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PRELIMINARY REPORT

CYTOLOGICAL CHANGES OF *ESCHERICHIA COLI* CAUSED BY POLYMYXIN E[1]

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Many reports on the mechanism of action of polymyxin E on susceptible organisms indicate that this compound may alter the bacterial cell surface.[1,2,3,4] Using high concentrations of polymyxin E, CHAPMAN[5,6] and SUGANUMA *et al.*,[7] reported cytological changes in *Escherichia coli* with formation of dense projects of the cell surface. They thought that these were produced by precipitation of polymyxin E with cellular components. In our preliminary experiments with a low concentration of antibiotic this did not seem to be the case. This paper reports the cytological changes in *E. coli* cells, presumably of the cell surface, caused by a low concentration of polymyxin E and those observed in ultrathin sections with an electron microscope.

Throughout the experiments *E. coli* strain B was used. Bacteria were cultured with shaking in Simmons' salts glucose medium. At the logarithmic phase of growth, cells were harvested, washed twice and resuspended in Simmons' salts medium and then incubated for the desired period with or without polymyxin E. For observation under an electron microscope (Hitachi HU 11-A model) specimens were prepared by the conventional double fixation technique with glutaraldehyde and osmium tetroxide. They were stained with 4% uranylacetate solution for 40 min, dehydrated with alcohol and then embeded in Epon 812 resin.[8] The block was sectioned with a Porter-Blum microtome.

When the cells were treated with 1–5 μg/ml of polymyxin E at 37°C for 20 minutes, formation of a number of blebs was seen, as shown in Fig. lb, c. In the control cells without polymyxin E no bleb formation was observed. (Fig. 1a) A similar observation has been made by KOIKE *et al.*[9,10] In our experiments, however, blebs were not produced at low temperature (2°C) even with 10 μg/ml of polymyxin E. To compare the effect of polymyxin E with other agents which are known to affect the bacterial cell surface, sodium dodecyl sulfate (0.2%), ehtylenediaminetetraäcetate (EDTA, 10^{-3} M, pH 7.4) and cetyltrimethylammonium bromide (10 μg/ml) were tested for their abilities to cause bleb formation in

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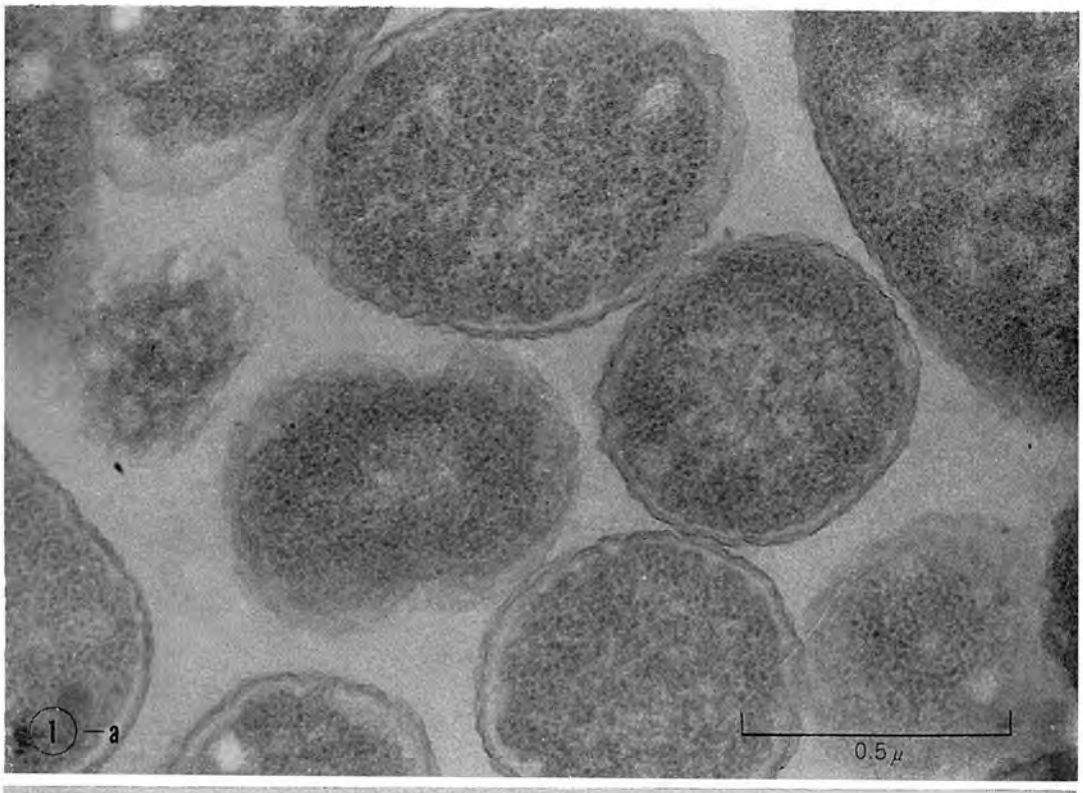
FIGURE 1a Ultrathin-section of control cells without polymyxin E at 37°C for 30 minutes, showing the typical structure of *E. coli* strain B cells. The smooth cell wall (W) is separated from the cytoplasmic membrane (CM), dense cytoplasm (C), and a fibrillar nuclear region (N). ×70,000.

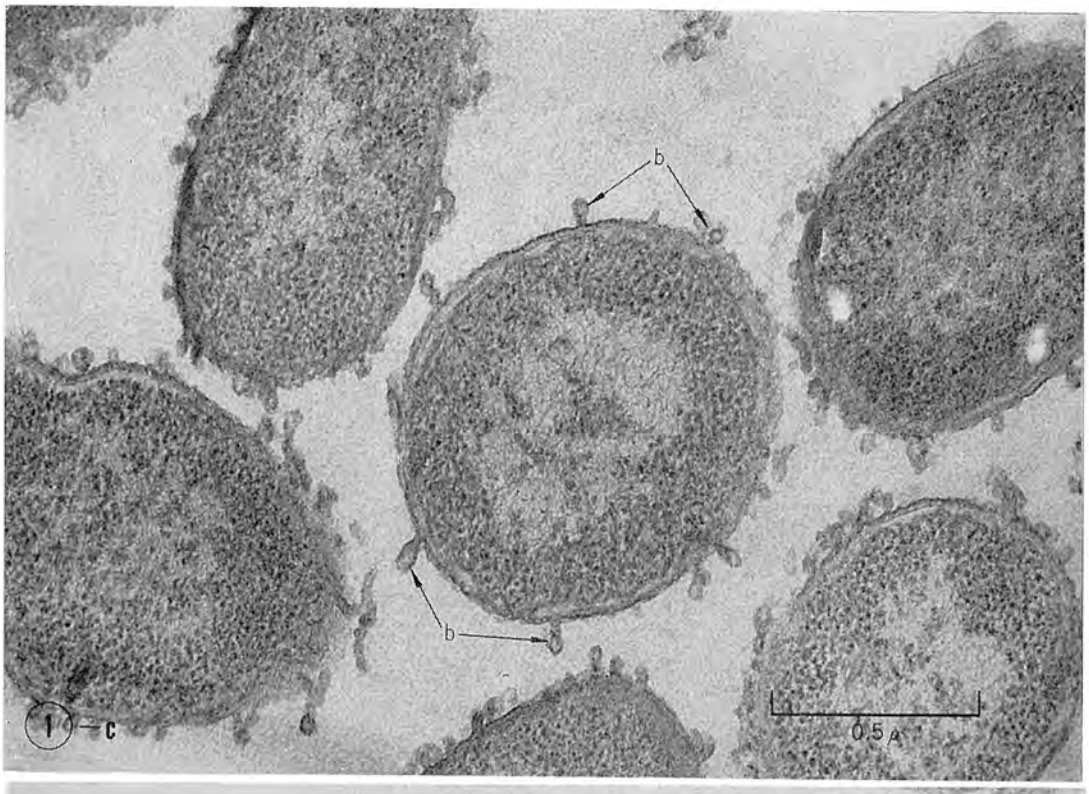
FIGURE 1b Ultrathin-section of *E. coli* strain B exposed to 1 μg per ml of polymyxin E at 37°C for 30 minutes, indicating that the multilayered cell wall (W) is forming blebs (b). ×70,000.


1 — a
0.5 μ


W
b
1 — b
b
0.5 μ

FIGURE 1c Ultrathin-section of *E. coli* strain B exposed to 5 μg per ml of polymyxin E at 37°C for 20 minutes. Numerous blebs (b) can be seen on the cell wall. ×70,000.

FIGURE 2 Ultrathin-section of *E. coli* strain B exposed to 10 μg per ml of polymyxin E at 2°C for 30 minutes. No blebs are seen on the wall. ×70,000.


b
b
1 — c
0.5 μ


2
0.5 μ

E. coli. None of these agents porduced blebs. However, similar morphological changes have been demonstrated in the absence of drugs by KNOX *et al.*[11] in a lysine requiring mutant of *E. coli* strain 12408 in a lysine limited culture. In their experiments numerous blebs were formed and the outer membranes of these were sometimes continuous with the outer triple membrane of the wall. They claimed that extracellular accumulation of lipopolysaccharide (LPS)-lipoprotein complex probably originated from these blebs. In their experiments surface defects were also suggested to be due to the action of lysozyme, which, at low concentration (10 μg/ml), lysed the lysine limited cells even in the absence of EDTA. Treatment with EDTA has been shown to cause a nonspecific increase in permeability of *E. coli* and release of cell wall LPS into solution.[12] In this respect they claimed that an altered permeability to lysozyme of lysine limited cells might be due to a defect in the outer LPS-containing layers of the wall. Cells are also rendered sensitive to lysozyme by polymyxin E.[4,9] We found that treatment of *E. coli* cells with 1–5 μg/ml of polymyxin E only made them sensitive to 10 μg/ml of lysozyme without EDTA. However, after treatment with lysozyme, these cells still retained blebs which suggests the noninvolvement of the rigid layer of the cell wall in bleb formation.

A characteristic feature of the action of polymyxin E is that cells do not lyse completely even with a high concentration of it (10–100 μg/ml). These cells always appear to have a membraneous outer structure under the electron microscope.

The significance of the damage of the cell surface caused by polymyxin E in relation with growth inhibition is supported by the fact that in a polymyxin E resistant strain of *E. coli* strain B (10 μg/ml) no blebs were produced at concentrations below 10 μg/ml of polymyxin E.

We observed that a cell wall preparation from mechanically disrupted cells produced blebs on treatment with polymyxin E at 37°C, while a cell wall preparation treated with trypsin did not.

Studies are planned on the possible participation of some enzymatic process in the cytological changes and alteration of cell permeability. Results will be published soon.

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