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Membrane Structures in Stable L-Forms of *Escherichia coli*

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Microtubular and lamellate membrane structures were observed at the log phase of growth in the stable L-forms of *Escherichia coli* cultured in the absence of penicillin.

Although there are a number of ultrastructural studies on the L-forms of gram-positive and gram-negative bacteria, little is known concerning the presence of intraplasmic membrane structures in these organisms (1, 4). Corfield et al. (5) observed microtubular structures in group D streptococcal L-forms and found them only in cultures growing in the presence of penicillin. Cole (3, 4) reported lamellate membrane structures and microtubules in the group A streptococcal L-forms. Cohen et al. (2) found core-like structures in protoplast of *Streptococcus faecalis*, and Hubert et al. (6) observed the similar structures in L-forms of *Pseudomonas aeruginosa*. In general, the membrane structures seem to be found most

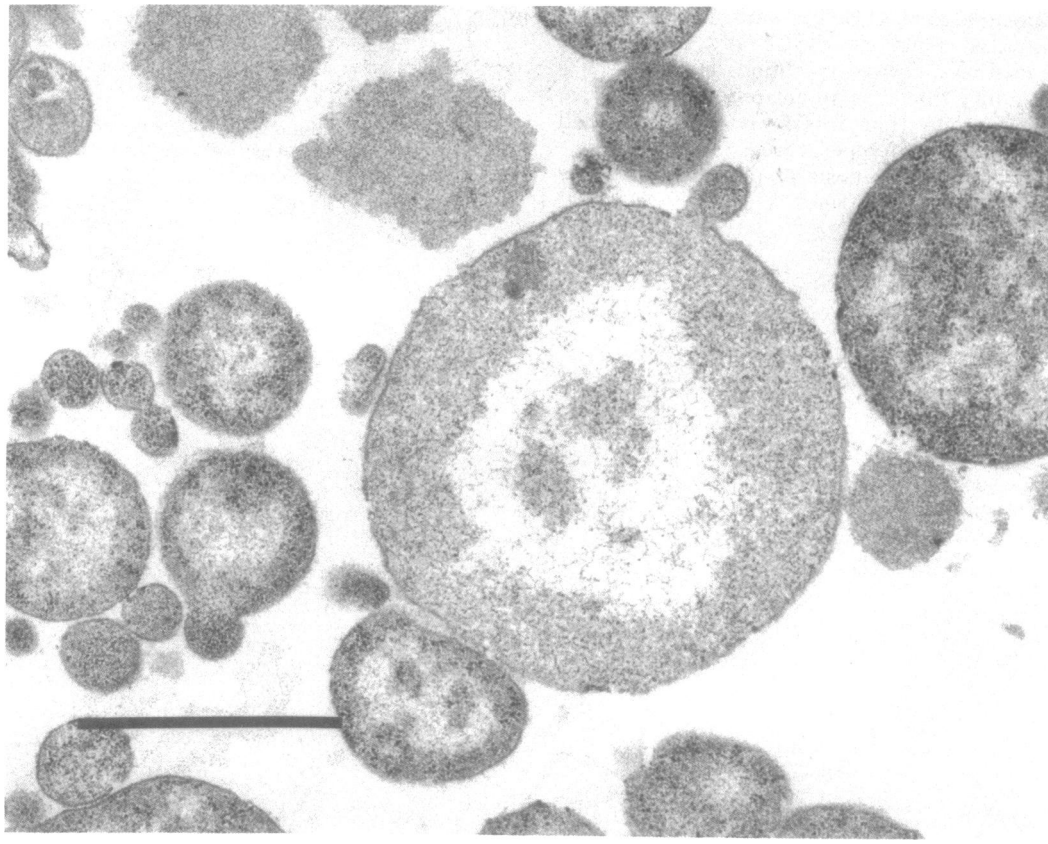

FIG. 1. *EcL strain of E. coli L-forms at the stationary phase of growth. Most of the cells are spherical in shape, lack a cell wall, and are surrounded by unit membrane. In the cytoplasm, ribosomal particles and nuclear materials are observed. No membrane structure is present. The bar represents 1 μm.*

commonly in L-forms of gram-positive cocci (4). There have been no reports of such structures in *Escherichia coli* L-forms.

In this report, we present the microtubular and lamellate membrane structures, which were found at the log phase of growth in the stable L-forms of *E. coli* cultured in the absence of penicillin.

EcL strain of *E. coli* L-forms was obtained from K. Shimizu, Department of Internal Medicine, Tsukuba University School of Medicine. The L-forms have been subcultured in L-form broth containing 3.7% brain heart infusion (Difco), 0.5% yeast extract (Difco), 4% NaCl, and 10% horse serum (Bio Test) for several years. This strain is stable in the absence of antibiotic and has not been found to revert during laboratory manipulations during several years. These L-forms reached maximum growth at 24 h in the L-form broth.

In the present investigation, the L-forms were grown in the L-form broth at 37°C. The cells were harvested at the log or stationary phase of growth by centrifugation at 10,000 rpm (Sorvall RC-2B) and prefixed with 2.5% glutaraldehyde in 0.1 M phosphate buffer, pH 7.2, for 30 min at 4°C. The fixed cells were centrifuged at 10,000 rpm at 4°C and washed three times with the same buffer in the cold. The cell pellets were postfixed with 1% osmium tetroxide in phosphate buffer, pH 7.4, for 2 h at 4°C. After dehydration in a graded series of acetone, the samples were embedded in Epon 812. Ultrathin sections were made with a Poter-Blum MT-1 ultramicrotome and stained with 1.5% uranium acetate and Reynolds lead citrate. The specimens were examined and photographed in a Hitachi HU-12AS electron microscope at 75 kV.

In the cells grown at the stationary phase, many photographs such as those shown in Fig. 1 showed that the EcL strain of *E. coli* L-forms varied in size ranging 250 nm to 2 μm. The

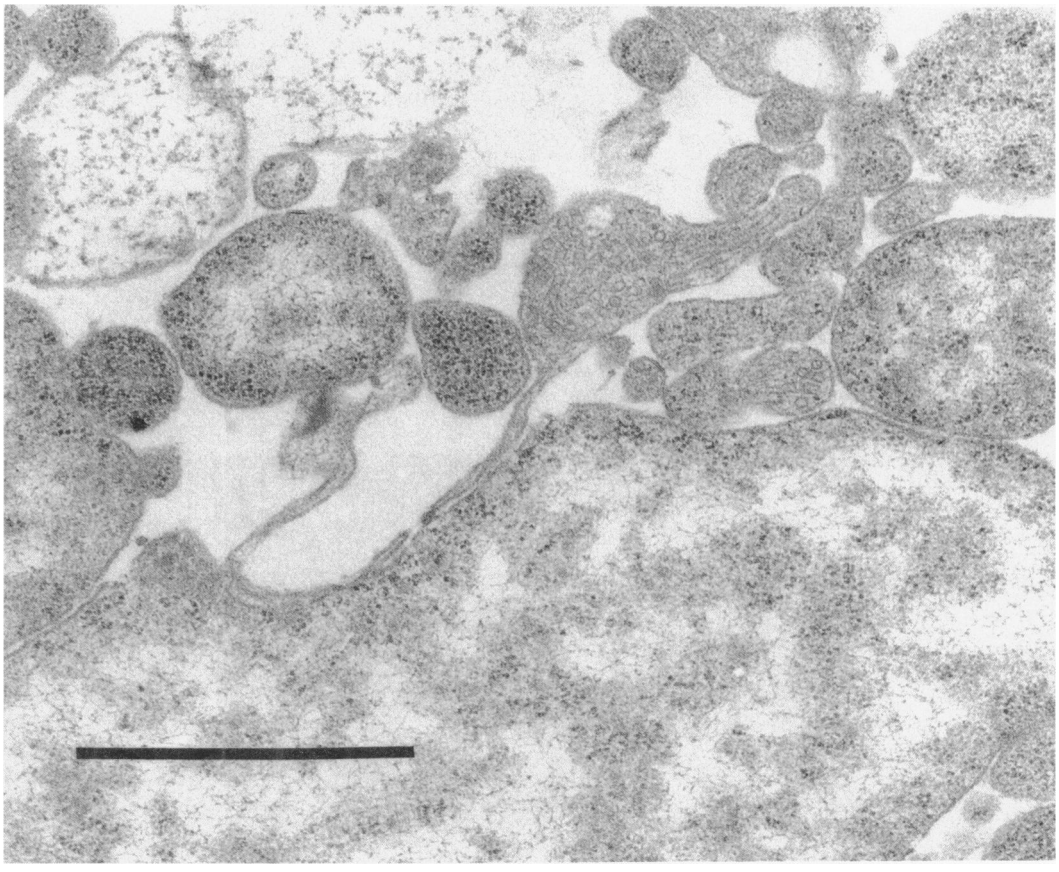

FIG. 2. *EcL strain at the log phase of growth. The cells are larger and more pleophormic. The microtubular structures are found in the cytoplasm of a pseudopod-like protrusion from a part of the cytoplasmic membrane. The bar represents 1 μm.*

individual cell was spherical in shape and bounded by a single unit membrane. The cell wall was completely absent. The cell had ribosomes and a centrally located fibrillar nucleoid material. No membrane structures could be found in the cytoplasm.

In the logarithmically growing culture, many cells were larger and more pleomorphic, and a filamentous structure protruding from a part of the cytoplasmic membrane was observed. In the cytoplasm of protrusion, the microtubular structures, which were arranged irregularly or sometimes in a bundle, were seen (Fig. 2, 3) and had a 15-nm internal diameter and 27- to 30-nm external diameter (Fig. 4). This structure seems to be different from core-like structure reported by Cohen et al. (2) or Hubert et al. (6) and closely resembles the microtubular structures shown by Corfield et al. (5) and Cole (4) because of its morphological similarity. In some of cells, the lamellate membrane structures similar to those reported by Cole (3, 4) were observed in the periphery of cytoplasm (Fig. 5).

In another experiment, we found also that the microtubular and lamellate membrane structure in the cells of group A streptococcal L-forms strain 124L harvested only at the log

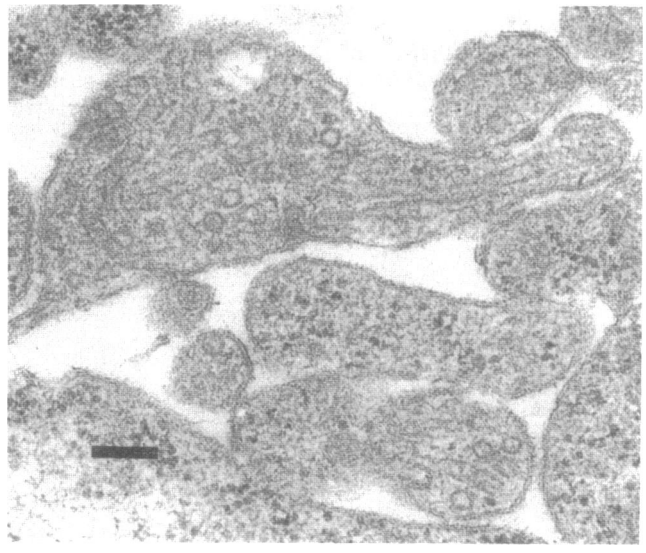

FIG. 3. *Higher magnification micrograph of the microtubular structures in the same cell shown in Fig. 2. Longitudinal and cross-sections are seen. The bar represents 100 nm.*

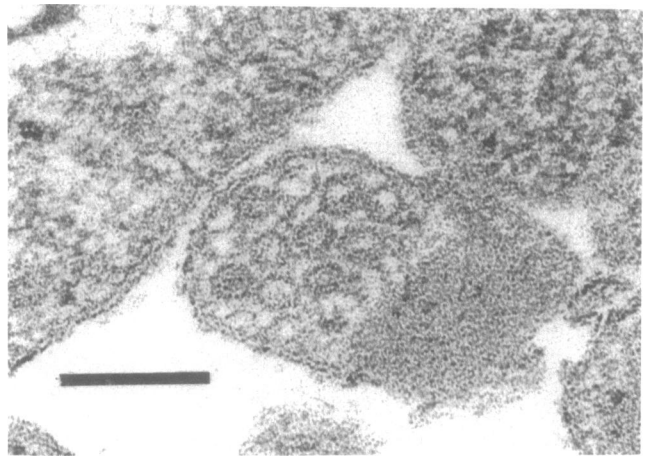

FIG. 4. *Cross-sections of the microtubular structures in the cell of EcL strain. The bar represents 100 nm.*

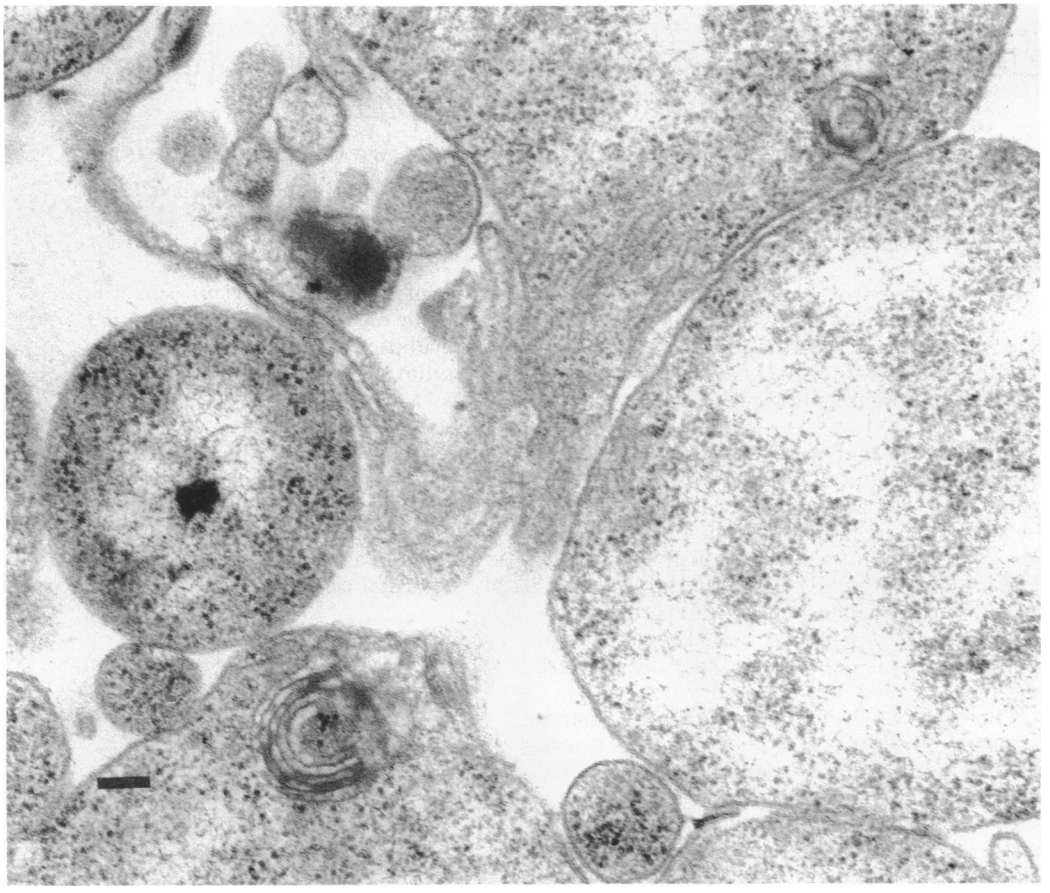

FIG. 5. *A lamellate membrane structure in EcL strain at the log phase of growth. The bar represents 100 nm.*

phase of growth, which was induced from naturally occurring group A *Streptococcus* (T-type 5) by aminobenzyl penicillin, and confirmed Cole's observation (3, 4). Our results suggest that these membrane structures correspond with a mesosome that exists in the ordinary bacteria because of its morphological similarity and are found universally in many of species of the L-forms if there are adequate conditions for their formation.

We wish to thank K. Shimizu, Tsukuba University, who kindly supplied EcL strain used in this study.

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