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Dimensional Rearrangement of Rod-Shaped Bacteria Following Nutritional Shift-up. II. Experiments with *Escherichia coli* B/r†

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The dimensions of *Escherichia coli* B/r (strain H266) in transition between two states of balanced growth, were determined from electron micrographs of fixed cells by sampling the culture at various times following nutritional shift-up from a doubling time of 72 min to one of 24 min. Mean cell length rises immediately and overshoots its final steady-state value, cell diameter increases monotonically; both approach their asymptotic levels only after several hours.

The results are compared with the dimensions predicted by each of two models of cell growth and morphogenesis in rod-shaped bacteria. The first attributes cell elongation to circular zones that double in number at a particular time during the cell cycle and which act at rates proportional to the growth rate; the second is similar, except that it considers surface growth rather than length extension as the active process, length being determined passively. Two possibilities are examined, that the zonal

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growth rate adjusts immediately to the new growth conditions, and that it does so gradually.

The experimental data appear consistent with the gradual response version of the surface growth model.

1. Introduction

Bacteria are bigger when they grow faster (Schaechter, Maaløe & Kjeldgaard, 1958). Upon transition to higher growth rates, the cell surface increases through changes in shape characteristic of the bacterial strain. In some species, such as *Bacillus subtilis*, this increase can be ascribed entirely to a change in length (Sedgwick & Paulton, 1974); in others, such as *Bacillus megaterium* (Herbert, 1958), *Salmonella thyphimurium* (Schaechter *et al.*, 1958) and *Escherichia coli* (Grover *et al.*, 1977), the cells extend in both length and diameter. This latter behavior has been attributed (Previc, 1970; Pritchard, 1974) to the fact (Sud & Schaechter, 1964) that envelope lags behind mass in the attainment of the new steady-state synthesis rates following nutritional shift-up (Kjeldgaard, Maaløe & Schaechter, 1958). The way in which the dimensions change during the transition must reflect not only the mechanical properties of the cell wall but also the mode of surface enlargement. Thus, measurements of cell geometry within this period should provide information on the mechanisms that control envelope growth and shape.

Three plausible models have been proposed to describe elongation during the bacterial cell cycle (Donachie & Begg, 1970; Zaritsky & Pritchard, 1973; Grover *et al.*, 1977). All three assume linear extension in a discrete number of growth zones (Donachie & Begg, 1970) that changes abruptly at a particular instant in the cycle. The models differ in the events with which these changes are identified and in the way the overall rate of envelope synthesis depends on growth rate.

In a recent study (Grover *et al.*, 1977) each of these models was tested by comparing predicted and experimental values of the mean length of *E. coli* B/r as a function of growth rate. The best agreement is obtained when the doubling is taken to occur a fixed time d prior to division and the elongation rate per zone to be proportional to the growth rate. This time, estimated at 17 min, may correspond to the termination of chromosome replication (Cooper & Helmstetter, 1968). Essentially the same data also fit an analogous model in which the rate of surface growth, rather than elongation, is proportional to the growth rate (Rosenberger *et al.*, 1978). Doubling is now estimated to take place about 49 min before division, corresponding to the middle of the chromosome replication cycle (Pritchard, 1974).

One possible way of discriminating between the elongation and the surface growth models (Rosenberger *et al.*, 1978) is to determine d directly, either through observations on individual cells or by the use of synchronous cultures (Donachie, Begg & Vicente, 1976; Meyer *et al.*, 1979). Alternatively, one can take advantage of the finding (Grover *et al.*, 1980) that the two models predict different transient behavior following transfer of the cells to conditions that support a higher growth rate. The present study is based on the latter approach, and uses *E. coli* B/r cells in transition between a culture doubling time of 72 min and one of 24 min.

2. Materials and Methods

(A) BACTERIAL STRAINS AND CULTURE CONDITIONS

Escherichia coli B/r strain H266 (obtained from P. G. de Haan, Laboratory of Microbiology, Utrecht) was used throughout. The cells were grown in minimal medium (Helmstetter & Cooper, 1968) supplemented either with L-alanine and L-proline at final concentrations of 400 μg/ml or with 0·4% glucose and 1% Casamino acids (Sigma). For each experiment, 100 ml of medium were inoculated with cells from a fresh agar slant kept at 5°C. All cultures were incubated in 500 ml flasks at 37°C and aerated vigorously for at least 10 generations. Growth was measured as the rise in absorbance at 450 nm (A_{450}), by means of a Gilford microsample spectrophotometer (model N300), or as the increase in cell concentration, by means of a Coulter Counter (model Z_B) with a 30 μm probe. Cultures were accepted as being in steady-state growth when length distributions from successive samples did not differ significantly at the 0·10 level, as determined by a Kolmogorov–Smirnov two-sample test (Siegel, 1956).

For nutritional shift-up, a pre-warmed mixture of concentrated glucose and Casamino acids was added to the alanine and proline culture (at $A_{450} \approx 0{\cdot}1$) to give the above concentrations. During postshift growth, the culture was periodically diluted about fivefold with pre-warmed medium in order to maintain the absorbance below 0·3.

(B) CELL DIMENSIONS

These were determined from electron micrographs of cells prepared by agar filtration (Kellenberger & Bitterli, 1976). The cells were first fixed in 1% (wt/vol) OsO_4 at a final concentration of 0·2% and then maintained at 5°C until applied to the agar filters; filtration was carried out by the modified procedure described previously (Grover *et al.*, 1977; Woldringh *et al.*,

1977). The electron micrographs were projected onto a transparent screen (Summagraphics digitizer connected to a Hewlett-Packard 9825A calculator via a HP Interface Bus) at a final magnification of between 10 000 and 12 000. Lengths and diameters were recorded in pairs, and the surface areas and volumes of the individual cells were then calculated on the basis of an idealized geometry of right circular cylinders with hemispherical polar caps (Errington, Powell & Thompson, 1965; Rosenberger *et al.*, 1978). Each sample consisted of 100–600 cells.

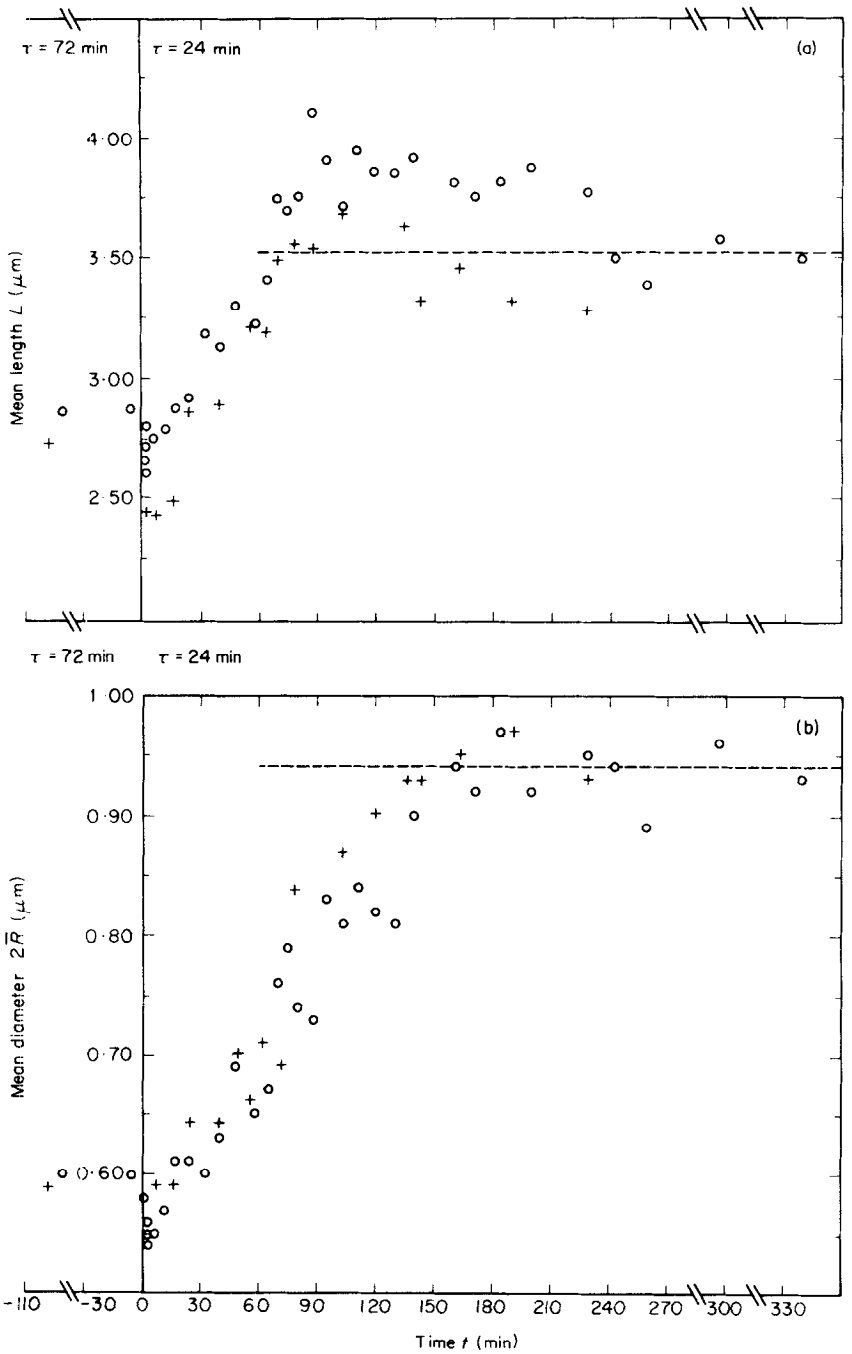
3. Results

The growth rate of *E. coli* B/r cells cultured under steady-state conditions with a doubling time τ of 72 min was raised threefold (to $\tau = 24$ min) by the addition of glucose and Casamino acids. The rate of mass increase accelerated immediately after the shift, as expected (Kjeldgaard *et al.*, 1958), reaching its new steady-state value within about 60 min (Bremer & Dennis, 1975). Cell division, on the other hand, maintained its pre-shift rate for 80 min or so, then increased gradually towards its new steady-state level (Kjeldgaard *et al.*, 1958).

The changes in mean cell length and diameter observed during the course of two independent shift-up experiments, are presented in Fig. 1. The length ($\bar{L}$) rises rapidly after the shift-up and overshoots its final steady-state value in about 75 min; this is then approached asymptotically during the subsequent 2·5 h [Fig. 1(a)]. In contrast to length, cell diameter ($2\bar{R}$) increases more slowly and attains its new steady-state level monotonically, in about 3 h [Fig. 1(b)]. (There was also an overshoot in the mean length of the constricted cells. In both experiments, the proportion of such cells remained at 10% throughout the first 50 min after the shift and then began to rise, levelling off at 35% some 60 min later.)

Length and diameter both decrease immediately after the shift (Fig. 1), probably because of the sudden rise in the osmolarity of the medium, from an initial value of 300 mosmol to 450 mosmol, when the glucose and Casamino acids are added. (That this is not sufficient to induce plasmolysis, can be readily ascertained by phase-contrast microscopy.) Such large and consistent shrinking, from 5–12% in five independent experiments, implies an envelope that is rather less than completely rigid.

FIG. 1. Cell dimensions as functions of time t following shift-up (at $t = 0$) from $\tau = 72$ min to $\tau = 24$ min. Circles and crosses refer to two independent experiments. Dashed lines: post-shift steady-state levels. (a) Mean cell length $\bar{L}$. (b) Mean cell diameter $2\bar{R}$.


τ = 72 min
τ = 24 min
(a)
Mean length L (μm)
4·00
3·50
3·00
2·50
τ = 72 min
τ = 24 min
(b)
Mean diameter 2R̄ (μm)
1·00
0·90
0·80
0·70
0·60
-110
-30
0
30
60
90
120
150
180
210
240
270
300
330
Time t (min)

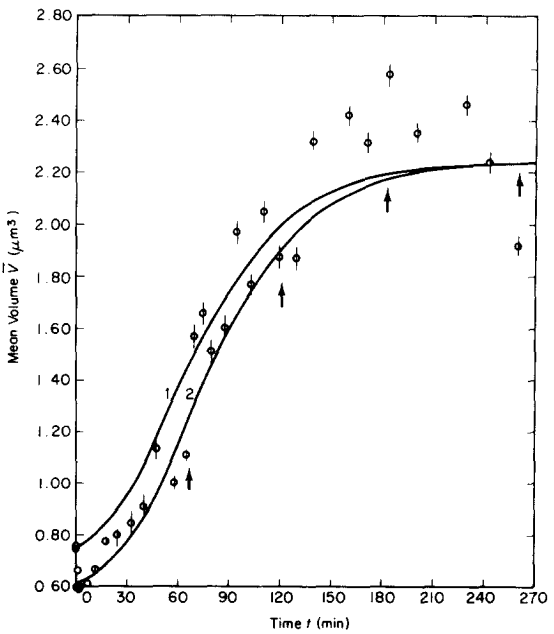


FIG. 2. Mean cell volume $\bar{V}$ as a function of time t following shift-up. Same experiment as in Fig. 1, circles. Vertical bars: 1 s.e. Arrows: times at which culture diluted. Curve 1, drawn to fit experimental data prior to shift-up and after steady state has been reached, under assumption of constant mean cell density: $C+D=56{\cdot}6$ min, $V_i=0{\cdot}628$ μm^3; curve 2, drawn to fit experimental data shortly after shift-up and after steady state has been reached, under assumption of constant mean cell density: $C+D=67{\cdot}3$ min, $V_i=0{\cdot}462$ μm^3.

In Fig. 2 we have plotted mean cell volume $\bar{V}$ as a function of time following shift-up, for one of the experiments (open circles) shown in Fig. 1. The vertical bars through each point represent one standard error in each direction. Theoretical curve 1 has been calculated as described in the preceding article (Grover *et al.*, 1980), the volume at initiation of chromosome replication V_i and the time between such initiation and the subsequent cell division $C+D$ having been chosen so that the curve fits the steady-state data; curve 2 was drawn to coincide with cell size after the initial shrinkage.

Figure 3 shows diameter and length distributions and photomicrographs of cells from the pre-shift and post-shift ($t=610$ min) steady-state cultures and from a sample in transition ($t=88$ min). During the transition period, an increased asymmetry in the site of constriction and in cell shape was found, many cells displaying a tapered pole [Fig. 3(b)]. (Comparable shapes are seen with phase optics and so cannot be attributed to preparation artifacts.)

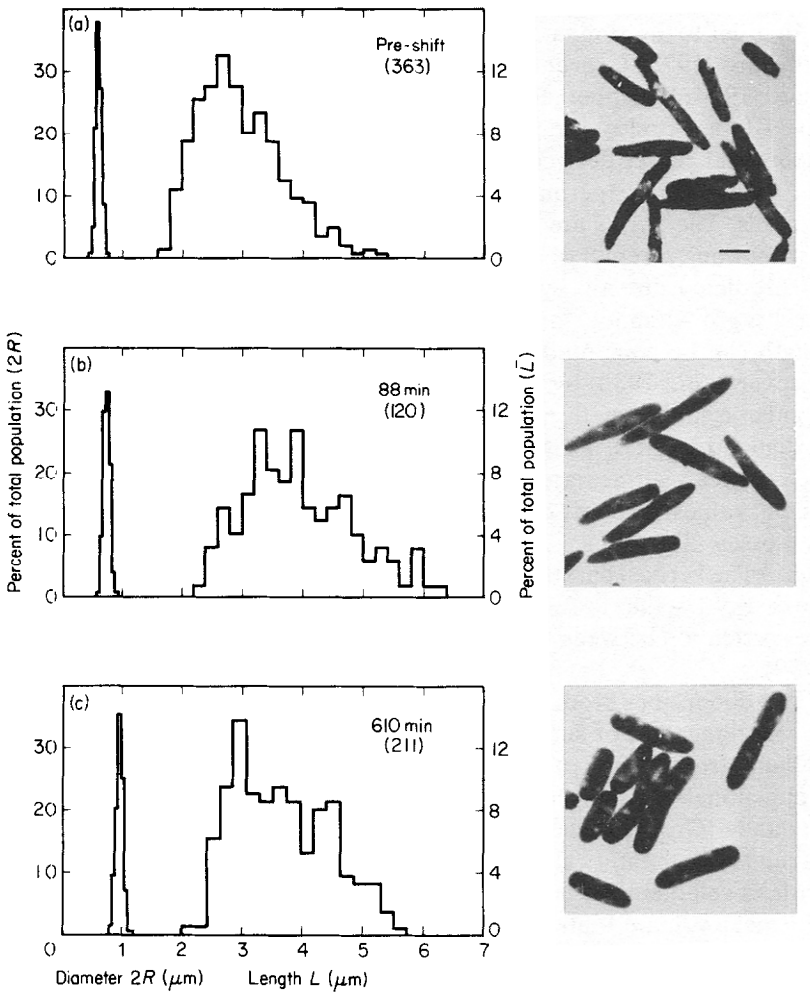


FIG. 3. Diameter and length distributions and electron micrographs of cells at various stages. Same experiment as in Fig. 1, circles. Numbers in parentheses: total cells measured. Magnification bar: 1 μm. (a) Pre-shift population. (b) 88 min after shift-up. (c) 610 min after shift-up.

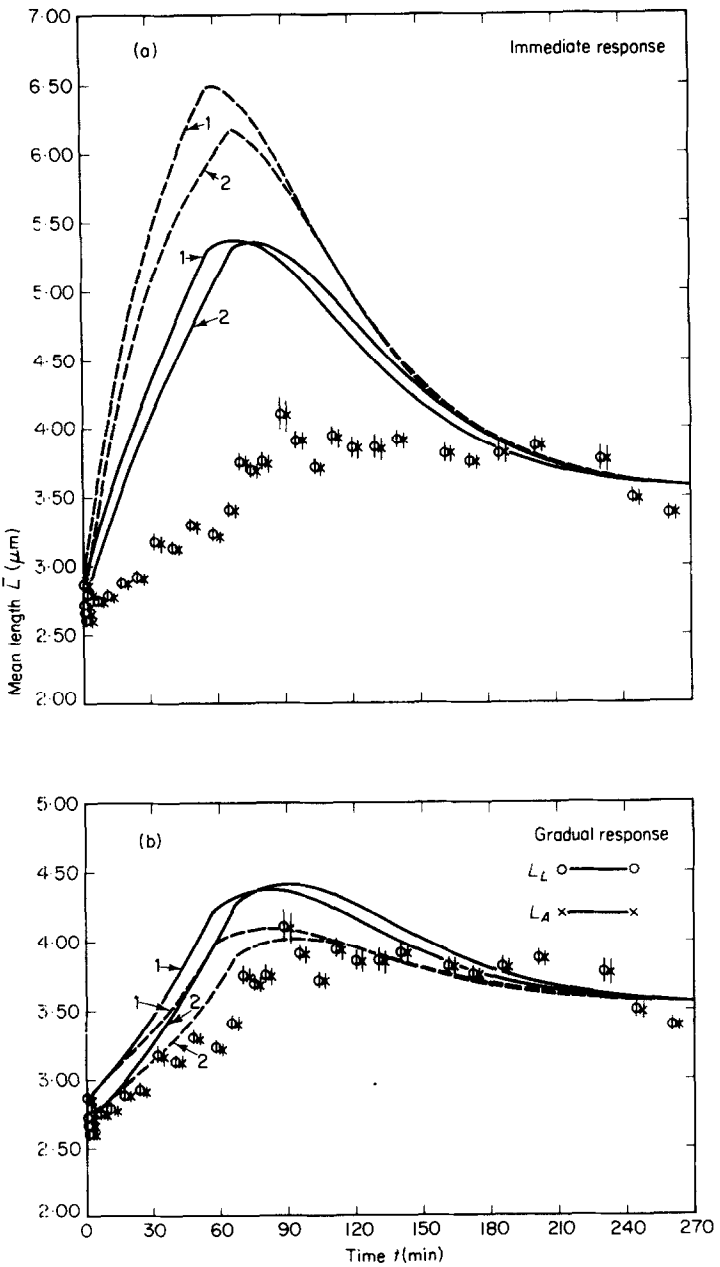
Apart from this, all the cells in the population seem to respond to the shift-up in a similar manner, if we are to judge from the shape of the distribution histograms, the coefficient of variation of cell length rising briefly from 24% to a maximum of 27% and that of cell diameter decreasing gradually from about 8% to 6%.

In order to discriminate between the length extension model (Zaritsky & Pritchard, 1973; Grover *et al.*, 1977) and the surface growth model (Pritchard, 1974; Rosenberger *et al.*, 1978), the data of one of the experiments in Fig. 1 (open circles) are plotted in Fig. 4 together with the corresponding theoretical curves derived as described in the preceding article (Grover *et al.*, 1980). The experimental data are now represented by two sets of symbols. The circles are the true mean lengths calculated from the length distributions, a replot of Fig. 1(a), and are to be compared with the lengths L_L predicted directly by the length extension model (solid curves). The surface growth model, on the other hand, predicts mean surface area. Cell length can be computed from this and the theoretical mean cell volume (Grover *et al.*, 1980) by assuming an idealized geometry (for *E. coli*, right circular cylinders with hemispherical polar caps). Such a mean length is designated L_A; it is, in effect, the expected length of a cell with mean volume $\bar{V}$ and mean surface area $\bar{A}$ (dashed curves). The corresponding experimental values, similarly derived from the measured $\bar{V}$ and $\bar{A}$, are indicated by crosses. (To enhance legibility, each cross has been displaced 3 min to the right.) The vertical bars through the data points represent standard errors; in the case of L_A, they are estimates based on the variance in V and in A and on the correlation between them, using the law of propagation of errors (Reed, 1959).

The dimensions predicted by the two models depend on whether the rate of cell elongation or surface synthesis is considered to change abruptly at shift-up from being proportional to the preshift growth rate to being proportional to the postshift growth rate, or whether it is taken to do so gradually (Grover *et al.*, 1980). The former assumption has been used to calculate the theoretical curves in Fig. 4(a) and the latter, in Fig. 4(b).

Mean cell diameter is presented in Fig. 5. Here R_L is the radius of a cell with mean volume $\bar{V}$ and mean length $\bar{L}$ as predicted by the length extension model, and R_A is the radius of a cell with mean volume $\bar{V}$ and mean surface area $\bar{A}$ from the surface growth model; the corresponding experimental points have been calculated accordingly.

FIG. 4. Mean cell length $\bar{L}$ as a function of time t following shift-up. Circles: $\bar{L}$ as in Fig. 1(a); crosses: $\bar{L}$ as computed from experimental mean cell volume and surface area, displaced 3 min to the right to enhance legibility. Vertical bars: 1 s.e. Solid lines: length extension model; dashed lines: surface growth model. Curves 1, drawn as in Fig. 2: $d = 10{\cdot}8$ min and $k = 1{\cdot}79$ μm (length extension model), $d = 34{\cdot}3$ min and $k = 2{\cdot}69$ μm^2 (surface growth model), $C + D = 56{\cdot}6$ min and $V_i = 0{\cdot}628$ μm^3 (both); curves 2, drawn as in Fig. 2: $d = 14{\cdot}0$ min and $k = 1{\cdot}63$ μm (length extension model), $d = 41{\cdot}2$ min and $k = 2{\cdot}20$ μm^2 (surface growth model), $C + D = 67{\cdot}3$ min and $V_i = 0{\cdot}462$ μm^3 (both). [The k's are constants of proportionality and have been defined in the preceding article (Grover *et al.*, 1980).] (a) Immediate response version. (b) Gradual response version.


(a)
Immediate response
(b)
Gradual response
L_L
L_A
Mean length $\bar{L}$ (μm)
Time t(min)
7·00
6·50
6·00
5·50
5·00
4·50
4·00
3·50
3·00
2·50
2·00
0
30
60
90
120
150
180
210
240
270

4. Discussion

The aim of the present study is to determine whether the rearrangement of cell dimensions after nutritional shift-up is able to discriminate between the two most plausible models put forward to date to describe growth and morphogenesis in rod-shaped bacteria. One of these (Grover *et al.*, 1977) is based on active length extension, the other (Rosenberger *et al.*, 1978) on elongation being determined by the rate of surface synthesis. Both models imply a passive role for cell diameter which, in addition, depends on the rate of total volume growth (Pritchard, 1974).

A comparison between the predictions of the models and the experimental results shows the immediate response version to be completely incompatible as regards both length [Fig. 4(a)] and diameter [Fig. 5(a)]. The gradual version is much closer to the measured values [Fig. 5(b)], although cell length is still a little high [Fig. 4(b)]; in all cases the surface growth model is superior.

The differences expected between the two models can be enhanced (Grover *et al.*, 1980) by transforming (Zaritsky, 1975) the original data into a dimensionless measure of cell geometry, the aspect ratio $\bar{f}$, defined as $\bar{L}/2\bar{R}$. A plot of $\bar{f}$ as a function of time after shift-up is presented in Fig. 6. Here too, the gradual response version of the surface growth model seems to describe the data best.

The observed changes in mean cell length [Fig. 4(b)] lag somewhat behind the predicted kinetics for the surface growth model, those in cell diameter [Fig. 5(b)] seem to precede it. These discrepancies can be lessened either by assuming a still slower approach of the zonal growth rate to steady state than was done in the gradual response version, or by suitably adjusting the parameters of the model. (Only the latter is applicable in the case of Fig. 2, because $\bar{V}$ is independent of the growth rate response.)

In order to derive an expression for mean cell volume as a function of time after shift-up (Grover *et al.*, 1980), we assumed that total cell volume is proportional to total cell mass and that the latter responds to the enriched growth medium according to Bremer & Dennis (1975), cell number then following cell mass by $C+D$ min (Cooper & Helmstetter, 1968). Values for $C+D$ were computed from the experimental steady-state levels of mean cell volume. But $C+D$ can also be estimated by measuring total cell mass and cell number in the steady-state cultures, with results that exceed the former by some 15–30 min. Such a difference can arise from an error in cell dimensions or in cell number, the determination of total mass being straightforward and reliable. If, for instance, cells grown at higher growth rates flatten less during air drying, so that their dimensions are

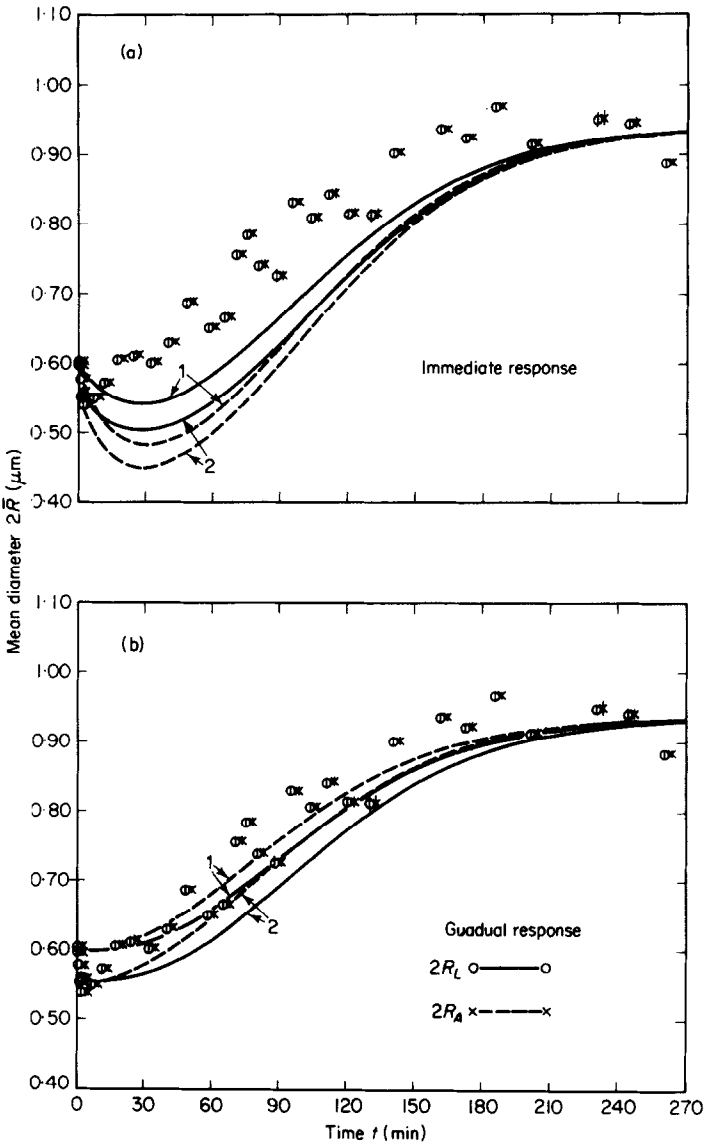


FIG. 5. Mean cell diameter $2\bar{R}$ as a function of time t following shift-up. Same experiment as in Fig. 1(b), circles. Circles: $2\bar{R}$ as computed from experimental mean cell volume and length; crosses: $2\bar{R}$ as computed from experimental mean cell volume and surface area, displaced 3 min to the right to enhance legibility. Vertical bars: 1 s.e. Solid lines: length extension model; dashed lines: surface growth model. Curves 1, as in Fig. 4, curves 1; curves 2, as in Fig. 4, curves 2. (a) Immediate response version. (b) Gradual response version.

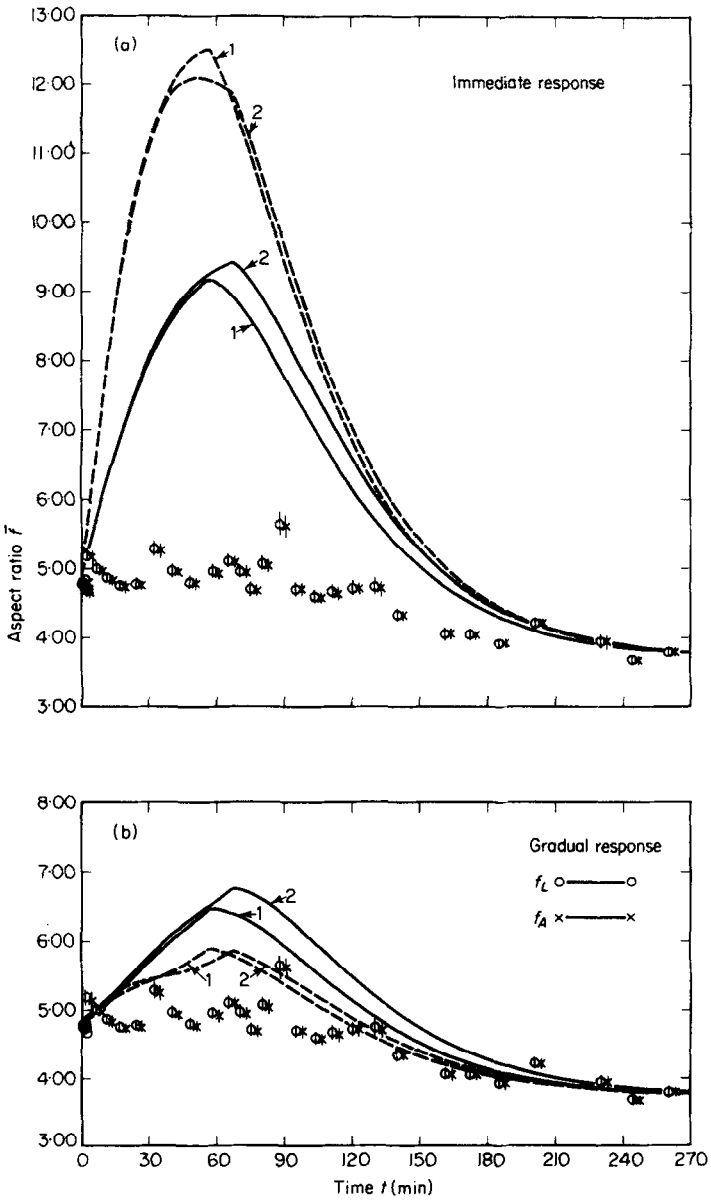


FIG. 6. Mean aspect ratio $\bar{f}$ as a function of time t following shift-up. Circles: $\bar{f}$ computed from the data of Figs 4 and 5, circles; crosses: $\bar{f}$ computed from the data of Figs 4 and 5, crosses. *Vertical bars: 1 s.e. Solid lines: length extension model; dashed lines: surface growth model.* Curves 1, as in Fig. 4, curves 1; curves 2, as in Fig. 4, curves 2. (a) Immediate response version. (b) Gradual response version.

underestimated, then the $C+D$ derived from $\bar{V}$ would be too low. This is not supported by observations using phase-contrast microscopy, where hydrated and air-dried cells are found to have essentially the same dimensions (Trueba & Woldringh, 1980). We are thus left with cell number. And indeed, it has been known for many years that measurements made with a commercial Coulter Counter, while highly reproducible, can be notoriously inaccurate (Grover *et al.*, 1969).

A striking feature of the dimensional rearrangement following nutritional shift-up is the overshoot in average cell length and the considerable period of time required to attain steady state. These observations are consistent with linear-log models in general (Previc, 1970; Pritchard, 1974) and support the fundamental assumption underlying the two tested here (Grover *et al.*, 1977; Rosenberger *et al.*, 1978): that the rate of cell elongation or of surface extension is proportional to growth rate. Such a relationship was originally based on results with thymineless mutants of *E. coli* under thymine limitation (Zaritsky & Pritchard, 1973). Although those results have since been shown to be fortuitous (Pritchard, Meacock & Orr, 1978; Zaritsky & Woldringh, 1978), some experimental evidence does still remain (Sargent, 1975; Donachie *et al.*, 1976).

If the rate of cell constriction were to depend on that of surface synthesis, then one would expect it to accelerate after the shift-up, increasing the rate of cell division and decreasing the proportion of constricted cells. That such behavior is not observed, implies that cell constriction and surface synthesis are not intimately associated processes.

The time course predicted for mean cell length and diameter during nutritional shift-up depends on the values of d and $C+D$ and their associated coefficients (Grover *et al.*, 1980). It would be interesting to compare these values with those obtained from a similar experiment using *E. coli* B/r *A*, a closely related strain in which, however, both $C+D$ (Helmstetter & Pierucci, 1976) and the dependence of cell dimensions on growth rate (Woldringh *et al.*, 1977) are different. In this way one could hope to extend the conclusions of the present article and perhaps even to address the question of coupling between growth zone doubling and the chromosome replication cycle.

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