

Growth Response of *Escherichia coli* to Nutritional Shift-Up: Immediate Division Stimulation in Slow-Growing Cells

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When *Escherichia coli* 15T⁻ cells growing exponentially at 70- to 80-min doubling times are subjected to a nutritional shift-up via glucose addition, cell division continues at the preshift rate for about 70 min (rate maintenance). The same cells growing at doubling times of 120 min or longer, however, begin to divide at a new faster rate immediately upon glucose addition. In both the rate maintenance and immediate division situations, cell mass, as measured by optical density (OD), begins to increase immediately upon shift-up. Consequently, the OD/cell pattern differs in the two growth-rate transitions. During rate maintenance, the OD/cell ratio increases dramatically for 60 to 70 min, and then slows appreciably and approaches the OD/cell characteristic of the new medium. During immediate division situations, the OD/cell increases only slightly for the first 180 ± min; then the rate of increase accelerates but does not stop at the OD/cell characteristic of the new medium. Immediate division upon nutritional shift-up apparently depends upon initial doubling times in excess of 115 to 120 min and provision of a readily metabolized carbon source supporting doubling times of about 40 min. Similar immediate division occurs in *E. coli* B/r and K-12.

Bacterial cells growing with doubling times of greater than 60 min have cell cycle features that differ markedly from cells with doubling times of about 40 min or less. At doubling times of 70 min or more, *Escherichia coli* 15T⁻ (1, 11, 19, 20, 25), *E. coli* B/r (7, 11, 14, 22), and *Salmonella typhimurium* (8) show periods in their cell cycle during which no deoxyribonucleic acid (DNA) synthesis occurs (DNA synthesis gaps). The same cells at 40-min doubling times or less show no such gaps. Slow- and fast-growing cells each replicate their chromosomes in a bidirectional manner (23), but the rate of initiation of Okazaki pieces (2) and, in some cases, actual chromosome synthesis times (1, 11, 22) differ. The number of chromosomes per cell also differs (4, 19, 20) in slow- and fast-growing cells, as does the number of replication forks per chromosome (8, 14). The period between chromosome termination and daughter cell separation (D period [7, 14]) has been reported to be similar (18, 19) in slow- and fast-growing cells by some workers and dissimilar (14, 22; J. Urban, unpublished observations) by others. Cell growth in minimal medium is unidirectional, whereas growth in complex media is bidirectional (10). Also differing in slow- and fast-growing cells is the cell mass per chromosome origin (12).

Although the examples above demonstrate

differences in the physiology of slow- and fast-growing cells, the early work of Kjeldgaard et al. (17) suggested that the transition from slow to fast growth rates occurred in a precise and reproducible manner. These workers shifted cells growing in a variety of media to new media that supported faster growth and measured synthesis of various cell components, optical density (OD), and colony counts. They found that transitions to faster growth occurred in a characteristic fashion. About 5 min after a shift-up to richer medium OD began to increase at a rate characteristic of the new medium, but cell number did not begin to increase at the new rate for 70 min. Cell number continued to increase at the preshift rate for 70 min, and then abruptly changed to the rate increase typical of the new medium. The transition pattern was termed "rate maintenance" by Kjeldgaard et al. (17) and has been repeatedly demonstrated (6, 14; M. J. Case and A. G. Marr, Abstr. Annu. Meet. Am. Soc. Microbiol., 1974, Abstr. G43, p. 27; Sloan and Urban, this report).

While extending the studies of numerous investigators who have attempted to describe regulatory events responsible for rate maintenance (2, 3, 4, 6), we discovered that rate maintenance is exhibited over only a rather narrow range of growth rates. When cells are grown at very slow growth rates and are exposed to cer-

tain shift-ups, a new rate of division is observed immediately. The pattern and nature of this immediate division, in addition to the conditions necessary for its appearance, are the subject of this report.

(Preliminary data leading to this study were presented at the 1975 Annual Meeting of the American Society for Microbiology [Abstr. I30, p. 121].)

MATERIALS AND METHODS

Strains. *E. coli* 15T⁻ (555-7) Arg⁻ Try⁻ Met⁻ Thy⁻ (20, 21), *E. coli* B/r (obtained from C. Helmstetter), and *E. coli* K-12 His⁻ (UTH 6182, obtained from T. S. Matney) were used in this study.

Media. The chemically defined medium M9 (16) was used in all experiments. The carbon sources used and their respective concentrations were 0.1% L-asparagine, 0.1% L-aspartic acid, 0.1% fumaric acid, 0.1% L-glutamic acid, 0.1% glyoxylic acid (all adjusted to pH 7.0 with NaOH before autoclaving), 0.4% D-glucose, 0.4% α -lactose, 0.4% D(-)-ribose, 0.4% D-(+)-xylose, 0.4% sucrose (lactose and sucrose were filter sterilized), and 0.45% succinic acid. Medium for *E. coli* 15T⁻ was additionally supplemented with L-arginine (100 μ g/ml), L-methionine (50 μ g/ml), L-tryptophan (50 μ g/ml), and thymine (4 μ g/ml). *E. coli* K-12 medium was supplemented with 100 μ g of L-histidine per ml. Carbon sources (except glucose and sucrose) and amino acids were obtained from Sigma Chemical Co., St. Louis, Mo.

Membrane filters (type B-6, Bac-T-flex) were obtained from Schleicher and Schuell, Keene, N.H.

Culture conditions. All cultures were bubble-aerated with water-saturated air and incubated at 37°C in culture tubes (38 by 200 mm). Cultures were kept in exponential phase, at cell densities of 2.5×10^7 to 1.5×10^8 , by dilution with fresh 37°C medium. All cultures were at steady state for at least two to three generations before being used in an experiment.

Media shifts. Normally, media shifts consisted of addition of 20 to 50 ml of exponentially growing cells to 0.01 volume (0.2 to 0.5 ml) of a new carbon source. In a limited number of control experiments, shifts were accomplished by filtration. Ten to twenty-five milliliters of cells was trapped on 25- or 50-mm membrane filters and washed with an equal volume of new medium. The washed cells were then suspended in new medium.

Cell counting. Cells were counted on a Coulter counter, model F_N, equipped with a 30- μ m orifice and a 50- μ l manometer. An attenuation setting of 0.5, an aperture current setting of 16, and a threshold setting of 6 were used for all counts.

Samples of culture, 0.1 or 0.2 ml, were diluted in 25 ml of filtered 0.15 M NaCl and counted within 5 min. The counter required 15 to 16 s to draw 50 μ l of the saline-cell suspension through the aperture orifice. Counts on filtered saline alone were always less than 500/50 μ l. Each experiment was performed with an individual saline stock; therefore, background counts for each sample in an experiment were constant. By maintaining culture densities within the range indicated and by using 0.1- or 0.2-

ml culture samples, actual particle counts per 50 μ l were kept between 10,000 and 30,000. Negligible coincidence occurs within this counting range on our instrument, as verified by sample dilution and saline volume manipulations. Viable counts were made on spread plates using M9-glucose dilution blanks and plating medium (1.5% agar).

OD measurements. Concurrent with cell number determinations, the OD of respective cell populations was measured with a spectrophotometer (Gilford model 240). Absorbance was measured at 450 nm in quartz cuvettes with 1-cm light paths. Respective uninoculated media served as blanks.

RESULTS

Nutritional shifts in *E. coli* 15T⁻ cells growing to 70- to 80-min doubling times. Glucose (to 0.4%) was added to cultures of *E. coli* 15T⁻ growing in M9-succinate (doubling time, 70 min), and cell number and OD were measured (Fig. 1). OD began to increase at a new rate almost immediately. The new faster rate of increase was maintained for about 80 min, and

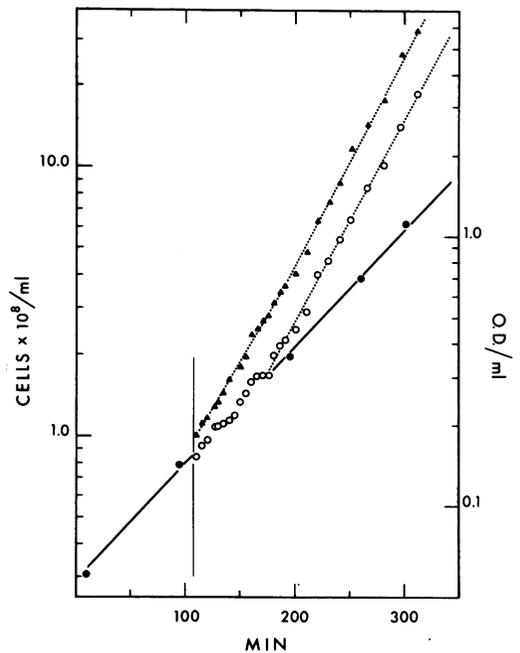


FIG. 1. Cell number and OD increases after addition of glucose to *E. coli* 15T⁻ growing in M9-succinate. At 107 min (thin vertical line in figure), glucose (0.4%) was added to part of the culture and at the indicated times cell number (Coulter counts) and OD (at 450 nm) were measured. The cells/ml and OD/ml were as indicated by the first data points after the shift-up. (See Materials and Methods for maintenance of population densities.) Symbols: (●) cell number, glucose-free culture; (○) cell number, glucose-supplemented cells; (▲) OD, glucose-supplemented cells.

then increased to an even faster rate, which was maintained for the duration of the experiment. Cell number, however, continued at the preshift rate for about 65 min. Then, an abrupt shift to a new doubling rate (doubling time, 40 min) was observed. This faster rate of doubling roughly paralleled the final rate of OD increase and was maintained for the duration of the experiment. This pattern and timing of mass and cell number increases is in agreement with published experiments (6, 14, 17) and has been described as "rate maintenance."

An increase in OD occurring before the ultimate increase in cell numbers suggests that cells are getting larger before assuming a new division rate (24), and Fig. 2 demonstrates this to be the case. Until the time that cell number began to increase at a new rate, the OD/cell increased dramatically. Once rapid division began, the dramatic increase in cell size is no longer observed. However, rather than leveling off at a new OD/cell as might be expected for the new medium (24), cell size continued to increase.

Figure 3 is another example of rate maintenance. $15T^-$ cells in M9-fumarate (doubling time, 76 min) were supplemented with glucose

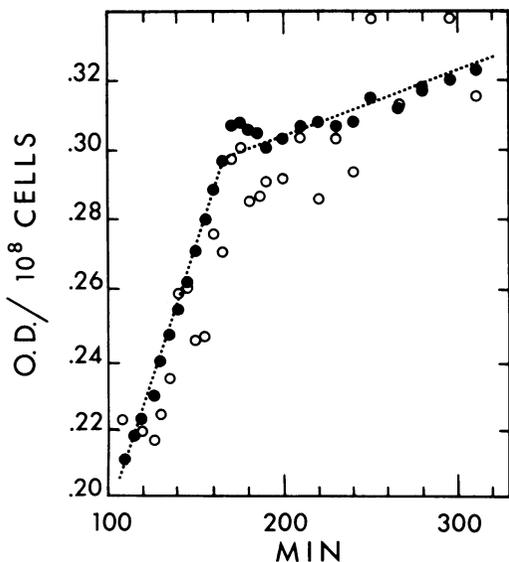


FIG. 2. OD/cell after addition of glucose to $15T^-$ growing in M9-succinate. The OD/ 10^8 cells was calculated from the data of Fig. 1. Symbols: (●) OD/ 10^8 cells calculated from extrapolated values (OD was extrapolated from the dashed line drawn through the OD values, and cell number values were extrapolated from [i] a continuation of the $15T^-$ in M9-succinate [solid] line from 110 to 170 min and [ii] the dashed line from 175 to 310 min); (○) OD/ 10^8 cells calculated from the actual data points shown in Fig. 1.

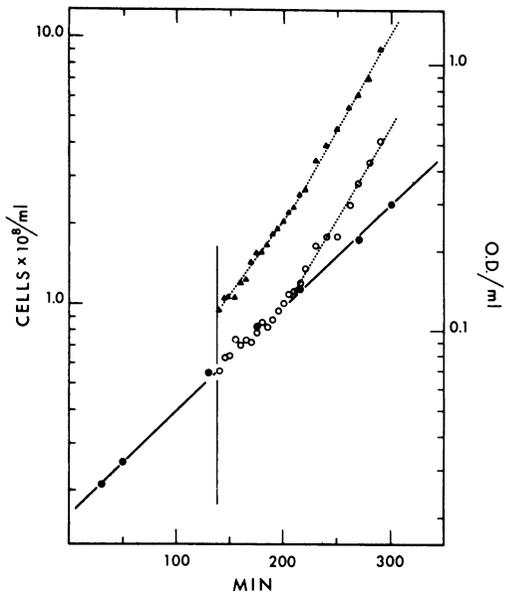


FIG. 3. Cell number and OD increases after addition of glucose to $E. coli 15T^-$ growing in M9-fumarate. At 139 min (thin vertical line), glucose (0.4%) was added to part of the culture, and at the indicated times samples were assayed and plotted as in Fig. 1.

(0.4%), and OD and cell number were monitored. Again, OD increased at a rapid rate immediately after the shift, and after about 70 min an even faster rate was assumed. As in Fig. 1, cell division continued at the preshift rate for 65 to 70 min before abruptly shifting to a doubling rate (doubling time, 43 min) roughly paralleling the final rate of OD increase. OD/cell data are not shown but closely mimic the data shown in Fig. 2.

Both M9-succinate and M9-fumarate supported $15T^-$ doubling times in the 70- to 80-min range, and in both media, rate maintenance was observed after glucose addition. In both media, the new rate of doubling was essentially that of $15T^-$ in M9-glucose medium.

Nutritional shifts in $E. coli 15T^-$ cells growing at doubling times of 120 min or more. $E. coli 15T^-$ cells growing at doubling times of 120 min or more did not display rate maintenance upon glucose addition. Strain $15T^-$ cells in M9-aspartate had a doubling time of exactly 120 min, and addition of glucose (to 0.4%) produced the division pattern shown in Fig. 4. Immediately, both OD and cell number began to increase (cell numbers increased at a rate corresponding to a 62-min doubling time, OD to a 58-min doubling time). After 200 min, the rate of increase in both cell number and OD again quickened (cell number to a 43-min doubling time; OD to 38 min). The disparity of mass and

cell number in doubling continued for at least 10 h, and at that time the OD/cell was $0.6/10^8$ cells, more than twice the size of $15T^-$ cells growing in M9-glucose.

The mass/cell pattern of the rapidly dividing cells in Fig. 4 is shown in Fig. 5. Rather than quickly attaining an OD/cell ratio more characteristic of the new medium (as in Fig. 2), immediately dividing cells maintained a size more characteristic of the cells before glucose addition (Fig. 5). However, after 200 min, the OD/cell ratio increased more dramatically and continued at the final rate for at least 10 h. The maximum cell sizes ultimately attained are under study and will be described elsewhere.

Figure 6 shows the division and OD/cell patterns after addition of glucose (0.4%) to $15T^-$ cells growing in M9-glyoxylate (doubling time, 185 to 195 min). Again cells began to increase in mass and divided at a rapid rate immediately upon glucose addition (cell number begins to increase at a rate corresponding to a doubling time of 61 min, OD to 60 min). After about 125 min, the rate of increase in OD changed to correspond to a doubling time of 54 min, but little or no change in division rate occurred.

When glucose was added to $15T^-$ cells growing in M9-asparagine or M9-glutamic acid (dou-

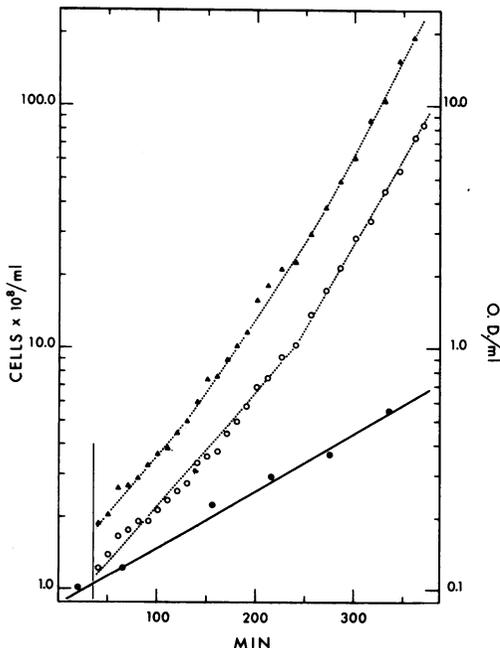


FIG. 4. Cell number and OD increases after addition of glucose to $15T^-$ in M9-aspartate. At 34 min (thin vertical line), glucose (0.4%) was added to part of the culture, and at the indicated times samples were assayed and plotted as in Fig. 1.

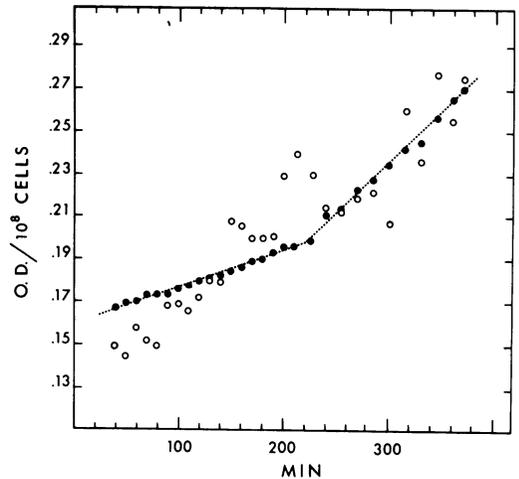


FIG. 5. OD/cell after addition of glucose to $15T^-$ growing in M9-aspartate. The OD/ 10^8 cells was calculated from the data of Fig. 4. Symbols: (●) OD/ 10^8 cells calculated from extrapolated values (OD and cell numbers were extrapolated from the dashed lines of Fig. 4); (○) OD/ 10^8 cells calculated from the actual data points shown in Fig. 4.

bling times, about 440 min in each medium), an immediate shift in division rate was also observed.

Non-rate maintenance division in other strains of *E. coli*. To determine if the immediate division pattern shown in Fig. 4 and 6 was unique to *E. coli* $15T^-$, glucose (0.4%) was added to *E. coli* B/r growing in M9-succinate (doubling time, 116 min) (Fig. 7) and *E. coli* K-12 growing in M9-aspartate (doubling time, 420 min) (Fig. 8). Each of the cultures displayed an abrupt shift to a faster rate of division upon glucose addition, with patterns similar to those in $15T^-$.

DISCUSSION

Although growth rate transitions have been used as a tool to identify and study parameters of growth regulation occurring in bacteria before and after a nutritional shift (for examples, see references 2, 3, 4, 6), these studies have not revealed some important regulatory patterns occurring in very slow-growing cells. Apparently, these patterns are such that upon nutritional shift-up very slow-growing cells can immediately assume both a new rate of mass increase and a new rate of division. In addition, these cells can experience some perturbation which precludes their attaining the mass/cell expected of the new medium.

Although our studies do not identify a specific growth rate that must be attained before an immediate shift can occur, our studies do

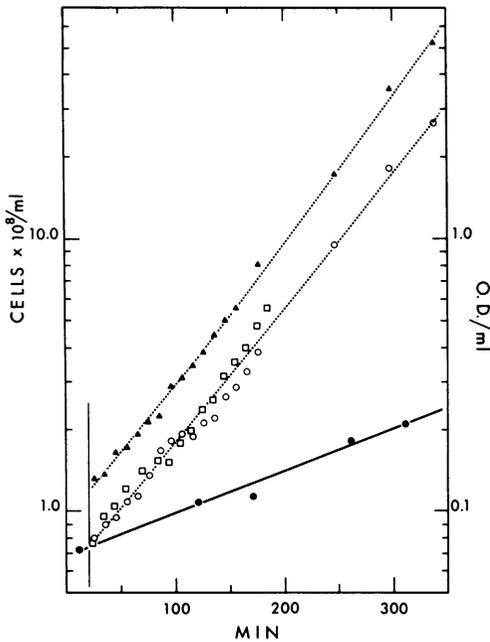


FIG. 6. Cell number and OD increases after addition of glucose to 15T⁻ growing in M9-glyoxylate. At 22 min (thin vertical line), glucose (0.4%) was added to part of the culture and at the indicated times samples were assayed for cell number and OD. Symbols: (●) cell number, glucose-free culture; (○) cell number, glucose-supplemented cells; (▲) OD, glucose-supplemented cells. The (□) symbols represent a duplicate set of data for cell number, glucose-supplemented cells.

suggest that such a specific rate might exist. Doubling times of 70 (Fig. 1) and 76 (Fig. 3) min yield rate maintenance division upon glucose addition, whereas 116- to 120-min doubling times (Fig. 4 and 7) demonstrate immediate shifts. In the course of doing more than 30 experiments with 15T⁻ growing in M9-aspartate (doubling time, 120 min) immediate shifts were always observed, but the postshift rate of cell doubling varied. In less than 10% of the experiments a doubling time of about 80 to 100 min was observed; in about 60% of the experiments a doubling time of about 70 to 75 min was attained, and in 30% of the experiments a doubling time of 55 to 65 min occurred. At doubling times in excess of 120 min an immediate shift and a very rapid doubling time (Fig. 6) is always observed. These observations suggest that about 120 min is a point of transition such that cells with a doubling time of 120 min have the potential for an immediate shift, but may have more or less potential for sustained rapid growth.

The immediate shift apparently depends

upon the presence of a readily metabolized substrate that can support doubling times of about 40 min. An immediate shift in 15T⁻ is also observed if ribose or xylose is used as a supplement. Lactose, a high-energy carbon source, the utilization of which requires enzyme induction, also stimulates an immediate shift. With lactose, however, shifts in OD and division have been delayed for several minutes, presumably as a consequence of the time required for enzyme induction. Lactose added to 15T⁻ in M9-fumarate produces a rate maintenance-type increase in cell number like that obtained with glucose (Fig. 3), but again the rise in OD is delayed. Additional experimentation should be done to positively correlate the delay in OD and cell number increases with actual utilization of lactose. Sucrose, a carbon source that is essentially non-metabolizable for 15T⁻, does not induce an immediate shift. Succinate added to 15T⁻ in M9-aspartate will not induce an immediate shift; instead, the culture continues to divide at the preshift rate.

The immediate shifts observed in Fig. 4 and 6 are not artifacts of electronic cell counting (Coulter counts). Dilution plate counts on M9-aspartate agar or nutrient agar verify Coulter

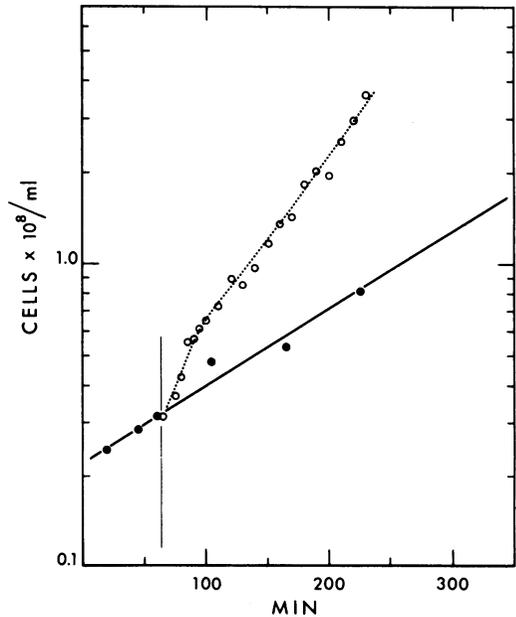


FIG. 7. Increase in cell number after addition of glucose to *E. coli* B/r growing in M9-succinate. At 65 min (thin vertical line), glucose (0.4%) was added to part of the culture and at the indicated times samples were assayed for cell number (Coulter counts). Symbols: (●) cell number, glucose-free cells; (○) cell number, glucose-supplemented cells.

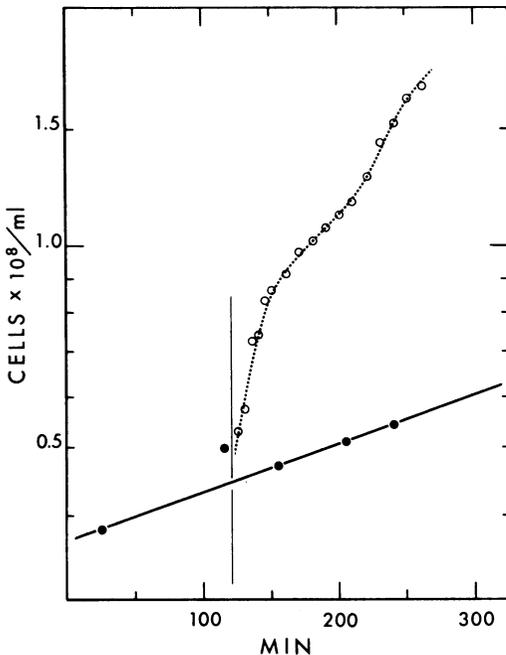


FIG. 8. Increase in cell number after addition of glucose to *E. coli* K-12 growing in M9-aspartate. At 122 min (thin vertical line), glucose was added to part of the culture, and at the indicated times samples were assayed and plotted as in Fig. 7.

counts under both rate maintenance and immediate shift conditions. It is clear, therefore, that immediate division after a shift-up is real, and that it is a function of the growth rate before and after the shift.

A division pattern like that shown in Fig. 4 and 6 is not totally without precedent. The original rate maintenance observations of Kjeldgaard et al. (see Fig. 1 of reference 14) show that colony counts experience a slight but immediate increase after a shift-up. This rate of increase, however, is not maintained. Instead, the rate of increase rapidly diminishes to the extent that the average increase is about equal to that of the pre-shift culture. This pattern has also been observed by others (14; Case and Marr, *Abstr. Annu. Meet. Am. Soc. Microbiol.* 1974, G43, p. 27; Fig. 1 and 3, this report) and characterized by Case and Marr (*Abstr. Annu. Meet. Am. Soc. Microbiol.* 1974, G43, p. 27). Cooper (6) hypothesized conditions under which cells growing according to the I + C + D model (7, 14) could undergo a growth rate transition described by an initial burst followed by a rapid decline. Our immediate shifts (Fig. 4, 6-8) show an extended initial burst of division followed by a slight decrease in growth rate. The decrease, however, is never extensive enough to ap-

proach the decrease seen in rate maintenance (Fig. 1 and 3).

The immediate shift may be an exaggeration of the normal rate maintenance curve, or it may be a manifestation of a completely different pattern of division. Which situation is occurring, and the nature of the division in either situation, is presently unknown. Either situation seems difficult to reconcile with the I + C + D model of cell division (7, 14). The model specifies that, in a nutritional shift-up, a time equivalent to part or all of C and all of D must pass before shifted cells can divide at the new medium-specific rate. Passage of such time clearly does not occur in the immediate shifts of Fig. 4 and 6-8.

Several explanations can be offered to account for division proceeding at a new rate within a time less than C + D: (i) some or all of the events prescribed by the I + C + D model need not occur in certain shift-ups; (ii) very slow-growing cells complete C + D events more rapidly than predicted by the growth rate, so that such events may be completed during a small fraction of their doubling time (5, 18, 19), allowing new rates of cell separation to be observed immediately upon shift-ups; or, (iii) division events after a shift-up are occurring at a rate(s) intermediate between those of pre- and postshift media.

An intermediate division rate (iii, above) could account for the aforementioned variations in doubling time after addition of glucose to 15T⁻ in M9-aspartate, the unusual OD/cell transition pattern, and the repeated observation that 15T⁻ cells continue to increase in size after an immediate shift. However, there is no clear rationale for the existence of such a mechanism. Likewise, possibility (i) is not easily rationalized. Recent literature supporting the basic cell cycle events described by the I + C + D model is extensive, and it is unlikely that very slow-growing cells deviate from the model extensively. Possibility (ii), however, seems plausible. Several studies suggest that C (5, 19) and D (18, 19) are of the same duration at a wide variety of growth rates. Such a situation could allow very slow-growing cells to be essentially "primed for division" during much of their cell cycle. Presumably, nutrient addition, and consequent increases in biosynthetic rates, would allow fruition of this primed state.

The question of why cells growing at doubling times of ± 70 min show rate maintenance upon shift-up and cells growing at more than 120 show immediate division cannot presently be answered, but is the subject of further study. Also unclear, but under intensive study, is why

OD/cell ratios behave as they do during immediate shifts.

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