NOTES

SPHEROPLAST-LIKE STRUCTURES IN *ESCHERICHIA COLI* DEMONSTRATED BY SCANNING ELECTRON MICROSCOPY

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Although available commercially since 1965, the scanning electron microscope has been little used by microbiologists. However, several studies have recently been reported on the scanning electron microscopy of antibiotic-induced alterations in the surface morphology of bacterial cells[1,2,3,4]. In our previous paper[5] we presented the results of the morphological investigation by electron microscopy on *Staphylococcus aureus* and *Escherichia coli* exposed to cephalexin. It is the purpose of this paper to report studies in which the scanning electron microscope was utilized to observe the effects of cephalexin on the surface morphology of *E. coli* NIH.

Heart infusion broth (Nissan) was inoculated with *E. coli* NIH and incubated at 37°C. Cephalexin was added to cultures at the 5 th hour of logarithmic phase of growth. Organisms were harvested by centrifugation at 3,000 r.p.m. for 5 minutes, washed three times in KELLENBERGER buffer solution, fixed for 16 hours in 1% solution of osmium tetroxide according to the method of KELLENBERGER *et al.*[6], and dehydrated with alcohol series. A drop of each suspension was air-dried on 5 mm microscope cover-slips. The cover-slips were coated with carbon and pure gold in a high-vacuum unit (JEOL JEE 4B) to obtain a uniform coating approximately 100 Å thick. A JEOL JSM-U3 scanning electron microscope operating at accelerating voltage of 9 kV was used.

Untreated *E. coli* NIH cells appeared to have smooth contours (Fig. 1).

The effects of cephalexin on the surface morphology of *E. coli* NIH are shown in Figs. 2, 3 and 4. The degree of morphological changes was found to be dependent on the concentration of cephalexin added. Exposure to 32 minimal inhibitory concentration (MIC) of cephalexin resulted in the formation of marked filamentous cells and spherical cells having multiple small saccular outpouchings, probably representing spheroplasts (Figs. 2 and 3). It appeared that the spherical cell became more and more enlarged and eventually ruptured (Fig. 4). It is noteworthy that the spherical cell had multiple small saccular outpouchings.

GREENWOOD and O'GRADY[1] studied the effect of aminobenzylpenicillin on the surface structures of *Staphylococcus aureus* and *Streptococcus pyogenes*. In addition, KLAINER and PERKINS[2] reported on the effects of penicillin G and cephalothin on the surface morphology of *S. aureus* and *E. coli*, respectively. But they did not observe such spheroplast-like structure as we indicate in this paper.

Acknowledgement

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References

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Fig. 1. Normal *Escherichia coli* NIH cells. (×8,000)

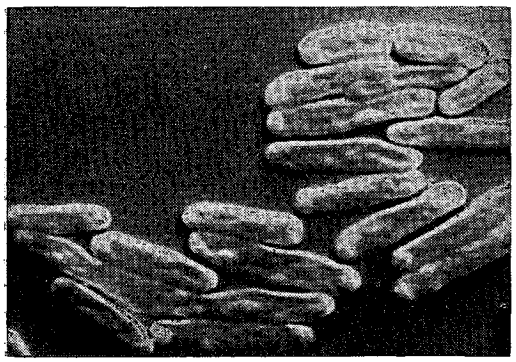


Fig. 2. Exposure to 32 MIC of cephalexin caused the formation of marked filamentous cells and spheroplast-like structure. (×6,400)

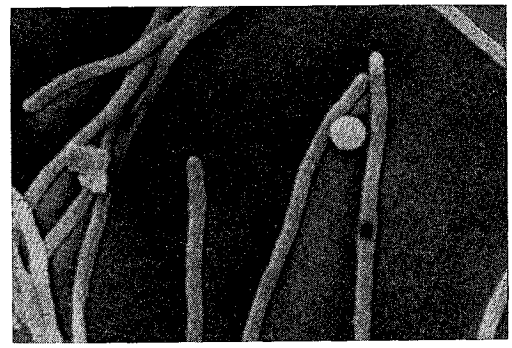
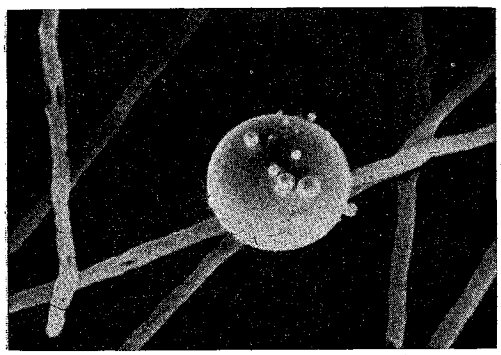


Fig. 3. Exposure to 32 MIC resulted in spherical cell having multiple small saccular outpouchings, presumably spheroplast. (×6,400 and ×4,800, respectively)

(a) (b)



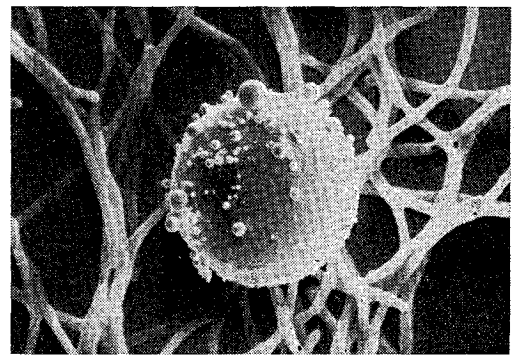
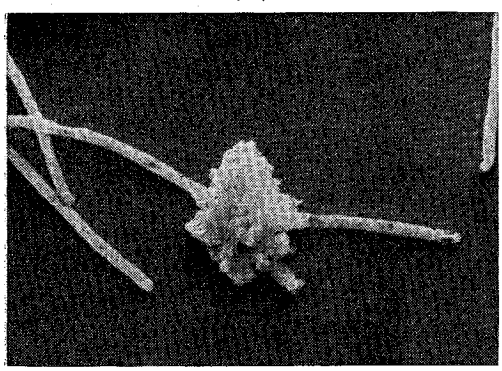
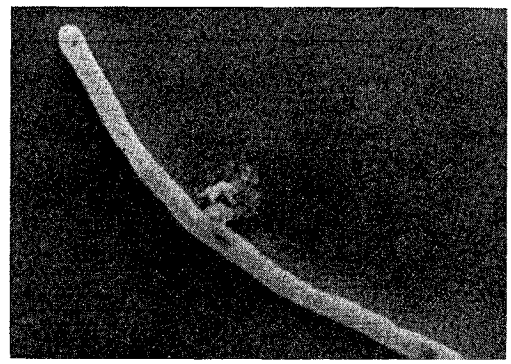


Fig. 4. The lysis of spheroplast-like structure was observed after treatment with 32 MIC. (×4,800 and ×9,600, respectively)

(a) (b)





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