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Dimensional Rearrangement of Rod-shaped Bacteria Following Nutritional Shift-up. I. Theory

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Theoretical expressions are derived for two models that describe average length and radius of a population of rod-shaped bacteria as a function of time following their transfer to a medium that supports a higher growth rate. The first attributes cell elongation to circular zones produced at a particular time during the cell cycle and which act thereafter at rates proportional to the growth rate; the second is formally identical but considers surface growth rather than length extension. Two possibilities are considered, that the zonal growth rate adjusts immediately to the transition, and that it does so gradually. The results are also displayed graphically, covering a broad range of each of the various parameters involved; values are chosen to permit a direct comparison between the models.

Average cell length is seen to undergo a large overshoot and to approach its steady-state value from above, while cell radius remains almost constant or even decreases somewhat before increasing monotonically towards its asymptotic level; both require a considerable period of time to reach steady state.

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The transient behavior predicted by the two models is found to be quite different even when the steady-state dimensions are identical; the differences between immediate and gradual response of the zonal growth rate are even greater. It is shown that using a dimensionless measure of cell geometry, the aspect ratio, can facilitate selection of the appropriate model.

1. Introduction

Many aspects of the growth and division of bacteria have been studied through observations on cultures in balanced, exponential growth in different media (Schaechter, Maaløe & Kjeldgaard, 1958; Dennis & Bremer, 1974). An extension of this approach that enhances our understanding of the bacterial cell cycle, considers transitions from one such state to another. The most common of these are the so-called shift-up experiments, in which the culture is transferred (at constant temperature) to a richer medium and its properties of interest monitored until the new steady state is attained (Kjeldgaard, Maaløe & Schaechter, 1958; Cooper, 1969; Bremer & Dennis, 1975).

Measurements of the dimensions and elongation rates of bacilliform bacteria under different steady-state growth conditions have led to a model that attributes cell extension to a circular zone produced at a particular time during the cell cycle and which acts thereafter at a rate proportional to the growth rate (Zaritsky & Pritchard, 1973; Sargent, 1975; Donachie, Begg & Vicente, 1976). Extensive measurements of the length of *Escherichia coli* B/r cells (strain H266) as a function of generation time, indicate that the doubling in the rate of elongation takes place about 17 min before cell division (Grover *et al.*, 1977). If, on the other hand, surface growth rather than length extension is considered, then essentially the same data imply a doubling some 49 min prior to division (Rosenberger *et al.*, 1978).

In principle, such a large difference could easily be used to distinguish between the length and surface area models provided, of course, that the time of doubling can be determined independently. Direct measurement using synchronous cultures (Hoffman, Messer & Schwartz, 1972; Churchward & Holland, 1976; Donachie, Begg & Vicente, 1976; Hackenbeck & Messer, 1977), however, have so far not proved successful. (This may be due to the difficulties of obtaining good synchrony without disturbing steady-state growth.)

Both models were fit to the same (steady-state) data and so, of necessity, both predict similar dimensions for cells in balanced growth. In the hope that their transient behavior would be different, we have turned to the shift-up design. In the original formulation of the models (Zaritsky & Pritchard, 1973; Pritchard, 1974; Grover *et al.*, 1977; Rosenberger *et al.*, 1978), the

question was left open as to whether, under such conditions, the *rate* of cell elongation (or surface synthesis) changes abruptly at shift-up from being proportional to the pre-shift growth rate to being proportional to the post-shift growth rate, or whether it does so gradually. In the absence of suitable guidelines from the literature, we have analyzed both cases.

In the present article, theoretical expressions are derived for each model that describe average length and radius of a population of cells as a function of time following their transfer to a medium that supports a higher growth rate. Actually, the only functions we are able to calculate directly are total cell volume and cell number and total cell length (for the length extension model) or surface area (for the surface growth model); the remaining dimensions are derived from these three basic quantities and the assumed geometry: right circular cylinders with hemispherical polar caps. The principal results are also displayed graphically. In the following article (Woldringh *et al.*, 1980), we present the experimental data and compare them with the theoretical predictions.

2. Theory

The approach we shall adopt is to fix on a population of cells that is in steady state prior to shift-up and follow its properties as a function of time t after shift-up (at $t = 0$) from a generation time of τ_1 to one of τ_2, $\tau_2 < \tau_1$. The notation is similar to that used previously (Grover *et al.*, 1977): C is the time for a replication point to traverse the genome, D is the time between the end of a round of replication and the subsequent cell division (Cooper & Helmstetter, 1968), $d (\leq C + D)$ is the time prior to this division at which the rate of length extension (or surface growth) doubles; $C + D$, d, τ_1 and τ_2 are average values over the cell population and assumed to be independent of time.

(A) TOTAL MASS

Total cell mass as a function of time after shift-up $M(t)$ is very nearly proportional (Maaløe & Kjeldgaard, 1966) to the amount of total protein $P(t)$ plus stable RNA $S(t)$. The latter increases exponentially with time and adjusts to the shift-up immediately (Bremer & Dennis, 1975):

$$S(t) = S(0)2^{t/\tau_1} \quad \text{for } t \leq 0$$
$$= S(0)2^{t/\tau_2} \quad \text{for } t \geq 0.$$

The rate of protein production, on the other hand, is proportional to the number of ribosomes. Since rRNA is a constant fraction of S (Dennis &

Bremer, 1974), we have that

$$P(t) = P(0) + \varepsilon_2 S(0)\tau_2(2^{t/\tau_2} - 1)/\ln 2 \quad \text{for } t \geq 0,$$

where $P(0)$ is given by the amount of protein produced during τ_1: $P(0) = \varepsilon_1 S(0)\tau_1/\ln 2$; the ε_j are constants proportional to the ribosomal efficiency at τ_j. Thus

$$\frac{M(t)}{M(0)} \approx \frac{P(t)+S(t)}{P(0)+S(0)} = \frac{(\varepsilon_2\tau_2 + \ln 2)2^{t/\tau_2} + (\varepsilon_1\tau_1 - \varepsilon_2\tau_2)}{\varepsilon_1\tau_1 + \ln 2} = 1 + v_1(2^{t/\tau_2} - 1),$$

where $v_1 \equiv (\varepsilon_2\tau_2 + \ln 2)/(\varepsilon_1\tau_1 + \ln 2)$.

(B) CELL LENGTH

According to the length extension model (Pritchard, 1974; Rosenberger *et al.*, 1978), the number of growth zones in a cell doubles a fixed time d prior to cell division. Since division takes place $C+D$ min after initiation of chromosome replication (Cooper & Helmstetter, 1968), the total number of zones in the culture Z lags behind the number of chromosome origins Φ by $C+D-d$ min: $Z(t) = \Phi(t-c)$, where $c \equiv C+D-d$. In addition, $\Phi(0)$ is equal to $N(0)2^{(C+D)/\tau_1}$, where $N(0)$ is the total number of cells at $t=0$ (Bremer & Churchward, 1977), and $\Phi(t)$ is proportional to total cell mass $M(t)$ (Donachie, 1968; Pritchard, Barth & Collins, 1969). Thus

$$Z(t) = \Phi(t-c) = \frac{M(t-c)}{M(0)}\Phi(0) = N(0)2^{(t+d)/\tau_1} \qquad \text{for } r \leq c$$

$$= N(0)2^{(C+D)/\tau_1}\{1 + v_1[2^{(t-c)/\tau_2} - 1]\} \quad \text{for } t \geq c.$$

The total length at any time t, $L(t)$, is given by

$$L(t) = L(0) + \int_0^t \alpha(\theta)Z(\theta)\,\mathrm{d}\theta,$$

where $\alpha(\theta)$ is the rate of cell elongation per zone at time θ.

The length extension model takes α to be inversely proportional to the doubling time τ or to the time it would take to double $M(t)$ at the current rate of growth, $\mathrm{d}M(t)/\mathrm{d}t$. In exponentially growing cultures, these assumptions are equivalent; under shift-up conditions, they are not: the former implies that the transition from being proportional to $1/\tau_1$ to being proportional to $1/\tau_2$ occurs abruptly at $t=0$, the latter implies that it takes place gradually as the new steady state is approached. These we now term the α and α' versions, respectively. Specifically, α goes from $k(\ln 2)/\tau_1$ at $t=0$ to

$k(\ln 2)/\tau_2$ in the α version and to $k(\ln 2)/\tau_2'(t)$ in the α' version, where

$$\tau_2'(t) = \tau_2(1 + v_2 2^{-t/\tau_2})$$

and $v_2 \equiv (1 - v_1)/v_1$. Thus, for the α version, by direct integration,

$$\frac{L(t)}{L(0)} = 1 + \frac{\tau_1}{\tau_2}(2^{t/\tau_1} - 1) \qquad \text{for } t \le c$$

$$= \frac{L(c)}{L(0)} + v_1 2^{c/\tau_1}\left[v_2 \frac{\ln 2}{\tau_2}(t-c) + 2^{(t-c)/\tau_2} - 1\right] \quad \text{for } t \ge c,$$

whereas for the α' version

$$\frac{L(t)}{L(0)} = 1 + \frac{\ln 2}{\tau_2}\int_0^t \frac{2^{\theta/\tau_1}}{1 + v_2 2^{-\theta/\tau_2}}\,d\theta \qquad \text{for } t \le c$$

$$= \frac{L(c)}{L(0)} + \frac{(\ln 2)2^{c/\tau_1}}{\tau_2}\int_c^t \frac{1 + v_1[2^{(\theta-c)/\tau_2} - 1]}{1 + v_2 2^{-\theta/\tau_2}}\,d\theta \quad \text{for } t \ge c$$

and cannot be expressed analytically unless τ_1 is an exact multiple of τ_2 (Courant, 1937).

It may be instructive to derive $L(t)$ by focusing on the behavior of individual cells rather than on total cell mass and number of origins. We have done this for the case in which cell mass is assumed to respond to the new growth rate immediately and not as found by Bremer & Dennis (1975). Even with so gross an oversimplification, the mathematics is still long and tedious. Details of the development can be found in the Appendix; the final result is identical with that found above to the same level of approximation ($v_1 = 1$).

(C) CELL NUMBER

A cell initiates chromosome replication when it has attained a constant mass per origin (Donachie, 1968; Pritchard *et al.*, 1969); it divides $C + D$ min later (Cooper & Helmstetter, 1968). The number of cells $N(t)$ is therefore directly proportional to total cell mass but with a time lag of $C + D$. Thus

$$\frac{N(t)}{M(t-c')} = \frac{N(0)}{M(-c')},$$

where $c' \equiv C + D$, and

$$\frac{N(t)}{N(0)} = 2^{t/\tau_1} \qquad \text{for } t \le c'$$

$$= 2^{c'/\tau_1}\{1 + v_1[2^{(t-c')/\tau_2} - 1]\} \quad \text{for } t \ge c'.$$

(D) SURFACE GROWTH

The expressions for total cell length $L(t)$ were derived on the basis of the linear elongation model. Completely analogous expressions are obtained for total surface area $A(t)$ in the case where cell envelope rather than length increases linearly with cell age, it is only necessary to replace $L(t)$ by $A(t)$ [and, formally, $\alpha(t)$ by $\beta(t)$, where $\beta(t)$ is the rate of surface growth per zone at time t].

(E) DERIVED DIMENSIONS

Mean cell mass and mean cell length (or surface area) follow directly from the corresponding total quantities by dividing by $N(t)$. In order to proceed further, we adopt the not unreasonable assumptions (Koch & Blumberg, 1976) of constant mean cell density as a function of τ, which enables us to equate total cell volume with total cell mass, and an idealized geometry: *E. coli* is taken to be a perfect rod-shaped bacterium with hemispherical polar caps. Mean cell volume $\bar{V}(t)$ and surface area $\bar{A}(t)$ can then be expressed in terms of mean cell length $\bar{L}(t)$ and radius $\bar{R}(t)$ to provide two simultaneous equations in two unknowns,

$$\bar{V}(t) = \pi\bar{R}^2(t)[\bar{L}(t) - 2\bar{R}(t)] + \tfrac{4}{3}\pi\bar{R}^3(t) = \pi\bar{R}^2(t)[\bar{L}(t) - \tfrac{2}{3}\bar{R}(t)]$$

$$\bar{A}(t) = 2\pi\bar{R}(t)[\bar{L}(t) - 2\bar{R}(t)] + 4\pi\bar{R}^2(t) = 2\pi\bar{R}(t)\bar{L}(t).$$

In the length extension model, the unknowns are $\bar{R}(t)$ and $\bar{A}(t)$, and the first of these expressions gives rise to a cubic equation in $\bar{R}(t)$ in terms of $\bar{V}(t)$ and $\bar{L}(t)$; in the surface growth model, the unknowns are $\bar{R}(t)$ and $\bar{L}(t)$, and both expressions are required in order to obtain the cubic equation in $\bar{R}(t)$ in terms of $\bar{V}(t)$ and $\bar{A}(t)$. In either case, there is always one and only one root in the range $0 < 2\bar{R}(t) < \bar{L}(t)$.

It should be borne in mind that $\bar{R}(t)$ as used here is not the true mean radius of the cells, in the accepted sense of the word, but rather the radius of a cell with mean volume $\bar{V}(t)$ and mean length $\bar{L}(t)$, for the length extension model, or mean volume $\bar{V}(t)$ and mean area $\bar{A}(t)$, for the surface growth model. To emphasize this point, we label these quantities R_L and R_A, respectively, and the corresponding lengths and surface areas, L_L, L_A and A_L, A_A. In part, this is done for consistency, as obviously $L_L \equiv \bar{L}(t)$ and $A_A \equiv \bar{A}(t)$. The various symbols and their origin are summarized below.

Dimension	Length extension model	Surface growth model
Length	$L_L = \bar{L}(t)$, from model	L_A, from $\bar{V}(t)$ and $\bar{A}(t)$
Area	A_L, from $\bar{V}(t)$ and $\bar{L}(t)$	$A_A = \bar{A}(t)$, from model
Radius	R_L, from $\bar{V}(t)$ and $\bar{L}(t)$	R_A, from $\bar{V}(t)$ and $\bar{A}(t)$
Volume	$\bar{V}(t)$, independent of model	$\bar{V}(t)$, independent of model

3. Results

Six families of curves are presented to illustrate the results of the calculations of the previous section. Mean cell length and radius are shown in turn as a function of time during the first 4 h following shift-up. All the curves were drawn by a computer-drive incremental x–y flat-bed plotter accurate to within 0·1% of one scale division. The values of the parameters have been chosen so as to permit a direct comparison between the models and to cover a broad range of experimental conditions.

In order to obtain such plots, it is first necessary to compute the steady-state dimensions explicitly. The appropriate expressions have been published before (Grover *et al.*, 1977; Rosenberger *et al.*, 1978), and we repeat them here for convenience:

$$\bar{V}(0) = (\ln 2) V_i 2^{(C+D)/\tau_1}; \qquad \bar{V}(\infty) = (\ln 2) V_i 2^{(C+D)/\tau_2};$$

$$\bar{L}(0) = \frac{k_L}{\ln 2} 2^{d_L/\tau_1}; \qquad \bar{L}(\infty) = \frac{k_L}{\ln 2} 2^{d_L/\tau_2};$$

$$\bar{A}(0) = \frac{k_A}{\ln 2} 2^{d_A/\tau_1}; \qquad \bar{A}(\infty) = \frac{k_A}{\ln 2} 2^{d_A/\tau_2}.$$

The coefficients V_i, k_L, k_A are as defined previously; the labelling has been modified slightly to conform to our present usage. It is also necessary to assign numerical values to the ribosomal efficiencies at τ_1 and τ_2. This was done using an approximate expression for ε extracted from the data of Bremer & Dennis (1975) by linear regression:

$$\varepsilon \approx 0{\cdot}1455 \qquad \text{for } \tau \leq 50 \text{ min}$$

$$\approx 0{\cdot}0066 + 7{\cdot}19/\tau \quad \text{for } \tau \geq 50 \text{ min}.$$

Mean cell length is depicted in Fig. 1. Nine pairs of curves are plotted, one member of each pair illustrating the dependence on time of L_L (solid lines) and the other of L_A (dashed lines). The various parameters were chosen as follows. Values for k_L and d_L were taken from an earlier article in which cell dimensions under steady-state conditions were described by the length extension model (Grover *et al.*, 1977); k_A and d_A are from a similar analysis based on the surface growth model and using essentially the same raw data (Rosenberger *et al.*, 1978). These four parameters allow us to compute $\bar{L}(0)$, $\bar{L}(\infty)$, $\bar{A}(0)$, $\bar{A}(\infty)$ from the above expressions for any given τ_1 and τ_2, and thence $\bar{R}(0)$, $\bar{R}(\infty)$, $\bar{V}(0)$, $\bar{V}(\infty)$, V_i and $C+D$. Thus L_L and L_A are completely specified, and in such a way as to coincide both before shift-up [with $\bar{L}(0)$] and after the new steady state is reached [with $\bar{L}(\infty)$]. [This form

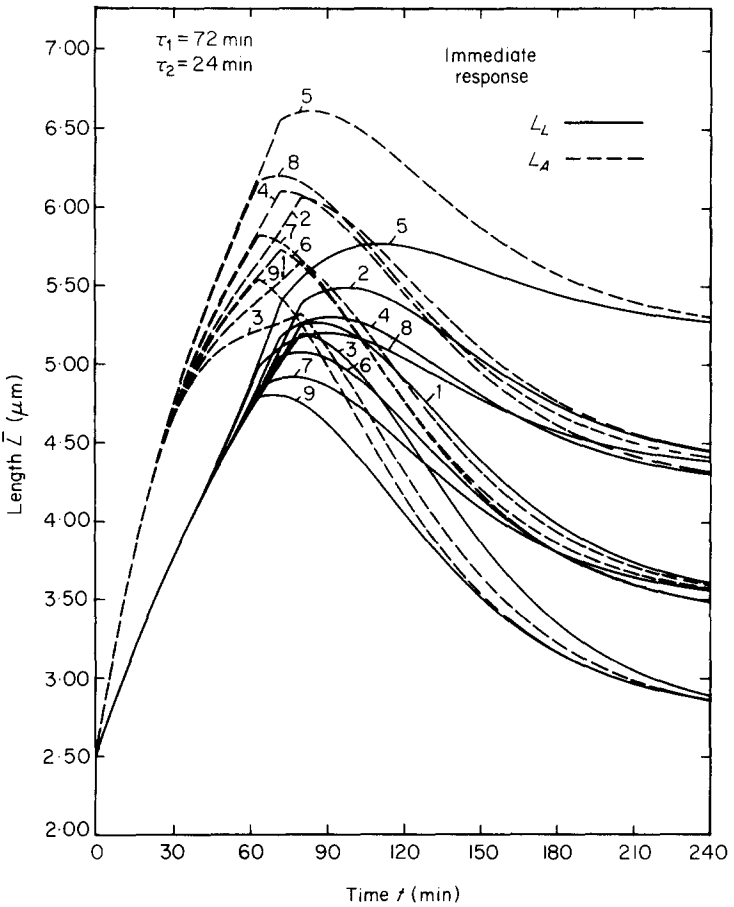

FIG. 1. Mean cell length $\bar{L}(t)$ as a function of time t following shift-up from $\tau_1 = 72$ min to $\tau_2 = 24$ min, for various combinations of $(C+D)$, d_L, d_A. Immediate response version. Solid lines: length extension model; dashed lines: surface growth model.

Curve pair	$C+D$ (min)	d_L (min)	d_A (min)
1	80·2	17·1	49·3
2		28·6	54·3
3		4·5	44·3
4	71·8	27·1	49·3
5		38·1	54·3
6		15·6	44·3
7	63·5	17·1	40·6
8		28·4	45·6
9		5·1	35·6

of graphic display is designed to facilitate comparison between the two models and will be used throughout this and the succeeding article (Woldringh *et al.*, 1980).]

The pre-shift dimensions and τ_1 and τ_2 are the same for all the curves in Fig. 1, the other parameters are not. Curve pair 1 uses the values derived above, pair 2 uses the same $C+D$ but its d_A has been increased by 5 min; in pair 3, d_A has been decreased by 5 min. For pair 4, d_A and k_A have been reset to those in pair 1 but d_L has been increased by 10 min. The new $C+D$ value obtained has then been used for the next 2 pairs of curves, their d_A values again being 5 min above and 5 min below that of pair 4. Pair 7 has the

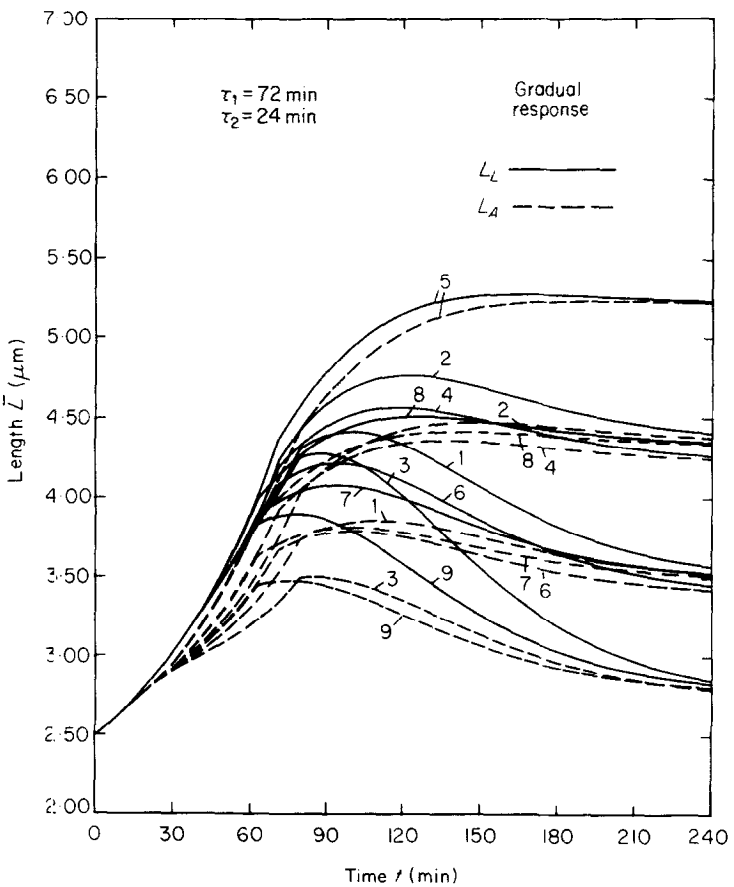

FIG. 2. Mean cell length $\bar{L}(t)$ as a function of time t following shift-up from $\tau_1 = 72$ min to $\tau_2 = 24$ min, for the same combinations of $(C+D)$, d_L, d_A as in Fig. 1. Gradual response version. Solid lines: length extension model; dashed lines: surface growth model.

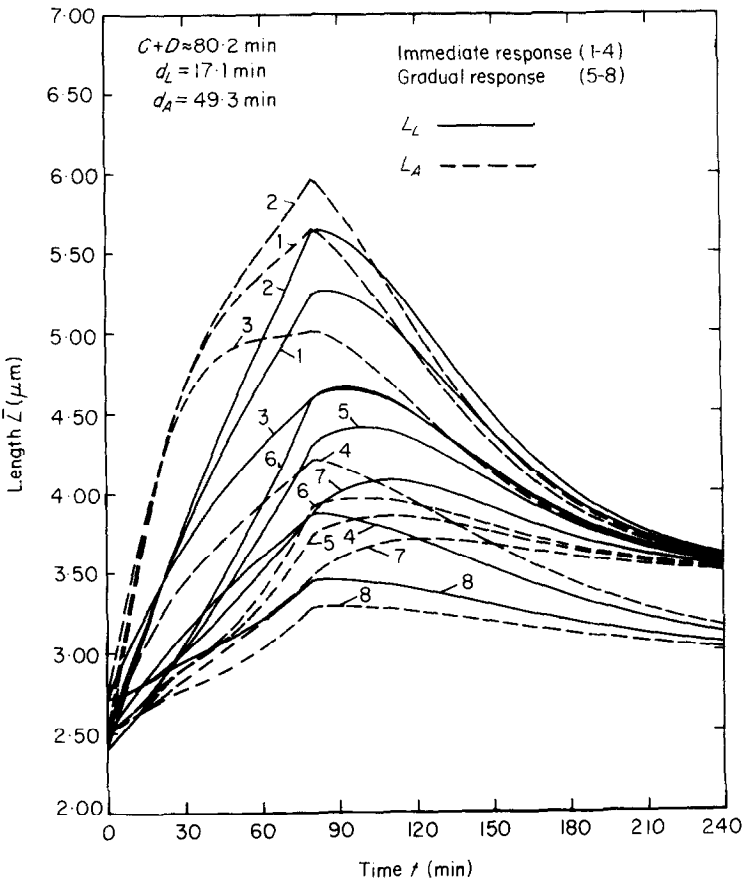

FIG. 3. Mean cell length $\bar{L}(t)$ as a function of time t following shift-up with $(C+D) = 80{\cdot}2 \pm 0{\cdot}1$ min, $d_L = 17{\cdot}1$ min and $d_A = 49{\cdot}3$ min, for various combinations of τ_1 and τ_2. Solid lines: length extension model; dashed lines: surface growth model.

Curve pair	Response version	τ_1 (min)	τ_2 (min)
1	Immediate	72	24
2		96	24
3		48	24
4		72	36
5	Gradual	72	24
6		96	24
7		48	24
8		72	36

same d_L and k_L as pair 1; its $C+D$ was chosen to be less than that of pair 4 by the same amount as the $C+D$ of pair 1 is greater. Pairs 8 and 9 again show the effect of changing d_A by 5 min.

The nine pairs of curves in Fig. 1 were calculated using the expressions developed for the α or immediate response version of the models; Fig. 2 contains the corresponding curves for the α' version.

Both versions appear in Fig. 3. The first four pairs of curves depict different combinations of τ_1 and τ_2 for the α version and the last four for the α' version, all eight having the same d_L, k_L, d_A, k_A used in pair 1 of Fig. 1. (Thus pair 1 here is a repeat of pair 1 in Fig. 1 and pair 5, of pair 1 in Fig. 2.)

Figures 4–6 are completely analogous to Figs 1–3, and with an identical set of parameters, but illustrate the behavior of R_L and R_A rather than L_L and L_A.

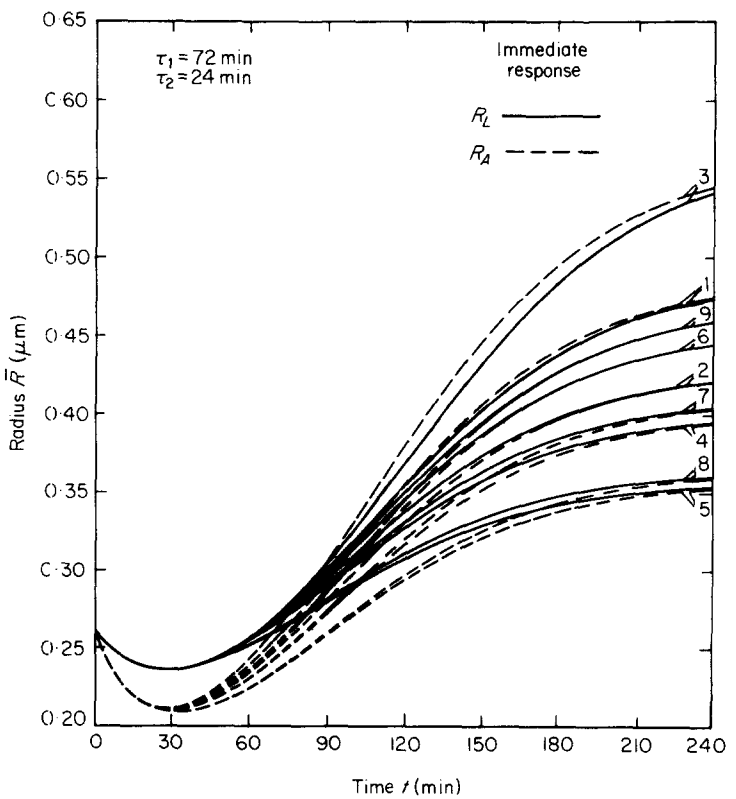

FIG. 4. Mean cell radius $\bar{R}(t)$ as a function of time t following shift-up from $\tau_1 = 72$ min to $\tau_2 = 24$ min, for the same combinations of $(C+D)$, d_L, d_A as in Fig. 1. Immediate response version. Solid lines: length extension model; dashed lines: surface growth model.

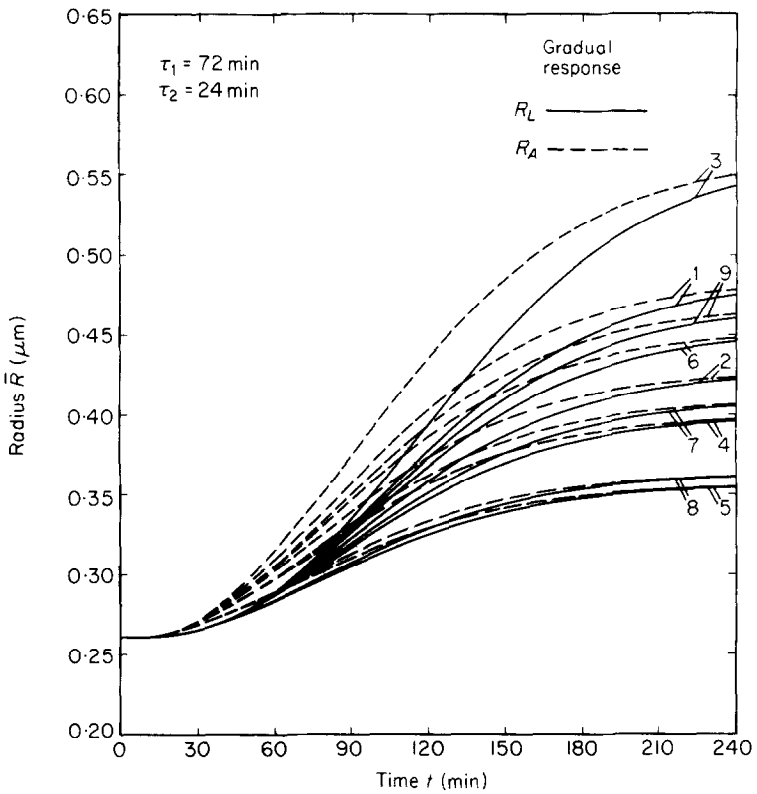

FIG. 5. Mean cell radius $\bar{R}(t)$ as a function of time t following shift-up from $\tau_1 = 72$ min to $\tau_2 = 24$ min, for the same combinations of $(C+D)$, d_L, d_A as in Fig. 1. Gradual response version. Solid lines: length extension model; dashed lines: surface growth model.

4. Discussion

The results presented here justify the approach proposed in the Introduction: the transient behavior predicted by the length extension model (Grover *et al.*, 1977) is indeed quite different from that expected on the basis of the surface growth model (Rosenberger *et al.*, 1978) even when the parameters are chosen to produce identical steady-state dimensions. This is particularly true in the case where the growth rate is assumed to change abruptly at shift-up but holds in the gradual response version as well, only to a lesser extent. Previous studies (Grover *et al.*, 1977; Rosenberger *et al.*, 1978) have shown that the experimental data require d to be greater in the surface growth model than in the length extension model. This implies that the latency period $c(\equiv C+D-d)$, during which the number of growth

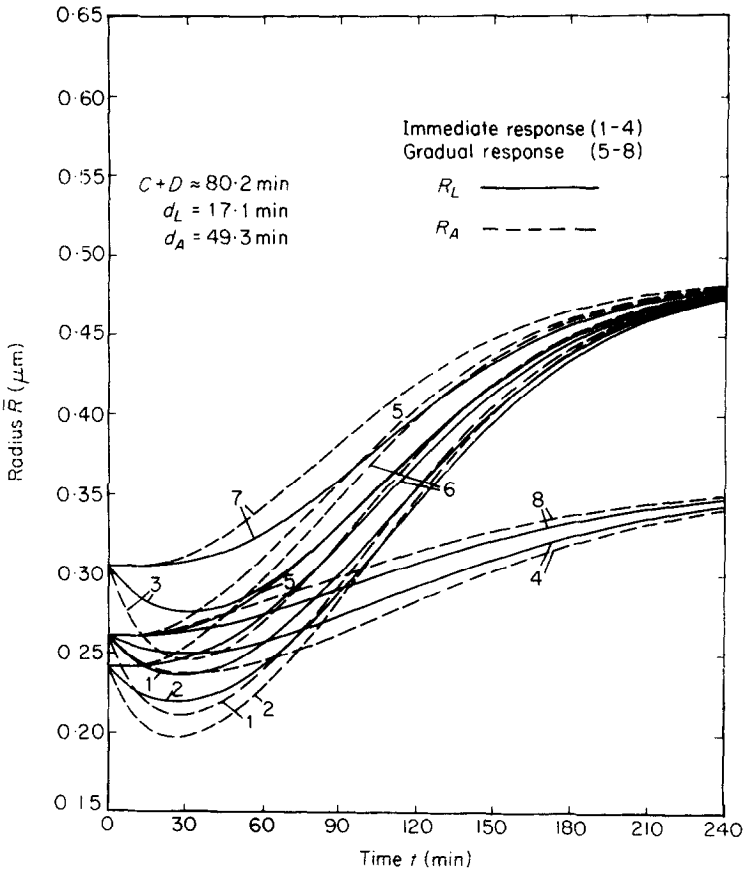

FIG. 6. Mean cell radius $\bar{R}(t)$ as a function of time t following shift-up with $(C+D) = 80{\cdot}2 \pm 0{\cdot}1$ min, $d_L = 17{\cdot}1$ min and $d_A = 49{\cdot}3$ min, for the same combinations of τ_1 and τ_2 as in Fig. 3. Solid lines: length extension model; dashed lines: surface growth model.

zones continues to increase at the pre-shift rate, is shorter with $A(t)$ the dimension under active control than with $L(t)$, and one would expect L_A to be greater than L_L, which it is, at least in the immediate response version. The moderating influence introduced by the α' version affects L_L less than it does L_A because the former, being under active control, is affected directly whereas the latter changes through its dependence on $A(t)$, which is greater than linear. Thus L_A drops below its level in the α version by much more than L_L does and, at any particular time, is actually less than the corresponding value of L_L.

The difference in the behavior of $\bar{L}(t)$ and $\bar{R}(t)$ is striking: $\bar{L}(t)$ undergoes a large overshoot and approaches its steady-state value from above, $\bar{R}(t)$

remains almost constant (α' version) or even decreases somewhat (α version) before increasing monotonically towards its asymptotic level.

Both dimensions take a considerable period of time to reach steady state. This should not be too surprising, perhaps, when one realizes that $N(t)$ continues to increase according to $2^{t/\tau_1}$ until $C+D$ min after the shift-up, so that only then can the dimensions begin their approach to steady state, a process of rearrangement that requires several generations because of the need to dilute out the excess length (or surface area) accumulated during the overshoot phase and the effect of the delay in protein production following shift-up. (This latter is responsible for the maximum overshoot occurring slightly beyond $t = C+D$.)

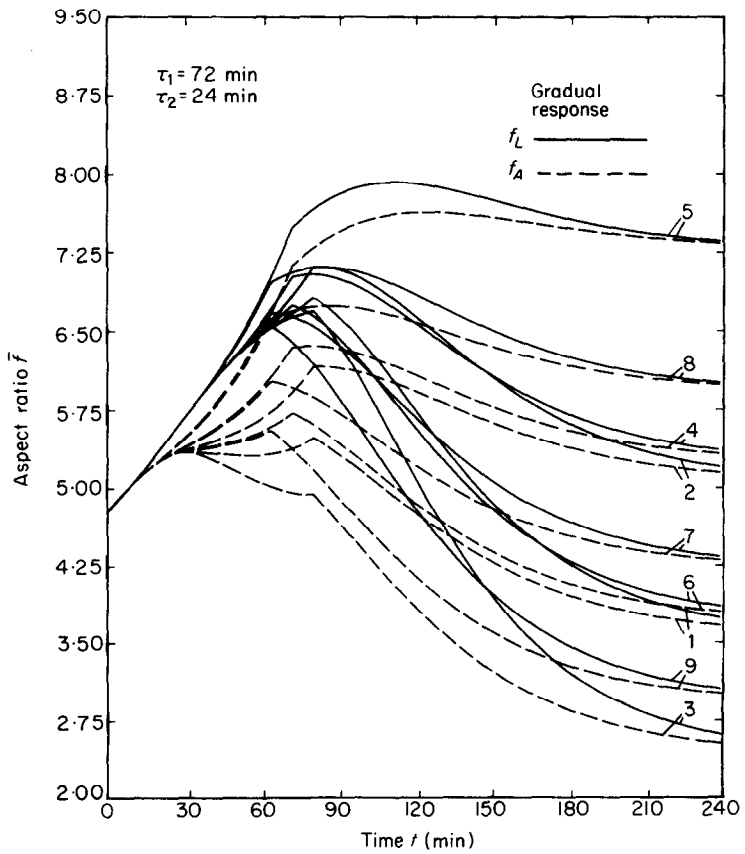

FIG. 7. Mean aspect ratio $\bar{f}(t)$ as a function of time t following shift-up from $\tau_1 = 72$ min to $\tau_2 = 24$ min, for the same combinations of $(C+D)$, d_L, d_A as in Fig. 1. Gradual response version. Solid lines: length extension model; dashed lines: surface growth model.

In the immediate response version, both R_L and R_A start to decrease right after the shift-up (the latter more rapidly) and remain below their pre-shift level for at least one hour; in the gradual response version, both increase from the beginning (albeit slowly at first) and continue to increase throughout the transition period. It should thus not be too difficult in practice to choose between the two, even without specifying the model. Choosing between the models is not as easy, and our ability to do so will depend in large measure on the magnitude of the predicted difference between them. Unfortunately, as we shall see in the following article (Woldringh *et al.*, 1980), the experimental results favor the version with the

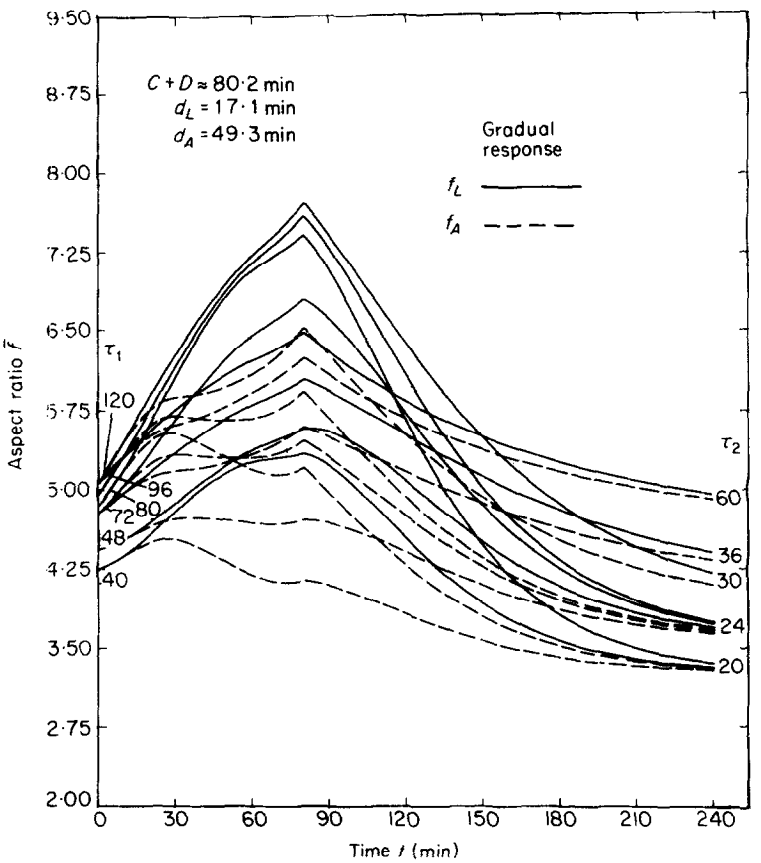

FIG. 8. Mean aspect ratio $\bar{f}(t)$ as a function of time t following shift-up with $(C+D) = 80{\cdot}2 \pm 0{\cdot}2$ min, $d_L = 17{\cdot}1$ min and $d_A = 49.3$ min, for the various combinations of τ_1 and τ_2 indicated (in min). Gradual response version. Solid lines: length extension model; dashed lines: surface growth model.

smaller difference, the α' version. In an attempt to enhance this difference, we turn to a dimensionless measure of cell geometry, the aspect ration $\bar{f}(t)$, defined (Zaritsky, 1975; Krasnow, 1978) as $\bar{L}(t)/2\bar{R}(t)$, so that $f_L \equiv L_L/2R_L$ and $f_A \equiv L_A/2R_A$. Figure 7 is a plot of this ratio as a function of time after shift-up using the data of Fig. 2 and Fig. 5. The separation between corresponding curves is indeed larger in the case of $\bar{f}(t)$ than for either of the original dimensions. (The aspect ratio possesses the added advantage of being independent of the absolute calibration of the experimental data.) Figure 8 illustrates the effect on this separation of various combinations of τ_1 and τ_2. These figures suggest that in a properly designed and carefully executed experiment, one could hope to be able to decide unequivocally between the contending models.

It should be pointed out that there is a fundamental difference between the two models as regards steady-state mean length: $\bar{L}(\infty)$ is a sensitive function of $C+D$ in the surface growth model but is completely independent of $C+D$ in the length extension model. Thus experiments designed (Zaritsky & Pritchard, 1973; Lane & Denhardt, 1975) to alter C could afford an independent test of the models provided D and d remain unchanged. Such measurements are now in progress and will be reported upon in a subsequent publication.

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APPENDIX

In order to develop an expression for total cell length based on the behavior of individual cells, we assume that $C+D$, d, τ_1 and τ_2 are not only independent of time, as before, but also that they are the same for all cells. In addition, we require that division give rise to two identical daughter cells and, perhaps most unrealistic of all, that cell mass respond to the new growth rate immediately rather than as found by Bremer & Dennis (1975). Since the approach presented here is meant to be of heuristic value only, such gross over-simplification need not be of too much concern; a less restrictive (and far less laborious) derivation is provided in the main Theory section of this article.

For convenience we treat cases in which age at initiation of chromosome replication a_0 precedes age at doubling $\tau_1 - d$, separately from those in which a_0 follows $\tau_1 - d$. The cells in each case are divided into three age groups:

$$0 \leq a \leq a_0, \quad a_0 \leq a \leq \tau_1 - d, \quad \tau_1 - d \leq a \leq \tau_1 \quad \text{for } a_0 \leq \tau_1 - d$$

$$0 \leq a \leq \tau_1 - d, \quad \tau_1 - d \leq a \leq a_0, \quad a_0 \leq a \leq \tau_1 \quad \text{for } a_0 \geq \tau_1 - d.$$

Consider first the group $a \leq a_0 \leq \tau_1 - d$, and let $p(\geq 1)$ represent the integer $(C+D+a_0)/\tau_1$. Then the time t_m at which doubling m takes place depends on p. For

$$p = 1,\ t_1 = (a_0 - a)\gamma + c,\ t_2 = t_1 + \tau_2, \ldots, t_m = t_1 + (m-1)\tau_2;$$

for

$$p = 2.\ t_1 = \tau_1 - d - a,\ t_2 = (a_0 - a)\gamma + c,\ t_3 = t_2 + \tau_2, \ldots, t_m = t_2 + (m-2)\tau_2;$$

and, in general,

$$t_1 = \tau_1 - d - a, \quad t_2 = t_1 + \tau_1, \ldots, \quad t_{p-1} = t_1 + (p-2)\tau_1,$$

$$t_p = (a_0 - a)\gamma + c, \quad t_{p+1} = t_p + \tau_2, \ldots, \quad t_m = t_p + (m-p)\tau_2;$$

where $\gamma \equiv \tau_2/\tau_1$ and $c \equiv C + D - d$.

The rate at which a cell extends is a function of t, the time since the shift-up, and a, the age of the cell at $t = 0$. It is possible to choose a series of time intervals in such a way that this rate alternates between doubling once for each cell and being completely independent of a; the actual sequence depends on p:

Sequence	Time interval	Rate of extension
1st, for $p \geq 2$	$0 \leq t \leq \tau_1 - d - a_0$	α for all cells
	$\tau_1 - d - a_0 \leq t \leq \tau_1 - d$	doubles at $t = \tau_1 - d - a$
2nd, for $p \geq 3$	$\tau_1 - d \leq t \leq 2\tau_1 - d - a_0$	2α for all cells
	...	
pth	$(p-1)\tau_1 - d \leq t$	$2^{p-1}\alpha$ for all cells
	$\leq p\tau_1 - d - a_0 = c$	doubles at $t = c + \gamma(a_0 - a)$
	$c \leq t \leq c + \gamma a_0$	
	...	
$(p+1+n)$th	$c + \gamma a_0 + n\tau_2 \leq t \leq c$	$2^{p+n}\alpha$ for all cells
	$+ (n+1)\tau_2$	
	$c + (n+1)\tau_2 \leq t \leq c$	doubles at
	$+ (n+1)\tau_2 + \gamma a_0$	$t = c + \gamma(a_0 - a) + (n+1)\tau_2$

When we combine all three groups of cells in the case $a_0 \leq \tau_1 - d$, the time intervals can be redefined so that within each interval the rate of extension doubles once, either for all cells with $a \leq \tau_1 - d$ or for all cells with $a \geq \tau_1 - d$, but not both:

Sequence	Interval	Rate for $a \leq \tau_1 - d$	Rate for $a \geq \tau_1 - d$
1st, for $p \geq 2$	$0 \leq t \leq \tau_1 - d$	doubles at $t = \tau_1 - d - a$	2α for all cells
	$\tau_1 - d \leq t \leq \tau_1$	2α for all cells	doubles at $t = 2\tau_1 - d - a$
2nd, for $p \geq 3$	$\tau_1 \leq t \leq 2\tau_1 - d$	doubles at $t = 2\tau_1 - d - a$	4α for all cells
		...	
pth	$(p-1)\tau_1 \leq t \leq c + \gamma a_0$	doubles at $t = c - (a - a_0)$ for $a \geq a_0$ and at $t = c + \gamma(a_0 - a)$ for $a \leq a_0$	$2^f\alpha$ for all cells
	$c + \gamma a_0 \leq t \leq c + \gamma(a_0 + d)$	$2^f\alpha$ for all cells	doubles at $t = c + \gamma(a_0 - a) + \tau_2$
		...	
$(p+1+n)$th	$c + \gamma(a_0 + d) + n\tau_2 \leq t \leq c + \gamma a_0 + (n+1)\tau_2$	doubles at $t = c + \gamma(a_0 - a) + (n+1)\tau_2$	$2^{p+n+1}\alpha$ for all cells
	$c + \gamma a_0 + (n+1)\tau_2 \leq t \leq c + \gamma(a_0 + d) + (n+1)\tau_2$	2^{p+n+1} for all cells	doubles at $t = c + \gamma(a_0 - a) + (n+2)\tau_2$

We can now calculate the total length at any time $L(t)$ by summing the contributions of all the cells. Again for the case $a_0 \leq \tau_1 - d$,

$$L(t) = L(0) + \int_0^{a_1} \alpha t \nu(a)\, da + \int_{a_1}^{\tau'} \alpha t_1 \nu(a)\, da + \int_{a_1}^{\tau'} 2\alpha(t - t_1)\nu(a)\, da + \int_{\tau'}^{\tau_1} 2\alpha t \nu(a)\, da \qquad \text{for } 0 \leq t \leq \tau'$$

and

$$L(t) = L(\tau') + \int_0^{\tau'} 2\alpha(t - \tau')\nu(a)\, da + \int_{\tau'}^{a_2} 2\alpha(t - \tau')\nu(a)\, da + \int_{a_2}^{\tau_1} 2\alpha(t_2 - \tau')\nu(a)\, da + \int_{a_2}^{\tau_1} 4\alpha(t - t_2)\nu(a)\, da \qquad \text{for } \tau' \leq t \leq \tau_1,$$

where $\tau' \equiv \tau_1 - d$, $a_i \equiv i\tau_1 - d - t$, and $\nu(a)$ is the number of cells at age a at $t = 0$ and is given by the product of $N(0)$, the total number of cells at $t = 0$, and $(2/\tau_1)$ (ln 2)$2^{-a/\tau_1}$, the frequency function of age (Powell, 1956). In general, for any closed interval $t_- \leq t \leq t_+$,

$$L(t) = L(t_-) + \int_0^{a_i} 2^{i-1}\alpha(t - t_-)\nu(a)\, da + \int_{a_i}^{\tau'} 2^{i-1}\alpha(t_i - t_-)\nu(a)\, da + \int_{a_i}^{\tau'} 2^i \alpha(t - t_i)\nu(a)\, da + \int_{\tau'}^{\tau_1} 2^i \alpha(t - t_-)\nu(a)\, da$$

or

$$L(t) = L(t_-) + \int_0^{\tau'} 2^{i-1}\alpha(t - t_-)\nu(a)\, da + \int_{\tau'}^{a_i} 2^{i-1}\alpha(t - t_-)\nu(a)\, da + \int_{a_i}^{\tau_1} 2^{i-1}\alpha(t_i - t_-)\nu(a)\, da + \int_{a_i}^{\tau_1} 2^i \alpha(t - t_i)\nu(a)\, da,$$

depending on which cells double their rate within that period. Here $t_i \equiv i\tau_1 - d - a$ and $i \equiv [(t_- + d)/\tau_1] + 1$. The results are surprisingly simple, and the same for both cases ($a_0 \leq \tau_1 - d$ and $a_0 \geq \tau_1 - d$):

$$\frac{L(t)}{L(0)} = 1 + \frac{\tau_1}{\tau_2}(2^{t/\tau_1} - 1) \qquad \text{for } t \leq c$$

$$= \frac{L(c)}{L(0)} + 2^{c/\tau_1}[2^{(t-c)/\tau_2} - 1] \quad \text{for } t \geq c.$$