JOURNAL OF BACTERIOLOGY, Feb., 1967, p. 605-617

Vol. 93, No. 2
Printed in U.S.A.

Kinetics of Growth of Individual Cells of *Escherichia coli* and *Azotobacter agilis*

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Received for publication 19 September 1966

ABSTRACT

Escherichia coli and *Azotobacter agilis* were grown in minimal media until a steady state was established. The distribution of cell size was determined electronically. From the equation of Collins and Richmond, the growth rate of individual cells was computed as a function of size. The main features of the growth of individual *E. coli* and *A. agilis* cells revealed by this work were: the specific growth rate decreased at the time of division, and both the absolute and specific growth rates increased between divisions. The frequency function of interdivision times was computed and was found to be positively skewed with a coefficient of variation of approximately 0.3. The results supported the hypothesis of Koch and Schaechter that the size of an individual cell at division is highly regulated.

The kinetics of growth of a bacterial population do not establish the kinetics of growth of the individual. In exponential balanced growth, all extensive properties of the population increase by the same factor during any given time interval (3). However, all extensive properties of an individual cell in this population need not increase in proportion during any arbitrary time interval. It is necessary that the mean value of all extensive properties of dividing cells should be twice the mean value for newly formed cells. The rate of increase in volume of the individual cell can be any function of time which permits the volume to double at the end of the interdivision period.

The method most commonly used for determination of the kinetics of growth of individual bacteria has been direct microscopic measurement of length or volume of cells in microculture as a function of time. Using this method, Adolph and Bayne-Jones (1) found the volume of *Bacillus megaterium* to be an exponential function of time. Growth of cells of *Escherichia coli* was approximately exponential, but the specific growth rate of an individual cell showed random fluctuations in time (2). A similar result was obtained with *B. cereus* by Knaysi (13). The rate of increase of volume of *Streptococcus faecalis* was found to decrease between divisions (14, 16). Using interference microscopy, Mitchison (16) found that the rate of increase in dry weight of *S. faecalis* showed a similar decrease.

In these microscopic measurements, the conditions in the microculture may not have permitted balanced growth. In many experiments it is obvious that growth was not balanced. In the more recent work of Schaechter et al. (20) and of Errington, Powell, and Thompson (6), the state of unrestricted, balanced growth in microculture has been verified. Errington et al. (6) found that the lengths of cells of *Pseudomonas aeruginosa*, *Serratia marcescens*, and *Proteus morganii* were not significantly different from an exponential function of time; however, several significant deviations from exponential increase were observed (6). Schaechter et al. (20) found that the increase in length of *E. coli* and *Salmonella typhimurium* was approximately exponential, but they recognized that microscopic measurement of bacterial cells is subject to a random error of sufficient magnitude to preclude distinguishing linear growth from exponential growth.

The results of direct microscopic measurements can be summarized by stating that the size of individual cells of most bacteria increases continously between divisions, but the exact form of the increase has not been determined.

An alternative to direct microscopic measurement of growth of single cells was developed by Collins and Richmond (4), who demonstrated that the kinetics of growth of individual cells determine the form of the size distribution of the cell population. A more rigorous development of

this relationship is given in Appendix A to this paper. From a measurement of the distribution of size of individual cells in the population, the rate of growth of these cells as a function of size may be calculated. The method avoids the limitations of the method of direct microscopic measurement.

This paper describes the kinetics of growth of *E. coli* and *Azotobacter agilis* determined from electronic measurement of the distribution of volumes of cells in unrestricted balanced growth.

MATERIALS AND METHODS

Symbols. Throughout this paper, the following symbols are used: v, volume of a cell; k, specific growth rate of the population; N, total number of cells in the population; $\lambda(v)$, frequency function of volume of extant cells; $\phi(v)$, frequency function of volume of cells which are dividing; $\psi(v)$, frequency function of volume of cells newly formed by division; $g(v)$, frequency function of volume at division of newly formed cells; $f(\tau)$, frequency function of life length, τ, of newly formed cells; $V(v)$, absolute growth rate of a cell of volume v.

Growth of bacteria. E. coli strain ML30 was grown in glucose-salts medium containing (in grams per liter): glucose, 1.0; NH_4Cl, 0.8; KH_2PO_4, 4.2; K_2HPO_4, 8.5; $MgCl_2 \cdot 6H_2O$, 0.2; Na_2SO_4, 0.2; KCl, 1.0; $CaCl_2$, 0.01; $FeSO_4 \cdot 7H_2O$, 0.0005.

E. coli B/r was grown in glucose-salts medium containing (in grams per liter): glucose, 2.0; NH_4Cl, 2.0; Na_2HPO_4, 6.0; KH_2PO_4, 3.0; NaCl, 3.0; $MgSO_4$, 0.013; Na_2SO_4, 0.011; $CaCl_2$, 0.002; $FeSO_4 \cdot 7H_2O$, 0.0005; $NaMoO_4 \cdot 2H_2O$, 0.000025.

A. agilis (*A. vinelandii* strain 0) was grown in Burks nitrogen-free medium containing (in grams per liter): sucrose, 3.5; KH_2PO_4, 0.41; K_2HPO_4, 0.52; $CaCl_2$, 0.02; $MgCl_2 \cdot 6H_2O$, 0.17; $Na_2MoO_4 \cdot 2H_2O$, 0.00025; $FeSO_4 \cdot 7H_2O$, 0.005; Na_2SO_4, 0.15.

Cultures were grown at 30 C in tubes (30 by 300 mm) containing 100 ml of medium and were aerated by sparging.

Measurement of growth. Periodically the cultures were sampled for measurement of optical density and cell numbers. Optical density was measured at 600 mμ by use of a Beckman model DU spectrophotometer with 1-cm absorption cells. Cell numbers were counted electronically by use of the apparatus previously described (8).

The specific growth rate, k, in hours^{-1} was computed by linear regression according to the equation:

$$\log_e x = \log_e x_0 + kt$$

where x is the optical density or the number of cells per milliliter at time t, and x_0 is the optical density or number of cells per milliliter at time zero.

Measurement of size distributions. Size distributions were measured with the apparatus described by Harvey and Marr (8). Pulses produced by the passage of cells through the Coulter transducer (W. H. Coulter, U.S. Patent 2,656,508, 1953) were differentiated and integrated, and the resulting pulses were measured by a pulse height analyzer. Samples were prepared for measurement by diluting to 10^5 to 2×10^5 cells per milliliter in a solution containing 0.85% NaCl and 0.04% formaldehyde. The size distribution of 2×10^4 to 3×10^4 cells was measured between 1 and 3 hr after sampling.

Measurement of volume by electron microscopy. Formaldehyde (0.04%) was added to a sample of culture, from which the cells were centrifuged, washed once in 0.04% formaldehyde, and resuspended in a solution containing 0.04% formaldehyde and 0.1% serum albumin to give a concentration of 2×10^8 cells per milliliter. The suspension was sprayed onto Formvar-coated grids and was air-dried.

Electron micrographs were made by use of an RCA EMU-3E microscope. Magnification was controlled to within ±0.75%. The micrographs were printed to give a final magnification of about 10,000 ×, and dimensions of cells were estimated from the prints to within ±0.2 mm. Measurement of length is sufficient to estimate relative volume of cells of *E. coli* (15), and was assumed to be sufficient for estimation of relative volumes of *A. agilis*.

RESULTS

Steady-state growth of E. coli and A. agilis. The equation of Collins and Richmond (4) is as follows:

$$V(v) = \frac{k}{\lambda(v)}\left[2\int_o^v \psi(x)\,dx - \int_o^v \phi(x)\,dx - \int_o^v \lambda(x)\,dx\right] \quad (1)$$

Before the equation can be applied to measured size distributions for calculation of growth rates, it must be established that the culture on which the measurement of size distribution is made is growing in a steady state. In this investigation, the steady state was defined by three criteria. (i) The specific growth rate of the population must be constant. (ii) The distribution of cell size must be independent of time. (iii) As a consequence of (ii), the specific growth rate computed from optical density must be identical with that computed from numbers.

These criteria should be met after growth of a bacterial population for many generations at relatively low cell densities. Both organisms were grown for approximately 20 generations before measurements were commenced. The cultures were periodically diluted with fresh medium so that the culture of *E. coli* did not exceed 5×10^8 cells per milliliter, and the culture of *A. agilis* did not exceed 5×10^6 cells per milliliter.

The population of *E. coli* was sampled at intervals of 0.5 to 0.25 generation over a period of 7 generations for the measurement of optical

density, numbers, and size distribution. Measurements of numbers and size distribution of *A. agilis* were made over a period of three generations. Optical density could not be measured at the low densities at which this organism was grown; growth at densities higher than 10^7 cells per milliliter does not approximate a steady state.

Specific growth rates of *E. coli* are compared in Table 1. By use of the *t* test, no significant difference was found between the specific growth rate estimated from numbers and the specific growth rate estimated from optical density. Analysis of variance of the measurements in three sequential periods showed that the specific growth rate did not vary significantly with time. Thus, the criteria of constancy and equality of the specific growth rates are satisfied in cultures of *E. coli*. Numbers of *A. agilis* also increased exponentially, satisfying the condition of constancy of specific growth rate.

The demonstration of the constancy of the frequency function of cell size, $\lambda(v)$, was based on examination of the parameters of this distribution as a function of time. Collins and Richmond (4) reported that the distribution of the logarithm of length of *B. cereus* was Gaussian; thus, the mean and variance of the logarithmic transform sufficiency defined the distribution. The logarithmic transforms of the measured volume distributions of *E. coli* and *A. agilis* were not Gaussian. The mean and variance are not sufficient to define the distribution because of the strong positive skewness. Arbitrarily, we decided to describe the distribution in terms of parameters based on the first four moments.

The mean, variance, g_1, and g_2 statistics (7) were calculated for each distribution measured. From replicate measurements on a single sample, the variance of the estimates of each parameter from measurement error (s^2_M) was calculated. The variance of the estimates of each parameter over the entire experimental period (s^2_P) was also calculated. If the value of a parameter did not change with time, the variance of its estimates will be due entirely to measurement error. Then the ratio $F = s^2_P/s^2_M$ should satisfy the *F* distribution.

The results of this analysis are shown in Table 2. For both organisms, the variances of mean, variance, g_1, and g_2 were no greater than expected from measurement error; any change with time of the parameters of the distributions was within the limits of measurement error, and the necessary condition of constancy of the size distribution was satisfied.

The measured distributions for each organism were pooled, and the resulting frequency functions are shown in Fig. 1 and 2. The parameters of the pooled distributions were not significantly different from the mean of the estimates of the parameters of the individual distributions. Thus, the pooling of data did not introduce bias.

Parameters of the distribution of size of dividing cells and of newly formed cells. The frequency function of size of dividing cells, $\phi(v)$, must be known or assumed in order to calculate growth rate as a function of size. Since the form of this function is not known, three representative types of functions were chosen, each of which is sufficiently described by the mean and coefficient of variation (CV). These were: (i) the Gaussian function, a symmetrical function; (ii) the Pearson Type III function (5), a positively skewed function; and (iii) a negatively skewed function obtained by a reflection of the variable of the Pearson Type III function. The parameters chosen for these functions are shown in Table 3. The basis for the choice of values for these parameters is given below.

TABLE 1. *Growth of Escherichia coli ML30*

Period of measurement[a]	Optical density		Numbers		*t* for difference	$P(t)$[b]
	Specific growth rate, *k*, hr^{-1}	Standard error	Specific growth rate, *k*, hr^{-1}	Standard error		
min						
72–125	0.6886	0.0167	0.6775	0.0132	0.521	>0.1
210–345	0.6700	0.0098	0.6446	0.0096	1.854	>0.05
356–502	0.6754	0.0064	0.6824	0.0079	0.686	>0.1
Value of *F* for constancy of *k*	0.544		3.951			
$P(F)$	>0.05		>0.025			

[a] The end of each period of measurement was dictated by the necessity for dilution of the culture.
[b] $P(t)$ and $P(F)$ refer to the probability that the observed value of *t* or *F*, respectively, is due to chance.

TABLE 2. *Parameters of size distributions of Escherichia coli and Azotobacter agilis*

Determination	Parameter			
	Mean, μ^3	Variance	g_1	g_2
E. coli ML30[a]				
Mean value	0.8285	0.0456	1.197	1.362
Variance	4.51×10^{-4}	1.02×10^{-5}	4.69×10^{-3}	7.16×10^{-2}
Measured sampling variance	1.60×10^{-4}	5.16×10^{-6}	8.57×10^{-4}	1.41×10^{-2}
F	2.819	1.977	5.472	5.078
$P(F)$	>0.05	>0.05	>0.05	>0.05
A. agilis[b]				
Mean value	5.351	1.961	0.825	0.707
Variance	1.64×10^{-2}	8.01×10^{-3}	2.73×10^{-3}	3.54×10^{-3}
Measured sampling variance	7.91×10^{-3}	5.06×10^{-3}	5.42×10^{-4}	4.63×10^{-3}
F	2.073	1.583	5.037	7.646
$P(F)$	>0.05	>0.05	>0.025	>0.01

[a] Eighteen distributions measured; total number of cells measured = 4.5×10^5.
[b] Nine distributions measured; total number of cells measured = 2.0×10^5.

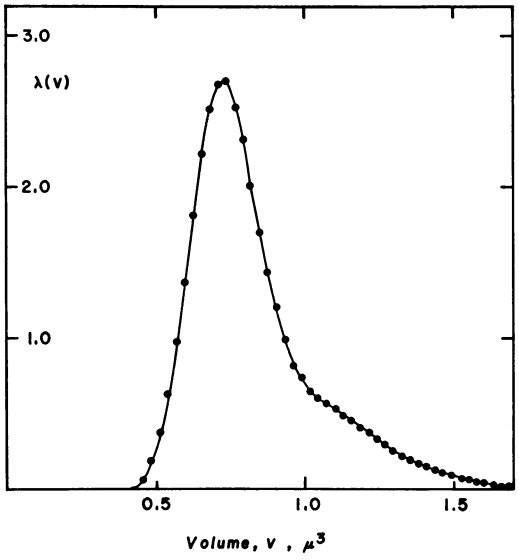

FIG. 1. *Frequency function of volume, λ(v), of Escherichia coli ML30. Values below 0.53 μ^3 were obtained by correcting observed values for background.*

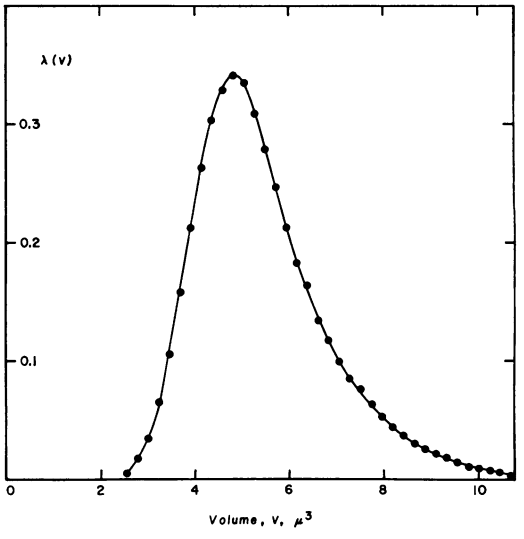

FIG. 2. *Frequency function of volume, λ(v), of Azotobacter agilis. Values below 3.3 μ^3 were obtained by correcting observed values for background.*

The coefficient of variation of size of dividing cells (CV) was found to be approximately 0.1 for *E. coli* B/r and *S. typhimurium* (20), for *B. cereus* (4), and for *E. coli*, *P. aeruginosa*, *S. marcescens*, and *P. morganii* (6). This value was confirmed for *E. coli* ML30 and *A. agilis* by examination of electron micrographs of air-dried cells from a steady-state culture. In each preparation, a small fraction of the cells were found to be deeply constricted and were judged to be approaching the size at division. For *E. coli* ML30, the CV of length of apparently dividing cells was 0.122; the range defined by the standard error was 0.087 to

TABLE 3. *Parameters of the distribution of size of dividing cells*

Determination	Best estimate	Range
Escherichia coli ML30		
Mean, μ^3	1.306	1.23–1.38
Coefficient of variation	0.12	0.09–0.15
Azotobacter agilis		
Mean, μ^3	8.13	7.68–8.58
Coefficient of variation	0.10	0.09–0.11

0.150. For *A. agilis*, the CV was 0.101, and the range defined by the standard error was 0.090 to 0.110. Since the cells observed had not yet divided but were in different stages of the final division process, the values for the coefficients of variation were overestimated and provided an upper limit to the range of the true value. It was assumed that the CV of size of dividing cells would fall within the range defined by the standard error of the estimate in each case.

The best estimate of the mean (m_ϕ) of $\phi(v)$ was determined from the relationship:

$$m_\phi = v_L/2 + v_S \qquad (2)$$

which is developed in Appendix B. The term v_S is the lower limit and v_L is the upper limit of the range of the distribution of size of cells. This estimate was based on the assumptions that $\phi(v)$ is symmetrical and that division is equal. The probable range of m_ϕ was obtained from the assumption that the function was either extremely negatively or positively skewed. The best estimate and the range of the mean of $\phi(v)$ for *E. coli* ML30 and *A. agilis* are in Table 3.

The validity of these estimates was supported by the distribution of volume of the cells in the effluent from the membrane filter device of Helmstetter and Cummings (9). The majority of such cells had just been formed by division. The frequency function of their volumes should be approximately $\psi(v)$. The value of the mode of the distribution of volume of the effluent cells should provide an estimate of m_ψ. The modal volume of *E. coli* B/r in the effluent was 0.49 μ^3, whereas the estimate obtained from equation 2 was 0.50 μ^3 with a range of 0.465 to 0.538 μ^3.

The ψ-distribution and ϕ-distribution are related; the relationship is a function of the inequality of division (18). The variable p is the ratio of the volume of daughter cell to volume of mother cell at the time of division, and $K(p)$ is the frequency function of the distribution of p. Powell (18) has demonstrated that, if $K(p)$ and $\phi(v)$ are known, $\psi(v)$ can be computed from the following equation:

$$\psi(v) = \int_o^1 \phi(v/p) \cdot \frac{K(p)}{p}\, dp \qquad (3)$$

For equal division, p is not distributed, giving $\psi(v) = 2\phi(2v)$. For *E. coli* ML30, the distribution of $K(p)$ was Gaussian with a CV of 0.04 (15). Similar measurements were made from the electron micrographs of *A. agilis* described above. For *A. agilis*, $K(p)$ was also Gaussian ($p_{\chi^2} = 0.23$) with a CV of 0.02. For most purposes, it can be assumed that division is equal in both organisms.

The act of division referred to throughout this paper is the act of physical separation of daughter cells. Physiological division, whereby the daughter cells become physiologically independent, must precede separation. The temporal relationship between these two processes is not known.

Calculation of growth rates. Growth rates of *E. coli* and *A. agilis* as a function of cell volume were calculated by use of equation 1. Figures 3 and 4 show the results of calculations with the use of the best estimates of the parameters of the distributions of dividing and newly formed cells. The rate of increase in volume in cubic microns per hour was plotted as a function of cell volume in cubic microns. The broken lines show the rates of growth expected if individual cells grew at all times with the same specific growth rate as the population.

Figures 5, 6, and 7 show the effects on the calculated growth rates of *E. coli* of different assumptions regarding the form, coefficient of variation, and mean, respectively, of $\phi(v)$. Results for *A. agilis* showed the same effects. Examination of the results showed that the rate of growth was substantially independent of $\phi(v)$ over a range of

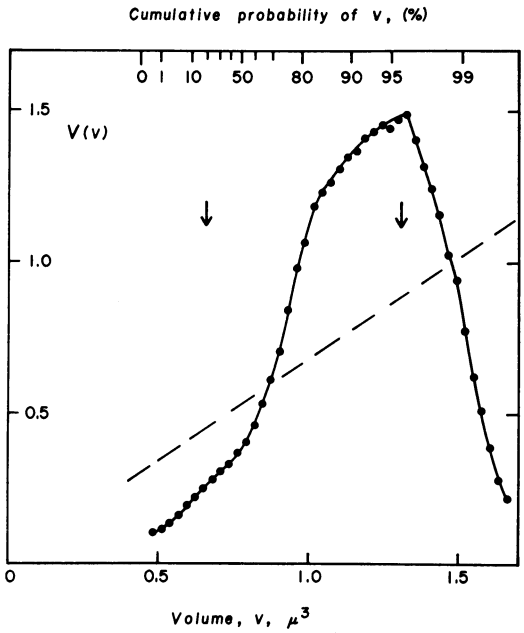

FIG. 3. *Rate of increase of volume, V(v), of Escherichia coli ML30 as a function of cell volume. The distributions of volume of dividing and newly formed cells are assumed to be Gaussian, with parameters as given by the best estimates shown in Table 3. The arrows indicate the best estimates of mean volume of newly formed and dividing cells. The upper scale on the abscissa shows the cumulative probability of the cell size distribution at the corresponding volume on the lower scale.*

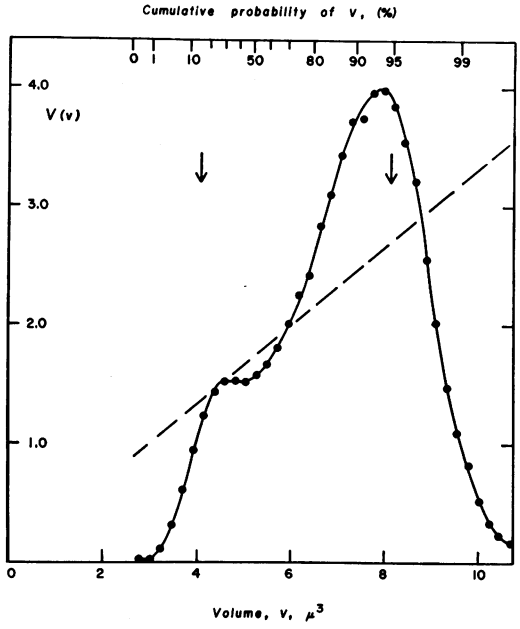

FIG. 4. *Rate of increase of volume, V(v), of Azotobacter agilis as a function of volume. The distributions of volume of dividing and newly formed cells are assumed to be Gaussian, with parameters as given by the best estimates shown in Table 3. The arrows and the upper scale have the same meaning as in Fig. 3.*

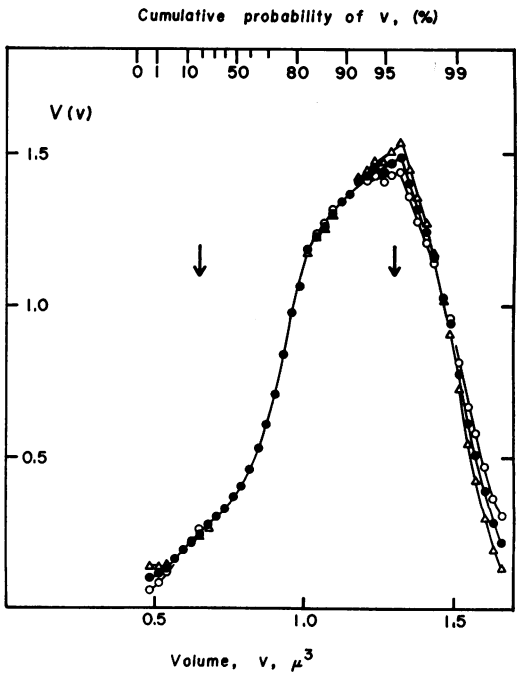

FIG. 5. *Effect of choice of function representing the distributions of volume of dividing and newly formed cells on the calculated values of V(v) for Escherichia coli ML30. The different functions are: ●, Gaussian; ○, Pearson Type III; △, reflected Pearson Type III. Parameters used are the best estimates given in Table 3. The arrows and the upper scale have the same meaning as in Fig. 3.*

cell volume containing approximately 50% of the population. Outside this range the assumptions did influence the calculated growth rates, but did not affect the general form of the functions.

The kinetics of growth computed for *E. coli* and *A. agilis* agreed closely with the kinetics of growth of *B. cereus* determined by Collins and Richmond (4) from the distribution of lengths measured by using light microscopy.

Calculation of distribution of interdivision time. The frequency functions of interdivision times, $f(\tau)$, of *E. coli* and *A. agilis* were calculated from the values of $V(v)$ by use of the equations given in Appendix C. The frequency function of size of dividing cells was assumed to be Gaussian with parameters as given by the best estimates in Table 3. The distribution of volume of newly formed cells was computed from $\phi(v)$, by use of equation 3, and from the experimentally determined parameters of $K(p)$. Hypothetical functions for $f(\tau)$ were calculated by use of the same $\phi(v)$, but assuming either exponential or linear kinetics of growth. Results of these calculations are shown in Fig. 8 and Table 4.

Similar results were obtained for *E. coli* and *A. agilis*. The function $f(\tau)$ calculated from the experimentally determined values of $V(v)$ for either *E. coli* or *A. agilis* was positively skewed and had a coefficient of variation of about 0.3. The function $f(\tau)$ calculated assuming either exponential or linear growth was symmetrical.

By direct numerical solution of the functions used in computing $f(\tau)$, the correlation coefficients of life lengths of mother and daughter and of sister and sister cells were computed. For these calculations, it was assumed that a mother divided to produce two sisters of equal size. The correlation coefficients for life lengths of mother and daughter cells were −0.307 and −0.408 for *E. coli* and *A. agilis*, respectively. The correlation coefficients of life lengths of sister and sister cells were 0.866 and 0.722, respectively. The negative correlation of life lengths of mother and daughter cells and the high positive correlation of life length of sister and sister cells were in general agreement with direct experimental measurements (17). The agreement between calculations and experimental results supported the hypothesis of Koch and Schaechter (11) that size controls the timing of cell division.

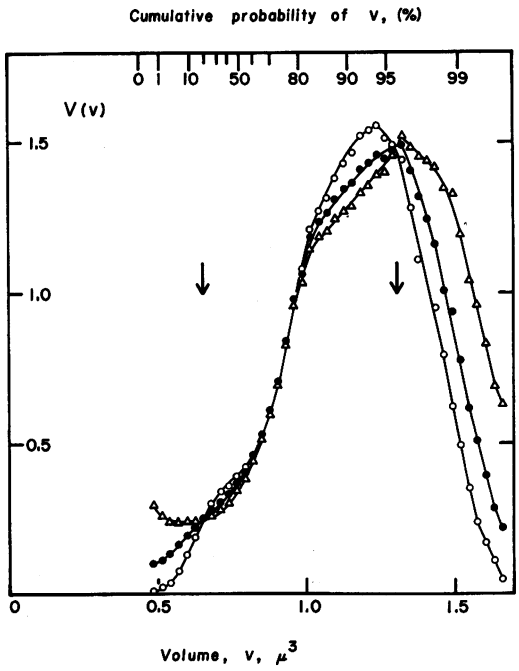

FIG. 6. *Effect of different coefficients on variation of the distributions of volume of dividing and newly formed cells on the calculated values of V(v) for Escherichia coli ML30. The distributions are assumed to be Gaussian, with means as given by the best estimates in Table 3, and coefficients of variation of:* ○, *0.09;* ●, *0.12;* △, *0.15. The arrows and the upper scale have the same meaning as in Fig. 3.*

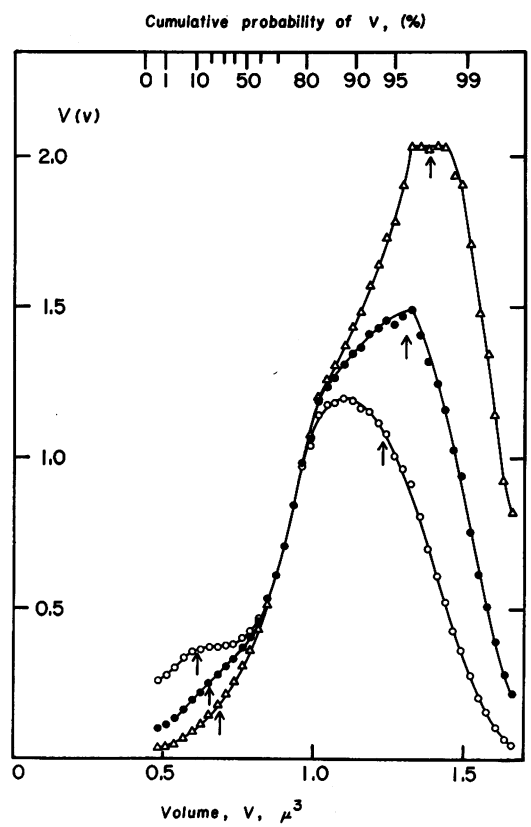

FIG. 7. *Effect of different means of the distributions of volume of dividing and newly formed cells on the calculated values of V(v) for Escherichia coli ML30. The distributions are assumed to be Gaussian, with coefficients of variation as given by the best estimates in Table 3, and values of:* ○, *1.231* μ^3; ●, *1.306* μ^3; △, *1.381* μ^3, *for the mean volume of dividing cells. The arrows indicate the mean volumes of newly formed and dividing cells for each curve. The upper scale has the same meaning as in Fig. 3.*

DISCUSSION

A growth rate, $V(v)$, is the average rate of increase in volume of those cells of volume v. If only size determines the rate of growth, all cells of volume v grow at rate $V(v)$; but, if other factors such as age are significant, the rate $V(v)$ is an average of a distribution of individual rates. The calculation is valid in either case.

From the results in Fig. 3 and 4, one can reconstruct the kinetics of growth of a typical individual if all cells of volume v grow at a rate $V(v)$. After formation at small volume, both the absolute and specific growth rates increased with increasing volume. Just prior to reaching the mean volume of dividing cells, the growth rate was maximal. At larger volumes, the values of $V(v)$ were strongly influenced by the form of the distribution of size of dividing cells, but it appears that individual cells which fail to divide before reaching the mean size of dividing cells grow at a continually decreasing rate.

Continuous exponential growth implies that immediately after division the average growth rate of the two daughter cells will be one-half the growth rate of the mother cell. This was not the case for *E. coli* and *A. agilis*. The growth rate $V(v)$ of cells of volume v was much less than one-half of $V(2v)$ for more than 99% of the population. The specific growth rate of a cell must decrease shortly before, during, or immediately after division. The decrease in growth rate which becomes evident at volumes larger than the mean volume of dividing cells could generally be true, but observable only if cells which are dividing constitute a large fraction of the cells in a given size class.

The kinetics of growth are more complex than predicted by the hypothesis of continuous exponential growth. The validity of this conclusion

depends upon the validity of the assumptions regarding the distribution of volume of dividing cells and upon the accuracy of measurement of the population size distributions. The mean and coefficient of variation of the distribution of volume of dividing cells can be determined with sufficient accuracy to conclude that reasonable values of these parameters are not consistent with the hypothesis of continuous exponential growth. Over the probable range, the values of these parameters do not affect the general form of $V(v)$.

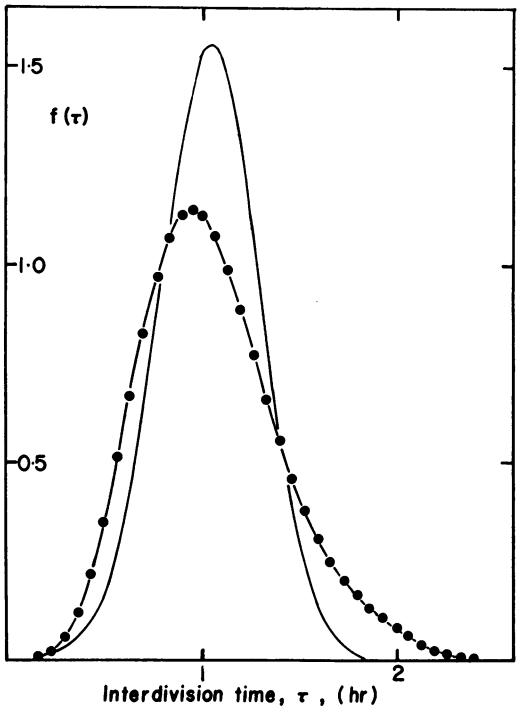

FIG. 8. *Frequency functions, $f(\tau)$, of the distribution of interdivision times. Symbols: ●, Escherichia coli ML30; solid line, hypothetical $f(\tau)$, calculated assuming exponential growth. In both cases, the distribution of volume of dividing cells was assumed to be Gaussian, with parameters given by the best estimates for E. coli ML30 in Table 3.*

The form of $\phi(v)$ is assumed, and it might be argued that there exists some frequency function of volume of dividing cells which is consistent with continuous exponential growth. That such a frequency function does not exist can be demonstrated by solving equation 1 for $\phi(v)$ and $\psi(v)$, giving the following equation:

$$2\int_o^v \psi(x)\,dx - \int_o^v \phi(x)\,dx = \frac{V(v)\cdot\lambda(v)}{k} + \int_o^v \lambda(x)\,dx \qquad (4)$$

Equation 4 can be solved numerically for $\phi(v)$ if $\lambda(v)$ is known, and the kinetics of growth are assumed. The solution is iterative, $\psi(v)$ being calculated from $\phi(v)$ by equation 3, by use of the known parameters of $K(p)$. Values of $\phi(v)$ were computed from the distributions of volume of *E. coli* and *A. agilis* shown in Fig. 1 and 2, with the assumption of continuous exponential growth, i.e., $V(v) = kv$. The result of these calculations for *E. coli* are in Fig. 9; results for *A. agilis* were similar. To be consistent with the measured volume distribution and continuous exponential growth, the function $\phi(v)$ must assume both positive and negative values and, hence, cannot be a frequency function. It follows that the measured volume distributions are inconsistent with the hypothesis of continuous exponential growth.

The form of the calculated distribution of interdivision times provides a powerful argument for the validity of the observed kinetics of growth and against the hypothesis of exponential growth.

TABLE 4. *Parameters of the computed distributions of interdivision times, $f(\tau)$*[a]

Organism	Kinetics of growth	Parameters of $f(\tau)$			
		Mean	CV	g_1	g_2
		hr			
Escherichia coli[b]	Experimental	1.020	0.336	0.443	0.028
	Exponential	1.044	0.246	−0.034	0.032
	Linear	1.046	0.259	−0.044	−0.080
Azotobacter agilis[c]	Experimental	2.037	0.257	0.901	1.442
	Exponential	2.114	0.201	−0.050	0.027
	Linear	2.074	0.214	−0.069	−0.065

[a] In all cases, the distributions of volume of dividing cells were assumed to be Gaussian, with parameters given by the best estimates in Table 3.
[b] Doubling time was 1.024 hr; coefficient of variation of $K(p)$ was 0.04.
[c] Doubling time was 2.094 hr; coefficient of variation of $K(p)$ was 0.02.

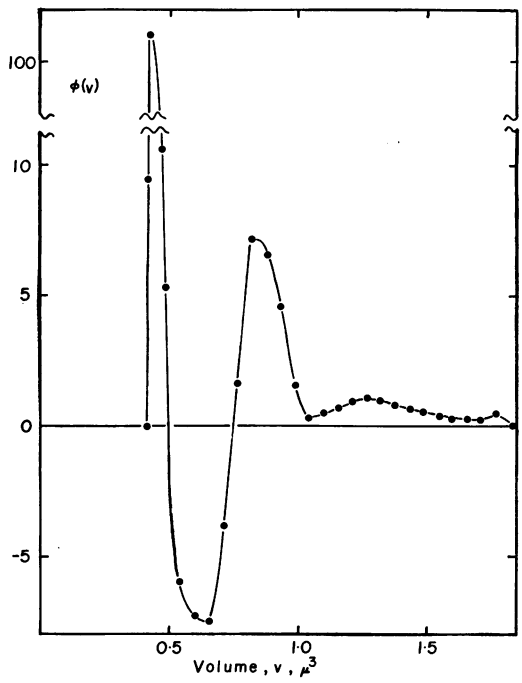

FIG. 9. *Theoretical function $\phi(v)$ computed from the measured distribution of volume of Escherichia coli ML30 assuming continuous exponential growth.*

Measurements of the distribution of interdivision times of a number of different organisms have shown that $f(\tau)$ is strongly positively skewed with a coefficient of variation of 0.15 to 0.5 (12, 17). The results in Table 4 are in good agreement with these observations, except that the coefficient of variation of $f(\tau)$ for *E. coli* is somewhat larger than values which have been observed. Powell (14) has asserted that, given exponential growth of individual cells, the function $f(\tau)$ will be symmetrical regardless of the symmetry of $\phi(v)$. We have established this symmetry by numerical analysis using equation C-1 in Appendix C. Since the present calculations of $f(\tau)$ and most previous measurements of this function show a strong positive skewness, it must be concluded that growth of individual cells cannot be strictly exponential. Positive skewness of $f(\tau)$ requires deviations from exponential growth.

Stochastic models based on the assumption that division occurs only after the completion of a number of random and independent cellular processes have been proposed to explain the positive skewness of the distribution of interdivision time (10, 19). Our calculations of $f(\tau)$ are based on the alternative postulate of Koch and Schaechter (11) that the *size* of a cell at division is under cellular and environmental control. The results support this more deterministic hypothesis.

The precision of determination of the kinetics of growth depends upon the fidelity of measurement of the distribution of size of bacteria. An examination of the accuracy of measurement of volume by the apparatus used in this investigation has been made by use of measurement by electron microscopy as the primary standard (7). The distributions of volumes both of spherical latex particles and of cells of *E. coli* were measured without detectable distortion. However, the necessarily small sample size and the possibility of uncontrolled errors in electron microscopy preclude the detection of minor distortions of the distribution. This applies particularly to errors in estimating moments higher than the second. As Koch (J. Gen. Microbiol., *in press*) has pointed out, accurate measurement of the higher moments of the size distribution is of primary importance in determining the kinetics of growth.

Although systematic errors may have influenced the estimates of $\lambda(v)$, the possibility can be eliminated that the deviations of the observed kinetics from exponential growth are due to random errors in measurement. Equation 1 can be solved for $\lambda(v)$ (*see* Appendix A, equation A-8). Distributions can then be calculated incorporating any assumptions regarding the kinetics of growth and the distributions of size of dividing and of newly formed cells. In Table 5 and Fig. 10, size distributions calculated assuming exponential growth are compared with the measured distributions of volume of *E. coli* and *A. agilis*. Distributions calculated assuming a constant growth rate between divisions (linear growth) are also shown. Figure 10 shows marked differences in the form of the distributions. The means, variances, and g_1 and g_2 statistics are compared by use of the t test in Table 5. The means and variances of the measured and theoretical distributions are significantly different in all but a few cases. The g_1 and g_2 statistics are markedly different, and the difference is significant in all instances. Random error in the measurement of the size distribution cannot, therefore, account for the deviations of the observed kinetics from either exponential or linear growth.

The results obtained by direct microscopic measurement of size of cells have been consistent with the hypothesis of continuous exponential growth; however, these results are not inconsistent with the kinetics of growth determined in this investigation. This becomes clear when the data of Fig. 3 and 4 are transformed to yield volume as a function of time, the form of data obtained by direct microscopic measurement. The

TABLE 5. *Comparison of measured size distributions with theoretical distributions calculated assuming exponential or linear growth*[a]

Determination	Assumed		Measured or calculated parameters			
	m_ϕ (μ^3)	CV_ϕ	Mean (μ^3)	Variance	g_1	g_2
Escherichia coli						
Measured			0.8285	0.0456	1.197	1.362
			$\pm 5.01 \times 10^{-3}$	$\pm 2.15 \times 10^{-2}$	$\pm 2.15 \times 10^{-2}$	$\pm 6.31 \times 10^{-2}$
Calculated assuming exponential growth	1.31	0.09	0.9073	0.0412	0.528	−0.376
	1.31	0.12	0.9073	0.0464*	0.569	−0.154
	1.31	0.15	0.9073	0.0532	0.588	0.045
	1.23	0.12	0.8556	0.0412	0.569	−0.154
	1.38	0.12	0.9949	0.0520	0.569	−0.154
Calculated assuming linear growth	1.31	0.09	0.9520	0.0436	0.354	−0.610
	1.31	0.12	0.9581	0.0500	0.409	−0.390
	1.31	0.15	0.9657	0.0576	0.452	−0.196
	1.23	0.12	0.9034	0.0444*	0.409	−0.390
	1.38	0.12	1.0126	0.0556	0.409	−0.390
Azotobacter agilis						
Measured			5.351	1.961	0.825	0.707
			±0.0427	±0.0298	±0.0174	±0.0628
Calculated assuming exponential growth	8.13	0.09	5.658	1.610	0.528	−0.376
	8.13	0.10	5.658	1.675	0.538	−0.300
	8.13	0.11	5.658	1.746	0.549	−0.221
	7.68	0.10	5.346*	1.500	0.538	−0.300
	8.58	0.10	5.971	1.860	0.538	−0.300
Calculated assuming linear growth	8.13	0.09	5.936	1.716	0.354	−0.610
	8.13	0.10	5.948	1.789	0.354	−0.523
	8.13	0.11	5.961	1.871	0.386	−0.446
	7.68	0.10	5.620	1.603	0.366	−0.523
	8.58	0.10	6.276	1.986*	0.366	−0.523

[a] The distributions of $\phi(v)$ were assumed to be Gaussian, and $\psi(v)$ was computed from equation 3.
* Not significantly different from the measured distribution at the 5% level of probability. All values not so marked are significantly different from the measured distribution.

results of such a transformation, representing the growth of approximately 90% of the population, are shown in Fig. 10 and 11. If the random error in microscopic measurement is only ±5%, the 95% confidence limits of measurement encompass both exponential growth and the observed kinetics for *E. coli*. For *A. agilis*, the confidence limits include linear growth as well.

Our results do not give information about the kinetics of synthesis of cell material. It is possible that the rate of synthesis is independent of the rate of change in volume. Dependence can be tested by pulse-labeling with a radioactive monomer (e.g., an amino acid) and determining the distribution of grains in an autoradiogram with respect to the length of the cells. Goldstein (*unpublished data*) has evidence that the specific rate of protein synthesis decreases at the time of division. A culture of *E. coli* was pulse-labeled with H^3-leucine, and the cells were subjected to autoradiography. If the pulse was of long duration, the large, dividing cells had more associated grains than did smaller cells. If the pulse was of short duration, the dividing cells did not have more associated grains.

If the rate of synthesis of protein and other macromolecules is not proportional to the rate of increase in volume of the cell, volume is more critical to cell division than the mass of macromolecules. If the rate of synthesis of protein is proportional to the rate of increase in volume of the cell (i.e., growth is balanced), some mechanism to control the efficiency at which ribosomes function must be envisaged.

APPENDIX A

Relationship between growth rate of individual cells and the size distribution of the population. The Collins-Richmond equation is a statement of conservation of cells that holds for any culture in exponential balanced growth, for which $\lambda(v)$ and $V(v)$ can be closely approximated by continuous functions and are independent of time or of the number of cells.

If the class T of all cells larger than some v is considered, the number of such cells is

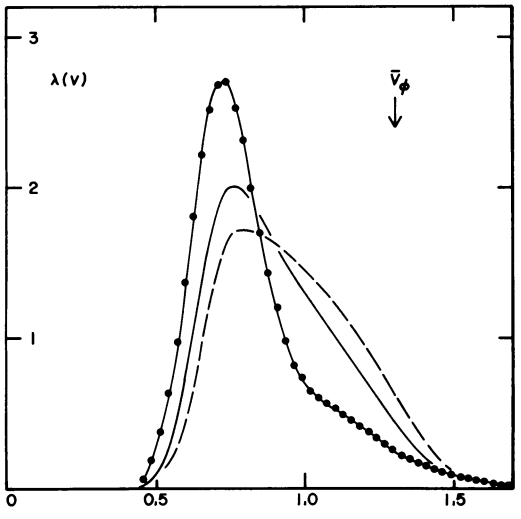

FIG. 10. *Comparison of measured frequency function of Escherichia coli ML30 (●) with theoretical functions calculated assuming continuous exponential growth (solid curve), and linear growth (broken curve) of all cells. In calculating the theoretical functions, the distributions of volume of dividing and newly formed cells were assumed to be Gaussian, with parameters as given by the best estimates for E. coli in Table 3. The arrow indicates the mean volume of dividing cells.*

$$N \int_v^\infty \lambda(x)\, dx$$

and the rate of increase in the number of cells in T is

$$kN \int_v^\infty \lambda(x)\, dx \qquad \text{(A-1)}$$

This rate has three components: cells lost by divisions in T, newly formed cells in T, and cells growing into T. The rate of loss of cells by division is

$$-kN \int_v^\infty \phi(x)\, dx \qquad \text{(A-2)}$$

Since two cells are formed from each dividing cell, the rate of formation of new cells in T is

$$2kN \int_v^\infty \psi(x)\, dx \qquad \text{(A-3)}$$

The rate of growth into T is seen to be

$$NV(v)\lambda(v) \qquad \text{(A-4)}$$

as follows: the number of cells in the interval $(v - dv, v)$ immediately below v is

$$N\lambda(v)\, dv \qquad \text{(A-5)}$$

Only the cells in the interval $(v - dv, v)$ can grow into T in time

$$dt = dv/V(v)$$

Dividing (A-5) by dt gives (A-4) the rate at which cells enter by growth. However, some of the cells in the interval may divide before growing into T. The The rate of divisions in this interval is

$$kN\phi(v)\, dv \qquad \text{(A-6)}$$

but dv can be as small as we like so that these divisions are an infinitesimal part of the rate $NV(v)\lambda(v)$. Thus, we can neglect the dividing cells, and a similar

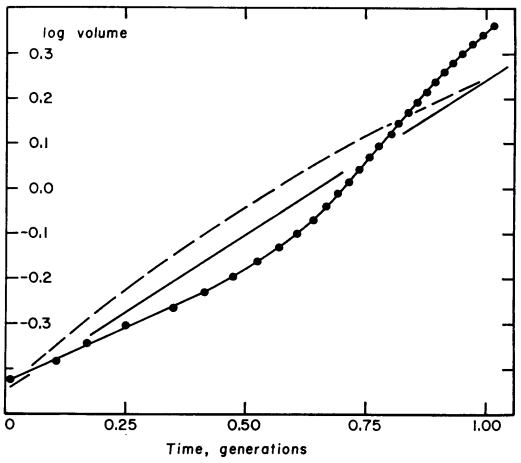

FIG. 11. *Growth of Escherichia coli ML30 expressed as logarithm of volume as a function of time. Symbols: ●, curve obtained by transformation of data shown in Fig. 3; solid line, theoretical curve for exponential growth; broken line, theoretical curve for linear growth.*

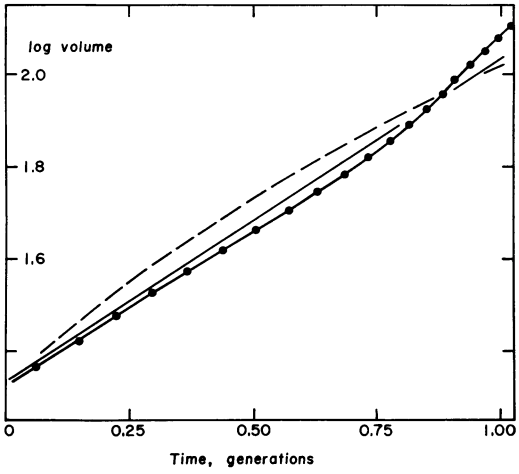

FIG. 12. *Growth of Azotobacter agilis expressed as logarithm of volume as a function of time. Symbols: ●, curve obtained by transformation of data shown in Fig. 4; solid line, theoretical curve for exponential growth; broken line, theoretical curve for linear growth.*

argument shows that we can neglect newly formed cells. The rate at which cells enter T by growth is thus given by the expression in A-4.

From A-1 through A-4, the argument above for the conservation of cells can be stated as

$$k\int_v^\infty \lambda(x)\,dx = V(v)\lambda(v) + 2k\int_v^\infty \psi(x)\,dx - k\int_v^\infty \phi(x)\,dx$$

By use of the fact that λ, ϕ, and ψ are frequency functions, and thus have integrals from zero to infinity equal to one, the familiar form of the equation of Collins and Richmond (4)

$$V(v) = \frac{k}{\lambda(v)}\left[2\int_o^v \psi(x)\,dx - \int_o^v \phi(x)\,dx - \int_o^v \lambda(x)\,dx\right] \quad \text{(A-7)}$$

can be obtained.

Differentiating A-7 gives a linear equation:

$$V'(v)\lambda(v) + V(v)\lambda'(v) = k[2\psi(v) - \phi(v) - \lambda(v)]$$

The solution of this equation is

$$\lambda(v) = \exp\left[-\int \frac{V'(v)+k}{V(v)}\,dv\right]\int\left(\frac{k}{V(v)}\cdot\exp\left[\int \frac{V'(v)+k}{V(v)}\,dv\right](2\psi(v) - \phi(v))\right)dv + C\exp\left[-\int \frac{V(v)+k}{V(v)}\,dv\right] \quad \text{(A-8)}$$

in which C is the constant of integration.

In deriving the Collins-Richmond equation, it is not necessary to assume that all cells of size v have the same growth rate. If, instead, their growth rates are distributed according to the frequency function $P(V)$, there are

$$NP(V)\,dV\lambda(v)\,dv \quad \text{(A-9)}$$

cells in the interval $(v - dv, v)$ that have growth rates between V and $V + dV$. The rate at which these cells enter T is obtained by dividing (A-9) by $dt = dv/V$, giving

$$NP(V)\,dV\lambda(v)V$$

Integrating this expression over V gives the total rate at which cells enter T by growth

$$N\lambda(v)\int_o^\infty VP\,(V)\,dV = N\overline{V}(v)\lambda(v)$$

where $\overline{V}(v)$ is the mean growth rate of cells of size v. Thus, the expression A-4 is valid whether or not the growth rate of cells of size v is distributed; if V is distributed, expression A-4 is independent of any parameter of the distribution other than the mean.

In the above derivation it is possible to show that, under reasonable conditions on V and λ (each has at most a finite number of discontinuities), V can have a discontinuity at v if and only if λ also has a discontinuity at v. Thus, if λ is continuous, V must also be continuous.

APPENDIX B

Estimation of the mean volumes of dividing and newly formed cells. The method used is a refinement of the method of Collins and Richmond (4). Let m_ψ and m_ϕ represent the means of $\psi(v)$ and $\phi(v)$, respectively, v_L the volume of the largest, and v_S the volume of the smallest cells observed. Since each cell at division forms two daughter cells, then

$$m_\phi = 2m_\psi$$

It is assumed that the probability of occurrence of a newly formed cell at size less than v_S and the probability of occurrence of a division at a size greater than v_L are approximately equal. If $\psi(v)$ and $\phi(v)$ are symmetrical functions, with identical coefficients of variation, C, then

$$\frac{m_\psi - v_S}{Cm_\psi} = \frac{v_L - m_\phi}{Cm_\phi} \quad \text{(B-1)}$$

and, from (B-1),

$$m_\phi = v_L/2 + v_S \quad \text{(B-2)}$$

The relationship in B-2 must be modified if $\psi(v)$ and $\phi(v)$ are not symmetrical functions, to give

$$m_\phi = A(v_L/2 + v_S)$$

The coefficient A is greater than unity if $\phi(v)$ is positively skewed, and less than unity if $\phi(v)$ is negatively skewed. A range of A of 0.67 to 1.5 will encompass most likely cases.

APPENDIX C

Calculation of $f(\tau)$ from growth rate as a function of size. The approach necessary for these calculations was developed for the case of continuous exponential growth by Powell (18). We present this general case.

The frequency function of interdivision time, $f(\tau)$, is the frequency function of the joint distribution of $\psi(v)$ and $g(v)$ after transformation of the variable from volume to time. Let $\psi_T(t)$ and $g_T(t)$ denote the transformed frequency functions such that

$$\psi_T(t)\,dt = \psi(v)\,dv$$

and

$$g_T(t)\,dt = \psi(v)\,dv$$

The time t corresponding to a volume v is

$$t = \int_{v_S}^v \frac{dx}{V(x)}$$

in which v_S is the minimal cell volume. Thus,

$$\psi_T(t) = \psi(v) \cdot V(v)$$

and

$$g_T(t) = g(v) \cdot V(v)$$

Assuming that $g_T(t)$ applies to each cell in $\psi_T(t)$, the frequency function of interdivision time is given by

$$f(\tau) = \int_o^\infty \psi_T(t) g_T(t + \tau)\, dt \qquad \text{(C-1)}$$

Conceptually, the function $g(v)$ determines $\phi(v)$, but in practice is calculated from the assumed $\phi(v)$. The probability of a cell of volume v dividing in the interval $(v, v + dv)$ is

$$\frac{g(v)\, dv}{\int_v^\infty g(x)\, dx}$$

The number of cells subject to this probability is the number growing through volume v in time dt, which from A-4 is

$$N\lambda(v)V(v)\, dt$$

The number of divisions in dt will be

$$\frac{N\lambda(v)V(v)g(v)\, dv\, dt}{\int_v^\infty g(x)\, dx}$$

The number of divisions in dt is also given by A-6; hence,

$$\phi(v) = \frac{\lambda(v)V(v)g(v)}{k\int_v^\infty g(x)\, dx} \qquad \text{(C-2)}$$

Equation C-2 can be rearranged and integrated to give

$$\ln \int_v^\infty g(x)\, dx = -\int_o^v \frac{k\phi(x)\, dx}{V(x)\lambda(x)}$$

or

$$\int_v^\infty g(x)\, dx = \exp\left[-\int_o^v \frac{k\phi(x)\, dx}{V(x)\lambda(x)}\right]$$

Differentiation gives

$$g(v) = \frac{k\phi(v)}{V(v)\lambda(v)} \exp\left[-\int_o^v \frac{k\phi(x)\, dx}{V(x)\lambda(x)}\right] \qquad \text{(C-3)}$$

ACKNOWLEDGMENTS

We wish to thank C. L. Squires for technical assistance.

The experimental work was supported by grants ARO-49-092-65-G95 and MD-49-193-65-G160 from the United States Army. Digital computations were financially supported by Public Health Service grant AI-05526 from the National Institute of Allergy and Infectious Diseases.

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