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Changes in Cell Size and Shape Associated with Changes in the Replication Time of the Chromosome of *Escherichia coli*

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Average cell mass is shown to be inversely related to the concentration of thymine in the growth medium of a *thy*⁻ strain of *Escherichia coli*. The kinetics of the transition from one steady-state average cell mass to another was followed in an attempt to determine the relationship between the chromosome replication time and the time between completion of a round of chromosome replication and the subsequent cell division. Differences in average cell mass are shown to be associated with similar differences in average cell volume. Changes in volume associated with changes in thymine concentration are shown to be due primarily to differences in the width of cells. It is proposed that extension in length of the cell envelope occurs at a linear rate which is proportional to the growth rate and which doubles at the time of termination of rounds of replication. Changes in volume not associated with a change in growth rate are therefore accommodated by a change in cell width. Conditions are described under which average cell mass can continue to increase in successive generations and no steady-state average cell mass is achieved.

The size and deoxyribonucleic acid (DNA) content of cells of *Escherichia coli* and *Salmonella typhimurium* may be described in terms of three parameters: (i) the mass (or volume) at which cycles of chromosome replication are initiated, (ii) the time (C) between initiation and completion of one round of chromosome replication, and (iii) the time (D) between termination of a round of chromosome replication and the subsequent cell separation. All three of these parameters appear to be constants at least in batch cultures with doubling times less than 70 min at 37 C (7, 8, 16, 34, 38).

A constant value for C at different growth rates had been anticipated by earlier work (27, 28, 31, 33) and could be the consequence of a single enzyme complex associated with a replication fork and a concentration of DNA precursors which is not rate limiting. A constant value for D is less easy to understand. If, as some evidence suggests (6, 17), completion of a cycle of replication sets in motion a succession of biosynthetic events leading to the synthesis of a cross wall and ultimately to separation of two sister cells, it is not obvious why the length of time required for these processes should be independent of the growth rate. It therefore seemed worthwhile to investigate the possibility of a more direct connection between C and D.

The discovery that the replication time of the chromosome can be varied without a concomitant change in growth rate, simply by changing the concentration of thymine in the growth medium in thy^- strains of *E. coli* (36, 42), provides a new method for investigating the relationship between C and D. Just as the kinetics of the transition from one cell size and composition to another after an increase in growth rate (23) have been shown to be those predicted on the basis of the three constants described above (7, 8, 34), so there should be corresponding changes in size and composition if C were varied and the growth rate kept unchanged. In this case, however, the magnitude and kinetics of these changes would depend on the length of D and its relationship to C.

In our initial studies with *E. coli* 15T⁻ (555-7) growing in synthetic medium with glucose as carbon source, we found that steady-state conditions in terms of mass per cell and DNA per cell could not be obtained (36). Both these

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values increased progressively during exponential growth, despite the fact that the rate of increase in mass and the rate of DNA synthesis were equal and the DNA/mass ratio consequently remained constant. This anomaly made it impossible to compare observed changes in mass per cell and DNA per cell with those predicted as a result of changes in C. The anomalous behavior of *E. coli* 15T⁻ (555-7) (which we also find in other *thy* strains) growing in glucose synthetic medium is described in more detail in this report. We subsequently found that, when this same strain is grown in synthetic medium with glycerol as carbon source, constant steady-state values for mass per cell and DNA per cell are obtained. Under these growth conditions, we have been able to determine the effect of changing C on cell size and DNA content and to follow the kinetics of the transition from one steady-state value to another. Although the results obtained are in qualitative agreement with the above assumptions (i–iii), they do not permit the relationship between C and D to be defined unambiguously.

One outcome of these studies on the change in cell size associated with a change in C, which we believe to be of considerable significance for an understanding of the mechanism of cell growth and division, is that it is achieved largely by a change in cell *width*. This suggests that either the rate of linear extension of the cell envelope or the rate of synthesis of an important component of the cell envelope is invariant at one growth rate.

MATERIALS AND METHODS

Strains, growth conditions, and measurements. Three strains of *E. coli* 15 were used: P245 *E. coli* $15T^+$ wild type (donated by T. R. Breitman); P178 *E. coli* $15T^-$ (555-7), *trp⁻ met⁻ arg⁻ thy⁻ drm⁻*(41); P178-2 *E. coli* $15T^+$, *trp⁻ met⁻ arg⁻ tpp⁻*. This strain is a *thy⁺drm⁺ tpp⁻* transductant of P178 (3).

M9 synthetic medium (1) was used in all experiments with either glucose (0.4%) or glycerol (0.4%) as sole carbon source. Required amino acids (50 μg/ml) were added as necessary. Thymine was supplemented at the concentration indicated in each experiment.

Medium was inoculated with a single colony of the appropriate strain, and the culture was aerated vigorously at 37 C in a New Brunswick gyratory shaker.

Absorbance of cultures was measured at 450 nm with a Gilford microsample spectrophotometer and particle number with a Coulter electronic particle counter (model B) with the use of a probe with a 30-μm orifice. The number of colony-forming units was measured in samples suitably diluted in phosphate buffer (pH 7.0), spread on plates containing M9 medium supplemented with thymine (10 μg/ml) and required amino acids, and solidified with agar (1.5%).

Relative amounts of DNA were determined by using ^{14}C-thymine (0.02 μCi/μg) in the growth medium and measuring the amount of label present in the acid-insoluble fraction. Cultures samples were added to an equal volume of cold (4 C) 10% trichloroacetic acid. At least 30 min later, each sample was filtered through a membrane filter (Sartorius; 0.45-μm pore size, 27 mm) and washed six times with about 5 ml of hot (95 C), distilled water. The filters were dried under an infrared lamp and placed with a constant orientation in small vials. These were filled with scintillation fluid (35), stoppered, placed in a standard Packard vial, and counted in a Packard liquid scintillation counter.

Changes in thymine concentration were achieved either by addition of a small volume of a concentrated solution of thymine or by an appropriate dilution into fresh prewarmed medium containing a lower concentration of thymine. Thymine starvation was achieved by depositing cells on a membrane filter (Millipore Corp.; 0.45-μm pore size, 47 mm), washing them on the filter with approximately the same volume of prewarmed unsupplemented M9 medium, and suspending them in the new medium by rapid shaking. This procedure usually took less than 3 min, and more than 90% of the bacterial mass was recovered.

In most experiments, the absorbance of the culture was kept within the range 0.05 to 0.3 by removing a proportion of the culture from the flask and replacing it with a similar volume of fresh, prewarmed medium. In no case was absorbance permitted to rise above 0.6 during an experiment. Under our experimental conditions, at least one more mass doubling could take place before the increase in absorbance ceased to be exponential.

Volume distribution. Volume distributions were determined on samples, fixed by adding formaldehyde (final concentration 0.25%), with a modified Coulter particle counter (13) connected to a multichannel pulse-height analyzer, and an orifice (manufactured by Swiss Jewel Co., Locarno) of 17-μm diameter and 75-μm nominal length. Fixed bacteria or polystyrene latex particles of known average volume (14), which were used as a standard, were suspended in 25.2% $MgSO_4$ as electrolyte.

Electron microscopy. Bacteria were fixed in M9 containing 0.25% formaldehyde, washed, and suspended in water. Samples were deposited on carbon-coated grids and air-dried. Photographs were taken to give a final magnification of approximately 16,700. Magnification was calibrated by using latex particles of known volume. Width was measured at the midpoint of each cell or at the midpoints of each of the halves of cells which were constricted.

RESULTS

Composition of glycerol-grown cells. Balanced (5) exponential growth of *E. coli* $15T^-$ (555-7) occurs in synthetic medium with glycerol as sole carbon source. Absorbance, cell number, and the amount of DNA all increase exponentially and at the same rate (Fig. 1a). The growth rate (doubling time ca. 60 min) is independent of the concentration of thymine in the growth medium, provided it is at least 0.2 μg/ml.

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Although the concentration of thymine does not affect the growth rate, it significantly alters cell composition (Table 1); the average cell mass increases and the DNA/mass ratio decreases as the concentration of thymine is reduced. In the experiment shown in Table 1, there was no significant change in the average amount of DNA per cell, but we usually observed a small increase in this parameter with increasing concentrations of thymine. The increase was consistently less than 10% over the concentration range 0.4 to 5.0 μg/ml.

Qualitatively, all of these changes in cell composition were those expected if changing the concentration of thymine in the growth medium affects the rate of addition of nucleotides to growing polynucleotide chains, thereby changing the replication time of the chromosome (36, 42). Quantitatively, however, they were not consistent with the assumption that the only effect of thymine concentration is on the replication time.

Average cell mass ($\overline{M}$), the amount of DNA per cell in genome equivalents ($\overline{G}$), and the DNA/mass ratio ($\overline{G}/\overline{M}$) are functions of C, D, and the doubling time (τ) in cultures in balanced exponential growth. Provided the description of the cell cycle given in the introduction to this paper is valid, the relationships are given by the following equations (7, 8, 34, 36):

$$\overline{G} = \frac{\tau}{C \ln 2} \left[2^{(C+D)/\tau} - 2^{D/\tau}\right] \quad (1)$$

$$\overline{M} = k2^{(C+D)/\tau} \quad (2)$$

$$\overline{G}/\overline{M} = \frac{\tau}{kC \ln 2} \left(1 - 2^{-C/\tau}\right) \quad (3)$$

where k is a constant.

The mean decrease in $\overline{M}$ over the concentration range 0.4 to 5.0 μg of thymine per ml from a number of experiments was found to be 28%. The mean increase in the $\overline{G}/\overline{M}$ was 25%, and the change in $\overline{G}$ was about 5%. If we compare these observed changes with those predicted by equations 1, 2, and 3, assuming that C is the only variable, the results are discordant. Thus the observed change in the DNA/mass ratio is that predicted if C is 45 min longer in 0.4 μg than in 5.0 μg of thymine per ml, but the change in average cell mass is that predicted for an increase in C of only 21 min, and the change in DNA per cell is that predicted for a change in C of less than 8 min (if the constant D is assumed to be 25 min, as it is in *E. coli* B/r). In addition, no value for D can be chosen which will reduce these discrepancies, provided it is assumed that it is constant and independent of C.

These discrepancies suggest that the notion of a period designated D which is constant and independent of C may not be valid in *E. coli*

TABLE 1. *Effect of thymine concentration on cell composition in P178*[a]

Time (min)	DNA/mass[b] (thymine concn)			Mass/cell[c] (thymine concn)			DNA/cell[d] (thymine concn)		
	5.0 μg/ml	1.0 μg/ml	0.4 μg/ml	5.0 μg/ml	1.0 μg/ml	0.4 μg/ml	5.0 μg/ml	1.0 μg/ml	0.4 μg/ml
0	1.76	1.63	1.51	1.80	2.19	2.14	3.16	3.57	3.24
20	1.75	1.65	1.39	1.91	1.93	2.37	3.36	3.19	3.29
40	1.81	1.66	1.45	1.79	2.03	2.35	3.25	3.36	3.41
60	1.80	1.70	1.51	1.78	1.94	2.08	3.18	3.30	3.15
80	1.83	1.65	1.41	1.77	2.00	2.16	3.24	3.29	3.03
100	1.81	1.63	1.46	1.90	1.98	2.35	3.46	3.23	3.44
120	1.84	1.67		1.88	2.06		3.47	3.44	
130	1.86	1.62		1.81	1.96		3.37	3.17	
140	1.79	1.66		1.77	2.02		3.16	3.35	
150	1.82	1.68		1.76	2.07		3.21	3.49	
160	1.81	1.67		1.82	2.04		3.30	3.42	
180	1.78	1.66		1.83	1.90		3.26	3.20	
200	1.86			1.76			3.29		
Avg	1.81	1.66	1.44	1.81	2.01	2.24	3.28	3.33	3.26

[a] Twofold dilutions were made approximately every 60 min.
[b] Expressed as 10^4 counts per min per unit of absorbancy of 450 nm.
[c] Expressed as absorbancy at 450 nm per 10^9 cells.
[d] Expressed as 10^{-5} counts per min per cell.

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15T⁻. They would disappear if it were assumed that there is a minimum period between initiation of a cycle of replication and cell division such that, although completion of the cycle is necessary to trigger the division process, it cannot do so until this minimum period has elapsed. In *E. coli* B/r (7), this minimum period would be about 70 min and the period designated D would be 70 min minus C (45 min), i.e., 25 min. If C were progressively increased, D would be correspondingly decreased, and the interval between completion and cell division might approach zero. In Table 2 we have calculated the change in cell composition which would be expected on such a model if C were doubled in length from 45 to 90 minutes. It can be seen that the differences expected are quite close to those obtained when the thymine concentration is reduced from 5.0 μg/ml to 0.4 μg/ml in *E. coli* 15T⁻. On the other hand we find no evidence for a period D as long as 25 min in cultures growing on high concentrations of thymine in the experiments described below.

Kinetics of changes in composition of glycerol-grown cells. Another method of investigating the relationship between C and D is to examine the kinetics of the change in average cell mass during a transition from growth on one thymine concentration to another. Such a transition, which we have termed a step-up or step-down (36) by analogy with the shift-up or shift-down experiments devised by Schaechter, Maaløe, and Kjeldgaard (37), is shown in Fig. 1b. One part of a culture in balanced exponential growth in medium containing 2.0 μg of thymine per ml was diluted into fresh medium to give a final concentration of thymine of 0.4 μg/ml (closed circles). This transition was followed by an immediate reduction in the rate of DNA synthesis and cell division and a gradual recovery to the previous rate. The rate of increase in optical density was not affected, however.

As a control, a second part of the culture was diluted similarly into medium containing the same concentration of thymine (open circles). In this case, no change was observed in the rate of DNA synthesis or cell division. In Fig. 2 we transformed the data by plotting the DNA/mass and mass/cell ratios as functions of time before and after the step-down. There was a gradual reduction in the DNA/mass ratio and increase in the mass/cell ratio which finally equilibrate at new values. No such changes occurred in the control culture.

Two features of Fig. 2 should be noted. First, the rate of change of the DNA/mass ratio is at a maximum immediately after the step-down,

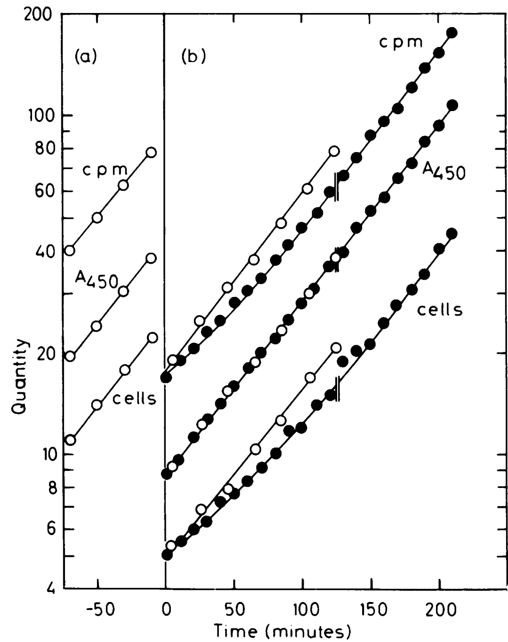

FIG. 1. *Effect of a reduction in thymine concentration on the kinetics of increase in DNA, absorbance, and cell number in P178. (a) Counts per minute (10^2/ml), absorbance (10^{-2} A_{450}), and cell number (10^7/ml) in a starting culture uniformly labeled with ^{14}C-thymine (2 μg/ml). At 0 min, the culture was diluted fivefold (b) into fresh prewarmed medium containing (○) the same concentration of labeled thymine or (●) no thymine to give a final concentration of 0.4 μg/ml. At 125 min, the latter culture was diluted twofold to maintain A_{450} below 0.4. The points after this time are corrected for the dilution.*

TABLE 2. *Predicted relative changes in cell composition for assumed values of C and D*[a]

Thymine concn	C (min)	D (min)	Mass/cell		DNA/cell		DNA/mass	
			Observed	Predicted	Observed	Predicted	Observed	Predicted
5.0	45	25	1.00	1.00	1.00	1.00	1.00	1.00
0.4	90	0	1.24	1.26	1.01	1.04	0.80	0.80

[a] Observed values are taken from Table 1. Predicted values were calculated from equations 1, 2, and 3.

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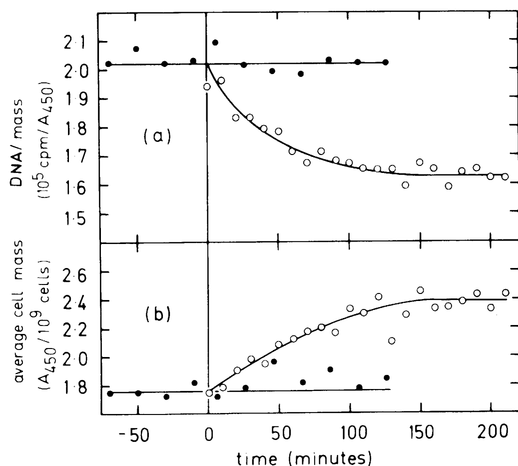

FIG. 2. *Effect of a reduction in thymine concentration on DNA/mass ratio (a) and average cell mass (b). Data are from Fig. 1; 0.4 µg of thymine per ml (O), 2.0 µg of thymine per ml (●).*

implying that the intracellular deoxynucleotide pools adjust very rapidly in response to the change in thymine concentration. Secondly, the new DNA/mass ratio is reached in 100 to 110 min. As we previously pointed out (36), the new equilibrium should be reached after a step-up or step-down when all replication forks in existence at the time of the transition have terminated, and the equilibrium time should therefore be equal to the new replication time. If we assume that the new replication velocity is achieved immediately after the step-down, then the data indicate a replication time of not more than 110 min in 0.4 µg of thymine per ml. This value is similar to that found for the same concentration of thymine in glucose-grown cultures of the same strain (36).

The average cell mass (Fig. 2b) also begins to change immediately after the step-down and comes to a new equilibrium at 110 to 120 min after the transition. This suggests that C + D has a value of not more than 120 min in 0.4 µg of thymine per ml.

In Fig. 3 we show the results of a reciprocal experiment in which a culture was stepped-up from 0.5 to 5.0 µg of thymine per ml. As before, there was no change in the rate of increase in optical density, but an immediate change in the rate of cell division. In this experiment the average cell mass attained its new equilibrium in about 60 min. This suggests that C + D in 5.0 µg of thymine per ml has a value of 60 min and consequently that C is no longer than this.

According to the model of Cooper and Helmstetter, completion of a round of replication signals a succession of events which lead to cell separation D minutes later. In a step-up or step-down experiment, we should therefore expect that the division of cells which were in the D period at the time of the transition from one concentration of thymine to another should not be affected, and a change in the rate of cell division should not occur until D min have elapsed. The data in Fig. 2 and 3 suggest that average cell mass (and hence the rate of cell division) begins to change very shortly after the step-down or step-up in thymine concentration. The accuracy of the data is such that it seems unlikely that there is a delay of more than 10 min before the rate of cell division begins to change and is consistent with there being no delay.

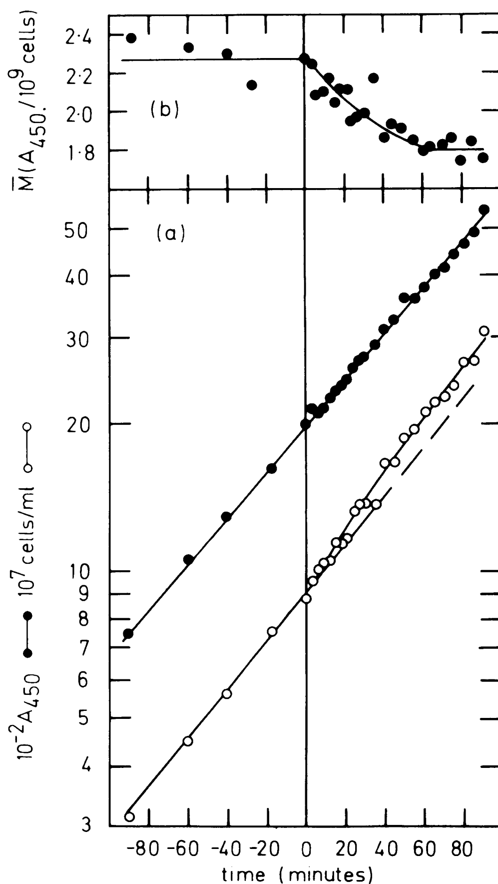

FIG. 3. *Effect of an increase in thymine concentration from 0.5 to 5.0 µg/ml on rate of cell division and average cell mass in P178. (a) Absorbance (●), particle number (O); (b) data from (a) replotted to show average cell mass before and after the transition at 0 min.*

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The rapid reduction in the rate of cell division after a step-down to a lower concentration of thymine suggests that total inhibition of DNA synthesis either by thymine deprivation or addition of nalidixic acid (NAL) should result in a total inhibition of cell division within a similarly short period of time. The effect of NAL on cell division is shown in Fig. 4c, and a comparison of the effect of NAL and thymine deprivation is shown in Fig. 4a and 4b, which shows that residual cell division gives an increment in cell number of not more than 10%. Also, there is no difference between the effect of NAL and that of thymine starvation.

The very small increment in cell number after NAL treatment or thymine starvation found with this *thy*⁻ strain of *E. coli* 15 growing in glycerol synthetic medium is in agreement with previous observations on this and related *thy*⁻ strains (9, 11) but differs from the behavior of *E. coli* B/r growing in glucose synthetic medium (6, 17). In *E. coli* B/r, cell division continues for 15 to 20 min and gives an increment in cell number of about 25%. The difference between the two strains might be due to a longer C period in 15T⁻ associated with the *thy*⁻ mutation. To examine this possibility, we determined the effect of NAL on the *thy*⁺ ancestor of 15T⁻ in both glycerol and glucose synthetic media. The increment in cell number and the length of time before division ceased was no different in either medium from that found with the *thy*⁻ derivative grown in glycerol (Fig. 5). The possibility is thus raised that this difference between *E. coli* B/r on the one hand and 15T⁻ on the other is not

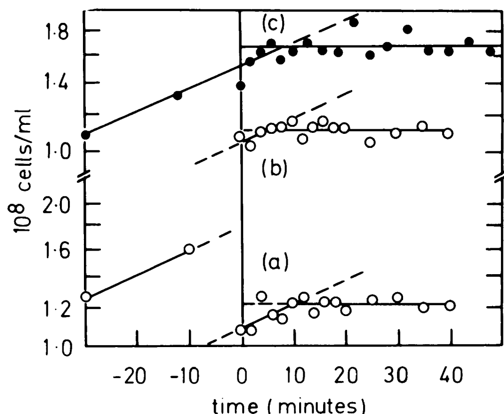

FIG. 4. *Effect of thymine starvation or NAL on cell division in P178. One culture* (O) *growing in glycerol M9 with 5.0 µg of thymine per ml was washed and suspended in medium without thymine* (a) *or with 5.0 µg of thymine and 50 µg of NAL per ml* (b). *A parallel culture* (●) *had NAL added without washing* (c).

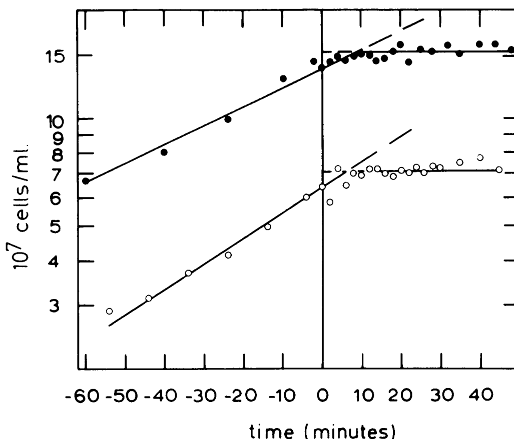

FIG. 5. *Effect of NAL on cell division in a thy⁺ strain (P245). NAL was added at 0 min. Culture in glucose M9* (O) *or glycerol M9* (●).

due to the presence of a *thy*⁻ mutation in the latter but to an inherent difference between these two strains of *E. coli*.

Absence of balanced growth in glucose-grown cultures. In contrast to glycerol-grown cultures, balanced growth of *E. coli* 15T⁻ (555-7) in synthetic medium with glucose as sole carbon source cannot be obtained (36). One aspect of the aberrant behavior of such cultures is shown in Fig. 6 and 7. In Fig. 6a, absorbance, particle numbers, and viable counts are plotted as a function of time for a glucose-grown culture containing 0.5 µg/ml thymine. Each of these parameters increased at a constant exponential rate with time, but the rates of increase in particle number and viable count were less than that of absorbance. The effect of this difference in rate is to cause a progressive increase in average cell mass with time. In Fig. 7b, average cell mass in such a culture is plotted as a function of time in an experiment in which exponential growth was maintained by successive twofold dilutions of the culture into fresh prewarmed medium for more than seven mass doublings. Average cell mass increased continuously over this period. With other thymine concentrations, this type of experiment has been continued for up to 12 mass doublings with similar results. Ultimately, the cultures appear to flocculate, preventing further measurements from being taken.

Failure to achieve balanced growth is associated with the thymine requirement of this strain since, in a *thy*⁺ transductant of it, a constant average cell mass is maintained. In addition (Fig. 6b and 7), the difference in the rates of increase of particle number and absorb-

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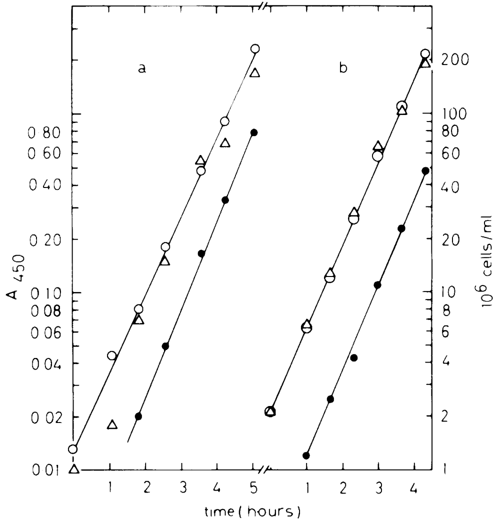

FIG. 6. *Effect of thymine concentration on rate of increase in absorbance (●), particle number (○), and colony-forming units (△) in P178 growing in glucose M9. (a) 0.5 μg of thymine per ml, (b) 5.0 μg/ml.*

ance (and hence the rate of increase in cell mass) becomes progressively smaller as the concentration of thymine in the growth medium is increased, and it is scarcely detectable at concentrations of 2 μg/ml or more. The very slight increase in mass observed at the two highest concentrations shown may in fact be due to a slight increase in growth rate which is sometimes observed during successive dilutions.

In contrast to average cell mass, the DNA/mass ratio maintains a constant value in these experiments (36), although, as in the case of glycerol-grown cells, this value changes as the concentration of thymine in the growth medium is altered. This can be seen from the step-down experiment (Fig. 8) in which the DNA/mass ratio moves from one steady-state value to another, although a constant mass/cell ratio is not found either before or after the step-down.

Provided the concentration of thymine in the growth medium is greater than 0.2 μg/ml, the rates of increase in absorbance and in the amount of DNA are equal (doubling time ca. 40 min) and independent of the thymine concentration. Although growth is not balanced under these conditions owing to the apparent aberration in cell division, we have defined it as "normal" to distinguish it from the clearly abnormal growth which occurs in media containing 0.2 μg of thymine or less per ml (42). We define growth as normal if (i) absorbance increases exponentially at a rate which is not altered significantly by changing the concentration of thymine, (ii) the DNA/mass ratio is constant, and (iii) the ratio of particle number to colony-forming units is constant and not significantly different from unity. Above 0.2 μg of thymine per ml, all three criteria are fulfilled. Below this concentration, none of them is.

We previously analyzed in detail the relationship between thymine concentration and the DNA/mass ratio in glucose-grown cultures and showed that, if it is assumed that the change in this ratio is a consequence solely of a change in C, the differences in C calculated in this way agree well with estimates of absolute and relative values of C obtained by three other methods (36, 42). We cannot estimate the values of C + D or D from cell composition in glucose-grown cultures, since steady-states with respect to mass per cell and DNA per cell are not obtained.

Several possibilities can be envisaged to account for the aberration in cell division which is reflected in the progressive increase in cell size which takes place during exponential growth of

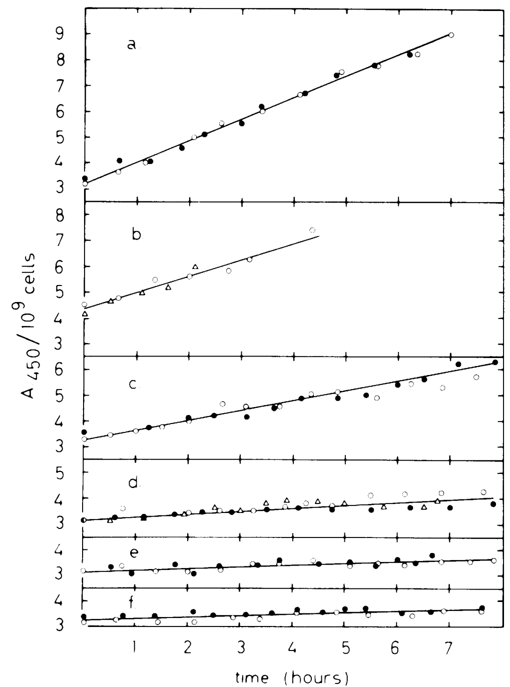

FIG. 7. *Rate of increase in average cell mass with time of P178 in glucose M9 containing the following concentrations of thymine: (a) 0.4 μg/ml; (b) 0.5 μg/ml; (c) 1.0 μg/ml; (d) 2.0 μg/ml; (e) 5.0 μg/ml; (f) 30 μg/ml. Different symbols in each section indicate different experiments.*

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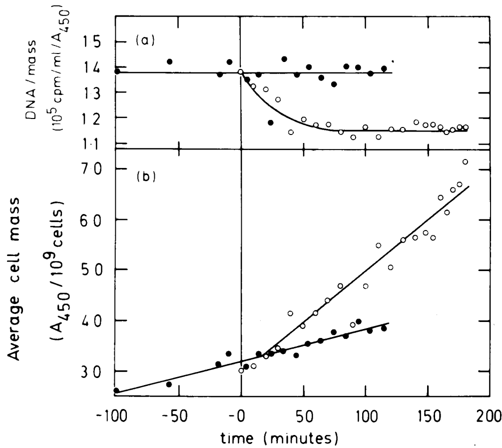

FIG. 8. *Effect of a decrease in thymine concentration on the DNA/mass ratio (a) and average cell mass (b) of P178 growing on glucose M9. The initial thymine concentration was 5.0 μg/ml. At 0 min, the culture was diluted fivefold with fresh medium containing 5.0 μg of thymine per ml (●) or no thymine to give a final concentration of 1.0 μg/ml (O).*

thy^- strains in glucose media. One possibility is that, with a low probability, cells fail to divide at the appropriate time and that the "lost" division is never recovered, so that some or all of the cells in the lineage derived from such cells are larger than normal. Such a hypothesis would predict that the distribution of cell size in the culture should become progressively broader than usual, but data presented below (*see* Table 3) suggest that this is not so and that the increase in cell size affects all cells uniformly. A second possibility is suggested by the fact that the aberration in cell division is not found in glycerol-grown cultures, which have a slower growth rate. Equation (2) shows that the faster the growth rate the greater will be the effect of a given change of C on cell mass. It is therefore conceivable that the larger adjustment in cell mass which would have to be made by glucose-grown cultures takes longer than one cell cycle and that the progressive increase that is seen represents a slow approach to equilibrium. The constant rate of increase in cell mass over many mass doublings (Fig. 7) does not support this hypothesis. A third possibility is that in glucose-grown cultures there is a progressive increase in C in medium containing a constant concentration of thymine and a corresponding progressive increase in cell size. This explanation seems unlikely for two reasons: first, a constant DNA/mass ratio is observed in glucose-grown cultures (Fig. 8) and would be compatible with a progressive increase in C only if this were associated with a corresponding decrease in the constant *k;* secondly, if the increment in DNA after amino acid deprivation is measured in glucose-grown cultures in successive generations, no difference is found (*unpublished data*), although a progressive increase would be predicted (36) if C were increasing.

If the replication time of the chromosome does not change during normal growth in glucose, then the increasing amount of DNA per cell which is associated with the increasing cell mass implies that the time between termination of rounds of replication and cell division is getting progressively longer with each succeeding generation. The data shown in Fig. 9 are in qualitative agreement with this supposition. In contrast to the effect of a step-up in glycerol-grown cultures (Fig. 3), there is a long delay before a detectable change in the rate of cell division is observed. The increment in cell number after NAL treatment is also greater in thy^- glucose cultures (Fig. 10) than in thy^+ cultures of the same strain (Fig. 5).

Effect of thymine concentration on cell size and shape. In this paper and in previous work (36, 42), we estimated relative cell mass by determining the absorbance at 450 nm per particle. In the majority of experiments, the absorbance of the experimental cultures was within the range 0.1 to 0.4. The assumption that under these conditions absorbance is proportional to dry weight and independent of cell size

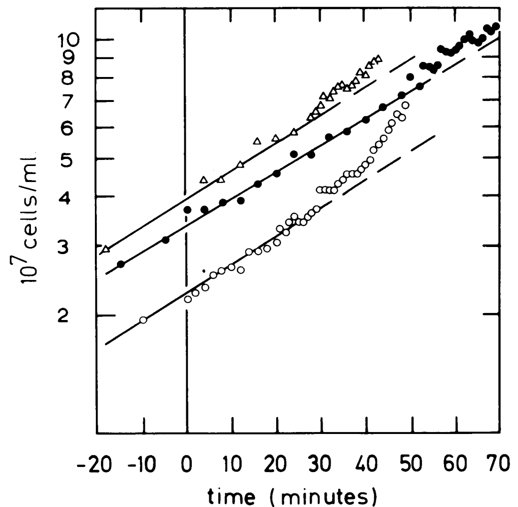

FIG. 9. *Effect of an increase in thymine concentration from 0.4 to 0.7 μg/ml (●), from 0.4 to 5.0 μg/ml (O), and from 1.0 to 5.0 μg/ml (Δ) on the rate of cell division of P178 in glucose M9.*

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seems justified (24, 37). The observed differences in cell mass could, however, reflect changes either in cell volume or cell density, and it therefore seemed necessary to determine more directly whether the observed changes in cell mass were associated with corresponding changes in cell volume. We attempted to determine relative cell volumes in cultures containing different concentrations of thymine from a comparison of pulse-heights generated by a modified Coulter particle counter (13, 14) and from direct microscope observations.

Table 3 summarizes the data obtained from the particle counter. Relative cell volumes were calculated from the mean of the pulse height distribution and its mode, setting the values for a thy^+ transductant of $15T^-$ 555-7 growing in glycerol medium at 1.00. The relative cell volumes calculated from the mean and mode of the pulse-height distributions are similar and in fair agreement with the measurements of average cell mass calculated from absorbance measurements. We therefore conclude that the major effect of changing the thymine concentration in the growth medium is on cell volume. The increase in cell volume caused by lowering the thymine concentration in the growth medium appears to affect all cells uniformly, since the coefficient of variation of the pulse-height distribution is similar in all cases.

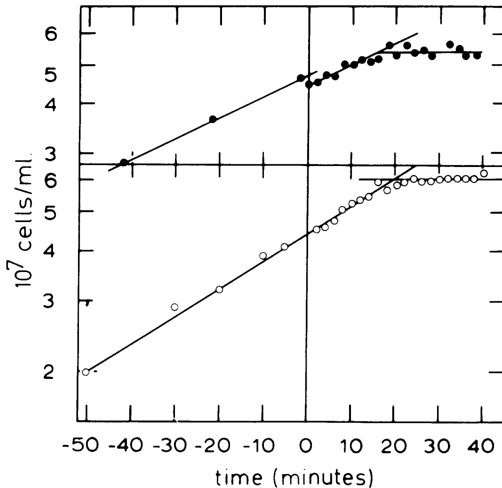

FIG. 10. *Effect of NAL (50 μg/ml) on cell division in P178 growing in glucose M9 containing 0.5 μg of thymine per ml (●) or 2.0 μg/ml (○).*

Our initial microscope observations were made on cultures of the thy^- strain growing in glucose medium by using phase-contrast optics. The results were striking. There appeared to be a large increase in cell diameter and little or no increase in cell length during the progressive increase in cell size which occurs during prolonged exponential growth of such cultures. The cells also appeared to have a more rounded shape as they increased in size. The appearance of cells after prolonged subculture in low concentrations of thymine was qualitatively different from that observed after growth in the total absence of thymine. Under conditions of thymine deprivation, the cells "snake" by increasing in length without changing significantly in width.

Examination of fixed preparations by electron microscopy confirmed that the major change was in cell diameter and permitted a more quantitative estimation of the effect of thymine concentration on cell dimensions. Cultures which had been fixed in 0.25% formaldehyde were washed, suspended in water, and deposited on grids which were then air-dried. The appearance of these unstained preparations is shown in Fig. 11. The method of deposition of suspensions on the grids was such that we could not ensure that the population of cells examined was a random sample from the culture, and we therefore measured only the diameter of the cells. It has been shown (30) that diameter is independent of cell age and, consistent with this, we found no difference between the aver-

TABLE 3. *Cell volume and mass of P178 under different cultural conditions*

thy	Carbon source	Thymine concn (μg/ml)	Relative vol calculated from		Coefficient of variation of size distribution	Relative mass[a] ($A_{450}/10^9$ cells)
			Mean	Mode		
+	Glycerol		1.00	1.00	30	1.00
−	Glycerol	2.0	1.07	1.07	28	1.05
−	Glycerol	0.3	1.56	1.54	30	1.40
+	Glucose		1.70	1.60	28	1.50
−	Glucose	5.0	2.20	2.17	30	1.9–2.0
−	Glucose	0.5	3.19	2.86	31	2.6–3.0

[a] Expressed as absorbancy at 450 nm per 10^9 cells. Data are from a different experiment. A range is given for the last two values because mass/cell is not constant in glucose M9 cultures.

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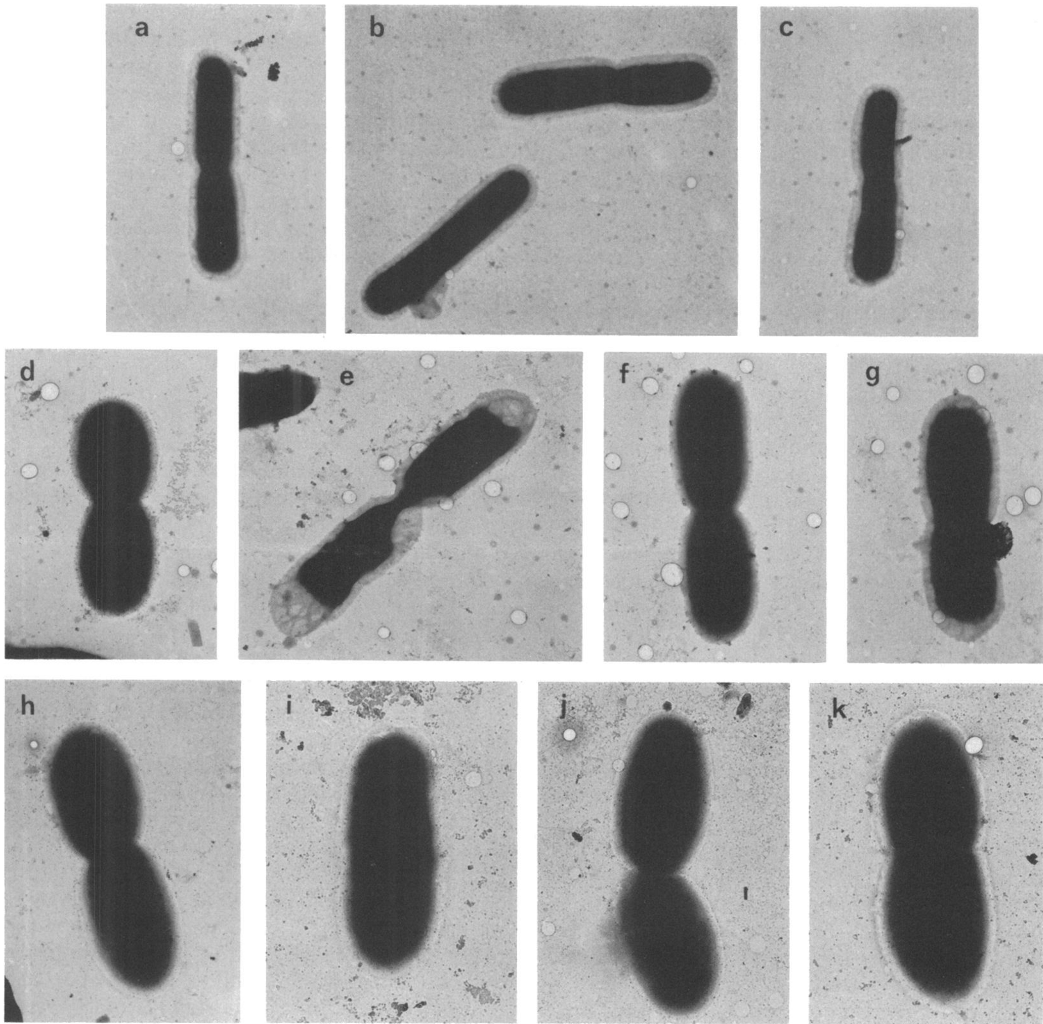

FIG. 11. *Electron micrographs of cells of P178 taken at three stages in the evolution of a culture growing in glucose M9; early (a–c), middle (d–g), and late (h–k). Plates e and g, cells from a preparation accidently subjected to plasmolysis due to insufficient washing.*

age diameter of cells with constrictions and those without. A summary of the measurements made on both glucose- and glycerol-grown cells is given in Table 4, each average being based on measurement of the diameter of 50 or more cells. Relative average cell lengths were calculated from the measured volumes in Table 3, and diameters assuming the cells to be cylinders (Table 4, column 4). If the relative lengths of the thy^+ cells in glycerol and glucose media are compared, it will be noted that the faster-growing cells are longer than the slower-growing ones. Increase in cell size as a result of an increase in C, on the other hand, seems not to be associated with any increase in cell length, the

TABLE 4. *Radii of cells of P178 growing under different cultural conditions*

thy	Carbon source	[T] (μg/ml)	Mean relative radius (± standard errors)	Relative mean length[a]
+	Glycerol		1.00 ± 0.01	1.00
−	Glycerol	2.0	1.17 ± 0.01	0.78
−	Glycerol	0.3	1.41 ± 0.02	0.78
+	Glucose		1.16 ± 0.01	1.23
−	Glucose	5.0	1.58 ± 0.02	0.88
−	Glucose	0.5	1.93 ± 0.04	0.81

[a] Calculated from relative mean radius and relative average cell volume from Table 3, assuming cells were cylinders.

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data suggesting that there may even be a reduction in length.

Too much reliance cannot be placed on the estimates of relative length from the relative volume and radius of cells of the glucose-grown *thy*$^-$ strain, because there is an obvious change in cell shape from a rod to an ellipsoidal form which will lead to an underestimation of relative cell length. The *thy*$^+$ strain and the *thy*$^-$ strain in glycerol M9 medium, however, were in all cases typical rods. The assumption here that they were cylindrical rather than, for example, prolate, or cylindrical with hemispherical ends will not lead to large errors. A more serious potential source of error is the possibility that fixation of cells prior to examination under an electron microscope seriously affects cell shape. This possibility needs further investigation. In one experiment, however, a formaldehyde-fixed preparation was air-dried in the presence of low concentrations of salt to induce plasmolysis. The appearance of such plasmolyzed cells is shown in Fig. 11e and 11g and suggests that the shape of the outer wall is not affected by retraction of the inner membrane from the cells envelope.

DISCUSSION

Cell composition. In this investigation we measured the effect of thymine concentration in the growth medium on mass per cell, DNA per cell, and the kinetics of the transition of average cell mass from one steady-state value to another in response to a change in thymine concentration. The results obtained are qualitatively those predicted by (i) Cooper and Helmstetter's model (7), (ii) the assumption that initiation of rounds of replication occurs at a constant mass per chromosome origin, and (iii) that the replication time of the chromosome is determined by the concentration of thymine in the growth medium in *thy*$^-$ strains of *E. coli*. An effect of thymine on the replication time of the chromosome has also been deduced from other data (29, 36, 42).

Unfortunately, the data do not provide unambiguous information concerning the relationship between C and D. The immediate change in the rate of cell division after a step-up or step-down in thymine concentration in glycerol-grown cultures implies either that D is very short and independent of C, or that D is proportional to C and is affected immediately by a change in thymine concentration. The small increment in cell number after inhibition of DNA synthesis in glycerol cultures of 15T$^-$ also implies that D is very short, and the similar behavior of a *thy*$^+$ relative of this strain suggests that the apparently short D period (compared with that found in *E. coli* B/r) is not due to the presence of the *thy*$^-$ mutation. On the other hand, the cell size and composition studies are in quantitative agreement with assumptions (i to iii) only if it is assumed that D is longer in the presence of high thymine concentrations than it is in low thymine concentrations. These discrepancies are unlikely to be resolved until experiments of the type described in this paper are performed on a strain like *E. coli* B/r in which D can be measured more directly.

Cell size and shape. It seems clear that changes in average cell mass resulting from changes in thymine concentration in the growth medium reflect, primarily, changes in cell volume. The cytological evidence presented in this paper also suggests that the observed changes in volume are due largely to alterations in cell diameter.

It has been shown that individual cells of *E. coli* increase in volume solely by extension in length under steady-state conditions (30). It therefore seems paradoxical that, when division is delayed as a result of an increase in C and cell volume is consequently increased, the additional volume should be accommodated by an increase in cell diameter rather than length. A solution to this paradox can be found by postulating that at a given growth rate the cell extends in length linearly at a constant rate which is independent of C and which doubles once during each cell cycle. Linear extension would occur if growth of the cell envelope took place at a constant rate from one or more discrete sites which double in number at a unique stage in the cell cycle (10), and average cell length would be determined by the average number of growth sites per cell. Since our data suggest that average cell length is independent of C, it would follow that the number of growth sites could not be identical to the number of chromosome origins or replication forks, the number of which per cell depends on C. The number of chromosome termini, on the other hand, is independent of C. Thus, if there is a connection between growth sites and chromosomes, the data are compatible only with the number of sites being determined by the number of termini. (This conclusion would be invalid if D is affected by changes in C. If D decreases when C increases, the number of growth sites could be equal to the number of chromosome origins. In view of the ambiguities in our data on this point, we will assume for the purpose of this discussion that D is constant.)

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In a step-down experiment termination and, on this hypothesis, the doubling in the linear rate of volume increase would be delayed by the reduction in replication velocity. Mass increase, on the other hand, would continue exponentially at an unchanged rate, and the cell would avoid a progressive increase in density only by increasing its width and reducing its surface/volume ratio. The increase in width could be a response to increasing hydrostatic pressure within the cell and would continue until the increment in volume for a given increment in length was sufficient to avoid an increase in density.

Cell size and shape are also influenced by growth rate (37). We should therefore consider whether the dimensions found are consistent with linear extension from specific sites, the number of which is proportional to the number of chromosome termini. In *S. typhimurium*, the diameter of cells at four growth rates has been determined (*see* 37). The average cell mass at these growth rates has also been measured (37). From these parameters, we calculated relative average cell length at the four growth rates, assuming the cells to be cylinders (identical relative values are obtained if it is assumed that the cells have hemispherical ends). Relative length is plotted against the specific growth rate (μ = generations per hour) in Fig. 12.

The average number of chromosome termini per cell in an exponential culture will be $2^{D/\tau}$(4, 40). If the rate of extension per site were constant, the average cell length would be a function of $2^{D/\tau}$. The τ and cell length would decrease as growth rate increased. This was not found (Fig. 12). If the rate of extension per site were not constant but proportional to the specific growth rate, then the predicted average cell length would be a function of $2^{D/\tau}$. The line drawn in Fig. 12 gives the predicted relationship between length and growth rate calculated from $2^{D/\tau}$, and is superimposed on the calculated value obtained for one doubling per hour in *S. typhimurium*. Also included in Fig. 12 are the two values for average cell length in *E. coli* 15T$^+$ given in Table 4, with the value at one doubling per hour again superimposed on the corresponding value found in *S. typhimurium*. The change in average length over the range of growth rates for which data are available is close to that predicted. The observed changes in length with growth rate are therefore consistent with linear extension from specific sites proportional in number to the number of chromosome termini, provided it is assumed that the rate of extension per site is proportional to the growth rate.

An alternative hypothesis (*see* 32), which leads to similar although not identical predictions to a growth site hypothesis, is that the rate of growth of the cell envelope is determined by the rate of synthesis of an envelope precursor which could be a protein specified by an unregulated gene. The rate of synthesis of this precursor would be proportional to the number of gene copies per cell (vide β-galactosidase; 15, 43) and would therefore be linear with discrete doublings in rate at the time the gene was replicated. As with the previous hypothesis, only if the gene were located near the chromosome terminus would the change in volume associated with a change in C be achieved largely through a change in cell width.

In accord with these hypotheses, it has been shown in *Bacillus megaterium* (39) that the rate of incorporation of wall and membrane precursors after a shift-up to a richer growth medium accelerates to match the new growth rate only after a delay of between 20 and 40 min. In addition, evidence was recently obtained from synchronous cultures of *E. coli* suggesting that incorporation of D-glutamic acid into the cell envelope occurs at a linear rate, with discrete doublings in rate occurring at about the time of termination of rounds of replication (20). The growth site hypothesis also implies that at least one component of the cell envelope should be laid down in discrete zones, but the weight of evidence appears to favor random intercalation of wall and membrane precursors in *E. coli* (*see* 26). Evidence has also been obtained in *E. coli* with synchronous cultures (25) which suggests that cell volume increases linearly with a doubling in rate at the predicted time of termination of rounds of replication. However, although we have assumed that all components of the cell envelope are synthesized at the same rate so that cell volume also increases linearly, similar predictions with respect to average cell size and

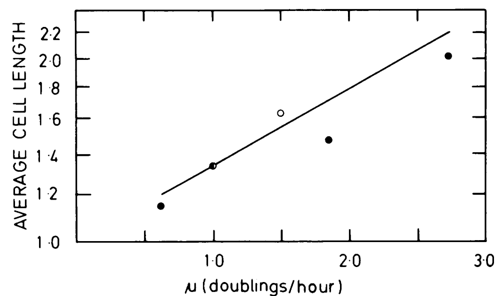

FIG. 12. *Average cell length in arbitrary units as a function of growth rate. Data from Schaechter et al. (37) in S. typhimurium* (●) *and from Table 4* (O).

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shape as a function of C and τ would be made if the outer layers of the cell envelope, and cell volumes, increased exponentially but an inner layer were synthesized in the manner described above (2). Under steady-state conditions, the inner layer would be synthesized at a faster rate than the outer layers in the period of the cell cycle immediately after termination, potentially leading to invagination of the membrane to form a septum and also to mesosome formation. The surplus mesosomal membrane would be depleted during the subsequent part of the cell cycle as the rate of volume increase accelerates.

The most attractive feature of models of the type we have outlined is that they suggest why septation is initiated at termination, why inhibition of DNA synthesis (and hence termination) prevents septation and leads to snaking, and why, in mutants which can terminate rounds but not initiate new ones, septation often continues leading to the formation of DNA-less cells (19, 38). The common feature of the models discussed above is that the rate of growth of at least one component of the cell envelope and the rate of increase in cell mass are not in phase during most of the cell cycle. Before termination, the rate of increase in mass is greater than the rate of increase of this component, and after termination the inverse situation exists. Invagination and septation could normally be initiated at termination because, at this time, the rate of synthesis of envelope material relative to the rate of increase in mass is at a maximum and is in excess of that required to maintain a constant internal hydrostatic pressure (*see also* 18). When DNA synthesis is blocked, either immediately by thymine starvation or NAL, or through inability to initiate new rounds of replication in thermosensitive initiation-defective mutants, mass increase continues exponentially for about one mass doubling before becoming linear in cultures growing in glucose synthetic medium (34; *unpublished data*). Since an immediate block in DNA synthesis will prevent any subsequent doubling in the rate of envelope synthesis, it will never be in excess of that required to maintain a constant hydrostatic pressure in the majority of cells, and no septation will be possible. In the case of mutants which can terminate rounds of replication, on the other hand, the rate of envelope synthesis will be twice that in a culture in which an immediate block in DNA synthesis has been imposed, and in many cells when mass increase has become linear the relative rates of mass and envelope synthesis will be similar to that in a normal cell at the time of termination. Repeated septation will occur.

Thus we suggest that there is no specific signal for septation. It occurs whenever the amount of envelope material synthesized in a given interval of time is greater than that required to accommodate the increment in mass which occurs during the same period without an increase in cell density. Whether or not inhibition of DNA synthesis will lead to continued septation and the production of DNA-less cells will depend not only on whether termination occurs but also on how rapidly the rate of mass increase becomes linear. There is thus no necessary conflict between the hypothesis and the fact that an immediate block in DNA synthesis in certain strains of *E. coli* also leads to continued septation and production of DNA-less cells (12, 21, 22).

Finally, it seems possible that the continuous increase in average cell mass during exponential growth of *thy*$^-$ strains in glucose medium may also be a consequence of the model of envelope growth and cell division discussed above. Average cell volume is a function of the ratio C/τ. With increasing growth rate and decreasing replication velocity, cell volume could reach a critical value above which the cell diameter is such that formation of a septum requires an increasing length of time. Above this value, D would be observed to increase and this increase would result in a further increase in cell volume leading to an even longer D period. Consequently, no steady-state could be established and cells would appear ellipsoidal when examined microscopically.

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LITERATURE CITED

1. Adams, M. H. 1959. Bacteriophages. Interscience Inc., New York.
2. Bazill, G. W. 1967. Lethal unbalanced growth in bacteria. Nature (London) **216**:346–349.
3. Beacham, I. R., K. Beacham, A. Zaritsky, and R. H. Pritchard. 1971. Intracellular thymidine triphosphate concentrations in wild type and thymine-requiring mutants of *E. coli* 15 and K12. J. Mol. Biol. **60**:75–86.
4. Bleecken, S. 1969. Duplication of the bacterial cell and its initiation. J. Theoret. Biol. **25**:137–158.
5. Campbell, A. 1957. Synchronization of cell division. Bacteriol. Rev. **21**:263–272.

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6. Clark, D. J. 1968. Regulation of deoxyribonucleic acid replication and cell division in *Escherichia coli* B/r. J. Bacteriol. **96**:1214-1224.
7. Cooper S., and C. E. Helmstetter. 1968. Chromosome replication and the division cycle of *E. coli* B/r. J. Mol. Biol. **31**:519-540.
8. Donachie, W. D. 1968. Relationship between cell size and time of initiation of DNA replication. Nature (London) **219**:1077-1079.
9. Donachie, W. D. 1969. Control of cell division in *Escherichia coli:* experiments with thymine starvation. J. Bacteriol. **100**:260-268.
10. Donachie, W. D., and K. J. Begg. 1970. Growth of the bacterial cell. Nature (London) **227**:1220-1224.
11. Donachie, W. D., D. G. Hobbs, and M. Masters. 1968. Chromosome replication and cell division in *E. còli* 15T$^-$ after growth in the absence of DNA synthesis. Nature (London) **219**:1079-1080.
12. Green, M. H. L., J. Greenberg, and J. Donch. 1969. Effect of a *recA* gene on cell division and capsular polysaccharide production in a *lon* strain of *Escherichia coli.* Genet. Res. **14**:159-162.
13. Grover, N. B., J. Naaman, S. Ben-Sasson, and F. Doljanski. 1969. Electrical sizing of particles in suspensions. I. Theory. Biophys. J. **9**:1398-1414.
14. Grover, N. B., J. Naaman, S. Ben-Sasson, F. Doljansky, and E. Nadav. 1969. Electrical sizing of particles in suspensions. II. Experiments with rigid spheres. Biophys. J. **9**:1415-1425.
15. Helmstetter, C. E. 1968. Origin and sequence of chromosome replication in *Escherichia coli* B/r. J. Bacteriol. **95**:1634-1641.
16. Helmstetter, C. E., and S. Cooper. 1968. DNA synthesis during the division cycle of rapidly growing *E. coli* B/r J. Mol. Biol. **31**:507-518.
17. Helmstetter, C. E., and O. Pierucci. 1968. Cell division during inhibition of deoxyribonucleic acid synthesis in *Escherichia coli.* J. Bacteriol. **95**:1627-1633.
18. Higgins, M. L., and G. D. Shockman. 1970. Model for cell wall growth in *Streptococcus faecalis.* J. Bacteriol. **101**:643-648.
19. Hirota, Y., F. Jacob, A. Ryter, G. Buttin, and T. Nakai. 1968. On the process of cellular division in *Escherichia coli.* J. Mol. Biol. **35**:175-192.
20. Hoffman, B., W. Messer, and U. Schwarz. 1972. Regulation of polar cap formation in the life cycle of *Escherichia coli.* J. Supramolec. Struct. **1**:29-37.
21. Inouye, M. 1969. Unlinking of cell division from deoxyribonucleic acid replication in a temperature-sensitive deoxyribonucleic acid synthesis mutant of *Escherichia coli.* J. Bacteriol. **99**:842-850.
22. Inouye, M. 1971. Pleiotropic effect of the *recA* gene of *Escherichia coli*: uncoupling of cell division from deoxyribonucleic acid replication. J. Bacteriol. **106**:539-542.
23. Kjeldgaard, N. O., O. Maaløe, and M. Schaechter. 1958. The transition between different physiological states during balanced growth of *S. typhimurium.* J. Gen. Microbiol. **19**:607-616.
24. Koch, A. L. 1970. Turbidity measurements of bacterial cultures in some available commercial instruments. Anal. Biochem. **38**:252-259.
25. Kubitschek, H. E. 1968. Linear cell growth in *E. coli.* Biophys. J. **8**:792-804.
26. Lin, E. C. C., Y. Hirota, and F. Jacob. 1971. On the process of cellular division in *Escherichia coli.* VI. Use of a methocel-autoradiograph method for the study of cellular division in *Escherichia coli.* J. Bacteriol. **108**:375-385.
27. Maaløe, O. 1961. The control of normal DNA replication in bacteria. Cold Spring Harbor Symp. Quant. Biol. **26**:45-52.
28. Maaløe, O., and N. O. Kjeldgaard. 1966. Control of macromolecular synthesis. Benjamin, New York.
29. Manor, H., M. P. Deutscher, and U. Z. Littauer. 1971. Rates of DNA chain growth in *E. coli.* J. Mol. Biol. **61**:503-524.
30. Marr, A. G., R. J. Harvey, and W. C. Trentini. 1966. Growth and division of *Escherichia coli.* J. Bacteriol. **91**:2388-2389.
31. Oishi, M., H. Yoshikawa, and N. Sueoka. 1964. Synchronous and dichotomous replications of the *B. subtilis* chromosome during spore germination. Nature (London) **204**:1069-1073.
32. Previc, E. P. 1970. Biochemical determination of bacterial morphology and the geometry of cell division. J. Theoret. Biol. **27**:471-497.
33. Pritchard, R. H. 1965. Structure and replication of the bacterial chromosome. Brit. Med. Bull. **21**:203-205.
34. Pritchard, R. H., P. T. Barth, and J. Collins. 1969. Control of DNA synthesis in bacteria. XIX. Microbial growth, p. 263-297. Symp. Soc. Gen. Microbiol.
35. Pritchard, R. H., and K. G. Lark. 1964. Induction of replication by thymine starvation at the chromosome origin in *E. coli.* J. Mol. Biol. **9**:228-307.
36. Pritchard, R. H., and A. Zaritsky. 1970. Effect of thymine concentration on the replication velocity of DNA in a thymineless mutant of *E. coli.* Nature (London) **226**:126-131.
37. Schaechter, M., O. Maaløe, and N. O. Kjeldgaard. 1958. Dependency on medium and temperature of cell size and chemical composition during balanced growth of *S. typhimurium.* J. Gen. Microbiol. **19**:592-606.
38. Spratt, B. G., and R. J. Rowbury. 1971. Cell division in a mutant of *S. typhimurium* which is temperature sensitive for DNA synthesis. J. Gen. Microbiol. **65**:305-314.
39. Sud, I. J., and M. Schaechter. 1964. Dependence of the content of cell envelope on the growth rate of *Bacillus megaterium.* J. Bacteriol. **88**:1612-1617.
40. Vielmetter, W., W. Messer, and A. Schütte. 1968. Growth direction and segregation of the *E. coli* chromosome. Cold Spring Harbor Symp. Quant. Biol. **33**:585-598.
41. Weatherwax, R. S., and O. E. Landman. 1960. Ultraviolet light-induced mutation and deoxyribonucleic acid synthesis in *Escherichia coli.* J. Bacteriol. **80**:528-535.
42. Zaritsky, A., and R. H. Pritchard. 1971. Replication time of the chromosome in thymineless mutants of *E. coli.* J. Mol. Biol. **60**:65-74.
43. Zeuthen, J., and M. Pato. 1971. Replication of the F'*lac* sex factor in the cell cycle of *E. coli.* Mol. Gen. Genet. **111**:242-255.

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