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NOTES

Ultrastructure of the Surface of *Rickettsia prowazeki* and *Rickettsia akari*

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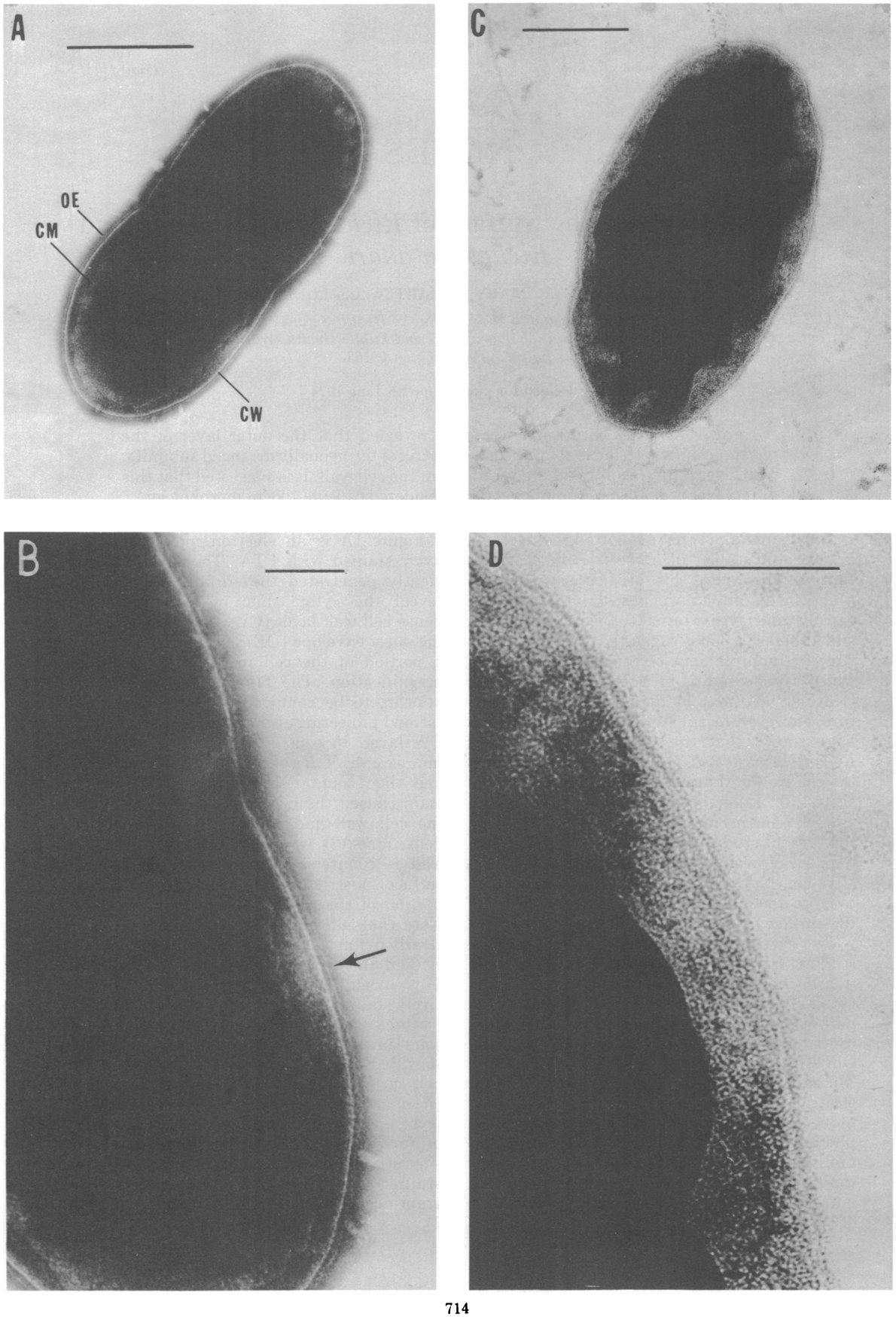
Negative-contrast electron microscopy revealed that the outer layer of the envelope of rickettsiae is composed of a matrix of tetragonally arranged subunits. The layer projects approximately 7 nm from the cell wall. It is suggested that this outer layer is analogous to the structure considered capsule-like in morphology.

Anacker et al. (1) have reported that electron microscopic studies of *Rickettsia prowazeki* in thin section show this organism to be surrounded by an amorphous, faintly staining, capsule-like layer external to the cell wall. They and others (4) have suggested that the group specific complement-fixation (CF) antigen which is released from rickettsiae after ether treatment (8) may be derived from this structure. However, recent studies in our laboratories have shown that the soluble CF antigen of *Rickettsia rickettsii* is derived primarily from the cell wall and has a complex protein composition (T. Tzianabos, E. Palmer, J. Obijeski, and M. Martin, manuscript in preparation). In the present study, preparations of purified free-lying rickettsiae were further examined by negative-contrast electron microscopy in an effort to more adequately delineate the topography of the surface of these organisms.

Two species of rickettsiae were used in these studies: *R. akari* (spotted fever group) and *R. prowazeki* (typhus group). They were propagated in embryonated chicken egg yolk sacs and purified by differential and density gradient centrifugation as described previously (3; J. F. Obijeski, E. L. Palmer, and T. Tzianabos, Microbios, in press). They were prepared for electron microscopy by the pseudoreplica technique (5) and stained with either 2.0% phosphotungstate (PTA), pH 7.0, or 0.5% aqueous uranyl acetate (UA), pH 4.5. Some preparations were doubly stained by floating grids which held entrapped organisms stained with UA on a drop of PTA for about 30 s.

Figure 1A is an electron micrograph of *R. akari* stained with PTA. The stain has sufficiently penetrated the surface of the cell to reveal the cytoplasmic membrane (CM), a dense cell wall layer (CW), and a faintly staining outer envelope (OE) layer. Figure 1B shows a portion of the cell in Fig. 1A at a higher magnification. The faintly staining OE layer appears to be composed of box-like subunits (arrow) projecting approximately 7 nm from the CW layer. In contrast, Fig. 1C is an electron micrograph of *R. akari* first stained with UA and then counterstained with PTA. The UA stain caused the cytoplasm to pull away from the cell wall and condense into an electron-dense mass (3), whereas the electron-lucent cell wall is counterstained by the PTA to reveal surface substructural detail. Figure 1D shows a portion of this cell at a higher magnification. Note that although the cell wall layer is not clearly defined, some details of the OE layer are evident and appear to be composed of subunits. This structure appears very similar to negatively stained cell wall fragments of *Chlamydia psittaci* (2). The matrix of the surface can also sometimes be seen by staining with PTA alone provided that the stain does not penetrate the cell surface. Figure 2A shows a rickettsial cell (*R. akari*) unpenetrated by PTA stain. The OE layer appears as a net-like structure composed of an array of tetragonally arranged subunits. This structure is strikingly similar to negatively stained cell wall preparations of another gram-negative bacterium, *Acinetobacter* sp. (6, 7).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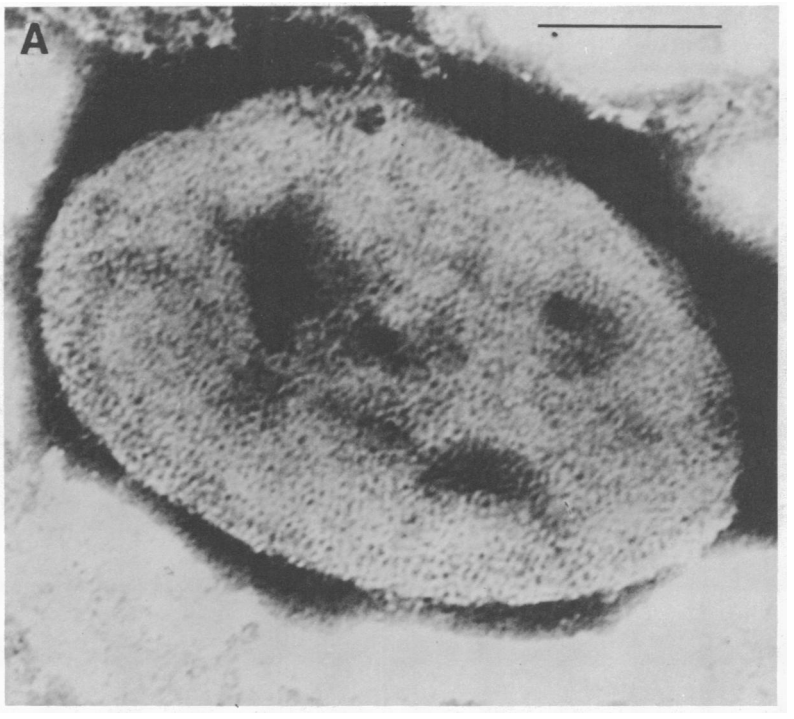

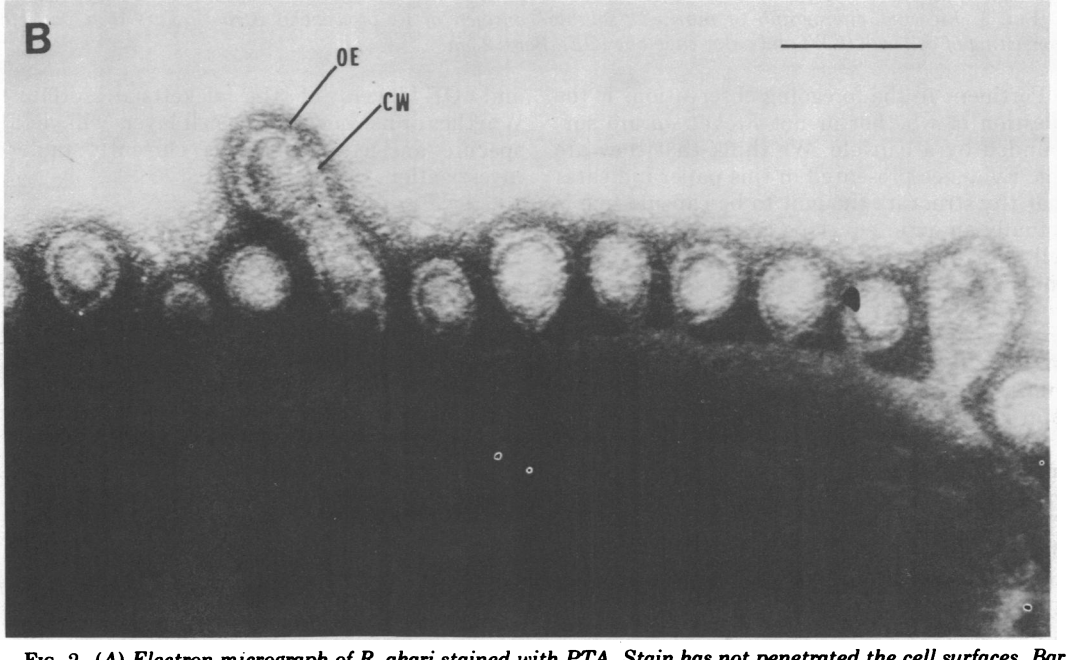

FIG. 2. *(A) Electron micrograph of R. akari stained with PTA. Stain has not penetrated the cell surfaces. Bar 0.2 μm. (B) Electron micrograph of R. prowazeki stained with PTA. "Soluble" antigen particles consisting of the outer envelope (OE) and dense cell wall layers (CW) are spontaneously forming. Bar 0.1 μm.*

FIG. 1. *(A) Electron micrograph of R. akari stained with PTA. (CW) cell wall, (OE) outer envelope, (CM) cytoplasmic membrane. Bar 0.4 μm. (B) Higher magnification of a portion of the cell shown in A. Arrow points to box-like subunits comprising the outer envelope. Bar 0.1 μm. (C) Electron micrograph of R. akari stained with UA and counter-stained with PTA. Bar 0.2 μm. (D) Higher magnification of a portion of the cell shown in C. Envelope is composed of box-like subunits. Bar 0.1 μm.*

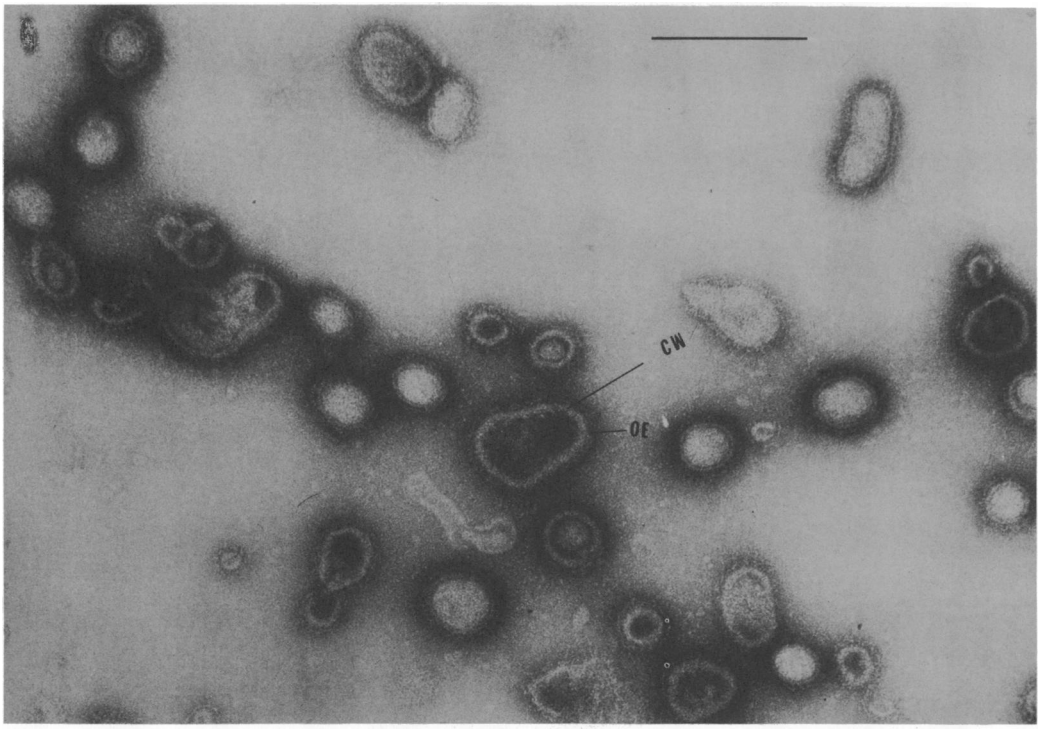

FIG. 3. *Electron micrograph of purified "soluble" antigen of R. prowazeki. Arrow points to a particle consisting of cell wall (CW) and outer envelope (OE). Bar 0.2 μm.*

Pertinent to the foregoing observations is the question of whether or not *Rickettsia* are surrounded by a capsule. We think that they are not. Evidence presented in this paper indicates that the structure thought to be capsule-like is actually an outer envelope layer external to the cell wall and is composed of tetragonal subunits. The subunits project approximately 7 nm from the CW layer, and previous studies with *R. prowazeki* indicate that they have a periodicity of 13 nm (3). This OE layer might be visible in thin sectioned preparations of rickettsiae as a faintly staining amorphous layer.

Further, we have found that the soluble CF antigen of rickettsiae is composed of, at least, the CW and OE layers of the cell integument. This is clearly seen in Fig. 2B which is an electron micrograph of a portion of *R. prowazeki* from whose surface soluble antigen particles are spontaneously forming. The CW and OE layers appear to round up to form the CF antigen particles. The OE layer remains firmly attached to the CW layer even after extensive purification of these particles. Figure 3 shows an electron micrograph of purified soluble antigen derived from ether-treated *R. prowazeki* (8). The particles are seen to possess both the CW and OE layers of the rickettsial surface. Whether or not one of these cell layers will yield specific antigen reagents is currently under investigation.

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