Electron Microscopy of *Proteus vulgaris* Exposed to Cephalothin

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Exposure of certain gram-negative bacteria to cephalothin and observation by light microscopy revealed the formation of long filaments and large bodies (T. W. Chang and L. Weinstein, J. Bacteriol. 88:1790, 1964). The degree of morphological change was related to the concentration of cephalothin used. This paper describes some abnormal morphological forms of *Proteus vulgaris* produced upon exposure to cephalothin and studied by electron microscopy.

To obtain morphological changes, filter paper discs saturated with 25 mg of cephalothin per ml (Eli Lilly & Co., Indianapolis, Ind.) were placed on Trypticase Soy Agar (BBL) plates inoculated with *P. vulgaris*. After incubation at 37 C for 15 hr, zones containing abnormal morphological forms were cut from the plates. Fixation was according to the method of E. Kellenberger, A. Ryter, and J. Sechaud (J. Biophys. Biochem. Cytol. 4:671, 1958). Specimens were embedded in Epon 812 (J. H. Luft, J. Biophys. Biochem. Cytol. 9:409, 1961). Sections were cut on a Porter-Blum MT-1 ultramicrotome, mounted on Formvar-coated copper specimen grids, and examined in an RCA-EMU-3G electron microscope.

*P. vulgaris* grew in three distinct concentric zones. The zone immediately surrounding the cephalothin disc was clear and contained no cells. The next zone appeared cloudy and contained large bodies. The third zone contained heavy growth in which long filamentous forms were found. The area surrounding this zone contained normal cells.

Electron microscopy of the long filamentous forms revealed no differences except in length between these forms and normal cells. The long forms (Fig. 1) attained lengths of over 20 times that of normal cells, and there was no evidence of a cell wall or cell membrane partitioning the long forms.

The large bodies, regarded as spheroplasts, had spherical shapes with diameters of 6 to 12 times that of normal cells (Fig. 2). Several smaller structures within the spheroplast were observed also. These were spherical or ellipsoidal and varied from 0.1 to 0.5 μ in diameter. Some of these structures contained densely packed granular material and seemed to form by a budding process from within the spheroplast (Fig. 3). Connections between these were observed frequently (Fig. 4). Other structures in the spheroplast contained little or no electron-dense material and seemed to form in toto within the spheroplast (Fig. 4).

The effects of cephalothin on *P. vulgaris* in the formation of long filamentous forms and large bodies appear similar to those produced by penicillin (A. Fleming, A. Vourek, J. R. H. Kramer, and W. H. Hughes, J. Gen. Microbiol. 4:257, 1950). In spheroplasts of *Escherichia coli* produced by penicillin, the antibiotic prevented the incorporation of mucopeptide into the cell wall with a resulting loss of rigidity and impairment in division (R. G. E. Murray, P. Steed, and H. E. Elson, Can. J. Microbiol. 4:547, 1965). In our study, where the concentration of antibiotic was low, the filamentous forms seemed to have an impairment in their division mechanism although they still retained their rigidity. Where the concentration of cephalothin was high, the cells also lost their rigidity and formed spheroplasts. The chemical nature of the smaller structures within the spheroplasts was not determined. However, in view of the similarities in modes of action of penicillin and cephalothin on cell-wall synthesis (P. E. Reynolds, p. 47, in B. A. Newton and P. E. Reynolds [ed.], Biochemical studies of antimicrobial drugs, Cambridge Univ. Press, London, 1966), the smaller structures could be accumulations of cellular components unable to be incorporated into the cell wall. P. Fitz-James and R. Hancock (J. Cell Biol. 26:657, 1965) observed in *Bacillus megaterium* grown in the...
FIG. 1. Longitudinal section of a long filamentous form with an impairment in its division mechanism. × 51,000.
FIG. 2. Cross section of several spheroplasts. X 15,000.
Fig. 3. Budding within the spheroplast of a smaller structure densely packed with granular material. × 80,000.

Fig. 4. Connection between a small structure densely packed with granular material and other small structures which have little electron-dense material and are formed in toto within the spheroplast. × 57,000.
presence of penicillin the accumulation of muropeptide, which they concluded was unorganized cell-wall material. In contrast, K. W. Knox, M. Vesk, and E. Work (J. Bacteriol. 92:1206, 1966) observed in a lysine-requiring mutant of E. coli grown in limited lysine the formation of blebs of lipopolysaccharide (LPS) that also resemble the smaller structures in our study. These investigators concluded that this LPS accumulated as a result of the unbalanced growth of the cell wall due to restricted protein synthesis imposed by lysine limitation. Our observations suggest that cephalothin is affecting the synthesis of the cell wall of P. vulgaris and support the finding of similarities in modes of action of penicillin and cephalothin.

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