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Sensitivity of *Escherichia coli* to Cephaloridine at Different Growth Rates

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Steady-state populations of *Escherichia coli* B/r were treated with cephaloridine at minimal inhibitory concentrations. The antibiotic sensitivity of the cells and the localization of spheroplast emergence along the cell surface were examined as a function of cell length and growth rate. In fast-growing populations (>1 division per h) the sites of cephaloridine interaction occurred preferentially at the cell pole in the smaller cells and at the cell center in dividing cells. At decreasing growth rates the cells became more resistant to cephaloridine, and a gradual shift from the cell pole toward the cell center was observed for the sphere position. A similar growth rate-dependent change in localization was found for sucrose-induced plasmolysis vacuoles.

β-Lactam antibiotics interact with a group of inner membrane proteins which may play a distinct role in the elongation, division, and shape maintenance of *Escherichia coli* cells (8). Depending on the exact function of these proteins and their binding properties, the above processes may be differently affected according to the nature and also the concentration of the antibiotic used (2, 9, 11). At low concentrations (16 to 50 IU/ml) penicillin only inhibits cell division, whereas at high concentrations (500 IU/ml) it induces cell lysis by inhibition of cell elongation and division (2, 6). Before lysis of a rod-shaped cell, a small sphere emerges at the cell surface by extrusion of the protoplast through the damaged peptidoglycan layer (10). Donachie and Begg (2) found that in penicillin-treated *E. coli* cells this extrusion occurred preferentially at sites of active cell division or at potential division sites. Moreover, the position of these preferential sites along the cell surface was dependent on cell length and was observed to shift during the division cycle from the youngest cell pole to the cell center while it remained at a distance of one "unit cell" length from the oldest cell pole (2). More recently, these observations were extended by Staugaard et al. (10) by electron microscopic examination of *E. coli* B/r cells treated with lytic concentrations of ampicillin (500 μg/ml). Although in newborn cells spheroplast emergence occurred preferentially at the cell pole, a shift in preferential sphere localization toward the cell center already became prominent early in the division cycle. During this transition no preferential sphere position could be detected (10). Therefore, these authors did not support the model of envelope growth described by Donachie and Begg (2) but came closer to agreement with the growth pattern derived from autoradiographic studies after the pulse labeling of *E. coli* murein sacculi with [^{3}H]diaminopimelic acid (5, 7).

Since both penicillin and ampicillin preferentially inhibit cell division (2, 6, 8), we took advantage of several reports on the high sensitivity of the cell elongation process to cephaloridine (8, 9, 11) to detect and localize elongation sites in the cell envelope. Steady-state populations of *E. coli* B/r were treated with cephaloridine at minimal inhibitory concentrations. This allowed a screening for fluctuations in the antibiotic sensitivity among the cells of a population according to their length or relative position in the cell cycle (12). In addition, antibiotic sensitivity and sphere localization were compared in populations grown at different rates (0.45 to 2.31 divisions per h) to detect possible variations in the pattern of envelope growth.

When an exponentially growing population of *E. coli* B/r was exposed to cephaloridine at its lowest effective concentration, cell growth became progressively inhibited (Fig. 1A). Exponential growth continued over approximately one doubling time before sphere formation could be detected. Further incubation in the presence of the antibiotic resulted in cell lysis which could not be adequately suppressed by lowering the pH of the growth medium (Fig. 1B) (3). Since these conditions allowed a distinction between the bacteriostatic and bactericidal effects of the antibiotic interaction, an examination of the relative susceptibility of the cells within a popula-

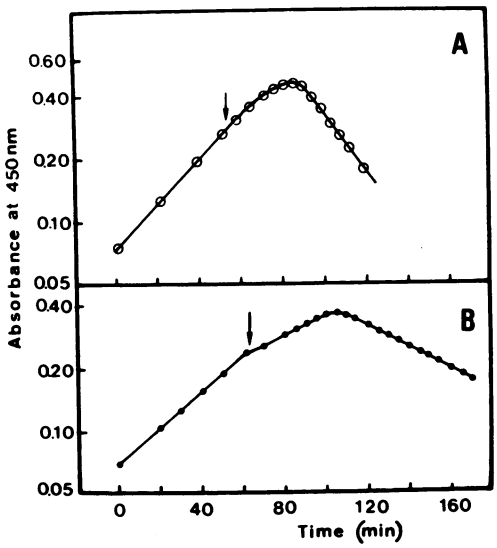

FIG. 1. *Inhibitory effect of cephaloridine on steady-state populations of E. coli B/r H266. Cells were grown in M9 mineral salts medium supplemented with 0.2% (wt/vol) glucose and 0.4% (wt/vol) Casamino Acids. At the time indicated by the arrow cephaloridine was added at a final concentration of 3 μg/ml. (A) Growth medium at pH 7.0. (B) Growth medium buffered at pH 5.0 with 0.1 M Tris and 0.01 M citric acid.*

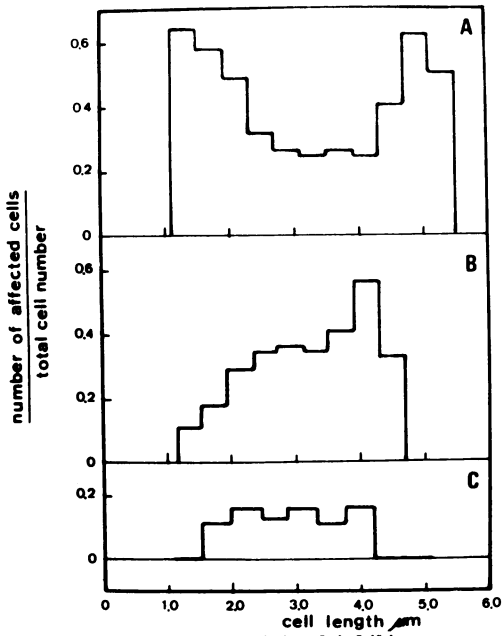

FIG. 2. *Sensitivity to minimal inhibitory concentrations of cephaloridine as a function of cell size for steady-state populations of E. coli B/r grown at different rates. The sensitivity was recorded by measuring the fraction of cells which revealed spheroplast emergence for the different classes of cell length as indicated. (A) E. coli B/r H266 grown in M9 medium supplemented with 0.2% glucose. The growth rate was 1.22 divisions per h. Cephaloridine was added at final concentration of 3 μg/ml. Sphere formation was examined by electron microscopy on cells prepared by agar filtration (10). At 60 min after the addition of cephaloridine, cells were fixed for 15 min in 0.1% OsO_4; the sampling time corresponded to the time when spheres were first observed in control samples examined by phase-contrast microscopy. The total number of cells observed was 2,260. Length measurements were calibrated with a line replica (1,152 lines per mm). (B) E. coli B/r K (kindly provided by C. Helmstetter) was grown in M9 medium supplemented with 0.2% alanine and 0.2% proline. The growth rate was 0.68 divisions per h. The B/r K strain, closely resembling B/r H266, was used since for this strain the exact position of the DNA replication cycle at various growth rates was known. Sphere formation was examined by phase-contrast microphotography. At 35 min after cephaloridine addition, samples of the population were rapidly scanned without any fixation to register the early effect of cephaloridine added at a final concentration of 10 μg/ml. The total number of cells observed was 1,285. Length measurements were calibrated with latex beads (diameter, 1.87 μm). (C) E. coli B/r K grown in M9 medium supplemented with 0.2% sodium acetate. The growth rate was 0.45 divisions per h. Cephaloridine was added at a final concentration of 5 μg/ml. The measurements were carried out as indicated for the alanine-proline populations in (B). The samples were taken at 70 min after the addition of cephaloridine. The total number of cells observed was 818.*

tion could be carried out. During the bacteriostatic phase several populations, grown at different rates, were screened for early detection of spheres arising at the cell surface. As Fig. 2 shows, the sensitivity to cephaloridine within a population was dependent on cell length and varied between cell populations grown at different rates. In fast-growing populations (>1 division per h) spheres were detected in all size classes but occurred more frequently among the smallest and largest cells of the population. Electron microscopic examination showed that the latter group consisted almost exclusively of dividing cells (Fig. 3). At lower growth rates (<1 division per h) the sensitivity to cephaloridine as a function of cell size was remarkably different (Fig. 2B and C). In a population of cells grown in the presence of alanine and proline, the sensitivity increased gradually with increasing cell length. When cells were grown in an acetate-supplemented medium, a marked decrease in sensitivity was observed at all cell lengths, and no spheres could be detected on cells of the smallest and largest size classes (Fig. 2C).

To detect any preferential sites of sphere formation along the cell surface, sphere position was measured as the distance from its center to the nearest cell pole and the cells were again classified by length. As expected from the data

of Fig. 2A, a clustering of spheres became apparent among the most sensitive cells of a fast-growing population (Fig. 3). Although for a given cell length the spheres could occur at numerous sites on the cell surface, in small cells spheres were preferentially found at the cell pole, whereas in larger (dividing) cells spheroplast emergence was more frequently observed at the cell center (Fig. 3). The same pattern of sphere localization was found for *E. coli* B/r H266 populations grown at 2.31 divisions per h (data not shown). At lower growth rates the clusters of spheres at the cell pole and cell center disappeared (Fig. 4A and B). In populations grown in the presence of alanine and proline, a less distinct clustering in sphere localization became apparent in cells of intermediate length and was spread around one-quarter of the cell length (Fig. 4A). In acetate-grown cells no significant clustering of sphere localization around preferential sites could be observed. Nevertheless, the spheres were not evenly distributed over the cell surface. The majority (77%) of the spheres were localized between the cell center and one-quarter of the cell length (Fig. 4B).

In addition to cephaloridine treatment, the weakest areas in the envelope of exponentially growing cells were also visualized by induction of plasmolysis vacuoles with sucrose. Similar to the position of antibiotic-induced spheres, the localization of the plasmolysis vacuoles was also dependent on growth rate (Table 1). In fast-growing cells they occurred predominantly at the cell poles. At decreasing growth rates the polar localization was strongly reduced, and vacuoles were preferentially found over the surface area extending from the cell center to one-quarter of the cell length.

Our data clearly indicate that the sensitivity of exponentially growing cells to cephaloridine depends on the growth rate (Fig. 2). Similarly the localization pattern of spheroplast emer-

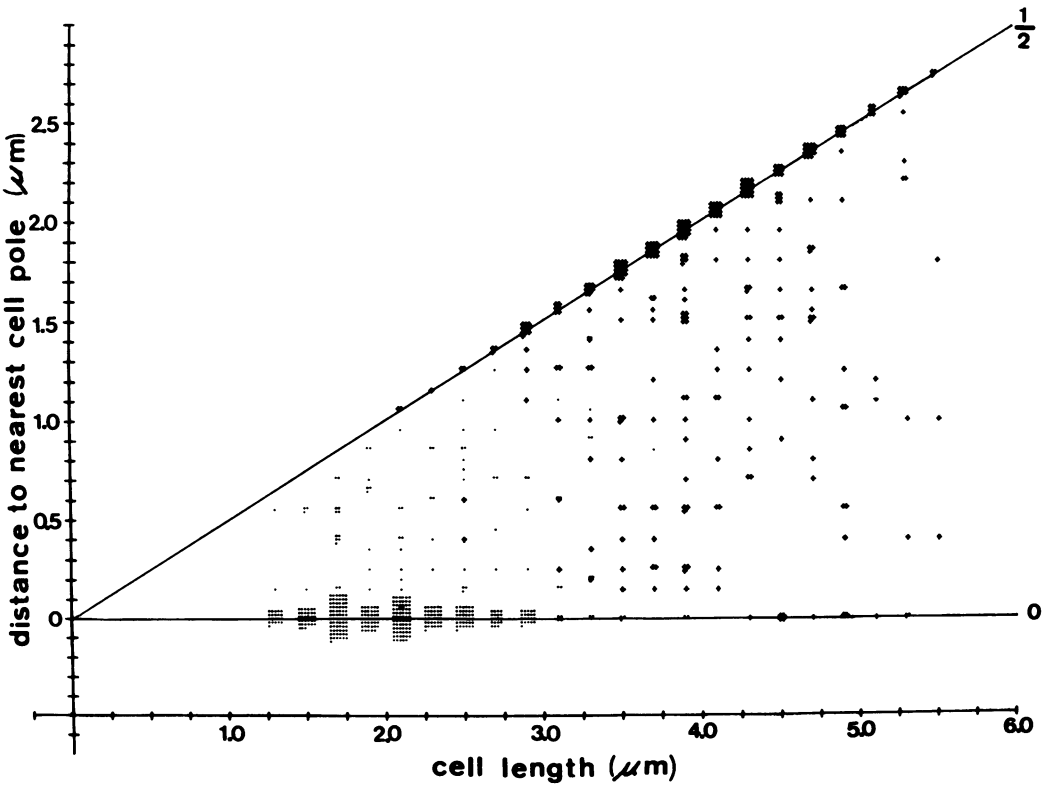

FIG. 3. *Sphere localization in the population of E. coli B/r H266 described in the legend to Fig. 2A. Sphere position was measured as the distance between the center of the sphere and the nearest cell pole. The line marked 0 indicates the position of the cell poles, whereas the line marked ½ indicates the position of the cell center for the cells of the various length classes. The total number of spheres measured was 774 and nearly equaled the number of affected cells since only occasionally was more than one sphere observed per cell. Symbols: ●, nondividing cells; +, dividing cells. The length measurements were calibrated by a line replica (1,152 lines per mm).*

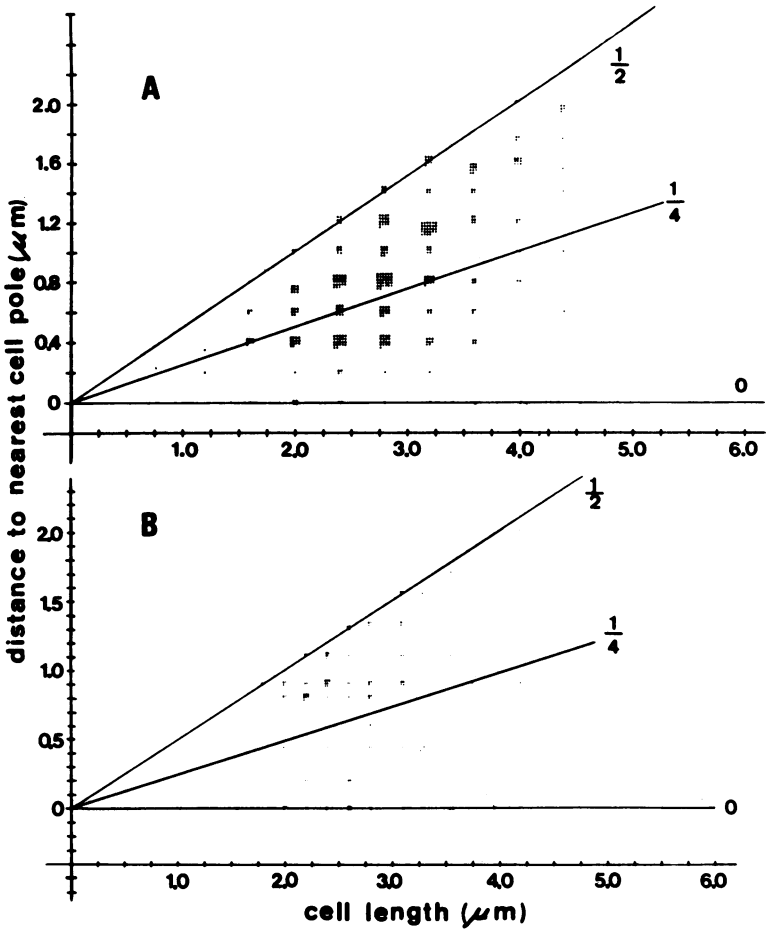

FIG. 4. *Sphere localization in populations of E. coli B/r K. (A) and (B) refer to the populations described in Fig. 2B and C, respectively. Since phase-contrast microscopy did not allow an accurate detection of cell division, no distinction between dividing and nondividing cells was made. The line marked ¼ indicates the position of one-quarter of the total cell length for cells of the various length classes. The total number of spheres measured was 396 (A) or 110 (B). The length measurements were calibrated by latex spheres (diameter, 1.87 μm).*

gence induced by cephaloridine changes with the growth rate of the cells (Fig. 3 and 4). This may have influenced the results of Donachie and Begg (2), who examined sphere localization on a mixture of cells derived from populations grown at different rates.

Nevertheless, preferential sites of sphere formation do exist. In fast-growing populations the cells at both ends of the length distribution were more sensitive to cephaloridine (Fig. 2A), and a clustering of spheres was observed at the cell pole and cell center (Fig. 3). Similar clusters have been described by Staugaard et al. for glycerol-grown populations of *E. coli* B/r (10). Since their populations were enriched in the smallest and largest cells by a synchronization procedure, no quantitative comparison with our data can be made. According to these authors, the clustering of spheres at the cell pole might be due to a retention by young cells of properties related to the previous cell division, whereas preferential sphere formation at the cell center could indicate an early recognition of the next division site (10). However, our electron microscopic observations on cephaloridine-treated cells revealed that the cluster of spheres at the cell center was exclusively related to dividing cells (Fig. 3). This suggests that cephaloridine, even at its lowest effective concentration, may still have a high affinity for sites of active cell division (Fig. 2A and B), although a cluster of spheres at the cell center was not observed for cells grown in the presence of alanine and proline (Fig. 4A). It is likely that the preferential sites

TABLE 1. *Localization of sucrose-induced plasmolysis vacuoles in E. coli B/r H266 at different growth rates*

Growth rate (divisions per h)[a]	Localization of vacuoles (% of total)[b]			No. of observations
	Polar	Area A	Area B	
1.8	52.41	69.58	30.42	332
1.03	3.50	26.10	73.90	656
0.65	4.80	21.83	77.10	417

[a] *E. coli* B/r H266 was grown in M9 mineral salts medium supplemented with 0.2% (wt/vol) glucose and 0.4% (wt/vol) Casamino Acids (1.8 divisions per h), 0.2% (wt/vol) glycerol (1.03 divisions per h), or 0.2% (wt/vol) alanine and 0.2% (wt/vol) proline (0.65 divisions per h). Plasmolysis was induced by 30% (wt/vol) sucrose at an absorbance of 0.25 at 450 nm. The cells were immediately fixed with 1% (final concentration) formaldehyde, and the distance between the plasmolysis vacuole and the nearest cell pole was measured on phase-contrast micrographs.

[b] Area A corresponds to the cell surface area extending from either cell pole to one-quarter of the cell length. Area B refers to the cell surface area extending from the cell center to one-quarter of the cell length. Length measurements were calibrated with latex beads (diameter, 1.87 μm). The percentage of vacuoles in area A includes the vacuoles at the cell pole. Similarly, the percentage in area B includes the vacuoles localized at the cell center.

of sphere formation at the cell pole arise from an enhanced rate of murein synthesis rather than from the detection of a potential division site (2) or from the retention of division properties by newborn cells (10). This conclusion seems to be justified by the observed variation in cephaloridine sensitivity as a function of cell length (Fig. 2A) and by the marked increase in transpeptidase activity which occurs shortly after cell division, as recently reported by Mirelman et al. (4) for synchronous glucose-grown *E. coli B ilvA* cells. Since our observations were carried out on asynchronous populations, no attempts were made to estimate the duration of this enhanced murein biosynthesis over the division cycle.

Our data on fast-growing populations suggest that preferential sites or zones of cephaloridine interaction, located at the cell pole(s) and cell center, are superimposed on a random network of insertion sites for murein precursors (Fig. 2A and 3). Consequently our results could support the model of envelope growth recently described by Begg and Donachie (1). Nevertheless, the sensitivity of the cell division process to cephaloridine (Fig. 2A and 3) does put limits on the usefulness of this probe for the detection of the mode of cell elongation. In addition, the early disappearance of the cluster of spheres at the cell pole(s) upon a lowering of the growth rate (Fig. 4A), together with the deviation from a random distribution of insertion sites at low growth rates (Fig. 4A and B), may seriously question the support of this model by our data. Therefore, we rather assume that the mode of surface growth may become tuned to the requirements set by the growth rate of the cell population.

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ADDENDUM IN PROOF

Recently the interdependence of the DNA replication cycle and cell surface replication was investigated in exponentially growing populations of *E. coli* B/r H266 (1.30 divisions per h). DNA synthesis was inhibited by the addition of 20 μg of nalidixic acid per ml. Shortly hereafter, during the remaining period of exponential mass increase, 4 μg of cephaloridine per ml was added. The sensitivity of these cells to cephaloridine was drastically reduced, and no preferential sites of sphere formation could be observed under these conditions.

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