in most instances (Fig. 1).

Whether the agreement obtained should be accepted as complete vindication of our assumptions remains, of course, a matter of opinion. Certainly their approximate truth has been established, as well as may be by measurements that are approximately correct.

An additional confirmation of hypothesis is given by the observation that population curves obtained with cells from aerated cultures (experiments 1 and 4, Fig. 1) show an abrupt transition from the latent period to the phase of logarithmic increase, as compared with cells from stationary broth or agar cultures (experiments 2 and 5). This peculiarity is less clear in curve 1, but has been observed in several additional experiments not shown. It is correlated with the remarkable uniformity of size revealed by microscopic examination, and perhaps by fractional sedimentation (3), of the aerated cultures, and should probably be referred to the homogeneous environment in which these cultures develop. The smaller absolute size of cells in aerated cultures is almost certainly the result of the virtually complete oxidative removal of food materials under these conditions.

Another observation bearing on the fundamental question of regulation

of size is that decreasing the growth rate by lowering the temperature diminishes the maximal size reached by *Bacterium coli*. Similarly, *Bacterium dysenteriae* apparently undergoes a smaller net change in size under the same conditions than the more rapidly growing organism. These suggestions are based on limited observations. In general it must be concluded that the mechanisms underlying the observed interdependence of growth rate, characteristic size, and environmental conditions (2), are unknown.

The values given in Table 1 include all measurements of L not previously reported. Experiment 4 represents the mean of three trials (L = 1.45, 1.53, and 1.53); experiment 5 of two (L = 0.95 and 1.02); the remainder are single observations. The data of Fig. 1 are also unselected, except that curves shown in the case of the duplicated experiments are those in which counting was continued longest.

Methods

The methods used were essentially those already reported (2). Population curves were obtained by agar-plate dilution counts, usually made at 15 minute intervals for accurate measurement of L. Dilutions were made in saline, with vigorous shaking, sufficient plates being poured to give 250 or more countable colonies (50 to 300 colonies per plate). Experimental variation has been reduced to an average of less than 10 per cent. Further improvement is apparently not feasible. Counts were conveniently made from cultures seeded with about 10^4 organisms per ml. The latent period obtained is little or not at all affected by the size of seeding within the stipulations noted (page 11). All cultures were incubated in a temperature-controlled water bath. A recording plate-counter (8) was indispensable.

Average values for the postulated constants, M and S_a, were used in all calculations. In the two experiments at the lower temperatures, these are averages of values observed at different times throughout the course of individual nephelometric experiments; in all others, they are averages of values obtained in numerous earlier experiments, for the most part manometric.

In current practice, minor technical improvements have been introduced leading to increased accuracy, particularly with the manometric method. For routine work, cells grown in day-old aerated broth cultures are being used to advantage, because these cultures are incapable of further growth, the oxidizable material available to the cells having been exhausted.

Aliquots of these cultures, for example, will give identical values for initial rate of O_2 consumption whether the period of equilibration is 20 minutes or an hour or more, prior to addition of fresh broth. Under the same conditions, the effect of oxygenation on stationary cultures is to initiate further growth, with or without added broth. Size is, therefore, measured nephelometrically, or after removing the cells from the culture medium.

The error resulting from inhibition of growth by removal of carbon dioxide during growth rate measurements (2) is largely avoided by placing the aliquot of the growing culture in the vessel for equilibration before adding the KOH. The vessel is then briefly removed from the bath for this purpose, after which readings can be made in 5 or 10 minutes.

The nephelometric method does not appear to be applicable to estimation of bacteria in day-old aerated cultures, giving values too high in relation to the viable count. The nephelometric index of size for these cultures is about 1.0, while the manometric size (0.85×10^{-7} mm.^{3}/cell hour) represents an index of 0.35 ($1.0 = 2.4 \times 10^{-7}$ mm.^{3}/cell hour). Since the latter figure agrees with microscopic observation,[4] we have given credence to the manometric value. Whether the excess turbidity is due to non-bacterial sediment, or dead bacterial cells, we have not determined. In either case, it could be expected to invalidate nitrogen determinations as well as optical measurements.

Improved measurements of growth rate with our strain of *Bacterium coli* at 37° C. have consistently given values between 3.0 and 3.3 doublings per hour, with an average of 3.1 (formerly (2) 2.8). This increase cannot be attributed to changes in the organism itself. Values for O_2 consumption given in this paper are based on new calibrations of the apparatus. Values previously reported are 16 per cent lower. This change is, of course, without effect on the values M and S_a/S_0.

[4] Mudge and Smith (9) report that *Bacterium coli* in aerated cultures is approximately one-third the length, at 24 hours, of the bacilli in stationary cultures. This is in accord with our own observation that the former are cocci, the latter bacilli about three times as long as broad, whose breadth equals the diameter of the cocci. Computing the volume of the bacillus as that of a cylinder 1×0.5 microns plus two hemispheres radius = 0.25 micron, the ratio of the volume of the bacillus to the coccus is 5.0. The corresponding ratio of manometric sizes is 2.4 to 0.85, or 2.8; of nephelometric sizes only 1.0. It may be recalled here that sizes reported by us are ultimately referred to nitrogen per cell, and presumably to volume; a bacillus about 1.5×0.5 microns = nephelometric index $1 = 2.4 \times 10^{-4}$ mm.^{3}/cell hour $= 3.5 \times 10^{-11}$ mg. nitrogen per cell, it being necessary to establish the validity of each method for each new condition studied.

SUMMARY

Simple assumptions have led to equations by which the latent period in multiplication and the bacterial numbers expected at any time during the phase of rapid growth may be predicted.

Experimental data obtained under rather diverse conditions have given satisfactory agreement with calculated values. Since the mathematical expressions contain no arbitrary constants, more than accidental significance must be attached to this agreement.

The hypotheses set forth appear completely to describe the early development of *Bacterium coli* and *Bacterium dysenteriae* in broth, without postulating differences other than size among individual cells, or cells obtained under different conditions.

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