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Elongation of Rod-shaped Bacteria

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Three models relating cell length to generation time are considered for rod-shaped bacteria growing under steady-state conditions; all three presuppose linear elongation. The first model assumes that the rate of elongation is proportional to the instantaneous number of chromosome replication forks per cell; the others, that it is inversely related to the generation time and doubles a fixed time prior to cell division. One of these (model 2) treats this relationship as continuous, with the doubling occurring during the last division cycle (at chromosome termination), while the other is a discrete model in which the doubling in rate takes place at chromosome initiation. Expressions are derived for mean cell length and length at birth in each case.

Comparison with experimental data on *E. coli* B/r using non-linear least-squares techniques results in an excellent fit for model 2 and unsatisfactory ones for the others, the best estimate for the time at which the rate doubles being 15·3 min prior to cell division and for the minimum length at birth (i.e., as the growth rate of the culture tends to zero), 1·47 μm.

The functional relationship between cell radius and generation time implied by model 2 is also presented. This model again produces a good fit to the experimental data and provides, for the first time, a direct

estimate of the volume/origin ratio at initiation of chromosome replication: 0·35 ± 0·05 μm^3 (S.E.).

The results obtained here are compared with various qualitative observations reported in the literature and with such numerical data as are available.

1. Introduction

Under steady-state growth, rod-shaped Gram-negative bacteria, such as *Escherichia coli* and *Salmonella typhimurium*, increase in length but maintain an approximately constant diameter (Marr, Harvey & Trentini, 1966). Faster growing cells, however, are not only longer but also thicker (Schaechter, Maaløe & Kjeldgaard, 1958); a change in cellular diameter must therefore occur during a limited period of time following transfer to a richer medium (Kjeldgaard, Maaløe & Schaechter, 1958) (i.e. a "shift-up"). This seems to be because the response of envelope synthesis is delayed relative to that of total mass increase after the transition (Previc, 1970; Pritchard, 1974). The resulting reduction in surface/mass ratio must be accompanied by a corresponding increase in the diameter/length ratio (Pritchard, 1974; Zaritsky, 1975) if the cells are to maintain a constant density.

One cannot readily conceive of a mechanism in which envelope is synthesized exponentially with the same rate-constant as that of mass while under steady-state growth but responds differently during the transition period. Furthermore, all macromolecules involved in the flow of information (DNA, RNA, protein) are synthesized with elongation rates that are independent of the doubling time of the cell (Maaløe, 1969) and so, most likely, of its age as well. The simplest description of surface extension should thus be based on constant elongation rates (Previc, 1970; Ward & Glaser, 1971; Pritchard & Zaritsky, 1972; Zaritsky & Pritchard, 1973). In order to maintain the steady state, it is of course necessary for each cell to double its elongation rate during its lifetime. (Such elongation can be envisaged as due to a fixed rate of deposition of envelope components along circular zones, the number of which change discretely.) Unfortunately, a model in which cells grow linearly, independently of growth rate, and double this rate once during their lifetime, is very obviously incompatible with the experimental evidence (Zaritsky & Pritchard, 1973). In order to improve the linear model, some type of dependence on growth rate must be introduced. Here we treat two simple cases, one continuous and the other discrete, including in each the provision that the cell division cycle be coupled to the chromosome replication cycle (Jacob, Brenner & Cuzin, 1963; Pierucci & Zuchowski, 1973). For continuous dependence, we take the rate of elongation to be inversely proportional to the doubling time of the culture τ and to double

a fixed time ($<\tau$) before division (at the termination of chromosome replication: Pritchard & Zaritsky, 1972; Zaritsky & Pritchard, 1973). In the discrete case, the number of zones is considered to double at chromosome initiation. That way the overall rate of elongation is made to depend on the set number (Cooper & Helmstetter, 1968), with the rate per zone remaining constant. We also analyse a more complex discrete model, one first proposed several years ago (Donachie & Begg, 1970; Donachie, Jones & Teather, 1973), in which the elongation rate is proportional to the instantaneous number of replication forks per cell.

In the following sections, the predictions of each of these three models are compared with experimental data of average cell length measured under different nutritional conditions, and least-squares estimates are obtained for the various parameters involved. For historical reasons, the three approaches described above are referred to here as models 2, 3 and 1, respectively.

2. Theory

All three models presuppose a constant rate of elongation (α); the latter two require that this rate double at some predetermined time d prior to cell separation (at age τ). If we define the length L as L_0 at birth and as $L_{\tau-d}$ at age $\tau-d$, it follows directly from the definition of d that

$$(L_{\tau-d}-L_0)/(\tau-d) = \alpha = \tfrac{1}{2}(2L_0-L_{\tau-d})/d,$$

whence

$$L_0 = \alpha(\tau+d). \tag{1}$$

Average cell length is defined in the usual way as

$$\bar{L} \equiv \int_0^\tau L(a)\nu(a)\,da \Big/ \int_0^\tau \nu(a)\,da,$$

where $\nu(a)\,da$ is the number of cells at age a; in normalized form (Powell, 1956), $\nu(a) = (2/\tau)(\ln 2)2^{-a/\tau}$. Upon substitution and integration we get

$$\bar{L} = \frac{\alpha\tau}{\ln 2}\,2^{d/\tau}. \tag{2}$$

MODEL 1

In this model, the mean length $\bar{L}$ is proportional to the average number of genome equivalents per cell $\bar{G}$, so that

$$\bar{L} = k_1\bar{G} = \frac{k_1\tau}{C\ln 2}\,2^{D/\tau}(2^{C/\tau}-1), \tag{3}$$

where C is the time for a replication point to traverse the genome and D is the time between the end of a round of replication and the subsequent cell

division (Cooper & Helmstetter, 1968). Length at birth can be obtained (Rasmussen, personal communication) by summing the contributions from all the replication forks in a cell, each weighted according to its lifespan in a single cycle (Cooper & Helmstetter, 1968),

$$L_0 = \frac{k_1}{C}\{2^n C + (2^n - 2^m)D - [(n-1)2^n - (m-1)2^m]\tau\}, \tag{4}$$

where

$$\begin{aligned} n &\equiv [(C+D)/\tau] \\ m &\equiv [D/\tau]. \end{aligned} \tag{5}$$

Thus $n(m)$ is the number of doublings that takes place from initiation (termination) of a round of chromosome replication to cell separation subsequent to completion of that round.

MODEL 2

Here the rate of elongation α is inversely proportional to the doubling time τ, $\alpha = k_2/\tau$, so that equation (1) becomes

$$L_0 = k_2(1 + d/\tau) \tag{6}$$

and equation (2),

$$\bar{L} = \frac{k_2}{\ln 2} 2^{d/\tau}. \tag{7}$$

[For the case $d \geq \tau$, which is of no interest here, equation (6) must be replaced by $L_0 = k_2(1 + d/\tau - m')$, where $m' \equiv [d/\tau]$; equation (7) remains unchanged.]

MODEL 3

Expressions for L_0 and $\bar{L}$ in terms of this model can be obtained directly by substituting $2^n k_3$ for α and $(C + D - n\tau)$ for d (Pritchard, Barth & Collins, 1969) in equations (1) and (2), giving

$$L_0 = 2^n k_3[(C+D) - (n-1)\tau] \tag{8}$$

and

$$\bar{L} = \frac{k_3\tau}{\ln 2} 2^{(C+D)/\tau}, \tag{9}$$

where k_3 is a constant.

MEAN SQUARE RADIUS

If we approximate the geometry of the cell by a perfect cylinder, then we can define the mean square radius $\overline{R^2}$ by $\overline{R^2} = \overline{V/\pi L}$, where V is the cell volume. Model 2 provides an expression for the length L as a function of

age:

$$\begin{aligned} L/L_0 &= 1 + \frac{a}{\tau+d} \quad \text{for} \quad 0 \le a \le \tau - d \\ &= 2\,\frac{a+d}{\tau+d} \quad \text{for} \quad \tau - d \le a \le \tau \end{aligned} \tag{10}$$

with L_0 given by equation (6). [The same expression holds for model 3, but in that case L_0 must be obtained from equation (8).] For exponential volume growth $V = V_0 2^{a/\tau}$ where V_0 is the cell volume at birth and is related to V_i, the volume/origin ratio at initiation of replication (Pritchard *et al.*, 1969; Donachie, 1968), by the equation

$$V_0 = \tfrac{1}{2} V_i 2^{(C+D)/\tau}. \tag{11}$$

After substitution and averaging, we get (for model 2)

$$\overline{R^2} = \frac{V_i 2^{(C+D)/\tau}}{2\pi k_2} \ln \frac{4\tau}{\tau+d} \ln 2. \tag{12}$$

For linear volume growth at a rate that doubles once during the cell cycle, coincident with that of elongation, $V(a)$ is given by an expression analogous to equation (10), and R is constant. As before, V_i is the value of V at $a = \tau - (C+D)$, so that (again for model 2)

$$\begin{aligned} \overline{R^2} = R^2 &= \frac{\tau V_i}{(\tau+\delta)\pi k_2} \quad \text{for} \quad \delta \le \tau \\ &= \frac{\tau V_i}{2\delta\pi k_2} \quad \text{for} \quad \delta \ge \tau \end{aligned} \tag{13}$$

where [see equation (5)]

$$\delta \equiv (n+1)\tau - (C+D) + d. \tag{14}$$

3. Materials and Methods

E. coli B/r (strain H266, Laboratory of Microbiology, Utrecht, The Netherlands) was grown in minimal salts medium (Helmstetter & Cooper, 1968) supplemented with either 0·04% alanine (τ = 160 min), 0·1% succinate (τ = 105 min), 0·04% alanine and 0·04% proline (τ = 72 min), 0·1% glycerol (τ = 60 min), 0·1% glucose (τ = 45 min), 0·4% glucose and 1·0% Bacto Casamino acids (Difco) (τ = 30 min) or 0·4% glucose and 1·0% Casein Hydrolysate (Sigma) (τ = 24 min).

Cells fixed in 0·1–0·2% OsO_4 were prepared for electron microscopy by the agar filtration technique (Kellenberger & Kellenberger, 1954). Agar (2%) was made up in distilled water and dried to about 80% of its original weight. A perforated plastic film was superimposed by pouring a solution of

0·4% Parlodion (Mallinckrodt) in amylacetate on the agar slant pre-cooled below the dew-point in order to induce the formation of tiny holes by water vapor condensation (Fukami & Adachi, 1965). Small drops of cell suspension were deposited on the film and allowed to drain away; those that failed to do so within 60 min were rejected.

Cell dimensions were obtained from electron micrographs projected to a final magnification of 12,000; at least 100 cells were measured at each growth rate. The length of new-born cells (L_0) was estimated from experimental length distributions by means of the expression (Woldringh, 1974; Harvey, Marr & Painter, 1967) $L_0 = \frac{1}{2}(L_{min} + \frac{1}{2}L_{max})$, where L_{min} and L_{max} are the minimum and maximum observed lengths, respectively.

4. Results

Experimental values of $\bar{L}$ as a function of τ are listed in Table 1, including four points gleaned from the literature. A non-linear least-squares analysis (Marquardt, 1963) was carried out for each of the three models using the above 11 points. The second model gives a very good fit ($P < 0{\cdot}1\%$) with $d = 17{\cdot}1$ min; the first fits the data best with $C < 0$ and $D = 42$ min. When restricted to positive parameters, the fit of model 1 remains good ($P < 1\%$) but with $C = 31$ min and $D \simeq 0$; when constrained to $C \geq 40$ min and

TABLE 1

Mean length $\bar{L}$ at various doubling times τ

τ (min)	$\bar{L}$(μm)	source
160	2·39	a
98	2·27	b
72	2·64	a
60	2·53	a
45	2·62	a
40	3·11	c
33	2·72	b
32	3·14	a
31	3·21	a
24	3·34	a
22	3·80	b

a This paper.

b Obtained as described in Table 1 of Zaritsky (1975) from data of Schaechter *et al.* (1958), normalized to 2·53 μm at $\tau = 60$ min.

c Obtained as described in Table 1 of Zaritsky (1975) from data of Zaritsky & Pritchard (1973), normalized as in (b) above.

$D \geq 10$ min, however, the fit deteriorates completely. It is clear that this model is unsatisfactory, the estimated values obtained for C and D lying far outside the range generally accepted in the literature (Cooper & Helmstetter, 1968; Pritchard *et al.*, 1969; Spratt & Rowbury, 1971; Kubitschek & Freedman, 1971; Kubitschek, 1974*a*). The regression equation based on model 3 is not able to explain any of the variance in $\bar{L}$ as a function of τ, from which we infer that this model too is not applicable.

The intrinsic variation in L_0 is much smaller than in $\bar{L}$ for any fixed value of τ, and so we turn to L_0 and equation (6) in order to improve our estimates of the parameters of model 2. The resulting (linear) regression line is shown in Fig. 1, together with the original data (circles). The fit is seen to be excellent ($r = 0{\cdot}99$, $P < 0{\cdot}01\%$), and a one-sample runs test (Siegel, 1956) is not significant, implying random deviations of the experimental points about the line. The least-squares estimate for d is 15·3 min [conservative joint 95% confidence interval (Brownlee, 1965): 11·9–19·3 min] and for k_2, 1·47 ± 0·07 μm.

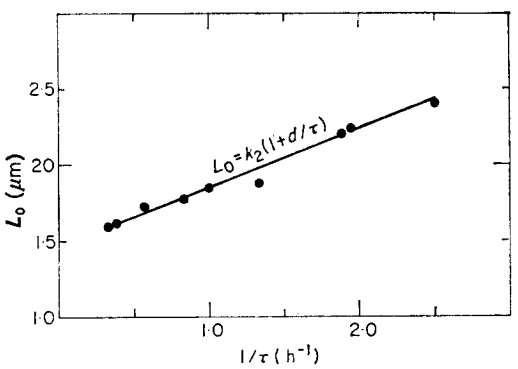

FIG. 1. Length at birth L_0 as a function of growth rate $1/\tau$. Circles: experimental data; solid line: linear least-squares fit ($r = 0{\cdot}99$) to data based on model 2, equation (6).

Having established reasonably precise values for d and k_2, we are now in a position to obtain estimates for $(C+D)$ and for V_i, the volume/origin ratio at initiation of chromosome replication. For this purpose we use measurements of mean square radius $\overline{R^2}$ at various values of τ. Table 2 lists the raw data and includes, for convenience, mean diameters as well. A non-linear, least-squares analysis (Marquardt, 1963) was carried out in order to fit equation (12) to these points. [Exponential rather than linear volume growth was used because the latter gives rise to a discontinuous function [equations (13) and (14)] and as such is not amenable to conventional least-squares analysis.] The best estimates obtained were for $(C+D)$, 78 ± 6 min (S.E.) and for V_i, 0·35 ± 0·05 μm^3.

TABLE 2

Mean square radius $\overline{R^2}$ *and mean diameter* 2R *at various doubling times* τ

τ(min)	$\overline{R^2}(\mu m^2)$	$2R(\mu m)$
160	0·047	0·430
105	0·064	0·500
72	0·053	0·460
72	0·073	0·538
60	0·059	0·483
47	0·087	0·592
45	0·097	0·620
31	0·182	0·845
24	0·213	0·920

4. Discussion

In this work, several aspects of the growth and division of rod-shaped bacteria were investigated. There are sound reasons for believing that such cells elongate at rates proportional to the number of growth zones they contain (Zaritsky & Pritchard, 1973; Donachie & Begg, 1970), and all models analysed were based on this assumption. Measurements were made of cell length in steady-state cultures of *E. coli* B/r (strain H266) under various nutritional conditions, the best fit to the experimental data being obtained under the hypothesis that the length increase produced by each of these zones is inversely proportional to the doubling time of the culture (model 2). Such a proposal was originally put forward by Zaritsky & Pritchard (1973) after studying the effects of thymine limitation on thymineless mutants in several strains of *E. coli*. This is also the conclusion arrived at as the result of volume and length determinations (Shannon & Rowbury, 1975) of a temperature-sensitive division mutant of the closely-related species *S. typhimurium*. The recent finding (Sargent, 1975*a*) in a Gram-positive organism (*Bacillus subtilis*) that there too the rate of elongation per site is inversely proportional to τ, indicates that such behavior may well be general to cylindrical bacteria. Extensive studies on unbalanced envelope mutants of *E. coli* K–12 (Normark & Wolf-Watz, 1974) have led to the construction of a very similar model. Further support is provided by the ability of this strain, when containing copy mutants of the R1 plasmid, to omit one cell division but still retain its capacity to divide one mass-doubling later (Engberg, Hjalmarsson & Nordstrøm, 1975).

The experimental results presented here (Fig. 1) are highly compatible with model 2 and predict a linear rate of elongation that doubles about

17 min before division. This seems to be consistent with the following independent observations. The rate of synthesis of a wall component (Hoffman, Messer & Schwartz, 1972) [or the activity of the relevant enzymes (Hakenbeck & Messer, 1974)] in synchronous glucose growing cultures of *E. coli* B/r doubles about 20 min prior to division. Time-lapse cinematography of various strains of *E. coli* growing on nutrient agar indicates (Adler, Fisher & Hardigree, 1969) that such cells elongate linearly during one-half to three-quarters of their life cycle and then increase their elongation rate; it is difficult to determine the exact age at which this increase takes place, because the doubling time of the culture was not reported. (A doubling time of 30 min would imply a value for d of 8–15 min.) Results obtained with several strains of *Bacillus* are in accord with a linear rate of envelope synthesis (Sud & Schaechter, 1964; Sargent, 1975*b*) that doubles around the time the chromosomes complete their replication. The distribution of inducible membrane markers in the progeny of fully-induced *E. coli* K–12 populations during growth and division in the absence of the inducer suggests a correlation between the number of nuclei, which doubles at termination of chromosome replication, and the number of growth zones in the membrane (Autissier & Kepes, 1971). Finally, electron-microscope measurements of dimensional changes of *E. coli* B/r during a shift-up (to be reported elsewhere) are also consistent with a constant rate of cellular elongation that is inversely proportional to τ and doubles once during the cell cycle.

Recent electron-microscope observations (Woldringh, 1974, 1976) show that the time c between initiation of visible constriction and the subsequent cell separation in certain *E. coli* B/r strains [where D is independent (Kubitschek, 1974*a*) of τ] is constant at 11 min while in others [where D is not independent (Helmstetter, Cooper, Pierucci & Revelas, 1968) of τ] it is proportional to τ for $\tau > 60$ min. The linear-log model for wall-cytoplasm synthesis (Pritchard, 1974) predicts that c be always less than d, since visible constriction is considered a consequence of the doubling of the rate of envelope synthesis (at age $\tau - d$). It is thus not clear why c appears (Woldringh, 1976) to exceed d at doubling times greater than 100 min. The excellent fit of the theoretical curve to the L_0 data ($P < 0{\cdot}01\,\%$) and the outcome of the test for randomness (not significant) argue against the possibility of an even slight dependence of d on τ. One should perhaps look for the source of this discrepancy in the design of the experiment: in our experience, it is not easy to maintain a steady state under slow growth conditions.

In addition to d, measurements of L_0 as a function of τ also provide an estimate of k_2. This parameter can be interpreted as the value of L_0 in a stationary-phase culture ($\tau \to \infty$)—namely, the smallest cell length at birth

possible. Others have defined it as a unit cell length (Donachie & Begg, 1970), with an estimated value (in *E. coli* 15 T^- JG151 and a variant of B/r) of 1·7 μm. This is about 15% greater than our result of 1·47 μm, a difference that is probably an artefact of the measuring technique used (light microscopy there, electron microscopy here) but may possibly represent a true difference between two bacterial strains.

In calculating mean square radius, it was assumed that the cells grow in volume exponentially. Whether this is so, or whether they grow linearly and double their growth rate at some point during the cell cycle, is still in debate (Anderson & Bell, 1971). Total mass increases exponentially with time (Kubitschek, 1970), so that if volume is to grow exponentially as well, then cell density must remain constant. The linear elongation pattern of model 2 then requires that the radius change (in a predictable manner) during the cycle. Data of mean square radius at various growth rates (Table 2) were used to compute estimates of both $(C+D)$ and V_i. This derivation of $(C+D)$ is a completely independent one and it is gratifying to note that the value obtained, 78 ± 6 min, falls within the accepted (Cooper & Helmstetter, 1968; Pritchard *et al.*, 1969; Spratt & Rowbury, 1971; Kubitschek & Freedman, 1971; Kubitschek, 1974*a*) range of 62–75 min.

In marked contrast to the situation with $(C+D)$, there have been, to the best of our knowledge, no previous direct experimental determinations of the volume/origin ratio V_i. On the other hand, the literature does contain other types of measurements, from which it is possible to calculate V_i indirectly. Unfortunately, such derivations reveal unsuspected difficulties.

Harvey *et al.* (1967) determined the average volume of *E. coli* cells with a modified Coulter counter (Harvey & Marr, 1966) and their results correspond to a V_i of 0·54 μm^3 [for a $(C+D)$ of 70 min]. As first pointed out by Kubitschek (1969), however, these data are probably not very reliable because the orifice used was too short (Grover, Naaman, Ben-Sasson & Doljanski, 1969).

Using a properly designed orifice (Kubitschek, 1969), Kubitschek (1974*b*) obtained values for mean cellular volume as a function of growth rate that result in a V_i of 0·55 μm^3 [or 0·78 μm^3 after correcting for particle shape (Grover *et al.*, 1969)]. But the same data also imply a $(C+D)$ of 44 min (or 42 min, corrected), which is far too low.

Schaechter, Maaløe & Kjeldgaard (1958) measured optical density of *S. typhimurium* as a function of growth rate. One can arrive at a value for V_i from the least-squares analysis of their results (Maaløe & Kjeldgaard, 1966) provided that the optical density data can be converted into mean cell volume. Since optical density (at 450 nm) was found to be proportional to dry weight regardless of cell size (Schaechter *et al.*, 1958), it is sufficient

to ascertain the constant of proportionality, the density of the cell, and the amount of water it contains. All three factors are known from the literature, albeit with different degrees of accuracy. The proportionality constant was reported by Schaechter *et al.* (1958) to lie between 170 and 180 μg/ml/OD-unit. Cell density, on the other hand, has been determined more precisely (Koch & Blumberg, 1976); it has a mean of 1·116 g/ml and varies very little ($\simeq 1\%$) over the range of growth rates of interest here. The last factor, water content, is the most difficult of all to measure; the accepted value for *E. coli* is that published by Winkler & Wilson (1966), about 73%, although the authors themselves suggest that this figure may be somewhat high. And indeed, the estimate for V_i obtained from the optical data with these factors is higher than that reported here: 0·66 μm^3 compared to 0·35 μm^3. Of course, at least part of this difference may reflect a true difference between the two bacterial species involved, *E. coli* and *S. typhimurium*.

It should be pointed out that the entire analysis presented here has been based on the tacit assumption that both τ and L_0 are uniform throughout the population; in other words, that all cells divide into two equal parts exactly τ min after birth. There is not much doubt, however, that certainly τ, and probably L_0 as well, do vary rather widely (Schaechter, Williamson, Hood & Koch, 1962; Kubitschek, 1970) even under steady-state growth conditions. Nevertheless, we believe that such variation will not materially affect the general conclusions arrived at in this paper.

In all the models discussed above, the rate of linear extension is looked upon as the parameter directly related to the growth rate and to events within the cell cycle. But because mean cell diameter is also affected by growth rate, this implies that wall synthesis is determined by these events only indirectly, through its dependence on linear extension. From some points of view, however, it may actually be much simpler to conceive of the rate of wall synthesis as determining length increase, rather than the other way around. Models in which the rate of wall synthesis is directly related to the cell cycle and to the growth rate provide quite different interpretations to the experimental data and will be considered in a subsequent publication.

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