JOURNAL OF BACTERIOLOGY, Nov. 1969, p. 1128-1130
Vol. 100, No. 2

Electron Microscopy of Effect of Polymyxin on *Escherichia coli* Lipopolysaccharide

J. LOPES AND W. E. INNISS
Department of Biology, University of Waterloo, Waterloo, Ontario, Canada

Received for publication 28 July 1969

Polymxyin treatment of isolated lipopolysaccharide from *Escherichia coli* resulted in the breakdown of its structure.

Polymixins exert their primary effect on the membranous structures of gram-negative bacteria (6). With electron microscopy, Chapman (1) observed that colistin caused such morphological changes as the loss of nuclear material and of granularity of the cytoplasm. Recently, Koike et al. (3) have shown that treatment of *Escherichia coli* and *Pseudomonas aeruginosa* with polymyxins results in changes in the cell wall as indicated by the formation of projections from the cell wall and the liberation of cytoplasm through cracks in the cell envelope. In addition to such structural effects, polymyxin has been found to prevent the lethal endotoxic activity of bacterial lipopolysaccharide [LPS (4, 5)]. The present report deals with an electron microscopic study of the effect of polymyxin B on the LPS isolated from *E. coli*.

Cells of an avian strain of *E. coli*, O18, were grown for 18 hr at 37 C in the synthetic medium of Davis and Mingioli (2) prepared without sodium citrate but to which 1% Trypticase (BBL) was added. After harvesting by centrifugation, the cells were extracted with 9% NaCl at 70 C for 30 min with continuous mixing. The cell suspension was then centrifuged at 37,000 × *g* for 10 min; the supernatant fraction was collected, dialyzed against distilled water, and lyophilized. The lyophilized material was suspended in distilled water and precipitated with four volumes of cold methanol. The precipitate was collected by centrifugation, washed twice with 80% methanol, dissolved in distilled water, and lyophilized. A 1% aqueous solution of the lyophilized product was subjected to the phenol-water extraction procedure for LPS by the method of Westphal and Jann (10). The LPS extract was again precipitated and washed with methanol as indicated previously, and then lyophilized. One gram of this material was separated from substances possessing molceular weights of 50,000 or less by column chromatography with Sephadex G-75 in a 7.5 by 75 cm column, and relyophilized.

To examine the effect of polymyxin, the LPS preparation was dissolved (2 mg/ml) in 0.1 M tris (hydroxymethyl) aminomethane buffer (Tris), *p*H 7.2, and 0.5-ml samples were placed in either 0.5 ml of polymyxin B sulfate in Tris buffer (final concentration of polymyxin, 25 μg/ml) or 0.5 ml of Tris buffer alone as control. After incubation at 37 C for 60 min, samples of the test mixtures were prepared for electron microscopy. A drop of 2% uranyl acetate was added to 0.3 ml of each sample for positive staining. After 15 min, one drop of the mixture was transferred to a carbon-coated copper grid which was previously treated with 0.1% bovine serum albumin to facilitate even spreading of the sample. The drop was rapidly removed by filter paper and the preparations examined with a Philips 300 electron microscope.

In the absence of polymyxin treatment, the LPS isolated from the avian strain of *E. coli* appeared as ribbon-like structures with frequent branching (Fig. 1). Also evident were areas in the structure consisting of two relatively dense outer lines with less dense inner material as reported by Shands et al. (7) for LPS from *Salmonella typhimurium*. When the LPS was exposed to polymyxin, the typical structure was broken down with only short sections or completely disaggregated material remaining (Fig. 2). Thus it would appear that the loss of endotoxicity caused by polymyxin (4, 5) may be due to the loss of structural integrity of the LPS. Tarmina et al. (8) have reported that the biological activity of endotoxin was diminished when disaggregated by treatment with sodium deoxycholate, although size alone was probably not the only factor (8, 9).

This investigation was supported by National Research Council of Canada grant A-1976.

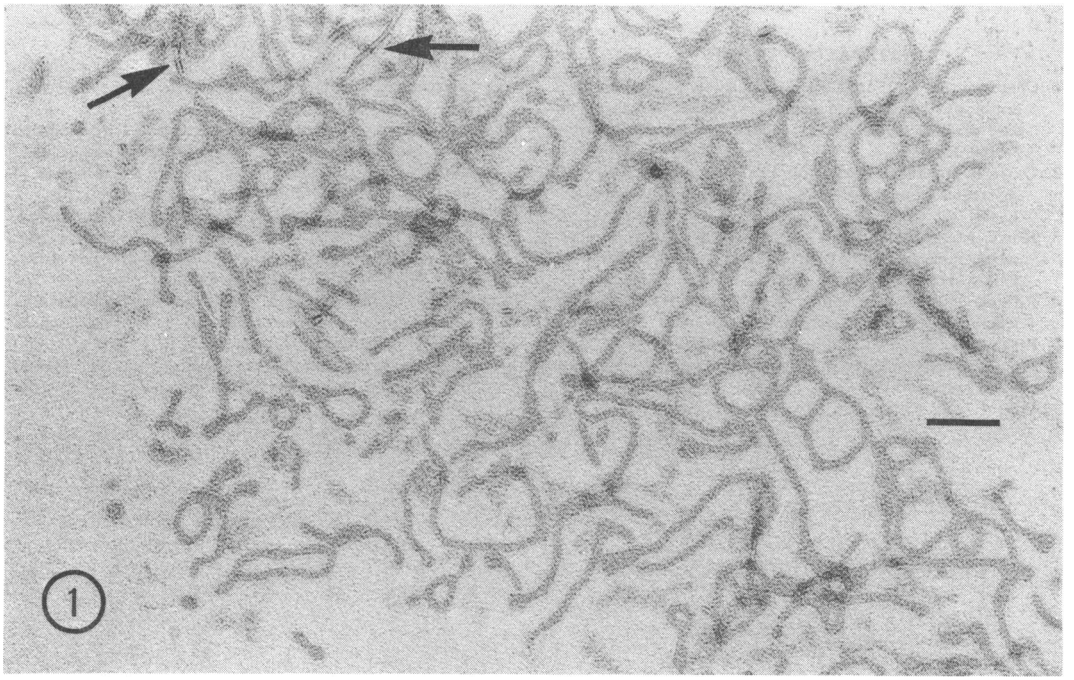

FIG. 1. *LPS isolated from E. coli O18 as ribbon-like structures with occasional areas consisting of relatively dense outer lines with less dense inner material (arrows). Marker represents 0.1 μm.*

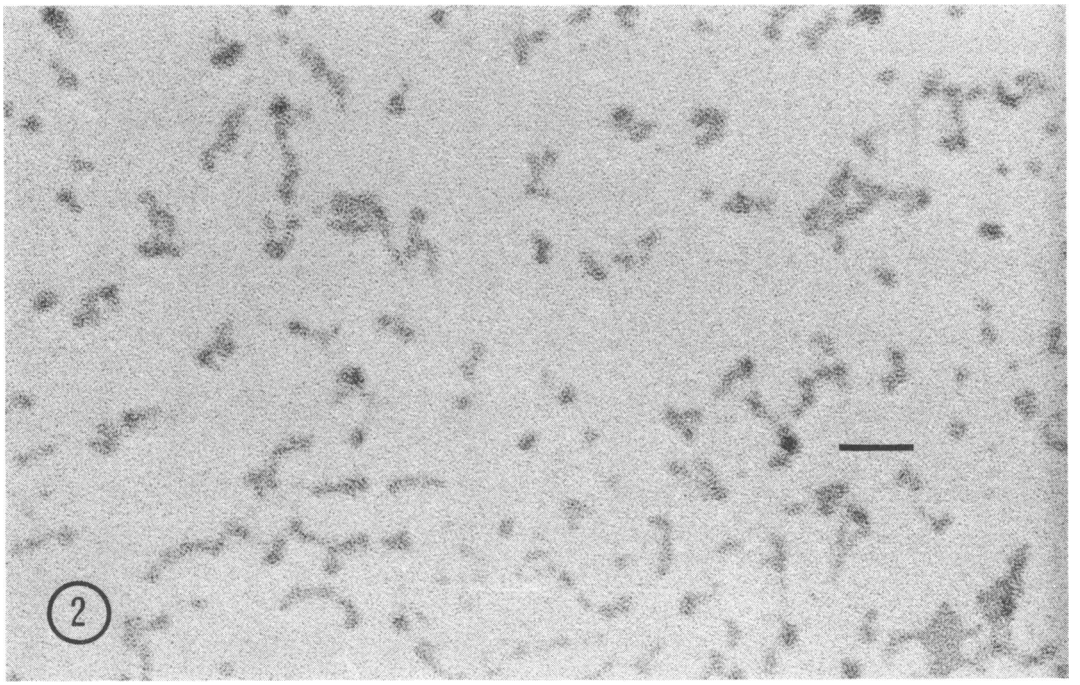

FIG. 2. *E. coli O18 LPS after exposure to polymyxin B (25 μg/ml) for 60 min. Marker represents 0.1 μm.*

We thank K. Zachariah for his help with the electron microscopy.

LITERATURE CITED

1. Chapman, G. E. 1962. Cytological aspects of antimicrobial antibiosis. I. Cytological changes associated with the exposure of *Escherichia coli* to colistin sulfate. J. Bacteriol. 84:169–179.
2. Davis, B. D., and E. S. Mingioli. 1950. Mutants of *Escherichia coli* requiring methionine and vitamin B_{12}. J. Bacteriol. 60: 17–28.
3. Koike, M., K. Iida, and T. Matsuo. 1969. Electron microscopic studies on mode of action of polymyxin. J. Bacteriol. 97:448–452.
4. Rifkind, D. 1967. Prevention by polymyxin B of endotoxin lethality in mice. J. Bacteriol. 93:1463–1464.
5. Rifkind, D., and J. D. Palmer. 1966. Neutralization of endotoxin toxicity in chick embryos by antibiotics. J. Bacteriol. 92:815–819.
6. Sebek, O. K. 1967. Polymyxins and circulin, p. 142–152. *In* D. Gottlieb and P. D. Shaw (ed.), Antibiotics, vol. 1, Mechanism of action. Springer-Verlag, New York.
7. Shands, J. W., Jr., J. A. Graham, and K. Nath. 1967. The morphologic structure of isolated bacterial lipopolysaccharide. J. Mol. Biol. 25:15–21.
8. Tarmina, D. F., K. C. Milner, E. Ribi, and J. A. Rudbach. 1968. Modification of selected host-reactive properties of endotoxin by treatment with sodium deoxycholate. J. Bacteriol. 96:1611–1616.
9. Tripodi, D., and A. Nowotny. 1966. Relation of structure to function in bacterial O-antigens. V. Nature of active sites in endotoxic lipopolysaccharides of *Serratia marcescens*. Ann. N.Y. Acad. Sci. 133:604–621.
10. Westphal, O., and K. Jann. 1965. Bacterial lipopolysaccharides. Extraction with phenol-water and further applications of the procedure, p. 83–91. *In* R. L. Whistler (ed.), Methods in carbohydrate chemistry, vol. 5, General polysaccharides. Academic Press Inc., New York.