JOURNAL OF BACTERIOLOGY, Jan. 1976, p. 248-257

Vol. 125, No. 1
Printed in U.S.A.

Morphological Analysis of Nuclear Separation and Cell Division During the Life Cycle of *Escherichia coli*

C. L. WOLDRINGH

Laboratory of Electron Microscopy, University of Amsterdam, Amsterdam, The Netherlands

Received for publication 7 October 1975

Quantitative electron microscope observations were performed on *Escherichia coli* B/r after balanced growth with doubling times (τ) of 32 and 60 min. The experimental approach allowed the timing of morphological events during the cell cycle by classifying serially sectioned cells according to length. Visible separation of the nucleoplasm was found to coincide with the time of termination of chromosome replication as predicted by the Cooper-Helmstetter model. The duration of the process of constrictive cell division (10 min) appeared to be independent of the growth rate for τ = 60 min or less but to increase with increasing doubling time in more slowly growing cells. Physiological division, i.e., compartmentalization prior to physical separation of the cells, was only observed to occur in the last minute of the cell cycle. The morphological results indicate that cell elongation continues during the division process in cells with τ = 32 min, but fails to continue in cells with τ = 60 min.

The study of chemical composition and rate of macromolecular synthesis in bacteria cultured at different growth rates has provided detailed information on the sequence of biochemical events that play a role in the regulation of the cell division cycle (8, 25, 27, 30).

For *Escherichia coli* B/r the process of growth and division can now be described in terms of deoxyribonucleic acid replication (8), protein synthesis (10), and envelope formation (19). To understand how these different reactions are integrated by the cell in both time and space, the sequence of biochemical events should be correlated with morphological data on size, shape, and structural organization of the cells.

In most studies a description of the cell cycle of *E. coli* in more physical and morphological terms has been based on either light microscope (9, 13, 24, 31) or wholemount electron microscope (4) observations, or on indirect biochemical determinations (5, 26).

The process of cell division in *E. coli* has been studied recently by Burdett and Murray at the ultrastructural level (2, 3). However, no quantitation on the occurrence of the structures observed was given, although their results were related to the cell cycle by observing cell samples from a synchronized culture (3).

In the present study quantitative electron microscope observations were performed on asynchronous populations of balanced growing cells. Timing of the morphological data was obtained by classifying cells from one sample according to length, and by establishing a relationship between length and age of the cells. This method allowed the determination of those events during the cell cycle which are difficult to characterize with the aid of biochemical or light microscope techniques. For instance, (i) the age at which nuclear separation occurs, (ii) initiation and duration of cell division, and (iii) the process of physical cell separation were determined in cells grown at different growth rates. The results are compared with biochemical observations.

MATERIALS AND METHODS

Organism and culture conditions. The organism used was *E. coli* B/r (strain H266, Laboratory of Microbiology, Utrecht, The Netherlands) kindly provided by P. G. de Haan. The bacteria were grown in the minimal salts medium used by Helmstetter and Cooper (17). To obtain different growth rates the basic medium was supplemented with either 0.1% glucose, 0.2% Casamino Acids, 50 μg of tryptophan per ml (doubling time, τ = 30 to 34 min), 0.5% glucose (τ = 45 to 47 min) or 0.1% glycerol (τ = 59 to 61 min).

More slowly growing cells were obtained with 0.2% acetate or succinate (τ = 100 to 105 min) or 0.025% alanine (τ = 150 to 210 min). For each experiment cells were grown in 100-ml batch cultures for about 16 h at 37 C with shaking. Subsequently the cells were cultured further at constant growth rate for at least 10 generations by periodical dilution of the culture, so that the cell concentration was maintained below 5×10^8 cells per ml (0.7 absorbance at 620 nm).

These culture conditions have been reported to result in balanced growth of the cells (14, 21). To ascertain this state of growth, the constancy of length distributions was determined as being one of the criteria for balanced growth (14). The length distributions obtained from cells fixed after different periods of exponential growth and prepared by agar filtration were analyzed by the contrast method of Sheffe (32). From cells with a doubling time of 32 or 60 min, constant length distributions were routinely obtained. Balanced growth of cells grown in acetate, succinate, or alanine appeared more difficult and was not always obtained.

Agar filtration. For the determination of length distributions cells were fixed by the addition of 1% OsO_4 to a final concentration of 0.1%. The fixed cells were applied without further manipulation to the agar filter and processed according to the agar filtration technique of Kellenberger (20). The length of 300 to 1,000 cells was measured on micrographs printed to give a final magnification of 12,000×.

Serial sections. Cells prefixed with 0.1% OsO_4 in the growth medium were fixed and embedded according to the method of Ryter et al. (29). Serial sections of about 70 nm thick were cut on an LKB Ultrotome and picked up with single-hole specimen supports covered with a carbon-coated Formvar film. Longitudinally sectioned cells were photographed in series at a final magnification of 36,000×. Reconstruction of the serially sectioned cells was performed by superimposing the drawings of nucleoplasmic and cellular outline from each section. Cell lengths were measured from these reconstructions.

Electron microscopy. Electron micrographs were taken with a Philips EM 300 operating at 80 kV. The magnification was calibrated with a line-grating replica (spacing 1.67 μm) or a cross-grating replica (spacing 464 nm).

RESULTS

Age determination by classification of cells according to length. The age of individual cells observed in electron microscope preparations can be determined if the size distribution and the doubling time (τ) of the population are known, and if a mode of growth for the individual cell is assumed as, for instance, a linear or an exponential increase in length between divisions (15).

As *E. coli* cells have been shown (22) to extend only in length and not in width at a given growth rate, cell length can be used as a measure of cell size. In Fig. 1A and B length distributions are shown of *E. coli* B/r cells cultured with a doubling time of 32 and 60 min, respectively. As cells in the process of division could easily be distinguished, the length distribution of dividing cells is shown as well (hatched areas in Fig. 1). From the minimal (l_{min}) and maximal (l_{max}) cell lengths obtained in the length distributions, the mean length of dividing cells (m_d) can be calculated from the relationship: $m_d = \frac{1}{2} l_{max} + l_{min}$ as given by Harvey et al. (14). Because each dividing cell results in two new-born cells, the mean length of these cells $m_n = \frac{1}{2} m_d$ (14). From the values of m_n and m_d (arrows, Fig. 1) and from the known doubling time it is possible to relate cell length to cell age (see time scales in Fig. 1). The interdivision ages corresponding to intermediary cell lengths can be derived after establishing either a linear or an exponential relationship between length and age of the cells.

The parameters of the length distributions (Fig. 1A and B) are summarized in Table 1. In general the shapes of the two distributions correspond with distributions shown in earlier publications and obtained by both microscopic measurement (6, 23) and the use of a Coulter counter (11, 14, 15).

Timing of nuclear separation. To determine the time in the cell cycle at which nuclear separation occurs, cells from the same cultures as those shown in Fig. 1A and B have been sectioned in series. From the serially sectioned cells it could easily be decided whether they contained one or two nucleoplasms. Only in three cases of the τ = 32-min cells did small extensions of nuclear material make it difficult to decide whether the nucleoplasms were still connected by a narrow bridge or had just separated; they were scored as cells containing an unseparated nucleoplasm.

Figure 2 shows representative examples of cells from the two cultures. The more rapidly growing cells are only slightly longer (see Fig. 1), whereas the diameter of these cells is distinctly larger. No variation in diameter with cell length was found in the serially sectioned cells of either culture (see reference 22).

In the thicker τ = 32-min cells, the nucleoplasm represents a relatively simple beanlike structure after its division, but changes into more complicated "W"- and "S"- shaped structures as deoxyribonucleic acid (DNA) replication proceeds (Fig. 2A; also see figures in C. L. Woldringh, Ph.D. thesis, University of Amsterdam, 1974). In the more slowly growing cells the nucleoplasm represents a cylindrical structure throughout the division cycle which elongates at the same rate as cellular length increases (Fig. 2B-F). In both cultures the nucleoplasm divides by constriction at its center and migrates after division in a gradual way to its new position in the daughter cell.

Figure 3A and B shows the length distributions derived from reconstructions of the serially sectioned cells. Indicated in these graphs is the

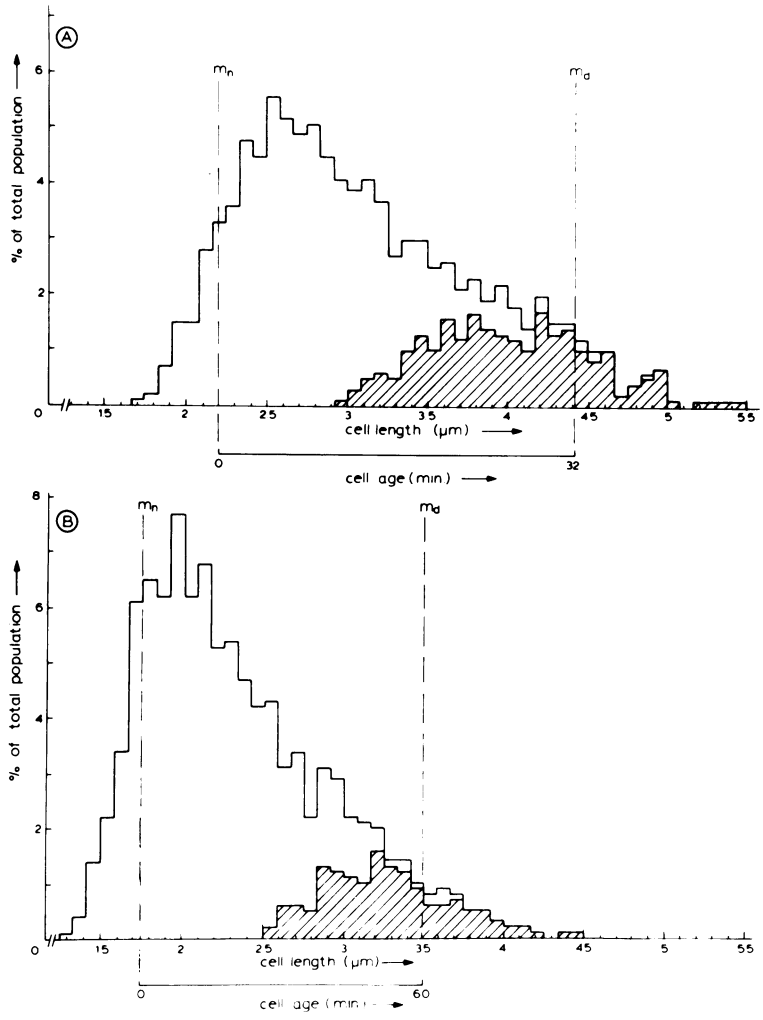

FIG. 1. *Length distributions of OsO_4-fixed cells prepared by agar filtration. (A) E. coli B/r growing with a doubling time of 32 min. (B) Doubling time 60 min. For the parameters of the length distributions, see Table 1. Hatched areas: Length distribution of cells in the process of constriction. Arrows m_n and m_d: Mean lengths of new-born and dividing cells, respectively. The values of m_n and m_d (see text for their determination) indicate age zero and the age of one doubling time, respectively, on a time scale for cell age.*

TABLE 1. *Parameters of length distributions from balanced growing cells prepared by agar filtration*[a]

Doubling time (min)	Total population				Dividing cells			
	No. of cells measured	m_n[b] (μm)	$\bar{m}$[c] (μm)	CV[c] (%)	Total population (%)	m_d[b] (μm)	$\bar{m}$[c] (μm)	CV[c] (%)
32	1,545	2.2	3.1	24	23	4.4	4.0	13
60	1,085	1.8	2.3	25	15	3.5	3.3	13

[a] See Fig. 1 for length distributions.
[b] See text for calculation of mean length of new-born (m_n) and dividing cells (m_d).
[c] Symbols: $\bar{m}$, Mean length of the population; CV, coefficient of variation.

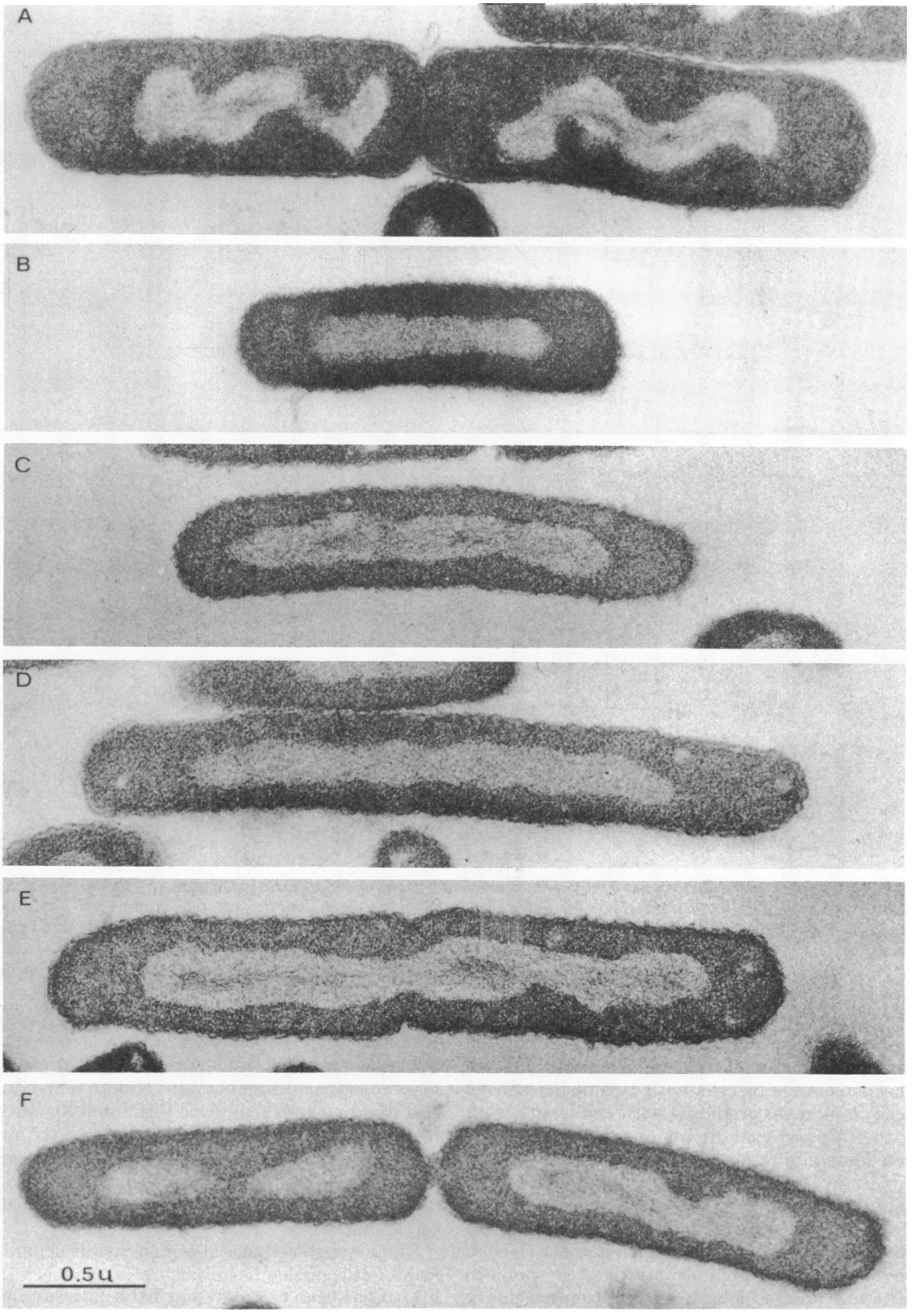

FIG. 2. *Examples of serially sectioned E. coli B/r cells fixed with OsO_4. (A) Dividing cell from the 32-min doubling time culture. Cell division proceeds by simultaneous invagination of both cell wall and cytoplasmic membrane; note the two rod-shaped nucleoplasms which are bent and twisted. (B–F) Simulation of the 60-min life cycle of E. coli B/r by representative cells from different length classes. The rod-shaped nucleoplasm gradually elongates with increasing cell length. In cell E, cell division has initiated before separation of the nucleoplasm. Magnification: ×36,000.*

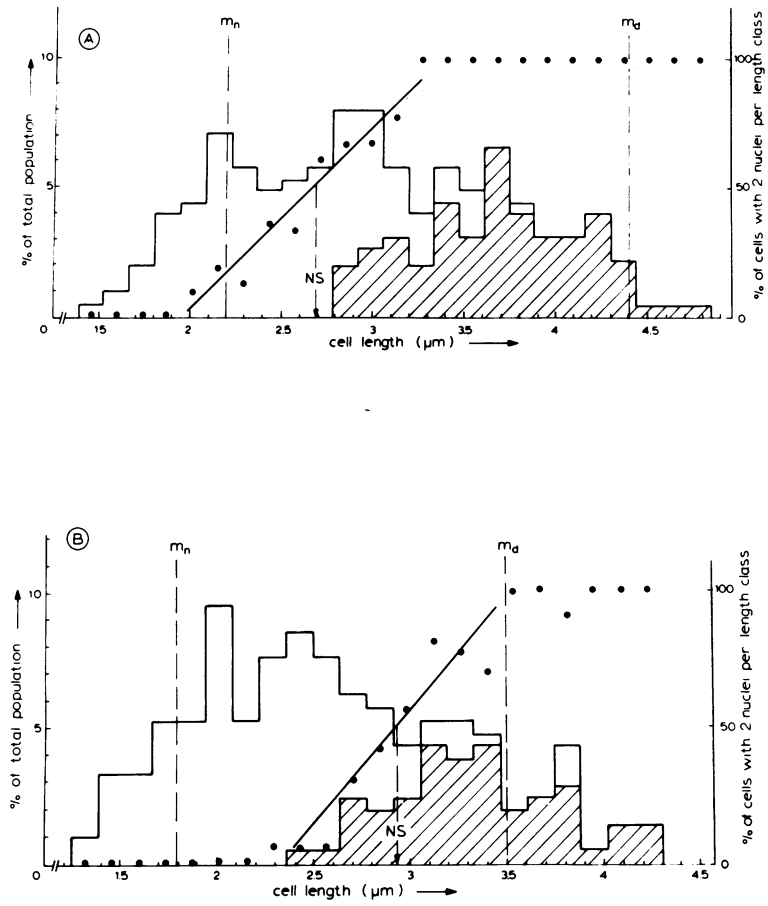

FIG. 3. *Length distributions determined from reconstructions of serially sectioned cells grown with doubling times of 32 min (A) or 60 min (B). (●) Percentage of cells in each length class containing two separate nucleoplasms. The straight lines indicate the transition of cells with one nucleoplasm to cells with two nucleoplasms as a function of cell length. The midpoint of this transition (arrows NS) indicates the cell length at which in the average cell nucleoplasmic separation occurs (see Table 2). The cell lengths m_n and m_d are those derived from the distributions in Fig. 1.*

fraction of cells in each length class containing two separated nucleoplasms. In both cultures the percentage of cells with two nuclei can be seen to increase gradually with cell length from 0 to 100%. The midpoint of this transition is considered to represent the cell length (l_{NS}) at which, in the average cell, visible separation of the nucleoplasm occurs.

The number of cells in serial sections (226 from the τ = 32-min population and 211 from the τ = 60-min population) was too small to obtain a reliable length distribution necessary for the calculation of m_n and m_d.

Therefore, these values have been derived from the length distributions of agar-filtered cells (Fig. 1A and B). In spite of the different way of preparation of the cells, this appears justified because there is a fair agreement between the two kinds of distribution with respect to the minimal lengths of both new-born and dividing cells (compare Fig. 1 and 3). With respect to the maximal cell length, the distributions in Fig. 3 appear to be incomplete. This can be explained by the smaller chance for long dividing cells to lie full-length in the plane of sectioning.

The average cell age at which visible separation of the nucleoplasm occurs (arrows NS in Fig. 3) has been calculated in Table 2, assuming both a linear and an exponential relation between length and age of the cells. It can be seen that with τ = 32 min nuclear separation coincides with the time of termination of DNA replication as derived in biochemical studies

TABLE 2. *Comparison of the time of nuclear separation with the time of termination of DNA replication as predicted by the model of Cooper and Helmstetter (8)*

Doubling time (min)	Cell length of nuclear separation[a] (μm)	Cell age (min) of nuclear separation assuming growth to be:		Time of termination (min)[c]
		Linear[b]	Exponential[b]	
32	2.7	7	10	8
60	2.9	39	43	36

[a] See arrows NS in Fig. 3A and B.

[b] Cell age of nuclear separation, $t_{NS} = T\ [(l_{NS} - m_n)/(m_d - m_n)]$, for a linear relationship between cell length and cell age. For an exponential relation the logarithm of cell lengths is used.

[c] For batch cultures the time of termination of DNA replication is assumed to occur 24 min (*D* period) before cell separation (see reference 8).

(8). On the other hand, in the τ = 60-min population, separation of the nucleoplasm lags somewhat behind termination of DNA replication, especially when assuming exponential growth of the individual cell (see below).

Duration of the process of cell constriction. In contrast to the volume distributions obtained with a Coulter counter, the length distributions obtained by microscopy measurement provide direct data on the fraction of dividing cells in the population. Moreover, from serial sections the degree of cell constriction of each individual cell can be estimated.

In the cultures with τ = 32 min and τ = 60 min, the dividing cells represented 15 and 23% of the total population, respectively (see Table 1). These fractions are comparable with those reported by Kubitschek (21): 15% of dividing cells in an *E. coli* B/r culture growing with a 60-min doubling time, and 28% in an *E. coli* THU culture growing with a doubling time of 20 min.

From the percentage of cells in the population which were seen to show cell constriction, the fraction of the cell cycle needed for the process of constriction, the T period (13), is given by: $T \ln 2 = \tau \ln(N_c/N + 1)$, T being the time needed for cell constriction and separation, τ the doubling time, N_c the number of cells seen to show cell constriction, and N the total number of cells in the population (compare with formula of the mitotic index in references 7, 33).

In Fig. 4 the T period has been determined for *E. coli* B/r cells grown at different growth rates. It can be seen that, in cultures with a doubling time of 60 min or less, the process of cell constriction takes a constant period of about 10 min independent of growth rate. In more slowly growing cells, however, the T period appears to increase in proportion with the doubling time, covering a fraction of about one-sixth of the cell cycle.

From each dividing cell sectioned in series it was possible to estimate the degree of cell constriction. In Fig. 5 cell constriction expressed as the distance over which the cell has invaginated (q) in percentage of the cell radius (r) has been plotted versus cell length. The average cell constriction per length class in the τ = 32-min population can be seen to increase with cell length (Fig. 5A), suggesting that the cells continue to elongate during the process of constriction. Figure 5B shows that there is no such length increase during constriction of cells grown with a doubling time of 60 min. This finding of a length increase during constriction of the τ = 32-min cells is in contrast to the observation by time-lapse photomicrography of Hoffman and Frank (18), who found no elongation during the division of cells grown with a doubling time of 21 min, but it confirms the observations of Adler et al. (1).

Duration of the process of cell separation. Measurement of the degree of cell constriction (Fig. 5) showed that all stages of division were present, including that of 100%, which indicates cells that are physiologically divided without as yet physical separation of the daughter cells (Fig. 2F). In Table 3 the fraction of dividing cells showing physiological division can be seen to be 4 to 5%. As the duration of visible cell constriction has been shown to be 10 min (Fig. 4), this low percentage indicates that the process of physical separation will not take longer than 1 min.

Initiation of cell constriction before nucleoplasmic separation. Another phenomenon observed in the serially sectioned cells and indicated in Table 3 is the initiation of cell division without as yet nuclear separation. This situation only occurs in 2% of the dividing cells in the τ = 32-min culture but in 15% of the dividing cells grown with a doubling time of 60 min (Fig. 2E). Preliminary observations on serial sections of alanine-grown cells (doubling time 180 min) showed that this phenomenon drastically increases to 50 to 65% in more slowly growing cells, suggesting that the time between nuclear separation and cell division remains short, whereas the T period increases (results to be reported elsewhere).

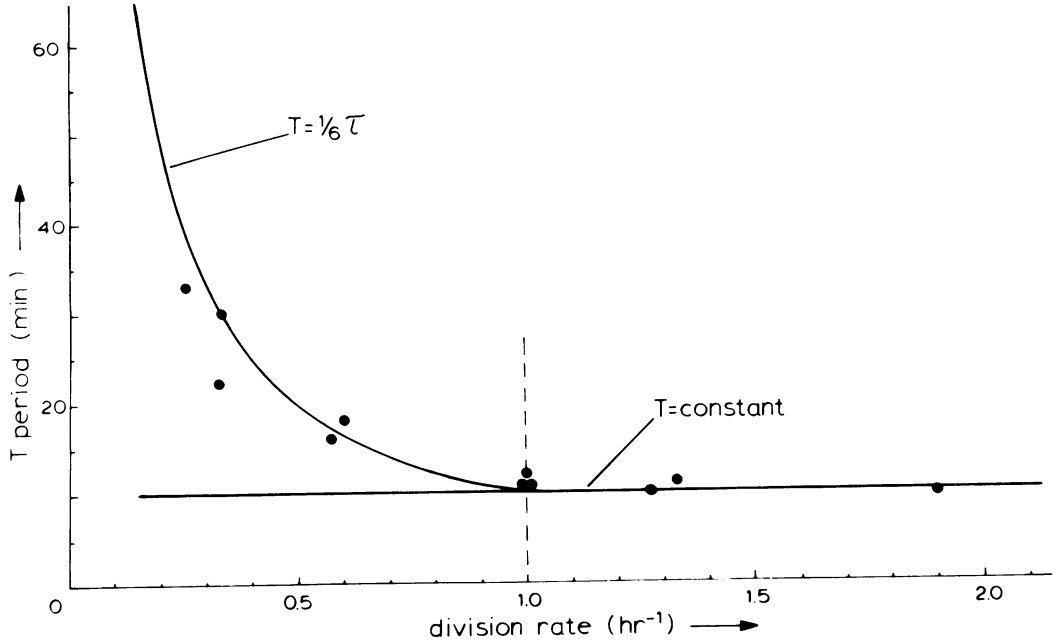

FIG. 4. *Duration of the process of visible cell constriction, the T period, in cultures of E. coli B/r grown at different growth rates. For cells grown with a doubling time of 60 min or less the T period appears to be 10 min, independent of growth rate. In more slowly growing cells the T period increases with decreasing growth rate. The horizontal line indicates a constant T period of 10 min. The curved line was calculated assuming $T = 1/6\ \tau$. Each point represents an independent growth experiment in which cells were fixed after exponential growth during at least three doubling times.*

DISCUSSION

The morphological events observed during the cell cycle of *E. coli* B/r cultured at two growth rates are summarized in Fig. 6. The moment of nuclear separation in rapidly growing cells (τ = 32 min) was found to coincide with the time of termination of DNA replication (Table 2). This result indicates that the newly synthesized DNA strands move apart during replication, so that at or shortly after termination the two genomes are also physically separated.

In more slowly growing cells with τ = 60 min, visible separation of the nucleoplasm follows somewhat later than termination of DNA replication (Table 2), especially when exponential growth of the cells is assumed. However, the observation that the τ = 60-min cells do not increase in length during the 10 min of constriction (Fig. 5B) suggests that the mean length of dividing cells (m_d in Fig. 1B) is already attained after 50 min. This would change the time scale and bring the cell age, which corresponds with the length of nuclear separation for exponential growth, to 36 min instead of the 43 min indicated in Table 2.

The process of cell constriction (Fig. 4) was found to take a constant period of 10 min in rapidly growing cells, but it appeared to increase to about one-sixth of the cell cycle in cells grown with a doubling time of more than 60 min. This phenomenon bears resemblance to the *D* period, the time between termination and cell separation, which has been shown to be a constant period of 24 min in rapidly growing cells (8), and to increase, at least in the case of *E. coli* B/r ATCC, to one-third of the cell cycle when growing with a doubling time greater than 60 min (16).

The observations on serially sectioned cells summarized in Table 3 and Fig. 5 indicated a very short period (1 min or less) between completion of cell constriction (physiological cell division) and physical separation of the daughter cells. This corresponds with the finding of Onken and Messer (26) that the two halves of a dividing cell are able to exchange DNA and protein molecules until near to the time of physical cell separation.

Our morphological results on balanced growing cells of *E. coli* B/r in the process of division showed a concomitant invagination of both cell wall and cytoplasmic membrane in all stages of

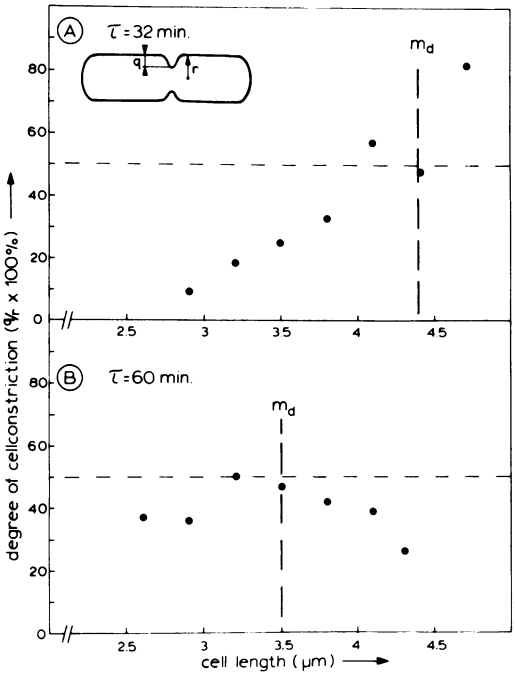

FIG. 5. *Cell constriction as a function of cell length in serially sectioned cells grown with a doubling time of 32 min (A) or 60 min (B). (●) The average degree of cell constriction per length class. The degree of constriction is expressed as the distance (q) over which the cell has invaginated in percentage of the cell radius (r).*

constriction. By contrast, Burdett and Murray (2, 3) observed the presence of real septa, composed of cytoplasmic membrane and mucopeptide layer, in 40 to 50% of the dividing cells of *E. coli* B/r. The authors ascribe the visualization of septa in *E. coli* and other gram-negative cells (12) to the use of 5 to 10% acrolein and 0.25% glutaraldehyde as a prefixative and regard constrictive division as observed after prefixation with either OsO_4 or glutaraldehyde as an artefact of fixation. Nevertheless, as all of their cells already show in the initial stage of septation some degree of cell wall invagination (see Fig. 7 to 12 in reference 3), cell division appears to initiate by constriction of the cell envelope.

TABLE 3. *Properties of serially sectioned cells in the process of division*

Doubling time (min)	Total no. of dividing cells measured[a]	Dividing cells showing (%): Physiological division (100% constriction)	Dividing cells showing (%): Unseparated nucleoplasm
32	91	5	2
60	62	4	15

[a] Compare with the length distributions in Fig. 3A and B.

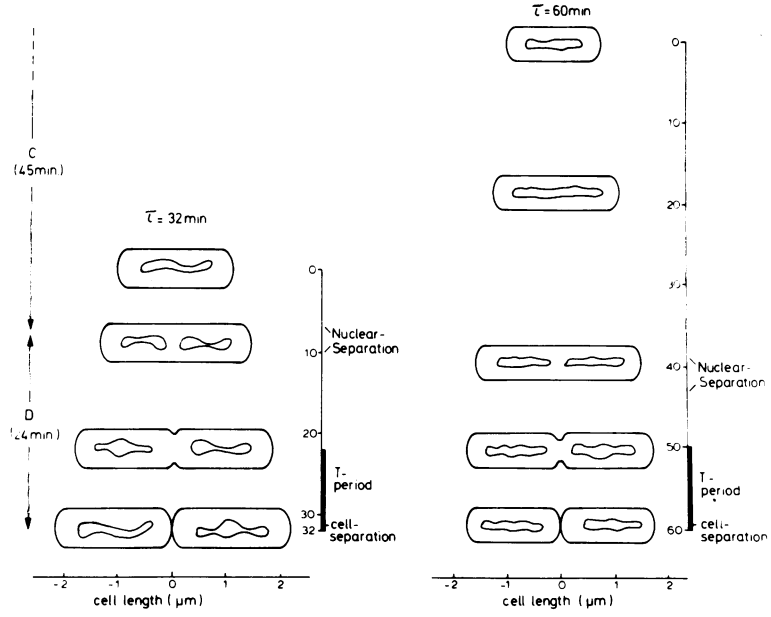

FIG. 6. *Summary of morphological events during the cell cycle of E. coli B/r grown with τ = 32 and 60 min. The timing of the morphological events is derived from the results in Table 2 and Fig. 4 and 5. Cell elongation has been assumed to be linear. Size and shape of cells and nucleoplasms have been derived from observations on serially sectioned cells.*

In this study OsO_4 fixation has been applied because it fixes the nucleoplasm in a contracted state which allows better observation of its division and migration than does dispersed DNA as it appears after aldehyde fixation (see reference 35). Moreover, OsO_4 (2) or glutaraldehyde (28) adequately preserves the septum which occurs in envelope mutants of *E. coli*, or in cells fixed after growth under unphysiological conditions (34). Preliminary observations further indicate that septa can be visualized in *E. coli* B/r prefixed with OsO_4 when the culture medium has been diluted (see reference 12) and the cells are fixed at a low cell density. These observations suggest that constrictive cell division may be more dependent on the physiological condition in which the cells are fixed than on the kind of fixative applied, as is emphasized by Murray and co-workers (2, 3, 12).

With respect to the timing of morphological events observed with the electron microscope, we applied the method of classification of cells from an asynchronous population. An alternative approach could have been to take samples from a synchronized culture at different times during the cell cycle (see reference 3). However, because of the variation in properties of individual cells, many cells have to be measured in each particular sample to determine the characteristic morphology of the average cell. As this would require studying many more cells, we preferred to classify cells of one single sample. Furthermore, this method is not dependent on the ability of the cells to grow on a membrane filter, and it excludes uncertainties introduced by unphysiological treatment of the cells during synchronization (15).

ACKNOWLEDGMENTS

I wish to thank P. C. Diegenbach and A. van der Stelt for their hospitality at the Zoological Laboratory (University of Amsterdam) and for advice and help with the statistical analyses. The technical assistance of S. P. Cohen-Hirschhaut and J. H. D. Leutscher is gratefully acknowledged. The final shape of the manuscript was appreciably improved by N. Nanninga.

LITERATURE CITED

1. Adler, H. J., W. D. Fisher, and A. A. Hardigree. 1969. Cell division in *Escherichia coli*. Trans. N. Y. Acad. Sci. **31**:1059-1070.
2. Burdett, I. D. J., and R. G. E. Murray. 1974. Septum formation in *Escherichia coli*: characterization of septal structure and the effects of antibiotics on cell division. J. Bacteriol. **119**:303-324.
3. Burdett, I. D. J., and R. G. E. Murray. 1974. Electron microscope study of septum formation in *Escherichia coli* strains B and B/r during synchronous growth. J. Bacteriol. **119**:1039-1056.
4. Chai, N-C., and K. G. Lark. 1970. Cytological studies of deoxyribonucleic acid replication in *Escherichia coli* 15T⁻: replication at slow growth rates and after a shift-up into rich medium. J. Bacteriol. **104**:401-409.
5. Clark, D. J. 1968. The regulation of DNA replication and cell division in *E. coli* B/r. Cold Spring Harbor Symp. Quant. Biol. **33**:823-838.
6. Collins, J. F., and M. H. Richmond. 1962. Rate of growth of *Bacillus cereus* between divisions. J. Gen. Microbiol. **28**:15-33.
7. Cook, J. R., and T. W. James. 1964. Age distribution of cells in logarithmically growing cell populations, p. 485-495. *In* E. Zeuthen (ed.), Synchrony in cell division and growth. Interscience Publishers Inc., New York.
8. Cooper, S., and Ch. E. Helmstetter. 1968. Chromosome replication and the division cycle of *Escherichia coli* B/r. J. Mol. Biol. **31**:519-540.
9. Donachie, W. D. 1968. Relationship between cell size and time of initiation of DNA replication. Nature (London) **219**:1077-1079.
10. Donachie, W. D., N. C. Jones, and R. Teather. 1973. The bacterial cell cycle. Symp. Soc. Gen. Microbiol. **23**:9-44.
11. Ecker, R. E., and M. Schaechter. 1963. Bacterial growth under conditions of limited nutrition. Ann. N. Y. Acad. Sci. **102**:549-563.
12. Gilleland, H. E., Jr., and R. G. E. Murray. 1975. Demonstration of cell division by septation in a variety of gram-negative rods. J. Bacteriol. **121**:721-725.
13. Gudas, L. J., and A. B. Pardee. 1974. Deoxyribonucleic acid synthesis during the division cycle of *Escherichia coli*: a comparison of strains B/r, K-12, 15 and 15T⁻ under conditions of slow growth. J. Bacteriol. **117**:1216-1223.
14. Harvey, R. J., A. G. Marr, and P. R. Painter. 1967. Kinetics of growth of individual cells of *Escherichia coli* and *Azotobacter agilis*. J. Bacteriol. **93**:605-617.
15. Helmstetter, Ch. E. 1969. Methods for studying the microbial division cycle, p. 327-363. *In* J. R. Norris and D. W. Ribbons (ed.), Methods in microbiology, vol. 1. Academic Press Inc., New York.
16. Helmstetter, Ch. E. 1974. Initiation of chromosome replication in *Escherichia coli*. I. Requirements for RNA and protein synthesis at different growth rates. J. Mol. Biol. **84**:1-19.
17. Helmstetter, Ch. E., and S. Cooper. 1968. DNA synthesis during the division cycle of rapidly growing *Escherichia coli* B/r. J. Mol. Biol. **31**:507-518.
18. Hoffman, H., and M. E. Frank. 1965. Time-lapse photomicrography of cell growth and division in *Escherichia coli*. J. Bacteriol. **89**:212-216.
19. Hoffmann, B., W. Messer, and U. Schwarz. 1972. Regulation of polar cap formation in the life cycle of *Escherichia coli*. J. Supramol. Struct. **1**:29-37.
20. Kellenberger, E. 1953. Les formes caractéristiques des nucléoides de *E. coli* et leurs transformations dues à l'action d'agents mutagènes-inducteurs et de bactériophages, p. 45-66. VIth Int. Congr. Microbiol. Fondazióne Emanuele Paterno, Rome.
21. Kubitschek, H. E. 1969. Growth during the bacterial cell cycle: analysis of cell size distribution. Biophys. J. **9**:792-809.
22. Marr, A. G., R. J. Harvey, and W. C. Trentini. 1966. Growth and division of *Escherichia coli*. J. Bacteriol. **91**:2388-2389.
23. Maruyama, Y. 1964. Synchrony of bacterial populations as established by filtration techniques, p. 421-432. *In* E. Zeuthen (ed.), Synchrony in cell division and growth. Interscience Publishers Inc., New York.
24. Mason, D. J., and D. M. Powelson. 1956. Nuclear division as observed in live bacteria by a new technique. J. Bacteriol. **71**:474-479.
25. Matsushita, T., and H. E. Kubitschek. 1975. DNA replication in bacteria, p. 247-327. *In* A. H. Rose and D. W. Tempest (ed.), Advances in microbial physiology, vol. 12. Academic Press Inc., London.
26. Onken, A., and W. Messer. 1973. Cell division in

Escherichia coli. Septation during synchronous growth. Mol. Gen. Genet. **127**:349-358.
27. Pardee, A. B., P. Ch. Wu, and D. R. Zusman. 1973. Bacterial division and the cell envelope, p. 357-412. *In* L. Leive (ed.), Bacterial membranes and walls, vol. 1. Marcel Dekker Inc., New York.
28. Rodolakis, A., P. Thomas, and J. Starka. 1973. Morphological mutants of *Escherichia coli*. Isolation and ultrastructure of a chain-forming envC mutant. J. Gen. Microbiol. **75**:409-416.
29. Ryter, A., E. Kellenberger, A. Birch-Andersen, and O. Maaløe. 1958. Etude au microscope électronique de plasmas contenant de l'acide désoxyribonucléique. I. Les nucléoides des bactéries en croissance active. Z. Naturforsch. **13**:597-605.
30. Schaechter, M., O. Maaløe, and N. O. Kjeldgaard. 1958. Dependency on medium and temperature of cell size and chemical composition during balanced growth of *Salmonella typhimurium*. J. Gen. Microbiol. **19**:592-606.
31. Schaechter, M., J. P. Williamson, J. R. Hood, Jr., and A. L. Koch. 1962. Growth, cell and nuclear divisions in some bacteria. J. Gen. Microbiol. **29**:421-434.
32. Sheffe, H. 1953. A method for judging all contrasts in the analysis of variance. Biometrica **40**:87-104.
33. Smith, C. L., and P. P. Dendy. 1962. Relation between mitotic index, duration of mitosis, generation time and fraction of dividing cells in a cell population. Nature (London) **193**:555-556.
34. Steed, M. T., and R. G. E. Murray. 1966. The cell wall and cell division of gram-negative bacteria. Can. J. Microbiol. **12**:263-270.
35. Woldringh, C. L. 1973. Effect of cations on the organization of the nucleoplasm in *Escherichia coli* prefixed with osmium tetroxide or glutaraldehyde. Cytobiology **8**:97-111.