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NOTES

Surface Projections of a Chlamydia-Like Parasite of Midge Larvae

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Projections are described in a chlamydia-like organism similar to *Rickettsiella chironomi*. These projections were present only on the edge of the disk-like elementary bodies; rod-shaped, they were found to be composed of repetitive subunits.

Recent electron microscopic studies on the envelope of intracellular procaryotes have shown that among the chlamydia-like microorganisms, some possess either an external layer or projections on their cell wall; *Rickettsiella* spp. are covered with a thin polysaccharidic layer (5), and hemispheric projections have been observed associated with the cell walls of *Chlamydia* spp. (3, 4, 6). Other chlamydia-like microorganisms described in arachnida and belonging to the genus *Porochlamydia* do not exhibit such external structures (7, 8). The present paper reports the presence of a peculiar structure composed of tubular projections at the surface of a microorganism similar to that previously known as *Rickettsiella chironomi* (1,2) which Federici (1) has proposed to transfer to the genus *Porochlamydia*.

Our strain of *R. chironomi* was studied in thin sections by negative staining. Fat bodies from *Chironomus dorsalis*-diseased larvae were fixed with 3% glutaraldehyde in 0.2 M cacodylate buffer, postfixed in 1% OsO_4, and embedded in Spurr resin. Thin sections were doubly stained with uranyl acetate and lead citrate solutions. Purified elementary bodies were obtained by repeated differential centrifugations at 1,000 and 9,000 × *g* for 20 and 30 min, respectively. The suspended unfixed pellets of the 30-min centrifugation were placed on carbon-coated collodion grids and negatively stained with 2% neutral sodium phosphotungstate.

Like those described in thin section in other midge larvae (1,2), the elementary bodies were disk-shaped with a diameter of 400 to 600 nm and a thickness of 60 to 80 nm. The particles were divided into two areas corresponding, respectively, to the nucleoid and to the cytoplasmic zones which were filled with ribosome-like particles in a paracrystalline array.

Observations of negatively stained elementary bodies confirmed their disk-like form (Fig. 1). The deposit of sodium phosphotungstate on the surface of the particles was unequal; it was greater over the nucleoid, which thus appeared to be crescent-shaped. Elementary bodies were

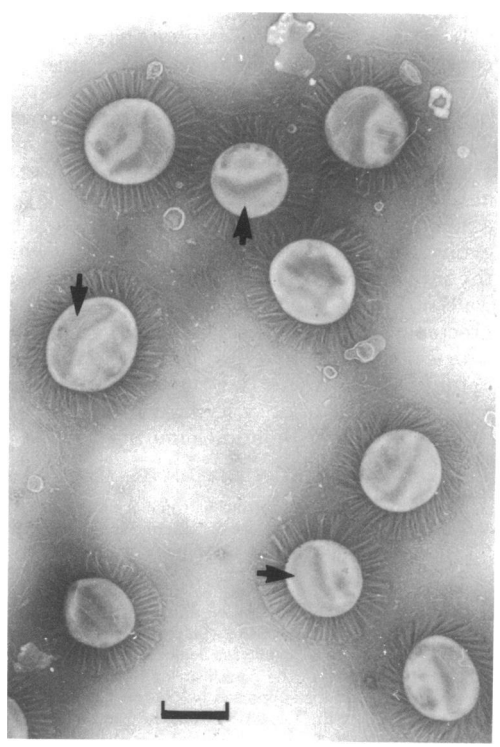

FIG. 1. *Electron micrograph of negatively stained R. chironomi elementary bodies. Note the deposit of sodium phosphotungstate over the nucleoid areas (arrows) and the corona of rod-like projections. Bar, 500 nm; ×18,000.*

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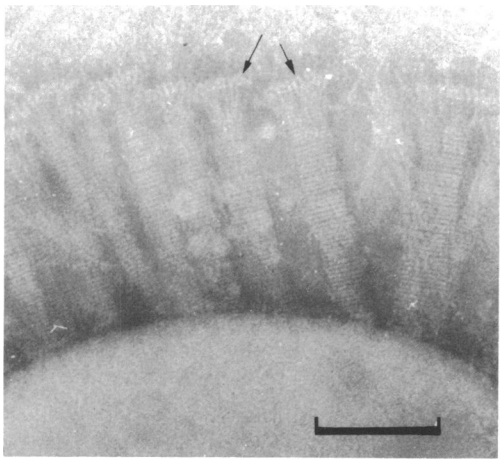

FIG. 2. *High magnification of rod-like structures showing their striated cylindrical part and the subunits of the enlarged tip (arrow). Bar, 100 nm; ×165,000.*

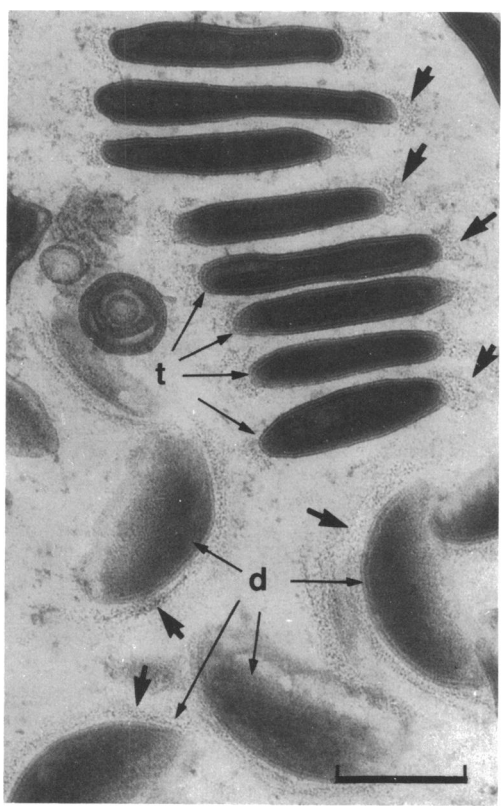

FIG. 3. *Thin section of R. chironomi elementary bodies. Note the amorphous electron dense material (thick arrows) around the disk-like sections (d) and on the extremities of the transversal sections (t). Bar, 250 nm; ×70,000.*

surrounded by a 200-nm width corona made up of 60 to 80 rod-like projections. Regularly disposed all around the edge of the disk, these structures were cylindrical with a diameter of 20 to 35 nm and presented an enlarged tip of 40 to 60 nm in width. At high magnification, these projections presented a striated appearance which seemed to result from a regular arrangement of subunits. The enlarged distal part of the projection apparently consists of another type of subunit disposed perpendicularly to the striations (Fig. 2).

On ultrathin sections, an amorphous material 25 to 50 nm thick was observed associated with elementary bodies. This material was located at both extremities on transversally sectioned particles and surrounds the particles on saggital sections (Fig. 3). It very likely corresponds to the projections more or less flattened on the cell wall within the developmental vacuoles.

These projections differ clearly from those described for *Chlamydia* spp (3, 6) and constitute a new type of differentation of the cell wall in chlamydia-like microorganisms. They could represent a special adaptation of *R. chironomi* to the liquid environment of its host. As for the glycocalyxes of bacteria, these structures could play a role in the attachment of the infectious particles, thus favoring the contamination of new hosts.

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