JOURNAL OF BACTERIOLOGY, Apr. 1978, p. 330-337
0021-9193/78/0134-0330$02.00/0

Vol. 134, No. 1
Printed in U.S.A.

Cell Growth and Length Distribution in *Escherichia coli*

J. CULLUM AND M. VICENTE†*
Department of Molecular Biology, University of Edinburgh, Edinburgh EH9 3JR, United Kingdom

Received for publication 12 December 1977

The length growth rate of an exponentially growing population of *Escherichia coli* B/r was calculated from the population length and birth length distributions. Cell elongation took place at a constant rate that doubled at a certain length. This change in rate was responsible for a sudden drop in the frequency of classes of cells longer than that length. Asymmetry in cell partition was able to generate cells both shorter and longer than the expected twofold range, but did not greatly modify the length distribution in between.

Exponentially growing populations of *Escherichia coli* are generally considered to be formed by cells that divide in two once in every generation time. The simplest model for the age frequency distribution of such a population follows an exponential curve as described by Powell (10). If cell length were a linear function of cell age, the length distribution of such an exponential population should fit an exponential curve as well. In contrast, real distributions of cell length are of a more complicated shape; they show a peak with a sharp drop after it, and they are not bound between a twofold range of lengths. Between birth and division, cells of *E. coli* elongate at a rate dependent on the mass growth rate of the culture. Several growth laws have been postulated for bacterial populations following linear or exponential growth (2). As far as elongation is concerned, it has been proposed by Donachie et al. (4) that the rate of elongation for a given growth rate doubles at a certain length that is the same for all growth rates. It has been recently postulated (1) that this increase in the rate of elongation takes place by the addition of new elongation sites when a certain length is reached. To determine whether elongation takes place with a constant, exponential, or stepwise growth law, we have calculated the rate of elongation during the cell cycle by means of a different, and in some respects more sensitive, approach than that used by Donachie et al. (4).

We have also devised a computer program that allows us to simulate bacterial elongation and division in exponentially growing populations following different growth laws and takes into account the proportion of unequal partition observed in natural populations (7-9). This explains some of the differences between the observed length distribution and that produced by simple models.

MATERIALS AND METHODS

Strain and growth conditions. *E. coli* B/r A, ATCC 12407, was used for all the experiments. Cultures were grown at 37°C in Oxoid nutrient broth no. 2 and kept at a cell density lower than 3×10^7/ml by dilution with prewarmed medium. For time-lapse photography, the same medium, solidified by the addition of 1.5% agar, was aerated after melting by being passed through a sterile Pasteur pipette; a thin smear was placed on a glass slide that was then warmed at 37°C before use and kept at this temperature throughout the experiment. Generation time (τ) was 20 min for liquid cultures (as measured by doubling in particles) and 22 min for cultures on agar slides (as estimated by doublings in total cell length).

Synchronous cultures were obtained by the membrane elution procedure of Helmstetter (6). The elution rate was fixed at 10 ml/min, and samples were taken for 1 min.

Particle counts. Particle counts were done in a Coulter Counter (model A).

Length measurements. Photographs of cells observed under phase-contrast optics were taken with a Zeiss Ultraphot microscope by the procedure of Donachie et al. (4). Samples from synchronous cultures were first concentrated 100-fold by centrifugation and suspension in a portion of supernatant fluid. Enlarged projections of the negatives were measured (3).

Computer program to model cell growth. The program to model cell growth was written in the IMP language and was run on an ICL4/75 computer. The culture being modeled was represented by a matrix. Each entry in the matrix represented the number of cells with a particular birth length that were of a particular age. Thus, the row of the matrix that an entry appeared in depended on its birth length, and the column depended on its age. At each time step, the entries corresponding to cells that divided were removed from the matrix, and the necessary numbers were added to the classes of newborn cells. (It was found that dividing the age into about 100 classes and the birth length into 150 classes gave satisfactory results.) The dividing-cell classes and the correct new-

† Present address: Departamento de Química Celular, Instituto de Biología Celular, Velázquez, 144, Madrid-6, Spain.

born-cell classes were selected according to the growth and division laws being used.

When outputs of the length distribution and newborn cell length distribution were required, they could be calculated from the matrix by using the growth law to find the length from the birth length and age.

The length distribution settled down to a fairly constant distribution after 5 to 10 generations, starting from various initial distributions.

Calculation of growth rate and length distributions. To calculate the growth rate and length distributions, we wrote two computer programs that used equation 1 below. The first program used the observed birth length and population length distributions to calculate the growth rate; the second used the observed birth length distributions and assumed growth law to calculate the population length distribution. In both cases, cell division was ignored, so the calculations are only valid for lengths less than those at which significant amounts of cell division occur. The length distributions were considered linear between the centers of the measured classes.

RESULTS

Calculation of length growth rate from length distribution and birth length distribution. Collins and Richmond (2) found an equation relating the growth rate [$g(x)$] of a cell of length x in an exponentially growing culture to the distribution of cell length (λ), length of newborn cells (ψ) and the length of dividing cells (ϕ). (λ, ψ, and ϕ are actually the probability density functions of those distributions.) This equation is:

$$g(x) = \frac{K}{\lambda(x)} \int_0^x [2\psi(y) - \phi(y) - \lambda(y)]\, dy \quad (1)$$

where K is the exponential growth rate constant. This assumes that the length growth rate is a function of the length alone and does not depend on the age or past history of a cell. The population must be growing exponentially with constant distributions of length and age.

The distribution of length of newborn cells was measured in samples from a membrane eluate (Fig. 1). Cell lengths were measured in samples from an exponential culture to obtain its length distribution (Fig. 2a). It was apparent from the lengths of cells with septa that very few cells divided at a length of less than 5 μm. This was also shown by the lengths of newborn cells (dividing cells must be approximately twice as long as newborn cells). We were able to ignore dividing cells (i.e., take $\phi \equiv 0$), therefore, and use the measured length and cell length distributions to estimate $g(x)$ (Fig. 2b). This gave a growth rate that was constant at lengths between 2 μm and 3.5 μm, doubled between about 3.5 μm and 4.5 μm, and was constant again at the new value until the neglect of dividing cells made the calculation invalid (about 5 μm).

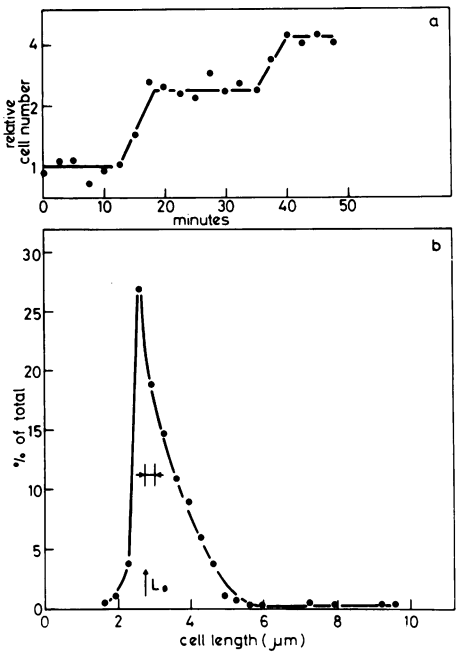

FIG. 1. *Length distribution of a population of newborn cells. One sample of the eluate from a membrane was collected for 1 min. A portion was used to follow the increase in particles (a) giving a synchrony index of 1.11 by using the equation of O. H. Scherbaum (J. Protozool. Suppl. 6:17, 1959) for the first division. Cell density at the time of elution was 1.5 × 10^5 cells per ml. Another portion was used to measure cell length (b); 448 cells were measured to calculate the distribution. The segment between the arrows shows the range of lengths expected for cells born at L_B and collected in the eluate during a sampling time of 1 min.*

This calculation of the growth rate supported models in which the growth rate doubles at a given length (here about 4 μm). The point at which the rate changed corresponded to the drop after the peak in the cell length distribution. A linear growth law would give a constant growth rate at all lengths, and an exponential growth law would give a straight line passing through the origin.

To find whether knowledge of the growth rate was sufficient to predict cell length distributions, a model was used in which all cells were born with length L_B, grew to length $2L_B$, and divided in half. For this, equation 1 became:

$$\lambda(x)g(x) = K\left[2 - \int_0^x \lambda(y)\, dy\right] \text{ for } L_B < x < 2L_B \quad (2)$$

this can be solved analytically for simple forms of $g(x)$. Figure 3 shows the solutions for the

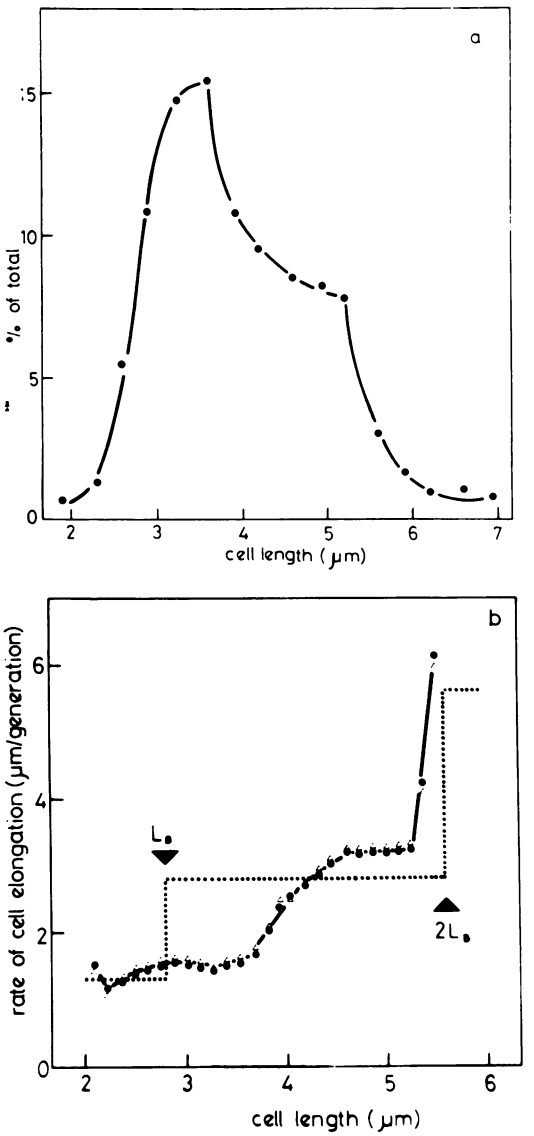

FIG. 2. *Rate of cell elongation during the cell cycle. The distribution of length in newborn cells shown in Fig. 1, the actual length distribution of an exponential culture shown in (a), and equation 1 (see text) were used to calculate the rate of elongation (b) for each cell length. Symbols:* ○, *values obtained considering the whole distribution in Fig. 1 as newborn cells;* △, *values obtained assuming that cells longer than 6.5 μm were not newborn and therefore were excluded from the calculation;* ——, *rate of elongation for each cell length;* · · · · ·, *course of rate of elongation as predicted by Donachie et al. (4) for* τ = 20 *min. As cell division was ignored for this calculation, the results are only valid up to about 5 μm.*

cases of linear growth, exponential growth, and growth that doubles its rate at about 4 μm.

None of the growth laws gave a very good fit to the observed distribution; this is not surprising, as the actual birth length distribution (Fig. 1b) showed a considerable spread. The result of a spread in birth lengths is to flatten the theoretical distributions. From equation 1, it can be seen that the value of λ will be smaller for shorter cells and larger for longer cells than that calculated on the assumption of constant birth length. This flattening means that the linear and exponential growth laws will not produce a clear peak, whereas the fit of the step growth law will be improved. We used equation 1 to calculate the population length distribution expected under the exponential and step growth laws when the observed spread in birth lengths is taken into account (Fig. 4). The birth length distribution in Fig. 1b was used, and cell division was ignored, so the calculation was not valid for lengths greater than 5 μm. As expected, the exponential growth law did not produce a high enough peak. The poor fit of the step growth law just after the peak probably reflected the fact that the actual change in growth rate (Fig. 2) is spread over 3.5 to 4.5 μm rather than being abrupt at 3.5 μm, as was considered for this calculation.

This simple model is useful for assessing qualitatively the effects of different changes in the growth law. We found that if the change in growth rate was spread over a range of lengths, rather than being instantaneous, then for a range similar to that of Fig. 2 (3.5 μm to 4.5 μm) a curve very similar to that in Fig. 3c was produced (except that the drop from the peak was not so abrupt), so the spread seemed to be of little importance. We also investigated in this way a variety of growth laws; one that gave a slightly better fit for the initial peak was a model in which the growth rate changed to 1.5 times its previous value rather than doubling (data not shown).

Asymmetric division. The deficiencies in the simple models that we have considered up to now suggested that any adequate model that aims at predicting the cell length distribution must have mechanisms to produce a spread in birth lengths. (As a consequence, it would also produce a tail of larger cells.) We thought that asymmetric division could be one such mechanism; when cells with a septum were examined, there were many in which the portions on either side of the septum were of different lengths.

The lengths of the two sister cells were measured in 205 dividing pairs of *E. coli* B/r in a sample of a population growing exponentially with a generation time of 20 min. Of these, 155 (76%) were symmetric by the criterion that the difference in length between the two sisters was smaller than 0.5 mm in the enlarged negative, that is, 0.33 μm in actual length. The remaining

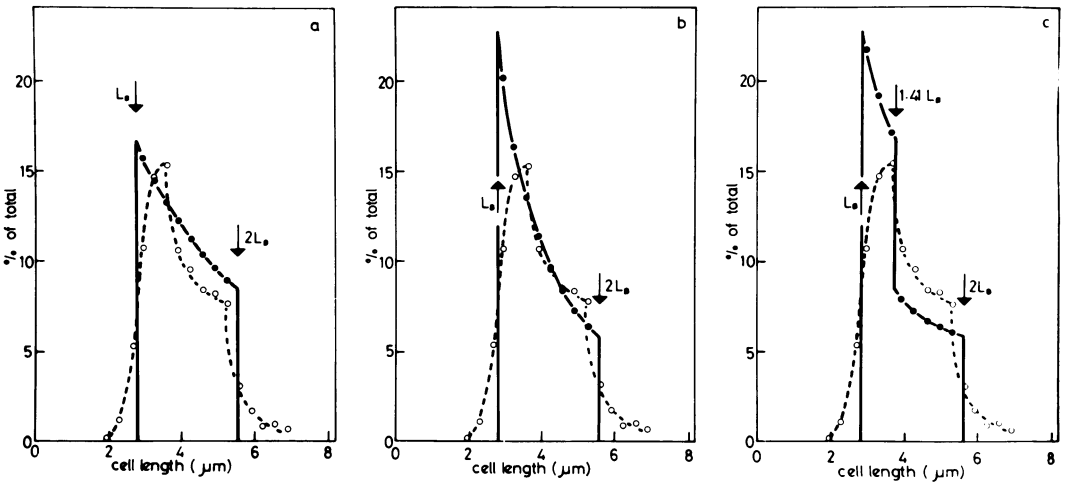

FIG. 3. *Comparison of theoretical and measured cell length distribution in E. coli populations. Symbols:* ○——○, *Observed cell length distribution (same as Fig. 2a);* ●——●, *theoretical distributions [λ (x)] calculated using equation 2 (see text) for the growth laws. (a) Rate of elongation is constant (linear growth law):*

$$\lambda(x) = \frac{4 \ln 2}{L_B} \exp\left[-\ln 2 \left(\frac{x}{L_B}\right)\right]$$

(b) Rate of elongation is proportional to length (exponential growth law):

$$\lambda(x) = \frac{2 L_B}{x^2}$$

(c) Rate of elongation is constant up to the length 2Λ, and then doubles (step growth law):

$$\lambda(x) = \begin{cases} \dfrac{2 \ln 2}{\Lambda} \exp\left[\dfrac{-\ln 2}{\Lambda}(x - L_B)\right] & \text{for } x < 2\Lambda \\ \dfrac{\ln 2}{2} \exp\left[\dfrac{\ln 2}{2}(2L_B - x)\right] & \text{for } x > 2\Lambda \end{cases}$$

We used values of birth length L_B = 2.77 μm and Λ = 1.95 μm.

50 pairs (24%) were asymmetric.

If we define the ratio of asymmetry as:

z = length of the long cell/length of the short cell

we find an experimental value of z = 1.17 (standard deviation, 0.08) for the measured asymmetric pairs.

Asymmetry has also been observed in cells from cultures growing at slower rates, but such measurements are less accurate because the mean cell length decreases as generation time increases (4).

Length measurements of dividing cells failed to show any preferential grouping of either the long or the short sister within asymmetric pairs with the length of cells in symmetric pairs. This suggests that asymmetry is more likely caused by inaccurate positioning of the septum within a dividing cell rather than as a consequence of abnormal elongation in one of the sisters. A computer model was used to study the effects of asymmetric division. A certain proportion (p) of cells was allowed to divide in the ratio of z:1, and the rest were allowed to divide symmetrically. These parameters for asymmetric division were considered to be the same for cells of all lengths.

Cell division was assumed to occur when cells had reached certain minimum length L_D and also reached a minimum age a_D. For the growth laws used, cells in the population that were born with length less than ½ L_D divided at length L_D at an age greater than a_D (i.e., had a longer generation time), and those with birth length greater than ½ L_D divided at age a_D with a length greater than L_D.

A growth law according to which the cell length growth rate doubled at lengths 2Λ, 4Λ, 8Λ, etc. was used. Results in Fig. 5 show the output of a computer run for two values of Λ. They have a defect similar to that in the distribution calculated for Fig. 3c; the peak is too

high. This was not due to the particular values of the parameters used for simulating asymmetric division, as the peak shape proved to be insensitive to variations in these parameters (results not shown). The birth length distribution generated by the model contains over 90% of the cells in two length classes, being therefore much more compressed than the observed one (Fig. 1b). Asymmetric division alone cannot account, then, for the observed cell length distributions, as it does not produce a large enough spread in newborn cell lengths; it does, however, account for some of the cells in the "tails" of the distribution at short and long lengths.

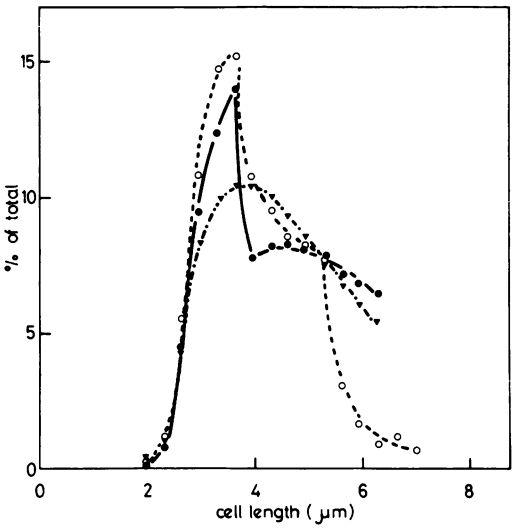

FIG. 4. *Calculation of cell length distributions assuming the observed birth length distribution and the exponential and step growth laws. The calculation is not valid for lengths greater than 5 μm because it ignores cell division. Symbols: ▼, Exponential law; ●, step law; ○, actual measured distribution.*

Varying the value of Λ changes the shape of the distribution; however, values of Λ much different from those in Fig. 5 will produce the drop from the peak at a length class quite different from that observed in real populations. One special case in which the doubling in rate occurs at birth, as suggested by Donachie et al. (4), is shown in Fig. 6. Here the minimum division length is 4Λ, so that the growth rate is constant over most of the cell cycle. In this case, the length distribution does not have a well-defined peak. A similar situation occurred when a simulation using an exponential growth law was run.

Higher limit for cell length. In our computer-modeled population, there was a lower limit for the length of a cell defined by the value

$$L_{min} = L_D/(1 + z)$$

that corresponded to the length of the short daughter of a cell that divides asymmetrically at

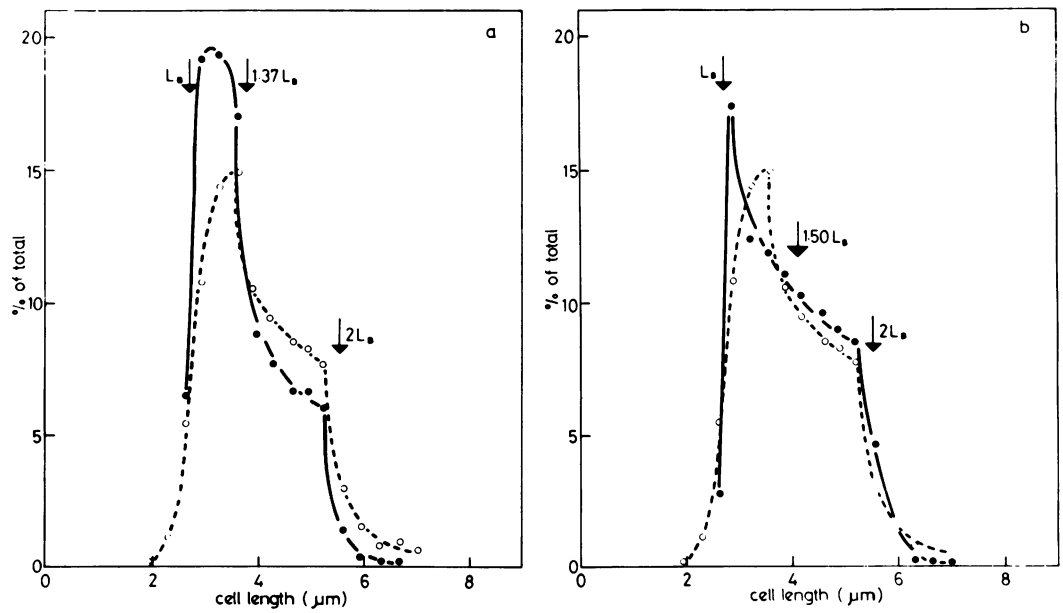

FIG. 5. *Distribution of length in a simulated population in which the rate of elongation was assumed to double at 3.8 μm when L = 1.37 · L_B (a) or when L = 1.50 · L_B (b). The simulation lasted 10 cycles. The computer was fed the values p = 24% and z = 1.17. Symbols: ●——●, distribution of length in simulation; ○- - -○, actual distribution of length measured in a population of E. coli B/r, shown for comparison.*

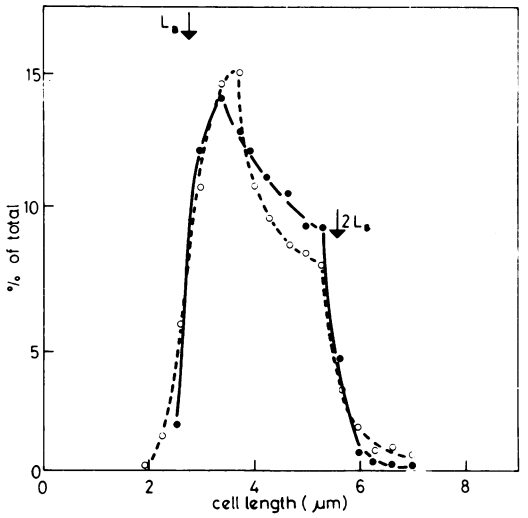

FIG. 6. *Distribution of length in a simulated population (●——●) in which the rate of elongation was considered to double at length 2Λ = 2.8 μm coincident with division and then at each successive doubling of total length. The simulation lasted 10 cycles. Parameters p and z were fixed as in Fig. 5. Distribution of length in actual population of E. coli (○- - -○) shown for comparison.*

length L_D. However, no upper limit was imposed in the model, as it could not be defined in terms of cell elongation alone. In any case, the proportion of cells longer than 8Λ after a simulation lasting 100 generations was less than 3% of the total, which made their contribution to the simulated distribution insignificant.

It seems possible, nevertheless, that although small increases in length will not shorten the generation time of a cell, increases in length large enough to contain additional units of initiation mass (5) can impose an upper limit to cell length. This follows from the reasoning that, in such long cells, additional rounds of DNA replication can be initiated and, once completed, will allow an additional division of the cell. These additional divisions could work as a compensating factor to limit the individual length of the progeny of very long cells.

One example of additional division is presented in Fig. 7, which shows a cell whose length at the time of cell separation was 13.50 μm instead of the minimum length at division of 5.30 μm for τ = 22 min (4). This cell divided for the second time 10 min after its first division; it divided for the third time approximately 20 min after the second division. At the time of the third division, some of the progeny lay within the length range of the control cell, which was dividing at approximately 22-min intervals, as expected.

DISCUSSION

The calculation of the rate of cell elongation shown in Fig. 2 agrees with the model of Donachie et al. (4) in that there is a doubling in rate at a certain cell length. They estimated, from a shift-up experiment with a population of homogeneous size, that the change occurs at 2.8 μm. However, our results indicate that such a change occurs between 3.5 and 4.5 μm. This spreading could be due either to a spread in each individual cell or to differences between the cells. It is not technically feasible to observe such spreading if the experimental approach of Donachie et al. (4) is used, which probably accounts for part of the discrepancy between the two results.

The results of Collins and Richmond for *Bacillus cereus* (2) are not incompatible with our model. They used a normal distribution for their distribution of newborn cells, which is not a good approximation when compared with our results in Fig. 1. They also introduced a mathematical smoothing step, thereby reducing the chances of finding abrupt changes in rate. Our results suggest that such changes in rate are responsible for the sudden drop observed in cell length distributions. In our simulations, some degree of smoothing was achieved by considering asymmetric divisions; this partially reproduced the tails at both ends of the distribution without affecting the peak and drop regions. Asymmetry in division was nevertheless insufficient to produce a spread in birth lengths as wide as the one found in a population from a membrane eluate (Fig. 1). It was found that, after a simulation lasting 100 cycles, 90% of the newborn cells fell within the two classes adjacent to the expected length at birth.

As the distribution of newborn cells is crucial in relating the growth laws to the distribution of exponential populations (Fig. 4), this failure to generate a large spread in the newborn population explains why our simulated populations do not show a good quantitative fit to the measured distribution. It is likely that some discontinuous events during the cell cycle, particularly at the the time of cell division, affect the patterns of partition or elongation in a way unaccounted for by our model, thus producing a wider spread in lengths at birth. In any case, asymmetry by itself can lead to fluctuation in the generation times of individual cells within a population. We explain these fluctuations by considering that cells born shorter than the normal birth length for the growth rate of the culture are delayed in division, whereas those whose birth length is the correct value or larger are not. Those cells longer than normal will not alter their generation times, at least for increases smaller than necessary to

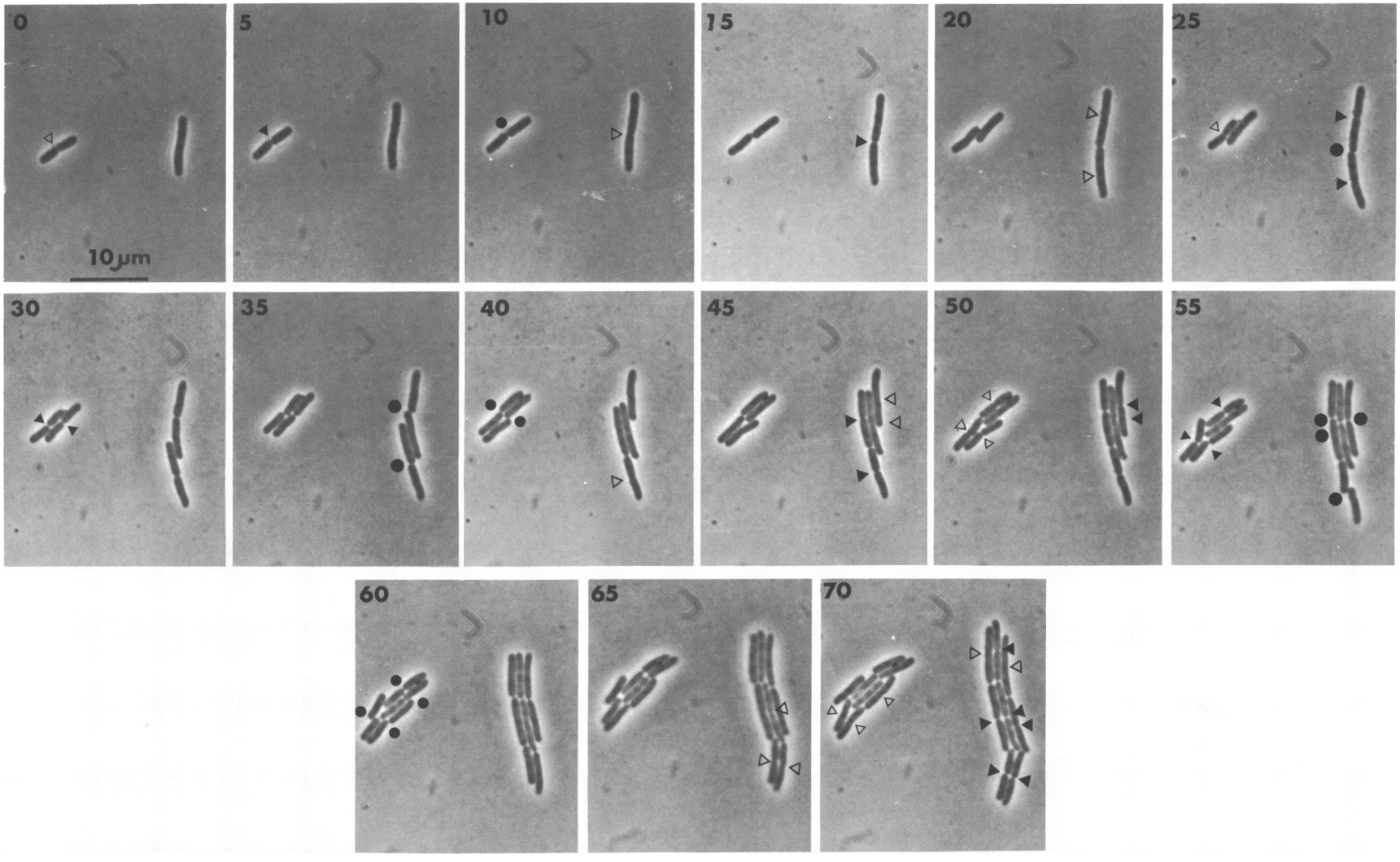

FIG. 7. *Division pattern of a cell whose length at division is 2.5 times the L_D predicted by Donachie et al. (4) for τ = 22 min (right-hand side of each frame) compared with a cell that falls within the predicted range of L_D (left-hand side of ea frame). Symbols (reflecting events observed in the enlarged negative rather than those visible in the print): ▽, Beginning of septum formation; ▼, completion of the septum; ●, cell separation. Frames were taken every 5 min.*

accommodate additional units of initiation mass. However, bigger increases could shorten the generation time for an individual and serve at the same time as a correcting mechanism to impose a higher limit to cell length (Fig. 7).

That cells of the same length can have different ages provides a theoretical explanation for why methods to obtain synchronous populations based on size selection give far-from-perfect results. It follows, as well, that even cultures derived from a single cell will eventually lose synchrony after a few generations, as they will then contain cells whose birth length is smaller than expected for the generation time of the culture. These cells will suffer a delay before they can divide. This agrees with experimental evidence derived from the analysis of cultures obtained from a single cell in which synchrony was lost after no more than 16 generations (P. Meacock, personal communication). The details of the division process are not yet well enough known to allow us an interpretation of asymmetry in division at the molecular level. It is possible that, if septum formation is regulated by some effector present in only a small number of molecules per cell, local differences in its concentration could produce individual diversity at the time of partition.

ACKNOWLEDGMENTS

We thank W. D. Donachie for very helpful criticism both at the experimental and writing stages of this paper and P. Broda for his encouragement. We also thank J. F. Collins for pointing out the relation between growth laws and length distributions.

LITERATURE CITED

1. **Begg, K. J., and W. D. Donachie.** 1977. Growth of the *Escherichia coli* cell surface. J. Bacteriol. **129**:1524-1536.
2. **Collins, J. F., and M. Richmond.** 1962. Rate of growth of *Bacillus cereus* between divisions. J. Gen. Microbiol. **28**:15-33.
3. **Donachie, W. D., and K. J. Begg.** 1970. Growth of the bacterial cell. Nature (London) **227**:1220-1224.
4. **Donachie, W. D., K. J. Begg, and M. Vicente.** 1976. Cell length, cell growth and cell division. Nature (London) **264**:328-333.
5. **Donachie, W. D., N. C. Jones, and R. Teather.** 1973. The bacterial cell cycle, p. 9-44. *In* J. M. Ashworth, and J. E. Smith (ed.), Microbial differentiation. Cambridge University Press, Cambridge.
6. **Helmstetter, C. E.** 1969. Methods for studying the microbial division cycle, p. 327-363. In J. R. Norris and D. W. Ribbons (ed.), Methods in microbiology, vol. 1. Academic Press Inc., New York.
7. **Koch, A. L.** 1966. On evidence supporting a deterministic process of bacterial division. J. Gen. Microbiol. **43**:1-5.
8. **Koch, A. L., and M. Schaechter.** 1962. A model for statistics of the cell division process. J. Gen. Microbiol. **29**:435-454.
9. **Marr, A. G., R. J. Harvey, and W. C. Trentini.** 1966. Growth and division of *Escherichia coli*. J. Bacteriol. **91**:2388-2389.
10. **Powell, E. O.** 1956. Growth rate and generation time of bacteria, with special reference to continuous culture. J. Gen. Microbiol. **15**:492-511.