Growth and Form in Bacteria

1. INTRODUCTION

Heterotrophic bacteria grow and multiply in a wide range of aqueous inorganic salt solutions augmented by a variety of carbon sources. The efficiency with which carbon can be utilized in the production of cell mass, however, varies considerably, depending on the particular compound involved. In addition, whatever building blocks (such as amino acids or nucleosides) are used to supplement the growth medium need no longer be made by the cell itself, allowing more resources to be channeled into the protein-synthesizing machinery.[41,42] As a result, different growth rates can be obtained by modifying the composition of the external medium, and are effected through corresponding changes in the macromolecular composition within the cells.[61]

Bacteria cultivated on richer media not only grow faster, they are also bigger.[19,61] That is because, at any given temperature, the concentration of chromosome origins at initiation of DNA replication[14,55] and the period between this initiation and the subsequent cell division[10] are both essentially constant, independent of growth rate. Bacteria grown in the same medium but at different temperatures, on the other hand, do not differ substantially either in average cell size or in the proportions of their constituent molecules, but only in their rate of multiplication[56,61] (also C. L. Woldringh, to be published; A. Zaritsky, to be published). Under steady-state conditions, rod-shaped bacteria grow by elongation[44] (with, per-

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haps, small concomitant oscillations in diameter[66]), and so one would expect faster growing cells to be longer but not broader. Spherical bacteria can be regarded as a special case of bacillary forms[53] and will not be considered. Some gram-positive rods do indeed behave this way[26] but the most thoroughly investigated gram-negative strains, *Escherichia coli* and *Salmonella typhimurium*, do not—they are both longer and broader. The implication, therefore, is that mean surface area in gram-negative bacteria increases proportionally less with growth rate than mean cell mass. During steady-state growth, on the other hand, mass and surface area increase at the same relative rate in all cases, by definition.[35] Thus, their syntheses must respond differently to transitions between steady states. Such divergence indicates that different controls operate, and considerable effort has been expended in recent years in an attempt to elucidate the mechanisms involved.[68]

One possible explanation for the different responses of mass and surface area to changes in growth rate[53,54] is supported by studies with synchronized cultures. These have suggested that while mass is synthesized exponentially throughout the cell cycle, cell extension is not; rather, it seems to proceed at a constant rate that doubles once during the cell cycle.[1,21,59]

Most models advanced to explain this behavior associate such discrete rate changes with specific events in the cell cycle.[15,58,72] Some of these models have been found inadequate on the basis of an analysis of cell dimensions of *E. coli* B/r (strain H266) under various growth conditions, including nutritional shiftup.[19,57,68] Among the remaining contenders, the most plausible one[57] attributes surface growth to circular zones produced at a particular time during the cell cycle and which act thereafter at rates proportional to the growth rate; rate doubling is considered to coincide with the duplication of a particular gene the product of which is rate limiting for surface growth.[54]

This proportionality between the growth rate of the surface and that of the culture[72] has been challenged by Donachie and Begg[15] and again by Pierucci,[51] the latter suggesting instead a model in which growth zones act at a constant rate independent of the culture doubling time and have a finite existence: from initiation of chromosome replication to the corresponding cell division.

Recently, evidence has come to light that is in apparent contra-

diction with the view that cell dimensions and the chromosome replication cycle are coupled,[16,56,73] and several alternative mechanisms have been proposed to account for the rate doubling in surface growth,[16,57,58] all of them predicting that it occur a fixed time prior to division irrespective of growth conditions.

Current methods of obtaining synchronous cultures are by no means perfect, however, and the expected differences between linear and exponential growth are small.[33] As a result, the data are not sufficiently precise to distinguish linear from exponential growth. Moreover, a different experimental design, one based on the steady-state size distributions of various cell populations during asynchronous growth, has led to the suggestion that cell extension may indeed be exponential after all.[31] If that be the case, then quite another explanation for the variation in surface area with growth rate must be sought. Pritchard *et al.*[56] have proposed that envelope synthesis may be regulated by catabolite repression. Another possibility is suggested by the linear correlation that has been observed[5] between outer membrane protein content and surface area of *E. coli* B/r cells growing under different conditions. If a substantial number of proteins are essential for the formation of new envelope and if their syntheses are not regulated coordinately, then envelope could be made exponentially in proportion to total protein, and would then change more slowly with growth rate than does mass.[6,61] Alternatively, a particular protein content could be necessary for proper assembly of the bacterial envelope[58] regardless of the actual species involved.[5,25,63]

In the present review, we reexamine all the models by comparing the predictions of each to published observations[68] of the dimensions of *E. coli* B/r during nutritional shiftup. We begin by deriving rigorous analytical expressions for mean cell surface area (or length) as a function of time following the shift. These are then fitted to the data by standard nonlinear least-squares techniques, from which an approximate analysis of variance is carried out and estimates for the various parameters obtained together with their standard errors. Next we analyze the quality of each fit by examining the distributions of the residuals, testing them for randomness in a variety of ways. Such a multistage approach permits us finally to eliminate all of the contending models but one, on which we then perform several checks for consistency.

The implications of these findings are considered in some detail, and so is the as yet unresolved question of coupling between cell surface growth and the chromosome replication cycle. Certain reservations that we have concerning the basic assumptions underlying a shiftup analysis of this type are also pointed out.

2. THEORY

Model LC (Linear, Coupled). This model presupposes a constant rate of surface synthesis under steady-state conditions that is proportional to the growth rate and which doubles when some hypothetical controlling gene undergoes replication[54] d min before cell division. Thus,

$$D \leqslant d \leqslant C + D,$$

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.[10] Equations describing the behavior of the total surface area $A(t)$ as a function of time t following nutritional shiftup from a medium with doubling time τ_1 to one with doubling time τ_2, have been derived before,[20]† and here we merely quote the relevant results:

$$\frac{A(t)}{A(0)} = 1 + \frac{\tau_1}{\tau_2}(2^{t/\tau_1} - 1) \qquad \text{for } t \leqslant C + D - d \equiv c$$

$$= \frac{A(c)}{A(0)} + \nu_1 2^{c/\tau_1}\left[\frac{\nu_2 \ln 2}{\tau_2}(t - c) + 2^{(t-c)/\tau_2} - 1\right] \qquad \text{for } t \geqslant c$$

or

$$\frac{A(t)}{A(0)} = 1 + \frac{\ln 2}{\tau_2}\int_0^t \frac{2^{\theta/\tau_1}}{1 + \nu_2 2^{-\theta/\tau_2}}\, d\theta \qquad \text{for } t \leqslant c$$

$$= \frac{A(c)}{A(0)} + \frac{(\ln 2)2^{c/\tau_1}}{\tau_2}\int_c^t \frac{1 + \nu_1[2^{(\theta-c)/\tau_2} - 1]}{1 + \nu_2 2^{-\theta/\tau_2}}\, d\theta, \qquad \text{for } t \geqslant c \quad (1)$$

† There is a minor printing error in the final table of that article (p. 438), f appearing twice as a superscript in place of p.

where $\nu_1 \equiv (\epsilon_2\tau_2 + \ln 2)/(\epsilon_1\tau_1 + \ln 2)$, $\nu_2 \equiv (1 - \nu_1)/\nu_1$, and the ϵ_j are constants proportional to the ribosomal efficiency at τ_j. The first set of expressions applies when the rate of surface synthesis is assumed to change *abruptly* at $t = 0$ from being proportional to the initial growth rate to being proportional to the final growth rate (version LCA); the second refers to the case where the transition is *gradual*, the rate of surface synthesis being proportional at any instant to the current relative growth rate (version LCG). The latter set cannot be expressed analytically[11] unless τ_1 is an exact multiple of τ_2.

In order to calculate the average surface area $\bar{A}(t)$, we must divide each of the above expressions by the total number of cells $N(t)$:

$$\begin{aligned} N(t) &= N(0)2^{t/\tau_1} && \text{for } t \leqslant C + D \\ &= N(0)\sigma_1[1 + \nu_1(2^{t/\tau_2} - \sigma_2)/\sigma_2], && \text{for } t \geqslant C + D \end{aligned} \qquad (2)$$

where $\sigma_j \equiv 2^{(C+D)/\tau_j}$ and is the exponential equivalent of the set number at τ_j as defined by Cooper and Helmstetter.[10] Under steady-state conditions ($t \to \infty$),

$$\bar{A} = k_1 2^{d/\tau}, \qquad (3)$$

where k_1 is a constant, as has been found before.[57,72]

If the hypothetical gene is situated at a distance c from the origin of chromosome replication, $c = C + D - d$, then $x \equiv c/C$, its relative marker position 4a, 9a, 49a, 65a, is independent of τ. Substituting for d in Eq. (3) we get

$$\bar{A} = k_1 2^{D/\tau} 2^{C(1-x)/\tau}. \qquad (4)$$

Model LU (Linear, Uncoupled). This is identical to the one above but places no restrictions on the value of d, so that the doubling in the rate of envelope synthesis can occur either before initiation ($d > C + D$) or after termination ($d < D$). In the range $d \leqslant C + D$, $\bar{A}$ is given as before by Eq. (3) for the steady state and by the ratio of Eq. (1) to Eq. (2) in the transient case. For $d > C + D$ it is necessary to modify the specifications of the model somewhat: that part of d which exceeds $C + D$ (that is, $-c$) cannot be independent of τ; if it were, then cells about to double their growth rate (at age $\tau - d$) just before shiftup would be well beyond that stage just after, the time between doubling and initiation hav-

ing contracted during this infinitesmal interval from $-c$ to $-c\tau_2/\tau_1$. Thus, in order to ensure an orderly sequence of events, so that such cells do not reach initiation before having doubled their growth rate, or slightly older ones do not double their growth rate a second time without an intervening initiation, $-c$ must remain the actual time it takes a cell to grow from its mass at rate doubling to its mass at initiation, and the ratio of these two quantities must be constant. Consequently, the total number of growth zones in the culture $Z(t)$ is proportional to total cell mass $M(t)$. We then have[20]

$$Z(t) = N(0)2^{d_1/\tau_1}[1 + \nu_1(2^{t/\tau_2} - 1)],$$

where d_1 is the value of d prior to shiftup, and so

$$A(t) = A(0) + \int_0^t \beta(\theta)Z(\theta)\, d\theta =$$

$$A(0) + N(0)2^{d_1/\tau_1} \int_0^t \beta(\theta)[1 + \nu_1(2^{\theta/\tau_2} - 1)]\, d\theta.$$

Here $\beta(\theta)$ is the rate of surface synthesis per zone at time θ. Again there are two versions. Mathematically, these equations are a special case of Eqs. (1) and can be derived from them directly, though less instructively, by setting $c = 0$.

$$\frac{A(t)}{A(0)} = 1 + \frac{\ln 2}{\tau_2}(1 - \nu_1)t + \nu_1(2^{t/\tau_2} - 1) \qquad \text{for } t \geqslant 0$$

or

$$\frac{A(t)}{A(0)} = 1 + \nu_1(2^{t/\tau_2} - 1), \qquad \text{for } t \geqslant 0 \qquad (5)$$

depending on whether β changes abruptly (version LUA) or gradually (version LUG) at $t = 0$. Thus for $d > C + D$ in the transient case, $\bar{A}(t)$ is given by the ratio of Eq. (5) to Eq. (2); for the steady state ($t \to \infty$),

$$\bar{A} = k_2 2^{(C+D)/\tau}, \qquad (6)$$

where k_2 is a constant.

Model LP (Linear, Pierucci). According to this model[51] new

growth zones are formed coincident with chromosome initiation and remain active until the corresponding cell division $C + D$ min later. Quantitatively, this requires that the increase in the number of cells over a period of $C + D$ min be equal to the number of functioning growth zones at the beginning of the period:

$$Z(t) = N(t + C + D) - N(t).$$

It follows directly that $Z(0) = N(0)(\sigma_1 - 1)$ and that

$$A(t) = A(0) + \int_0^t \beta(\theta)\,[N(\theta + C + D) - N(\theta)]\,d\theta.$$

In addition, model LP takes β to be constant and so, by substituting for $N(t)$ from Eq. (2), the above expression can be integrated to give

$$\begin{aligned} A(t) &= A(0) + N(0)\beta\nu_1[\nu_2(\ln 2)\sigma_1 t + \tau_2\sigma_1(2^{t/\tau_2} - 1) \\ &\qquad - \tau_1(2^{t/\tau_1} - 1)/\nu_1]/\ln 2 \qquad \text{for } t \leqslant C + D \\ &= A(C + D) + N(0)\,\beta\nu_1\tau_2\sigma_1(\sigma_2 - 1)(2^{t/\tau_2} - \sigma_2)/\sigma_2 \ln 2, \\ &\qquad \text{for } t \geqslant C + D \end{aligned} \tag{7}$$

where

$$A(0) = N(0)\,\beta\tau_1(\sigma_1 - 1)/\ln 2. \tag{8}$$

The transient behavior is obtained by dividing Eq. (7) by Eq. (2); for the steady-state case ($t \to \infty$),

$$\bar{A} = k_3\tau[2^{(C+D)/\tau} - 1], \tag{9}$$

where k_3 is a constant.

Model E (Exponential). This model assumes that envelope growth is exponential, proportional to total protein synthesis, and that the delay between mass increase and surface extension found during nutritional shiftup[65,68] is the same as that between mass increase and protein synthesis (version EO). The rate of protein synthesis is proportional to the number of ribosomes, and since rRNA is a constant fraction of the amount $S(t)$ of stable RNA,[13] the total protein content $P(t)$ as a function of time t after the shift is[6,20]

$$P(t) = P(0) + \epsilon_2 S(0)\tau_2(2^{t/\tau_2} - 1)/\ln 2,$$

where $P(0) = \epsilon_1 S(0)\tau_1/\ln 2$ is the amount of protein produced dur-

ing τ_1. Average protein content per cell is obtained by dividing by $N(t)$:

$$\frac{\bar{A}(t)}{\bar{A}(0)} = \frac{P(t)/N(t)}{P(0)/N(0)} = \frac{1 + \nu_3(2^{t/\tau_2} - 1)}{2^{t/\tau_1}} \qquad \text{for } t \leqslant C + D$$

$$= \frac{1 + \nu_3(2^{t/\tau_2} - 1)}{\sigma_1[1 + \nu_1(2^{t/\tau_2} - \sigma_2)/\sigma_2]}, \qquad \text{for } t \geqslant C + D \quad (10)$$

where $\nu_3 \equiv (\epsilon_2\tau_2)/(\epsilon_1\tau_1)$; under steady-state conditions ($t \to \infty$)

$$\bar{A} = k_4 \frac{\epsilon\tau}{\epsilon\tau + \ln 2} 2^{(C+D)/\tau}, \qquad (11)$$

where k_4 is a constant.

It has been observed[68] that mean cell length decreases slightly shortly after shiftup. This could be caused by an immediate acceleration of the constriction process, by an increase in the osmolarity of the medium upon shiftup, or by the cells preferentially synthesizing rRNA, ribosomal proteins,[9,62] and the mRNA coding for them.[4] Thus, the accumulation of proteins participating in envelope assembly might lag behind mass increase more than total protein does. In order to account for a possible delay between operative shiftup and its effective onset (version Eδ), an additional parameter δ is introduced. During the first δ min after the shift, the rate of surface protein production is considered to be fixed at its preshift level:

$$dP/dt = \epsilon_1 S(0) 2^{t/\tau_1} \qquad \text{for } t < \delta$$

$$= \epsilon_2 S(0) 2^{t/\tau_2} \qquad \text{for } t \geqslant \delta$$

These give

$$\bar{A}(t)/\bar{A}(0) = 1 \qquad \text{for } t \leqslant \delta$$

$$= \frac{2^{\delta/\tau_1} + \nu_3(2^{t/\tau_2} - 2^{\delta/\tau_2})}{2^{t/\tau_1}} \qquad \text{for } \delta \leqslant t \leqslant C + D \quad (12)$$

$$= \frac{2^{\delta/\tau_1} + \nu_3(2^{t/\tau_2} - 2^{\delta/\tau_2})}{\sigma_1[1 + \nu_1(2^{t/\tau_2} - \sigma_2)/\sigma_2]} \qquad \text{for } t \geqslant C + D$$

which, of course, reduce to version EO and Eq. (10) for $\delta = 0$; Eq. (11) remains unchanged.

3. RESULTS

Experimental values of $\bar{A}(t)$ were calculated from the lengths and diameters of individual *E. coli* B/r cells (strain H266) measured[68] during shiftup from $\tau_1 = 72$ min to $\tau_2 = 24$ min, by assuming the cells to be right circular cylinders with hemispherical polar caps. The fit of the various models to the data was carried out by standard nonlinear least-squares techniques.[43] Because the equations that describe $\bar{A}(t)$ all have discontinuous derivatives at at least one point $C + D$ (in addition, model LC has a second discontinuity at $t = C + D - d$ and model $E\delta$ at $t = \delta$), we chose an algorithm that is derivative free.[7] The entire curve-fitting procedure was repeated using analytical derivatives and segmentation (Naaman and Grover,[76]) with essentially identical results. In all cases, it was decided not to compute $\bar{A}(0)$ directly from the experimental measurements at $t = 0$ but rather to treat it as one of the parameters of the model, to be estimated along with the others. An overall analysis of variance was performed for each in the usual way. These can only be approximate, of course, since the models are nonlinear. In addition, the residuals were thoroughly tested for randomness. This was done both nonparametrically, by the single-sample runs test[18] (the sign test and testing the number of runs up and down and the length of the longest run and the longest up-and-down run having been found not very sensitive), and parametrically, by mean-square successive differences[8] (or serial correlation, which is asymptotically equivalent and not much different even for moderate degrees of freedom); the Durbin–Watson statistic was also computed, because of its suitability to small samples,[29] but the existence of only very limited tables severely restricts its usefulness. The distributions of the residual deviations were also examined for normality, by testing their skewness and kurtosis.[17] The results of the analysis are summarized in Table I and plotted together with the experimental points in Figure 1.

The method of least squares is eminently suited to providing estimates for the parameters and their standard errors but is almost totally incapable of distinguishing among the models themselves: in each of the cases presented in Table I, for instance, the significance of the variance ratio is well below 10^{-10}, which only indicates

TABLE I

Results of fitting various surface growth models to shiftup data

Model	Parameter estimate ± standard error[a] $C + D$(min)	$\bar{A}(0)(\mu m^2)$	Other[b] (min)	Residuals[c]	Analysis of residuals Runs[d]	MSSD[e]	D–W[f]
LCA	92.1 ± 9.5	3.93 ± 0.13	50.7 ± 2.5	7.04	0.04	0.04	⟨5⟩
LCG	75.5 ± 8.0	4.80 ± 0.12	41.5 ± 2.0	5.01	0.47	0.27	>5%
LUA[g]	57.2 ± 2.8	3.51 ± 0.15	—	10.89	0.23	0.002	<1%
LUG[g]	44.4 ± 2.2	4.63 ± 0.16	—	6.83	0.18	0.04	⟨5⟩
LP	60.6 ± 2.7	5.52 ± 0.15	—	7.63	0.02	0.01	<5%
EO	47.7 ± 2.1	4.79 ± 0.16	—	6.68	0.18	0.05	⟨5⟩
Eδ[h]	43.9 ± 3.1	5.16 ± 0.29	21.2 ± 9.4	6.54	0.18	0.06	⟨5⟩
ES[i]	49.2 ± 5.5	5.01 ± 0.38	8.7 ± 8.3	6.54	0.18	0.05	⟨5⟩

[a] Obtained by inverting the Jacobian matrix.[3]
[b] Estimate of d for LC models, δ for model Eδ, s' for model ES.
[c] Residual sum of squares, as a percent of total sum of squares.
[d] Exact probability of runs being randomly distributed.
[e] Asymptotic probability of mean-square successive differences test.
[f] Probability bounds of the Durbin–Watson test (⟨5⟩: inconclusive at the 5% level).
[g] Refers to $d > C + D$ only; for $d \leqslant C + D$, LU and LC models are formally identical.
[h] Improvement obtained by adding δ is not significant ($p > 0.40$).
[i] This model is discussed in Section 4A.

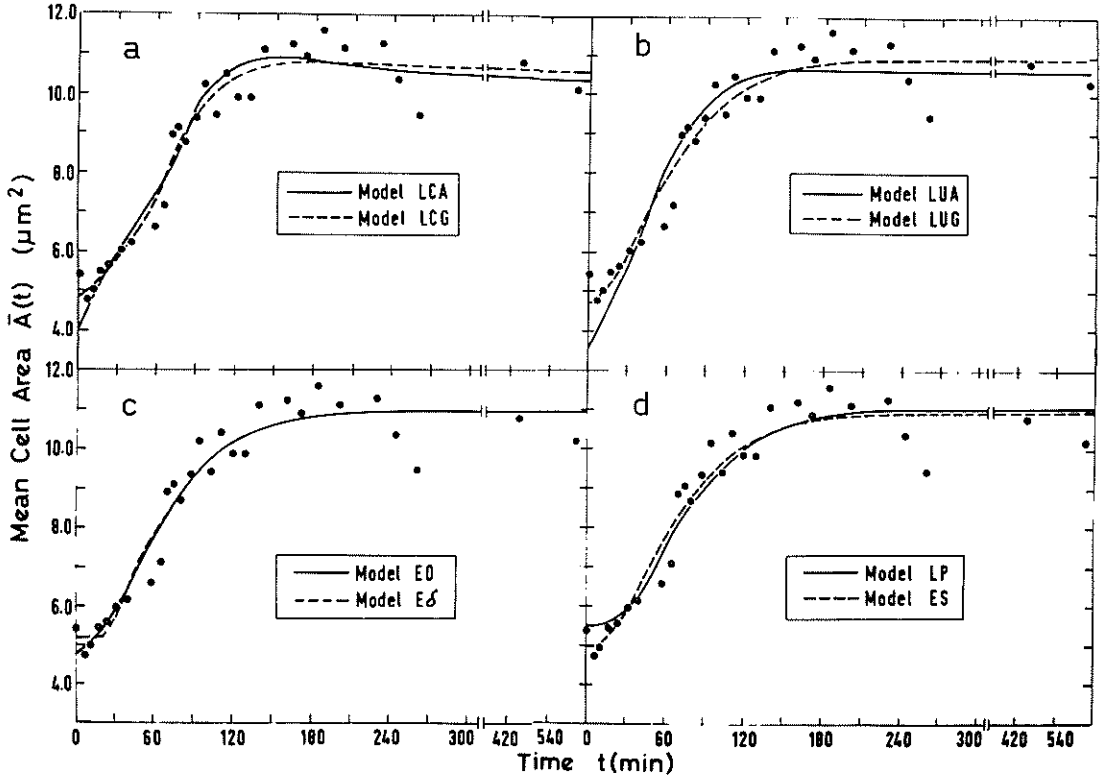

FIGURE 1 Mean cell area $\bar{A}(t)$ as a function of time t following shiftup. A steady-state culture of *E. coli* B/r (strain H266) grown by shaking in alanine–proline minimal medium at 37°C was shifted up at $t = 0$ by the addition of glucose and Casamino acids; absorbance (at $\lambda = 450$ nm) was kept below 0.3 by periodic dilution.[67] Dots: electron micrographic data[19] computed from measured lengths and diameters of individual cells on the basis of an idealized geometry of right circular cylinders with hemispherical polar caps. Lines: best fit of models to data as determined by nonlinear least-squares analysis. (Model ES is discussed in Section 4A.)

that the scatter of the data points about the predicted curves is considerably less than about their own mean (or that even the poorest of the models is much better than no model at all).

Four of the models, the three exponential ones (including ES, which is discussed in section 4.A below but listed here for the sake of completeness) and LUG, can be rejected outright on the grounds that the $C + D$ values they predict are much too low[12,23,37] (below 50 min). Testing the residuals for randomness allows us to eliminate three more: model LP, in which all the tests show significant nonrandomness at the 5% level; model LUA, in which the runs test does not but the other two do, and at the 1% level (note that the residual variance for this model exceeds 10%); and model LCA, in which the runs test and the mean-square successive differences test are both significant at the 5% level while the Durbin–Watson test is inconclusive (the $C + D$ estimate here is also rather high, above 90 min).

We are left therefore with the gradual version of model LC, and our choice appears sound. Thus model LCG has the smallest residual variance of any model and shows the least probability of nonrandomness as calculated by all three tests; in fact, it is the sole model in which the Durbin–Watson test is unequivocally nonsignificant. And qualitatively, only the LC models are capable of explaining the substantial overshoot in mean cell area following shiftup that is so prominent a feature of dimensional rearrangements.[68]

It would seem that our original expectations[20] were justified: the changes in dimensions during nutritional shiftup predicted by any one of these models do differ sufficiently from those predicted by any other to allow the desired discrimination, although the differences are by no means large.

When the models are interpreted in terms of active control of cell elongation[15,16,19,31,60,67,72] rather than of surface extension, all of them predict $C + D$ values below 50 min and all of them result in residual variances well in excess of 10%; not one is able to pass the tests for randomness.

Analysis of a second set of shiftup data,[68] although consistent with the first, was less conclusive and several models survived the various tests. This we attribute to the paucity of observations (15) and to the fact that the experiment was terminated at $t = 190$ min, before the cells had reached their final steady-state dimensions.

The discussion that follows is therefore limited to the results obtained with the initial data set.

4. DISCUSSION

The rather attractive concept[28] of an envelope growth zone serving in the segregation process of replicated chromosomes, in analogy with the mitotic spindle apparatus in eukaryotes, has gained wide support from physiological,[27,52] biochemical[40,64] and morphological[15,48] studies; for a recent exhaustive review, see Helmstetter *et al.*[24] Since the establishment of the chromosome replication cycle and its connection with growth and division,[10,14,55] several models have been proposed to explain the changes that the dimensions of gram-negative bacilliform bacteria undergo under steady-state growth conditions and during nutritional shiftup.[15,16,19,20,51,53,54,57,58,60,72] The various models differ among themselves in the event within the cell cycle that is assumed to trigger production of new growth zones and in the manner in which the activity of such zones is dependent on the growth rate of the culture.

Attempts to reduce the list of competing models by quantitative comparisons between predicted and measured dimensions of rod-shaped bacteria have in the past been confined to steady-state data, and with a certain measure of success.[19,57] There is, however, a major drawback to this approach: degeneracy. Very different models exhibit the identical functional dependence on τ. Thus, not only are the gradual and abrupt versions of models LC and LU totally indistinguishable, but so are LC and LU themselves, and so are EO and Eδ (and, for that matter, ES and LC); our eight models have been reduced to three, with a concomitant loss of information. Furthermore, such analyses are in general less likely to differentiate among the contending models than are comparable analyses using shiftup data.[20] A case in point is model LP, which could not be eliminated on the basis of the steady-state data[51] but was rejected here by all three tests of randomness.

Our first shiftup study was quite limited. The theoretical derivations,[20] although rigorous, were confined to model LC and its variations. The companion article[68] presented the experimental points and the predicted curves on the same grid, but no attempt was

made to fit the models or to analyze the residuals. The conclusions were somewhat weak: that the gradual version is probably superior to the abrupt and that surface extension rather than cell elongation appears to be the element under active control. In the present article, we have reexamined a large number of models and variations of models, using the same shiftup data as before, and arrived at conclusions that are more solid and more general. In particular, we have confirmed the role of cell surface and found that the gradual version of model LC provides the only quantitatively valid description of the experimental data.

A. Exponential Models

Although model E has been eliminated, this should not be taken to imply rejection of exponential surface growth as such, only that it does not follow total protein. Equation (3), which is a very satisfactory description of how $\bar{A}$ varies with τ under steady-state conditions,[57] may have another interpretation altogether (other than in terms of model LC, that is): it could mean that cell *surface area* reaches a constant value, independent of τ, at a certain time before division s, also independent of τ, for if that were the case, then Eq. (3) would now apply (with d replaced by s) when the surface area is an exponential rather than a bilinear function of cell age. During shiftup, this model (version ES) predicts that

$$\frac{A(t)}{A(0)} = 2^{t/\tau_1} \qquad \text{for } t \leqslant s'$$

$$= 2^{s'/\tau_1}[1 + \nu_1(2^{(t-s')/\tau_2} - 1)], \qquad \text{for } t \geqslant s' \tag{13}$$

where $s' \equiv C + D - s$ and is always positive. It can easily be shown that $C + D$ must be greater than s for R to increase with decreasing τ, as is generally observed in practice. The value estimated for $C + D$ (Table I) is not much better than the others from model E and, in the absence of any clear specifications as to the way in which exponential surface area extension might proceed, other than as tested here and found wanting, we are inclined to remain with the linear-log mechanism[53,54] and model LCG.

B. Coupling to Chromosome Replication

Model LU was proposed in order to distinguish between a presumed doubling in the rate of surface growth that is associated

with a concomitant replication of a specific segment of the bacterial genome, and one that is not.[54,72] Note, however, that it is formally identical to model LC, even for $d > C + D$, and it is only the interpretation of d that differs: in the latter, we would expect it to lie in the range $D \leqslant d \leqslant C + D$ and to provide a value for x independent of τ, as in Eq. (4); in the former, any positive value less than $C + D$ would be acceptable. Of course, there is no way of estimating $-c$, the amount by which d exceeds $C + D$ (if indeed it does), even in the transient case. This question therefore remains open; in order to settle it unambiguously, it is necessary to measure the dimensions and the C and D values of cells in which the timing of events in the chromosome cycle has changed. The predicted behavior of $\bar{A}$ would then be quite different for the two models, which should thus be readily distinguishable with sufficiently accurate data. Several such systems exist: (a) the variation of C and D with temperature,[50] but there all the reaction rates change in the same proportion[61] (A. Zaritsky, to be published); (b) strains in which C or D (or both) depend on τ, but existing data[23,51,67] are far too scanty and too scattered to permit precise quantitative analysis; (c) thymine limitation of Thy$^-$ strains,[45,72] but the validity of this system has recently been challenged[56,71,73]; (d) a mutation (*rep*) that slows down the replication velocity[38,39,73]; (e) the dependence on temperature of the initiation mass in[56] *dna A* and in other *ts dna* initiation mutants.

These last two systems are of special interest because they seem to preserve the normal physiology of the cell and because the dimensional changes that occur are not associated with changes in the growth medium. However, the assumption that a single mutation results in a single phenotypic modification (which has probably been misleading even in system (c), although thymine metabolism is supposed to be well established[49,54]) could pose a particular problem in systems such as (d) and (e) that are involved with processes as complex as propagation and initiation of chromosome replication. Thus, in both these systems the finding that coupling of surface synthesis to chromosome replication is unlikely[56,73] should be interpreted with caution. If in the end it does turn out to be the coupled model, then the results of our analysis (Table I) would place the hypothetical gene somewhere in the third quadrant of the marker map, at around $x = \frac{2}{3}$.

C. Zonal Growth Rate

We are left with the gradual response versions of model LU, $d \leqslant C + D$, and model LC. Both presume linear surface growth at a rate per zone that is proportional to the growth rate of the culture, the number of zones doubling once during each life cycle. The original model proposed by Pritchard[54] was more general: the relationship between surface growth rate and culture growth rate was not confined to strict linearity, which has no biological preference over any other sort of dependence. In fact, it has been suggested (K. Nordstrøm; R. H. Pritchard, personal communications) that the rate of envelope synthesis per growth zone β may actually be proportional to cell girth, and so depend on τ only indirectly. In order to examine the implications of such a modification to model LC (or LU), we derive Eq. (3) as before, but without substituting for β. The result is $\bar{A} = \beta\tau 2^{d/\tau}$. If we now put $\beta \propto 2\pi R$, then $\bar{A} \propto R\tau 2^{d/\tau}$ or $\bar{L} \propto \tau 2^{d/\tau}$, since for cylindrical cells with hemispherical polar caps, $\bar{A} = 2\pi R\bar{L}$. This is formally equivalent to a length extension model in which the number of growth zones doubles at some point during the chromosome replication cycle and the rate per zone remains constant, independent of τ. Such a model has already been tested and rejected[19] and we are thus spared the necessity of developing corresponding expressions for the transient case.

The question of the relationship between surface growth rate and τ can be handled in a more general manner by replacing k_1 by $k_1'\tau^{1-n}$ in Eq. (3) and carrying out a nonlinear least-squares analysis of the modified model with k_1', d and n as adjustable parameters. We have done this using previously published steady-state data[58] of length and radius at various values of τ; the results, $n = 1.06 \pm 0.23$, show that n is statistically indistinguishable from unity ($p > 0.80$), so that surface growth rate and culture growth rate would indeed seem to be directly proportional. The corresponding modification for the shiftup equations is rather difficult to handle and was not attempted.

5. IMPLICATIONS

The analysis presented here assumes that surface area is a faithful reflection of the amount of envelope material in the cell and that

cell shape is cylindrical with hemispherical polar caps. The surface densities of both the peptidoglycan layer[75] and the outer membrane envelope proteins[2,5] seem to be fairly constant in gram-negative rod-shaped bacteria growing under steady-state conditions; we have supposed this to be the case during shiftup as well. There is much less information available on cell shape during shiftup[68]; nevertheless, here too we have taken it to be the same as in steady state.

Having adopted an idealized geometry, we are in a position to examine the behavior of mean cell density during nutritional shiftup by comparing cell mass to cell volume. Total mass is given by[6,20]

$$\frac{M(t)}{M(0)} = 1 + \nu_1(2^{t/\tau_2} - 1) \tag{14}$$

so that mean cell mass $\bar{M}(t)$ is just this divided by Eq. (2); mean cell volume $\bar{V}(t)$ can be computed directly from the experimental values of cell length and diameter.[68] The least-squares fit of $\bar{M}(t)$ to $\bar{V}(t)$ is shown in Figure 2. The results (Table II) are excellent, with no detectable trend in the residual distribution, implying that mean cell density (and shape) remains effectively constant throughout the shiftup, as it does over a wide range of steady-state growth conditions.[36,69]

The above analysis provides us with means of testing the $\bar{L}CG$

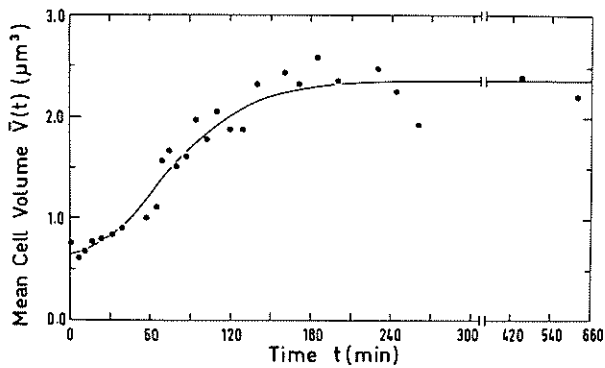

FIGURE 2 Mean cell volume $\bar{V}(t)$ as a function of time t following shiftup. Dots: experimental data, as in Figure 1. Line: best fit of predicted mean cell mass to data as determined by nonlinear least-squares analysis.

TABLE II

Results of fitting predicted mean cell mass to measured mean cell volume[a]

Analysis of variance		*Randomness of residuals*	
Variance ratio[b]	= 200.2	Runs	= 0.43
Residuals	= 6.2%	MSSD	= 0.26
Probability	$< 10^{-14}$	D-W	> 5%
Normality of residuals		*Estimation of parameters*	
Lack of skewness	$p = 0.24$	$C + D$ (min)	= 66.5 ± 3.0
Kurtosis	$p = 0.61$	$\bar{V}(0)(\mu m^3)$	= 0.653 ± 0.031

[a] Symbols as in Table I.
[b] Degrees of freedom = 2 and 26.

(or LUG, $d \leqslant C + D$) model for consistency. The value of $C + D$ estimated from $\bar{M}(t)$ (66.5 ± 3.0 min) is completely independent of the growth models and so can be compared with that from model LCG: the two are statistically indistinguishable ($p > 0.30$). Furthermore, now that we have an independent estimate for $C + D$, we can introduce it into the expressions for model LCG, keep it fixed, and fit for the remaining parameters. Again the results are highly satisfactory, the differences between the two sets of parameters being quite insignificant (Table III).

Having established that mean cell density remains constant, we can use mean cell mass to compute mean cell length and diameter from the predicted mean cell area at any time following the shift-up. The results for model LCG with $C + D$ fixed at 66.5 min are plotted in Figure 3 together with the experimental data[68]; the ratio of mean cell length to mean cell diameter, the so-called aspect ratio,[32,70] is also shown. Actually, these are not the true mean cell lengths and diameters but rather the length and diameter of a cell with area equal to the mean cell area and volume equal to the mean cell volume.

It is an implicit requirement of all linear-log models for surface-mass growth that cell diameter vary with cell age if the density is to remain constant.[54] Any such oscillations are expected to be quite small[34] and none were detected for a long time,[44] but recent studies[66] suggest that they may indeed occur more or less as predicted. It is obvious, however, that the assumption of constant cell shape during the cell cycle is incompatible in principle with steady-state growth, at least as far as cylinders with hemispherical polar caps

TABLE III

Fit of surface growth model LCG to shiftup data comparison between $C + D$ fixed and $C + D$ as adjustable parameter[a]

	$C + D$ fixed	$C + D$ free
Analysis of Variance		
Variance ratio	219.3	156.8
Residuals	5.6%	5.0%
Probability	$<10^{-14}$	$<10^{-14}$
Normality of Residuals		
Lack of skewness, p	0.13	0.27
Kurtosis, p	0.77	0.61
Randomness of Residuals		
Runs	0.47	0.47
MSSD	0.16	0.27
D-W	>5%	>5%
Estimation of Parameters		
$C + D$	66.5 fixed	75.5 ± 8.0
$A(0)$	4.75 ± 0.13	4.80 ± 0.12[b]
d	42.3 ± 1.9	41.5 ± 2.0[c]

[a] Symbols as in Table I.
[b] Not significantly different ($p > 0.80$).
[c] Not significantly different ($p > 0.75$).

are concerned, for a cell cannot maintain such a shape throughout its life cycle and still give rise on division to two daughter cells identical to itself. Indeed, constriction implies a transition in cell shape from that of a single cylinder with hemispherical polar caps to two, a period during which the geometry is not completely specified. Neglecting this leads to a contradiction: the precise equality in volume and surface area that we require to obtain between dividing and newborn cells not only does not ensure a corresponding relationship as regards cell length and diameter, it actually precludes it. Quantitatively the effect is small, as can be seen in Figure 4. Here we have plotted cell length, cell diameter, and the aspect ratio as functions of age for various growth rates, as predicted by model LCG. The results illustrate the point that the length of a newborn daughter cell appears to be somewhat greater than that of its mother at birth and the diameter, somewhat less. This artifact of our oversimplification is magnified by the aspect ratio but applies only to individual cells; when we consider average dimensions, as we have been doing until now, the discrepancy is of course

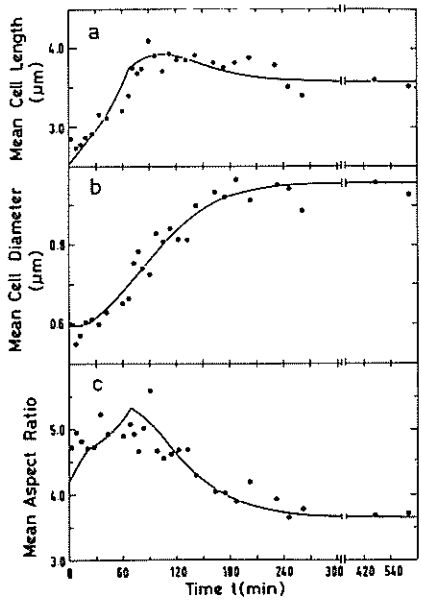

FIGURE 3 Derived cell dimensions as a function of time t following shiftup. Dots: experimental data, as in Figure 1. Lines: computed from mean cell mass and the mean cell area predicted by model LCG. (a) Mean cell length; (b) mean cell diameter; (c) mean aspect ratio.

much less and can almost certainly be ignored for all practical purposes.

6. RESERVATIONS

The use of steady-state data to discriminate among the many models designed to explain the observed changes in the dimensions of an *E. coli* B/r cell at different growth rates, has been inconclusive[16,46,51]; by turning to a widely studied perturbation, the nutritional shiftup, we were able to reject all but one (or two) of them quite convincingly. It should be borne in mind, however, that the assumptions underlying most of these models have never been

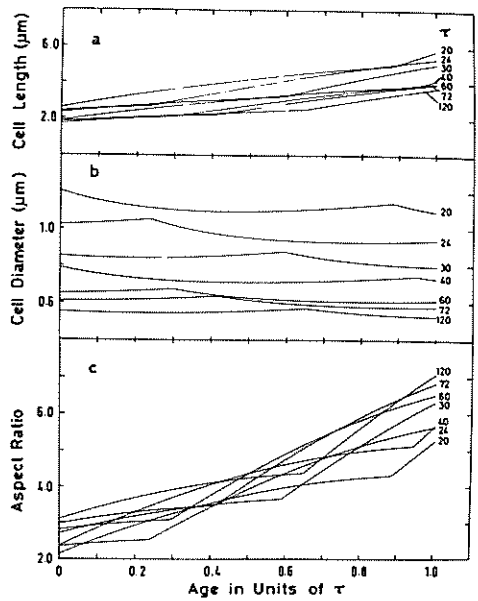

FIGURE 4 Derived cell dimensions as a function of relative cell age for the values of doubling time τ indicated (in min). Lines: computed from cell mass and the cell area predicted by model LCG. (a) Cell length; (b) cell diameter; (c) aspect ratio.

shown to hold during such a transition between steady states, and one should not be too surprised if, for instance, some of the parameters presumed to remain rigidly constant (at least as far as their means are concerned) were actually found to vary. In fact some variation has been reported for[23] C and D and, very recently, for[74] mass at initiation of chromosome replication (Bremer and Chuang; Crossman, Woldringh and Ron; Zaritsky and Zabrovitz—all of the EMBO Workshop[76]) but much more comprehensive measurements would be required before the effect on the various models could be evaluated. It is not inconceivable that such a rigorous study would invalidate most of the conclusions arrived at here and that the true nature of bacterial morphogenesis has yet to be elucidated[22,30,47] (Koch, Higgins and Doyle; Mendelson—both of the EMBO Workshop[76]).

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