LOCALIZATION OF POLYSACCHARIDE COMPONENTS IN POLYMYXIN B TREATED CELLS OF *SERRATIA MARCESCENS*

Sir:

Polysaccharide localization has been performed in thin sections of bacteria using the silver proteinate method of THIERY[1]. This technique involves oxidation by periodic acid (PA) of 1 : 2 glycol linkages to yield aldehydes, which on reaction with thiosemicarbazide (TSC) form hydrazones. Subsequent treatment with silver proteinate, which is reduced by the thiol groups of the hydrazones, results in deposition of silver at the site of original 1 : 2 glycol linkages. We have applied this method to localize the polysaccharide components in cells of *Serratia marcescens* before and after polymyxin B treatment.

When whole cells or isolated cell envelopes of Gram-negative bacteria are treated with polymyxin B, blebs are generally formed on the surface[2,3]. Although several suggestions have been presented to explain this bleb formation, the exact origin and the chemical characteristics of the blebs remain unclear. It has been suggested the formation of blebs might be attributed to aggregations of polymyxin B with the lipopolysaccharide (LPS) and/or the phospholipid components of the outer membranes of Gram-negative bacteria[4]. This suggestion is supported by the findings of TSANG *et al.* (1976) who provided evidence for the complex formation between polymyxin B and LPS from *S. marcescens.*[5] In this study we attempted to localize the LPS components in the blebs after polymyxin B treatment of cells of *S. marcescens.*

Materials and Methods

The strains of *S. marcescens* used in this study have been previously described[3]. Cells were grown in an enriched medium with aeration at room temperature and harvested at an optical density between 0.50 and 0.55 units. The *in vivo* treatment of whole cells by polymyxin B has been described previously[4]. Cells from 1 liter of culture medium were treated with 20 mg of polymyxin B sulfate (Burroughs Wellcome) in 75 ml of 0.9% NaCl solution, pH 7.3 for 1 minute at 37°C.

Ultrathin section of the cells before and after polymyxin B treatment were placed on either copper or gold grids. All specimens on copper grids were post sectioned stained with uranyl acetate-lead citrate. Those sections on gold grids were floated on a 1% PA solution for 60 minutes at ambient temperature. After washing 3 times in distilled water, they were floated on a 1% TSC solution for 120 minutes. The sections were again washed 3 times and floated on a 1% solution of silver proteinate in the dark for 45 minutes and washed 3 times in distilled water. All sections were examined in either an Hitachi HU-11A or an RCA-EMU-3G electron microscope at 50 kV.

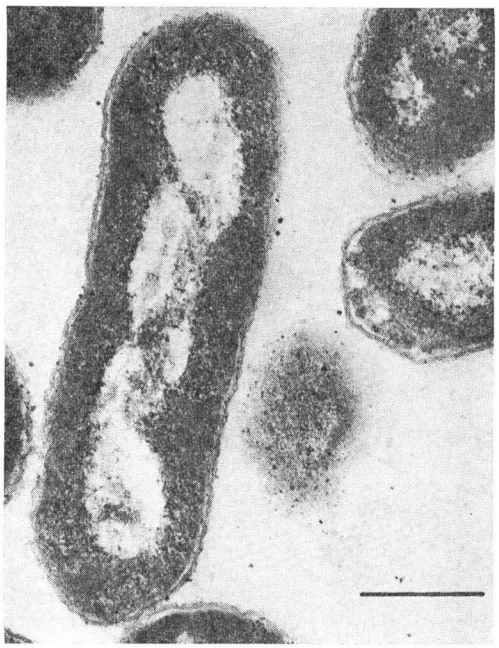
Results and Discussion

This report represents a continuation of the studies of the effects of polymyxin B on sensitive and resistant cells of *S. marcescens.* The presence of extensive bleb formation and protrusions on the surface of cells of *S. marcescens* after polymyxin B treatment has been previously observed by the negative staining technique. When whole cells of strain 08 and Bizio stained with uranyl acetate/lead citrate were examined (Figs. 1-A and 1-B), the normal double track layer of the outer membrane of the cell envelope was clearly shown. After treatment with polymyxin B, the resistant strain 08 (Fig. 1-C) shows some blebs, which appear to originate from the outer membrane. Despite the presence of these projections, the outer membrane remains almost intact. When the sensitive strain Bizio (Fig. 1-D) is examined after treatment, there are disruptions of the entire outer membrane as well as extensive bleb formation.

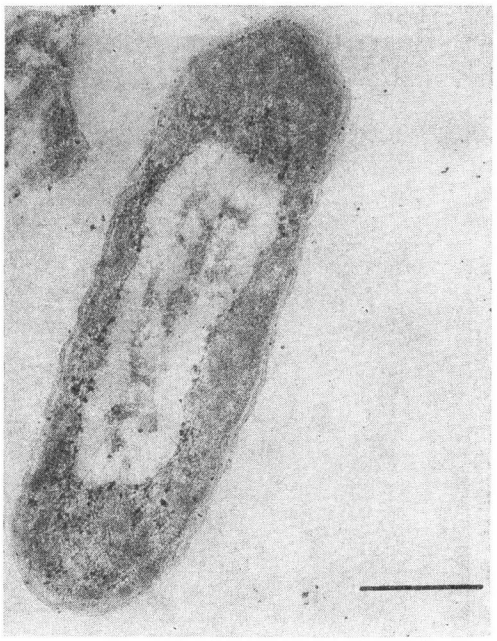
When untreated whole cells of both strains (Figs. 2-A and 2-B) were observed after the TSC-Ag staining, distinct deposition of silver is seen around the periphery of the cells corresponding to the regions where polysaccharides (lipopolysaccharides) are found. After similar staining of polymyxin B treated cells of the resistant cells (08) (Fig. 2-C), numerous distinct sites were shown to contain polysaccharide components. The few blebs present contain not only the double track membranes structure but also the polysaccharide sites which resemble those in the outer membrane proper (Fig. 2-C). However, the polysaccharide material of the sensitive cells is no longer present in a continuous manner along the periphery as in the outer membrane of cells before treatment. The areas of the polysaccharide material appeared to be distributed in a non-

Fig. 1. Morphological appearance of whole cells of *Serratia marcescens* before and after polymyxin B treatment as shown by uranyl acetate-lead citrate staining cells.

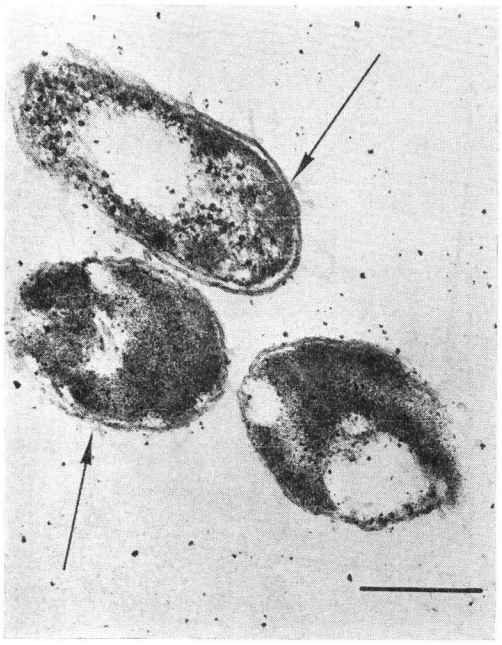
A) Untreated whole cells of resistant strain 08.



B) Untreated whole cells of sensitive strain Bizio.



C) Polymyxin B treated resistant strain 08: note the blebs (arrows), but that the outer membrane is intact.



D) Polymyxin B treated sensitive strain Bizio: note the blebs (arrow) as well as the projections of the outer membrane itself (large arrows). The marker bars in all micrographs represent 0.2 μ.

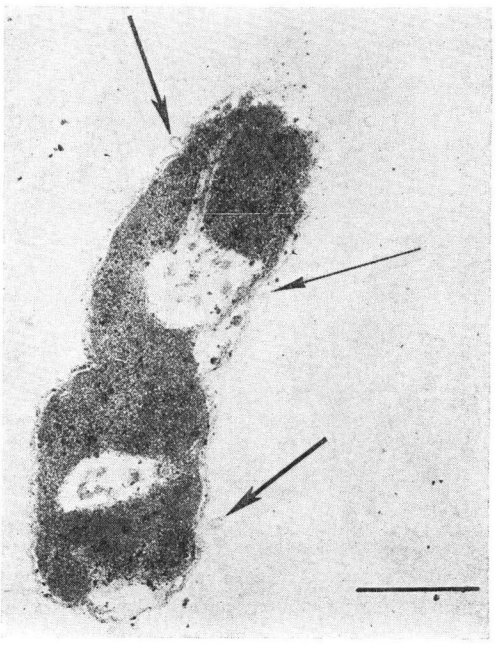
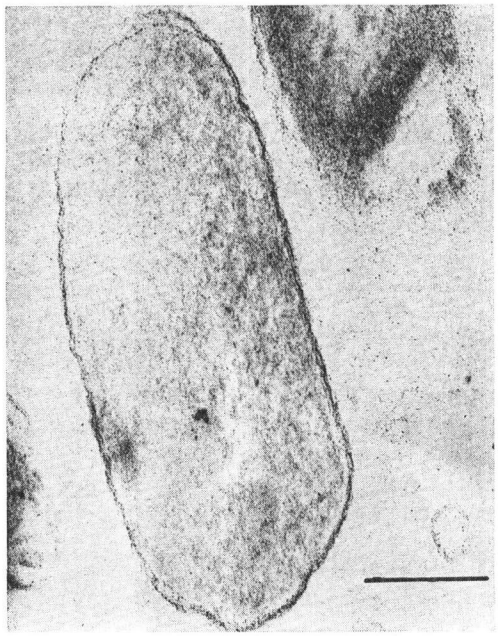
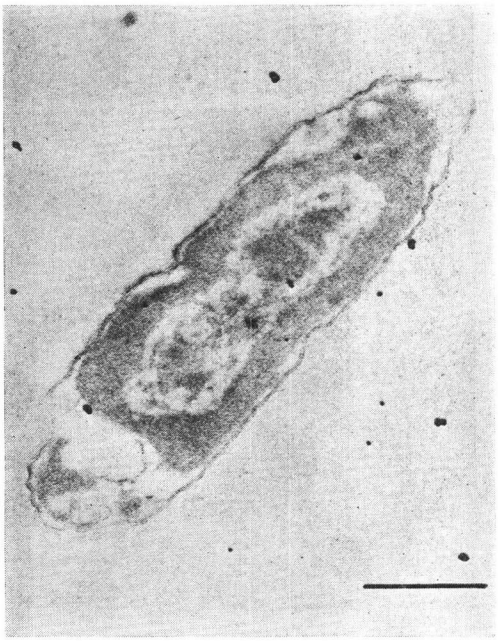


Fig. 2. Morphological appearance of whole cells of *Serratia marcescens* before and after polymyxin B treatment as shown by periodic acid-thiosemicarbazide-silver proteinate staining.

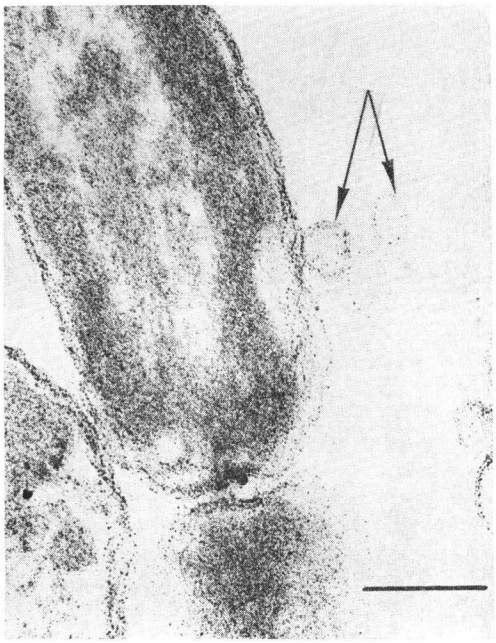
A) Untreated whole cells of resistant strain 08: note the intense staining of the outer membrane.



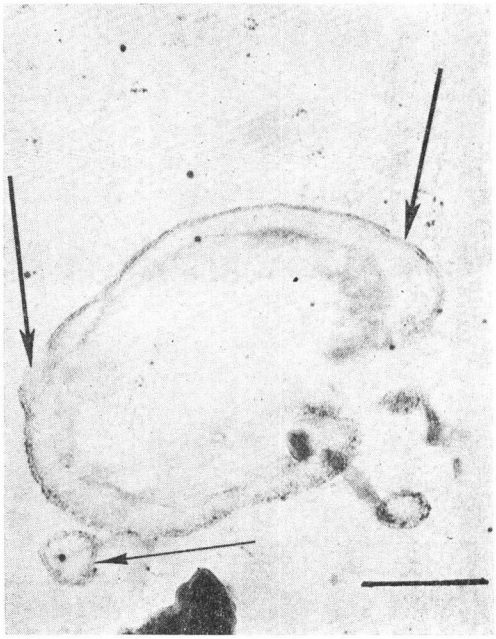
B) Untreated whole cells of sensitive strain Bizio: note the intense staining at the site of the outer membrane.



C) Polymyxin B treated resistant strain 08: note the presence of positively stained polysaccharide granules in the blebs from the outer membrane. (arrows)



D) Polymyxin B treated sensitive strain Bizio: note the loss of integrity of the outer membrane, the presence of positively staining granules in the blebs from the outer membrane (arrows) and the absence of staining in intermittent points along a portion of the membrane (large arrows).



random fashion, interspersed with areas of varying staining intensity. The blebs protruding from the surface of the outer membrane are clearly shown to contain carbohydrate components in the double track membranous structure.

In both the sensitive and resistant strains of *S. marcescens*, PA-TSC-Ag technique has demonstrated that polymyxin B indeed releases the polysaccharide components, most likely in the form of lipopolysaccharide from the outer membrane. The lipopolysaccharide components are found in the free form, as well as in the blebs[6].

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