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# Morphological Analysis of the Division Cycle of Two *Escherichia coli* Substrains During Slow Growth

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Morphological parameters of the cell division cycle have been examined in *Escherichia coli* B/r A and K. Whereas the shape factor (length of newborn cell/width) of the two strains was the same at rapid growth (doubling time, $\tau$, less than 60 min), with decreasing growth rate the dimensions of the two strains did change so that B/r A cells became more rounded and B/r K cells became more elongated. The process of visible cell constriction ($T$ period) lasted longer in B/r A than in B/r K during slow growth, reaching at $\tau$ = 200 min values of 40 and 17 min, respectively. The time between termination of chromosome replication and cell division ($D$ period) was found to be longer in B/r A than in B/r K. As a result, in either strain completion of chromosome replication seemed always to occur before initiation of cell constriction. Nucleoplasmic separation did not coincide with termination as during rapid growth but occurred in both strains within the $T$ period, about 10 min before cell division.

Two different patterns of chromosome replication have been proposed for *Escherichia coli* B/r cells growing with doubling times exceeding 60 min. Helmstetter and co-workers (5, 14) originally suggested that during slow growth the time ($C$) needed for a round of deoxyribonucleic acid (DNA) replication, increases with the doubling time ($\tau$), so that $C = 2/3\,\tau$. Kubitschek and Freedman (21), on the other hand, concluded that in chemostat cultures the replication time and the time ($D$) between termination of DNA replication and cell division remain constant at slow growth. This would result in an increasing period devoid of DNA synthesis at the beginning of the cell division cycle.

Gudas and Pardee (10) compared *E. coli* strains B/r, K-12, 15, and 15T$^-$ after synchronization of the cells by sucrose gradient centrifugation. They failed to find a period devoid of DNA synthesis at the beginning of the cell cycle during slow growth, which suggested that $C$ is not constant. On the other hand, Chandler et al. (4) found evidence for a constant $C$ period in slow-growing *E. coli* K-12 cells. This is in accordance with the finding of Kubitschek (20), who, comparing batch-cultured cells of *E. coli* B/r and K-12 and *Salmonella typhimurium*, found the $D$ period to be, in all strains, independent of growth rate. This controversy has been ascribed to either the use of different cell lines of *E. coli* B/r (12) or the application of different methods of culturing or cell selection (4, 20). Recently, however, Helmstetter and Pierucci (16) have found significant differences in DNA replication patterns during slow growth of the substrains B/r ATCC 12407, B/r F 26, and B/r K, the strain used by Kubitschek.

In previous work (30), classification according to length of cells from an asynchronous population has been used to determine, during rapid growth of *E. coli* B/r H266, the time of nuclear separation and the duration of the process of visible cell constriction. In the present study this analysis has been applied to slow-growing populations of *E. coli* B/r ATCC 12407 and B/r K, to explore whether the biochemical differences found for the two substrains (16) are reflected in the morphological parameters of the cell cycle.

## MATERIALS AND METHODS

**Bacteria and culture conditions.** The organisms used were *E. coli* B/r A (ATCC 12407), obtained from C. E. Helmstetter, and *E. coli* B/r K, obtained from H. Kubitschek. Reference is made to *E. coli* B/r H266, which has been analyzed in previous work (30). All three strains proved to be B/r, as judged from the absence of filamentation in response to ultraviolet irradiation (tests kindly performed by H. J. W. Wijsman, Genetical Institute, University of Amsterdam). The bacteria were grown in the minimal salts medium of Helmstetter and Cooper (13) containing, in 1 liter of distilled water: NaCl, 3 g; $Na_2HPO_4$, 6 g; $KH_2PO_4$, 3 g; $NH_4Cl$, 2 g; and $MgSO_4$, 0.25 g. To obtain different doubling times ($\tau$), batch cultures were supplemented with the carbon sources summarized in Table 1. For each experiment, 100 to

TABLE 1. *Average doubling times (τ) of E. coli B/r A and K grown in different media*

| Growth medium | B/r A | | B/r K | |
|---|---|---|---|---|
| | τ (min) | SD[a] | τ (min) | SD[a] |
| Batch cultures | | | | |
| 0.1% glucose + 1% Casamino Acids | 25, 27 (2)[b] | | 27, 35 (2)[b] | |
| 0.1% glucose | 45 (2) | | 50, 54 (2) | |
| 0.04% L-alanine + 0.04% L-proline | 69 (8) | 7 | 104 (9) | 12 |
| 0.2% sodium succinate | 70, 90 (2)[c] | | 70, 80 (2)[c] | |
| 0.2% acetate | 105, 123 (2) | | 135, 140 (2) | |
| 0.04% L-alanine[d] | 122 (9) | 15 | 177 (8) | 19 |
| Chemostat cultures[c] | 175 | | 175 | |

[a] SD, Standard deviation.

[b] Number in parentheses indicates number of independent growth experiments in which a constant τ was obtained.

[c] In these experiments constancy of length distributions could not be obtained.

[d] In early experiments a concentration of 0.025% has been used, and doubling times up to 220 min have been obtained for both B/r A and K (cf. Table 3). In some of these experiments no constancy of length distributions could be found, but a steady state of growth was nevertheless obtained, as indicated by a constant mass/cell ratio.

150 ml of minimal medium was inoculated and incubated at 37°C for about 10 generations in 500-ml flasks, aerated by shaking vigorously in a water bath. The cell concentration was maintained below $10^8$ cells/ml by periodical dilution of the culture.

Growth was monitored by measuring the absorbance of the cultures at 450 nm with a Gilford microsample spectrophotometer, and in some experiments by measuring cell number with a Coulter counter model $Z_B$ equipped with a probe having an aperture diameter of 30 μm. The steady state of growth was verified by either obtaining a constant mass/cell ratio or obtaining constancy of length distributions in two consecutive samples. The two samples were considered to come from the same population if their cumulative length distributions (cf. Fig. 1B) appeared not to differ significantly after application of the Kolmogorov-Smirnov two-sample test (29), at a level of significance of $\alpha = 0.10$. In some cases the maximal difference ($D_{max}$) between the distributions and the critical difference ($D_{crit}$) with the corresponding value of α (see Table M in reference 29) are given (see Results).

**Exposure to chloramphenicol.** At a 450-nm absorbance of 0.1, the culture was divided, and chloramphenicol was added to part of it at a final concentration of 200 μg/ml. Increase of absorbance stopped immediately. As the cell number was found to increase for about 1 h, the extent of residual division was determined after treatment with chloramphenicol for at least 2 h.

**Agar filtration.** The original method introduced by Kellenberger and Arber (17) was modified so that small drops of bacterial suspensions could be filtered directly without spreading (see also reference 18). For this purpose the agar surface was covered with a holey film prepared according to the method of Fukami and Adachi (9) in the following way. Agar slants containing 30 ml of agar (2%) made up in distilled water were dried at 37°C to about 90% of their original weight. The slants were cooled at −15°C for 2 to 4 min, covered with 2 ml of a solution of 0.4% Parlodion (Mallinckrodt) in amyl acetate, which was poured off immediately, and placed upside down at an angle of about 30° in a chamber in which the relative humidity was kept at 50 to 60% (room temperature, 20°C). During evaporation of the amyl acetate, small water droplets condensed on the covered agar, precooled below the dew point, causing tiny holes in the Parlodion film. Size and number of the holes could be adjusted in conjunction with the time of cooling (cf. reference 9). Small drops of cells prefixed with 0.1% $OsO_4$, containing 0.002% sodium dodecyl sulfate to promote spreading, were placed on the filtering film and allowed to drain into the agar in an atmosphere of saturated water vapor. The optimal drainage time was about 30 min for 10-μl drops. After filtration, a small piece of agar below a drop was cut out, and the plastic film was floated off on distilled water and picked up from below with a grid covered with a thin Formvar film. Cell dimensions were measured from electron micrographs projected onto a transparent screen at a final magnification of 12,000. The minimal number of cells measured for determining length and width was 150 and 50, respectively.

**Critical-point drying.** Prefixed cells, from the same suspensions as used for agar filtration, were washed by centrifugation in distilled water and dehydrated by passing through increasing concentrations of ethyl alcohol (50, 75, 90, and 96%). After suspension in 100% ethyl alcohol (dried with molecular sieve, BDH), small drops of cells were placed on the plastic side of Formvar-carbon double films, whereupon the cells were allowed to settle down for 30 to 60 min. Subsequently, the grids were placed in wire baskets fitting the Polaron critical-point drying instrument. Processing through the critical-point cycle was done according to the method described by Bartlett and Burstyn (1; cf. their path no. 1), using carbon dioxide as transitional fluid.

**Prefixation and electron microscopy.** Prefixation

was carried out in the growth medium by adding $OsO_4$ (1%, wt/vol) to a final concentration of 0.1% and keeping the suspension at room temperature (20°C) for about 15 min. For thin sectioning, the prefixed cells were postfixed with 1% $OsO_4$ and processed further according to the method of Ryter and Kellenberger (26). Critical-point-dried cells were either observed in a Philips EM 300 operating at 80 kV or in a Cambridge M 2A scanning electron microscope.

## RESULTS

**Cell growth and cell shape.** To determine the change in cell shape as a function of growth rate, length and diameter distributions were made of *E. coli* B/r A and K, cultured in different media. In most media *E. coli* B/r A grew faster than B/r K, which made it difficult to compare the two substrains at the same growth rate (Table 1). Two distributions from alanine-grown cells are shown in Fig. 1, and the parameters of these and other distributions are summarized in Table 2. Although the range of cell lengths differs considerably between the two substrains (Fig. 1A), the cumulative plots in Fig. 1B show that the shape of the two distributions is very similar. Distributions obtained from *E. coli* B/r A and K cells cultured with similar doubling times in, respectively, alanine

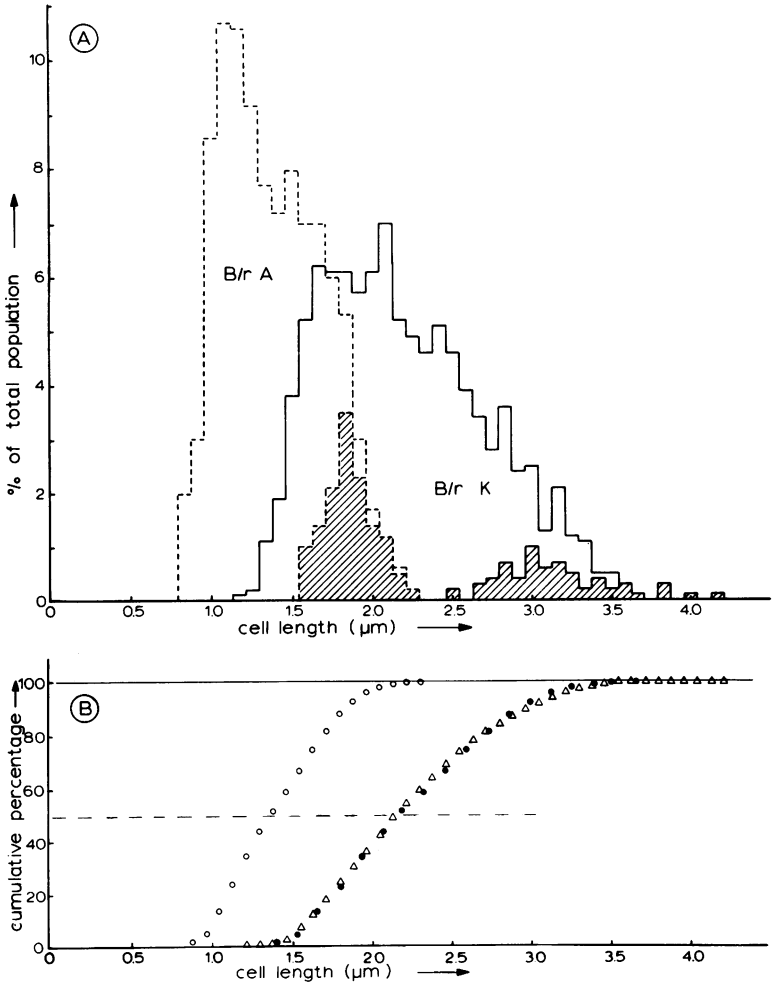


FIG. 1. *Length distributions of E. coli B/r A and K (τ = 160 and 180 min, respectively) prepared by agar filtration. (A) The two populations differ considerably as to length range and percentage of constricted cells (hatched area; see also Table 2). (B) Cumulative plot of the two distributions. Multiplication of the length classes of E. coli B/r A by 1.59, the ratio of the average cell lengths in the two populations, shows that the length distributions of the two substrains have a similar shape. Symbols:* (○) *B/r A;* (●) *B/r A* × *1.59;* (△) *B/r K.*

TABLE 2. *Parameters of length distributions obtained from batch- and chemostat-cultured cells, prepared by agar filtration*

| Culture | $\tau$ (min) | $\bar{L}$ ($\mu$m) | CV[a] (%) | $L_0$[b] ($\mu$m) | Cells showing constriction | | | Cell width ($\mu$m) | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | | % | $\bar{L}_c$[c] ($\mu$m) | CV (%) | 2$R$ ($\mu$m) | SD[d] ($\mu$m) |
| B/r A | | | | | | | | | |
| Batch[e] | 160 | 1.4 (1,025)[f] | 23 | 1.0 | 14 | 1.9 | 8 | 0.65 | 0.04 (144)[f] |
| Batch[g] | 126 | 1.6 (632) | 24 | 1.1 | 14 | 2.2 | 8 | 0.64 | 0.03 (69) |
| Chemostat | 175 | 1.5 (974) | 23 | 1.1 | 9 | 2.1 | 8 | 0.56 | 0.04 (112) |
| B/r K | | | | | | | | | |
| Batch[e, g] | 180 | 2.2 (1,140) | 24 | 1.6 | 6 | 3.1 | 11 | 0.46 | 0.04 (119) |
| Chemostat | 175 | 2.4 (955) | 25 | 1.6 | 8 | 3.2 | 15 | 0.52 | 0.04 (127) |

[a] CV, Coefficient of variation.
[b] The length of newborn cells ($L_0$) was estimated from the distributions using the formula (11): $L_0 = {}^1/_2 (L_{min} + {}^1/_2 L_{max})$, where $L_{min}$ and $L_{max}$ are, respectively, the minimal and maximal cell lengths observed.
[c] $\bar{L}_c$, the average length of cells showing constriction, is usually somewhat smaller than $2 \times L_0$.
[d] SD, Standard deviation.
[e] See Fig. 1 for length distributions.
[f] The number in parentheses indicates the number of cells measured.
[g] Cultures used for analysis of nuclear separation in critical-point-dried cells (cf. Table 4).

medium ($\tau$ = 109 min) and alanine-proline medium ($\tau$ = 100 min) also appeared to have the same shape, as indicated by the Smirnov-Kolmogorov test.

In spite of this similarity of distributions, the B/r K populations were always found to contain more very short and very long cells, as reflected in Table 2 by the larger coefficient of variation of cells showing constriction. A similar difference between the two strains was also found by Helmstetter and Pierucci (16), who showed that in *E. coli* B/r K the variation in the timing of initiation of DNA replication between individual cells and the variation in cell ages during membrane elution are larger than in B/r A.

In Fig. 2A and B the lengths of newborn cells ($L_0$), and the average cell width (2$R$) of *E. coli* B/r A and K are presented as a function of growth rate. During rapid growth the two substrains have about the same shape (Fig. 2C), i.e., a shape factor, $a$ ($a = L_0/2R$; 32), of 2.3. At slower growth rates the $L_0$ of B/r A cells decreases far more than the $L_0$ of B/r K (Fig. 2A), whereas the average width of B/r A decreases less than that of B/r K (Fig. 2B). As a result, at slow growth, B/r A cells become almost round ($a$ = 1.5), whereas B/r K changes gradually into a thin rod ($a$ = 3.2). These shape differences are shown in the two agar filter preparations of Fig. 4A and B.

Because results have been reported (21) on *E. coli* B/r K cells cultured in a chemostat, cells grown under conditions of glucose restriction ($\tau$ = 175 min; see Table 1) were compared with batch-cultured cells. In chemostat cultures it has been observed that cell properties change less with growth rate than in batch cultures (27) and that cell volume depends on the nature of the limiting nutrient (28). Deviating cell shapes could therefore be expected. However, Fig. 2 (double-circle symbols) and Table 2 show that chemostat cells of the two substrains have the same cell shapes as batch-cultured cells.

Donachie et al. (7) recently suggested the existence of a minimum unit cell with a length at birth ($L_0$) of 1.4 $\mu$m at zero growth rate, which applies both to an *E. coli* K-12 strain and to *E. coli* B/r A. By contrast, our data (Fig. 2A) show this theoretical minimum length to be 0.8 $\mu$m for *E. coli* B/r A and 1.5 $\mu$m for B/r K. The latter value is close to the 1.47 $\mu$m obtained for *E. coli* B/r H266 (N. B. Grover, C. L. Woldringh, A. Zaritsky, and R. F. Rosenberger, J. Theor. Biol., in press), another substrain that closely resembles B/r K in both dimensions and cell shape.

Although our measurements have been performed on cells prepared by agar filtration, i.e., cells that have been flattened during air-drying (Fig. 4C), unpublished observations show that the width and average cell length of the agar-filtered and dried cells equal those of the living cell observed by light microscopy (measurements performed with a measuring magnifier on photographs at a magnification of ×4,000). Therefore, cell volume can be estimated from the data in Fig. 2, assuming the cells to be cylinders. This shows that the volume of the newborn cell is similar for the two substrains over the range of growth rates measured.

***T* period.** From the percentage of cells showing constriction in the agar filter preparations,

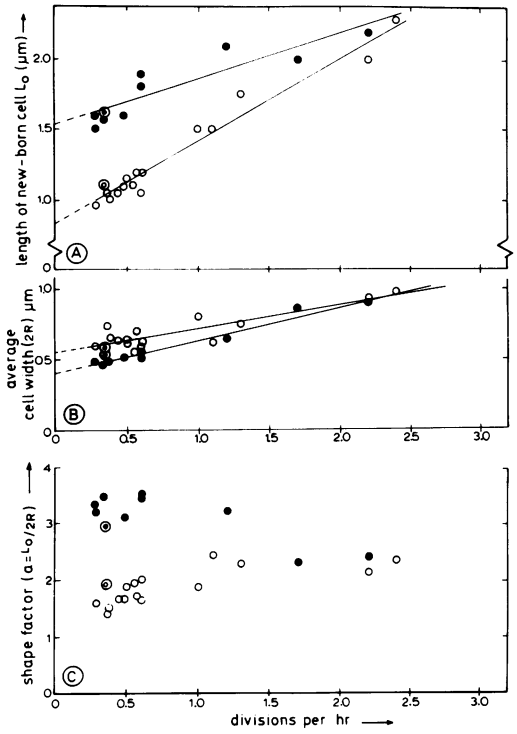


FIG. 2. *Cell length (A), cell width (B), and cell shape (C) of E. coli B/r A (○) and K (●) as a function of growth rate. The lengths of newborn cells were calculated as indicated in the footnotes to Table 2. Double-circle symbols represent chemostat cultures. Solid lines represent linear least-squares fit (r > 0.87).*

the duration of the process of cell constriction ($T$ period) can be calculated (24, 30). In Fig. 3 the $T$ period is plotted as a function of the growth rate. During rapid growth ($\tau < 60$ min) the $T$ period is almost a constant at about 10 min. In previous work a similar result was obtained with another substrain, *E. coli* B/r H266 (30). At slow growth the $T$ period slightly increases in B/r K: from 10 to 12 min to 15 to 17 min. However, in *E. coli* B/r A, $T$ increases drastically to almost 40 min (Fig. 3).

**$D$ period.** If the time $D$ between termination of DNA replication and cell division would remain constant at slow growth (4, 20), and the $T$ period were to increase as is the case for *E. coli* B/r A at slow growth (Fig. 3), cell constriction would be initiated long before termination of DNA replication. To investigate this possibility, the $D$ period was determined in both substrains by the method of residual division after exposure of the cells to chloramphenicol as described by Kubitschek (20).

In Table 3 the observed values of the $D$ period (column 4) and the corresponding $T$ period (column 3) are presented. It can be seen that, with one exception, $D$ is equal to $T$, or larger than $T$. The $D$ values found for B/r A do not amount to the value of one-third of a doubling time, as found by Helmstetter et al. (14, 16) for this

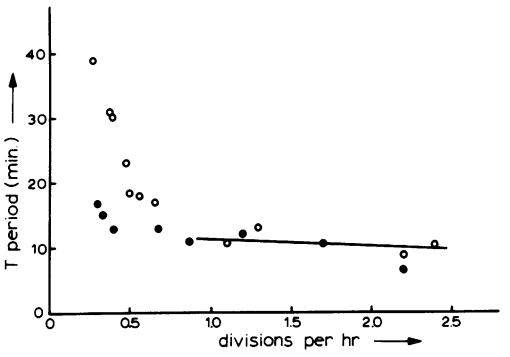


FIG. 3. *Duration of the process of visible cell constriction (T period) in E. coli B/r A (○) and K (●), grown at different growth rates. During rapid growth the T period is the same for both substrains and almost constant. At slow growth the T period increases considerably in B/r A, in contrast to a slight increase in B/r K.*

TABLE 3. *Comparison of the T and D periods in E. coli B/r A and K*

| Substrain | $\tau$ (min) | % Cells showing constriction | $T^a$ (min) | $D^b$ (min) | % Constricted cells after chloramphenicol | $D^c$ corrected[c] (min) |
|---|---|---|---|---|---|---|
| B/r A | 117 | 14 (450)[d] | 23 | 38 | 1 (500)[d] | 39 |
| | 120 | 10 (819) | 18 | 30 | 2 (449) | 33 |
| | 166 | 14 (391) | 30 | 22 | 5 (264) | 34 |
| | 220 | 13 (681) | 36 | 42 | 4 (931) | 53 |
| B/r K | 150 | 6 (634) | 13 | 16 | 2 (396) | 21 |
| | 200 | 6 (749) | 17 | 17 | 1 (227) | 20 |

[a] $T$, the duration of the process of visible cell constriction, was calculated from the percentage of cells showing constriction by: $T \ln 2 = \tau \ln (1 + N_c/N)$, $N_c$ being the number of constricted cells and $N$ the total number of cells.

[b] $D$, the time between termination of DNA replication and cell division, was calculated from the percentage of cells capable of completing division in the presence of chloramphenicol by: $D \ln 2 = \tau \ln (1 + N_{CAM}/N_0)$, $N_{CAM}$ being the number of cells giving residual division and $N_0$ the initial number of cells.

[c] $D$ corrected was calculated by increasing the percentage of cells giving residual division (see footnote *b*) by the percentage of constricted cells in the chloramphenicol-treated culture as observed by agar filtration.

[d] Number in parentheses indicates number of cells counted.

strain during slow growth. Also, the *D* found for B/r K proved to be somewhat lower than the value given for this strain by Kubitschek (20).

In our experiments cell numbers were found to increase until about 60 min after addition of chloramphenicol. Although this inhibitor has not been found to cause significant retardation of DNA replication in *E. coli* B/r (23), chloramphenicol can be expected to decrease the rate of the cell constriction process. Therefore, agar filter preparations were made after 2 h of exposure to chloramphenicol. To our surprise these preparations still contained cells showing constriction (Table 3). Presumably, these cells having terminated DNA replication at the time of addition of the inhibitor could initiate cell constriction but were unable to complete cell division (see Discussion). If these cells had divided, the extent of residual division, and thus the value of the *D* period, would have been higher. Correction for these cells has been carried out (Table 3, last column). From the corrected *D* values it became clear that in all cases, and in both strains, the *D* period is longer than the *T* period, i.e., that termination of DNA replication occurs well ahead of initiation of cell constriction.

**Timing of nucleoplasmic separation.** After establishing different *D* periods for *E. coli* B/r A and K during slow growth, we wanted to find out whether the time of nucleoplasmic separation would also differ in the two substrains. In a previous analysis on serially sectioned cells of *E. coli* B/r H266, it was found that, contrary to the situation in rapidly growing cells, nucleoplasmic separation in slowly growing cells does not coincide with the termination of DNA replication (30). Although the nucleoplasm is visible in the flattened cells obtained after agar filtration (3, 18), the resolution was not sufficient to permit analysis of nuclear separation (Fig. 4A and B). To avoid the laborious task of making serial sections, the analysis was performed on critical-point-dried cells. We found that the nucleoplasm could be visualized with the same accuracy as in serially sectioned cells (unpublished observations; see Fig. 4D and E).

To compare the dimensions of critical-point-dried cells (Fig. 4E and F) with those of cells prepared by agar filtration, cell lengths have been corrected for the considerable shrinkage that appeared to occur during dehydration. As compared with the dimensions of the agar-filtered cell, the average critical-point-dried cell has shrunk to 74% of its length in the case of *E. coli* B/r A and to 63% in the case of B/r K. A similar reduction holds true for embedded cells (Fig. 4G and H), as has been emphasized by Fuhs (8).

After correction for shrinkage, the length distribution obtained from critical-point-dried *E. coli* B/r A cells did not differ significantly from the distribution obtained after agar filtration ($D_{max}$ = 8%; $D_{crit}$ = 12.7%, for $\alpha$ = 0.10). For *E. coli* B/r K cells a significant difference was obtained for the distributions of the total population, probably because of greater interference of tilted cells measured in the critical-point-dried preparation (cf. Fig. 4D). However, no significant difference was found for the distributions of constricting cells ($D_{max}$ = 25%; $D_{crit}$ = 30.9%, for $\alpha$ = 0.10). Consequently, in both substrains cells showing constriction in the critical-point-dried preparations can be considered to represent a reliable sample of the total population as measured after agar filtration. Because in both *E. coli* B/r A and K every cell found to contain two separate nucleoplasms also appeared already to have initiated cell constriction, the period NS, during which the average cell contains two nucleoplasms, could be calculated as a fraction of the *T* period from the percentage of constricted cells showing nuclear separation. The results in Table 4 show that, in spite of the long *D* period found in *E. coli* B/r A, nuclear separation occurs even later in the cell cycle than in B/r K.

## DISCUSSION

***T* and *D* periods.** At doubling times exceeding 60 min, *E. coli* B/r A and K have been shown to adopt widely different cell shapes (Fig. 2 and 4). Whereas B/r K cells become thinner and remain relatively long, B/r A cells become shorter and their width remains relatively large. At the same time, the *T* period increases in both strains with decreasing growth rate, but more so in B/r A than in B/r K. From these observations it can be deduced that the duration of the cell constriction process depends on both growth rate and the width of the cell. It can be envisaged that during rapid growth, increase in width and growth rate compensate each other, resulting in an almost constant *T* period (cf. reference 30). With decreasing growth rate the width of B/r A cells remains relatively large, which might explain the observed lengthening of the *T* period (Fig. 3).

In addition to the *T* period, the *D* period, as determined from residual division, was also found to differ during slow growth for the two strains (Table 3). This result corresponds with the observations of Helmstetter and Pierucci (16), who determined the *C* and *D* periods in *E. coli* B/r A, F-26, and K after slow growth ($\tau$ = 100 min) in sodium acetate medium. That in our experiments *D* was always found to exceed

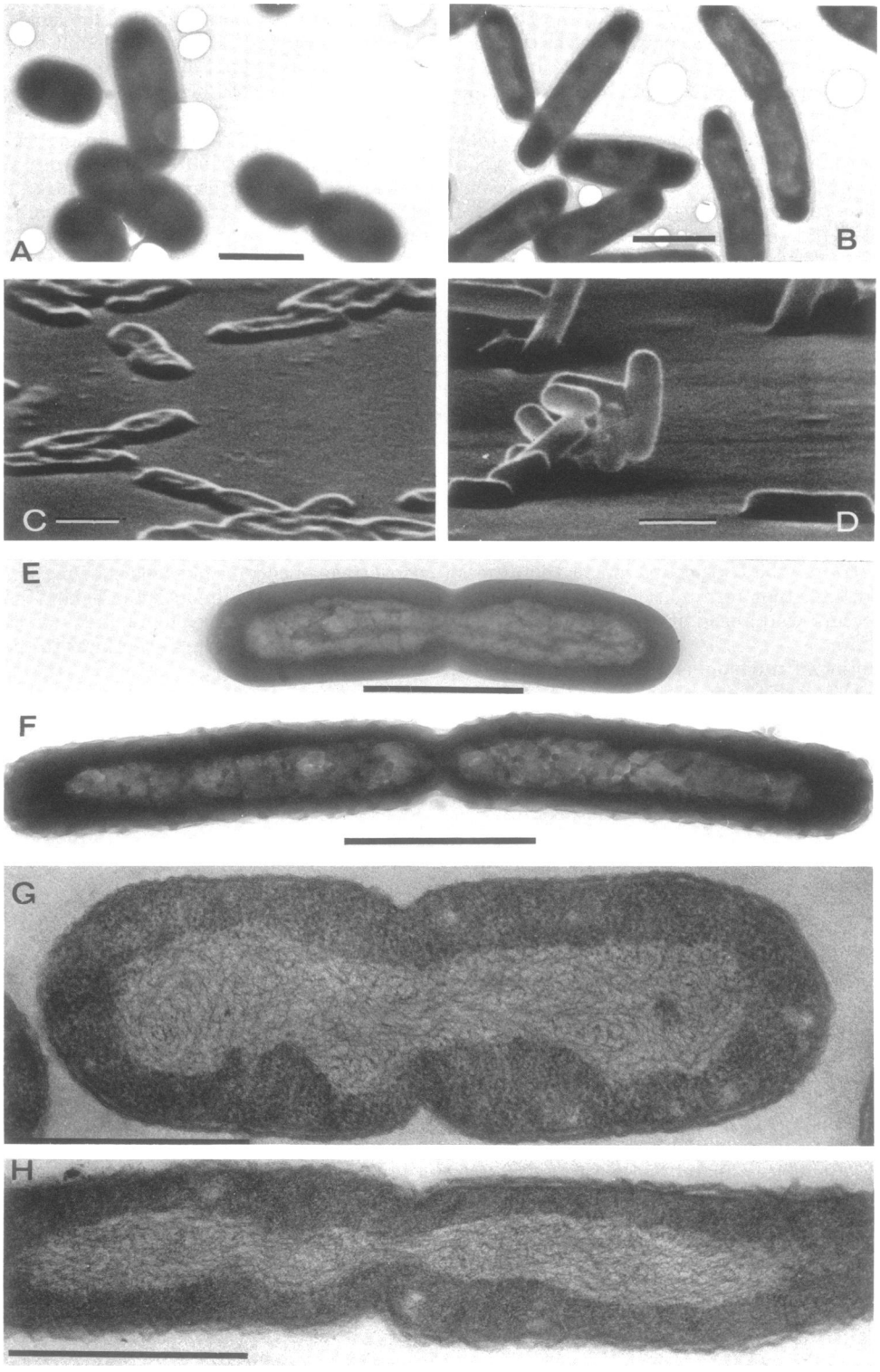


FIG. 4.

TABLE 4. *Period (NS) during which the average cell contains two separated nucleoplasms as determined after critical-point drying*

| Substrain | $\tau$ (min) | $T$ period[a] (min) | Constricted cells after critical-point drying | | NS[d] (min) |
|---|---|---|---|---|---|
| | | | No. of cells measured ($N_c$)[b] | % Cells showing nuclear separation ($N_{ns}/N_c$)[c] | |
| B/r A | 126 | 23 | 29 | 31 | 7 |
| B/r K | 180 | 15 | 20 | 65 | 10 |

[a] The $T$ period was derived from the population of agar-filtered cells. See footnotes to Table 3.

[b] The number of constricted cells ($N_c$) measured in the critical-point-dried preparation can be considered a reliable sample of the total population (see text) and corresponds, therefore, to the given value of the $T$ period.

[c] All cells showing nuclear separation belonged to the population of constricted cells.

[d] $NS = T N_{ns}/N_c$.

$T$ fits into the concept that the onset of the cell division process is dependent on DNA termination (13, 15).

At variance with the results of Helmstetter and Pierucci (16) is our finding that the volume of the newborn cell of B/r A is similar to that of B/r K at all growth rates. A similar volume was also found for newborn cells of *E. coli* B/r F26 and B/r H266 (unpublished observations). These results imply that cell volume at initiation may be different in the various substrains.

**Nucleoplasmic separation.** The time of nucleoplasmic separation did not appear to be related to completion of DNA replication (Table 4). In a previous study using slowly growing *E. coli* B/r H266, nuclear division likewise occurred after termination. The two events coincided, however, during rapid growth (30). The present results obtained with slowly growing *E. coli* B/r A and K confirm the short time of nucleoplasmic separation (NS in Table 4) and indicate that nuclear separation is not essential for initiation of the cell constriction process. These data are in accordance with the finding of Chai and Lark (3) that during slow growth of *E. coli* 15T$^-$ cells DNA segregation occurs late in the division cycle.

Because DNA concentration increases with decreasing growth rate (6, 25), the volume of the nucleoplasm will increase relative to cell volume (see Fig. 2 in reference 30). One can envisage, therefore, that during slow growth space for nucleoplasmic separation and movement is only available at the end of the division cycle.

In addition, the geometry of the cell may play a role. In rounded cells like B/r A there is less space for the nucleoplasm to move in the long axis of the cell than there is in the more filamentous B/r K cells (see Fig. 4). Perhaps this is the reason why nuclear separation occurs later in the cycle of B/r A than in that of B/r K cells (Table 4). Moreover, the dependency of nuclear separation on cell growth could explain why not all cells in the $D$ period were capable of completing cell division after inhibition of protein synthesis (Table 3). In cells with one continuous nucleoplasm the constriction process can very well be initiated, but the yet unseparated mass of DNA could inhibit completion of the constriction.

The presence of an unseparated nucleoplasm in cells that have already initiated cell constriction (Fig. 4E and H) is difficult to reconcile with the suggestion of Burdett and Murray that a cell septum is formed before initiation of cell constriction (2). Although in thin sections of rapidly growing *E. coli* B/r A cells the plasma membrane could sometimes be found to invaginate ahead of the outer cell wall layer (22), no such structures could be observed in the slowly growing cells described here (Fig. 4G and H).

**Concluding remarks.** The different shapes of *E. coli* B/r A and K and the differences in the duration of the respective $C$ and $D$ periods suggest a relationship between shape and DNA replication. Such a relation has been described

FIG. 4. *E. coli B/r A and K cells prepared by different techniques. (A and B) B/r A and K cells, respectively, grown with a doubling time of 160 and 200 min, prepared by agar filtration. (C) Scanning electron micrograph (SEM) of an agar-filtered preparation of E. coli B/r A ($\tau$ = 126 min) tilted at 70°. It is clearly seen how the cells have become flattened during air-drying. (D) SEM picture (tilt, 70°) of critical-point-dried cells of the same preparation as in (C). The cells have retained their three-dimensional shape but have shrunk considerably as a result of dehydration in alcohol. (E) Transmission electron micrograph of a critical-point-dried B/r A cell ($\tau$ = 126 min). Within the transparent cell the nucleoplasm is clearly visible. As the cells were only fixed with 0.1% $OsO_4$, the DNA coagulated into coarse fibers (cf. reference 31). (F) E. coli B/r K ($\tau$ = 180 min) prepared as in (E). (G and H) Thin sections of B/r A ($\tau$ = 90 min) and B/r K ($\tau$ = 70 min), showing the unseparated nucleoplasm in cells that have already initiated constriction. Bars = 1 μm for A through D and 0.5 μm for E through H.*

by Zaritsky and Pritchard (32, 33) in a model in which cell length and cell width are proportional to, respectively, $2^{D/\tau}$ and $2^{C/2\tau}$. A consequence of the model is that cell shape will remain constant as long as $C = 2D$ (32). Because this relationship has been shown to hold during slow growth for both *E. coli* B/r A and K (16), the shape changes estimated from the data in Fig. 2 cannot be explained by the model of Zaritsky and Pritchard (32, 33).

The observed similarity in the shape of length distributions may indicate, according to Koch (19), similar kinetics of cell length extension. However, although the length distributions of *E. coli* B/r A and K show no marked difference (Fig. 1), according to recent analyses (cf. reference 7; Grover et al., J. Theor. Biol., in press) the two lines relating length at birth with growth rate (Fig. 2A) suggest that elongation may proceed differently in the two substrains.

In a search for general rules governing bacterial growth, strain differences with respect to parameters of the division cycle have not often been emphasized (cf. reference 16). However it is hoped that further analysis of the different patterns of elongation and of macromolecular synthesis will throw some light on the possible coupling between chromosome replication and cell growth.

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