

Details of the Ultrastructure of *Rickettsia prowazekii* Grown in the Chick Yolk Sac

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The fine structure of *Rickettsia prowazekii* as revealed in thin sections of infected monkey kidney cells, yolk sac of embryonated chicken eggs, louse gut, or tick hemocytes has recently

M. Shestopalova, *Acta Virol.* 10:260, 1966; R. G. Bird, N. Kordová, and J. Rěháček, *Acta Virol.* 11:60, 1967). In our studies, we have observed three additional morphological features of the

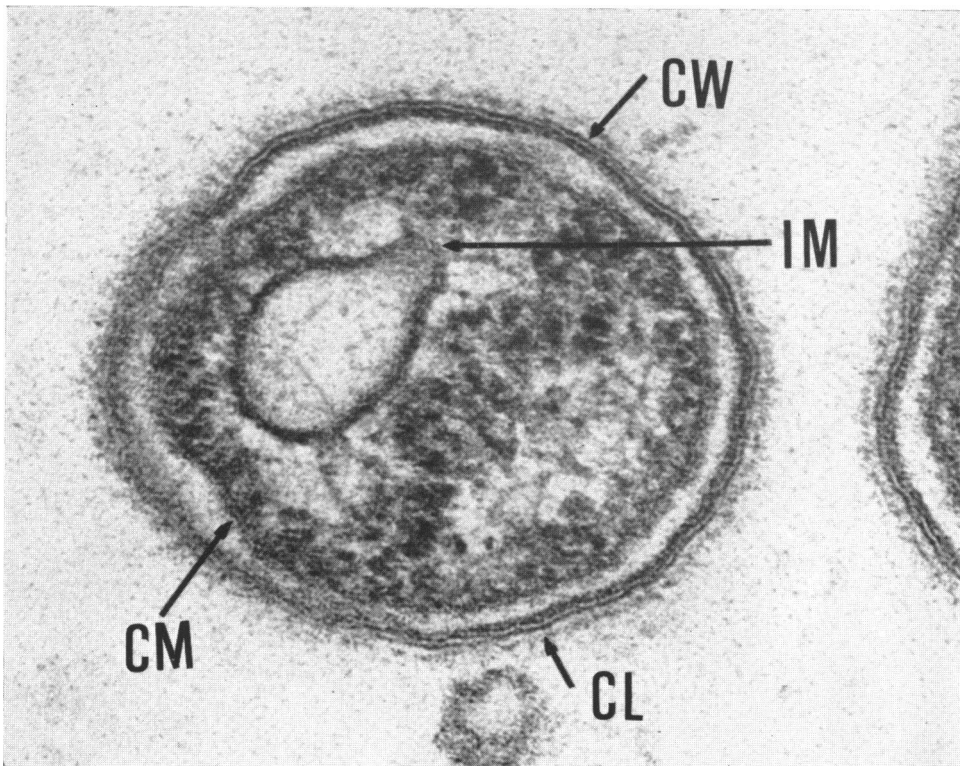


FIG. 1. Thin section of *Rickettsia prowazekii* in the chick yolk sac fixed in Formalin and then in buffered osmium tetroxide, embedded in Epon, and stained with aqueous uranyl acetate and lead citrate. The capsule-like layer (CL), cell wall (CW), cytoplasmic membrane (CM), and intracytoplasmic membrane (IM) are denoted by arrows. $\times 185,000$.

been described by workers in three laboratories (D. R. Anderson et al., *J. Bacteriol.* 90:1387, 1965; L. Ya. Shkolnik, B. G. Zatulovsky, and N.

typhus rickettsia: a capsule-like layer, a five-layered cell wall, and an intracytoplasmic membranous organelle.

Two rickettsial preparations were examined, *R. prowazekii*-infected chick yolk sacs and partially purified rickettsial suspensions. Yolk-sac fragments from embryonated eggs inoculated with

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the Madrid strain of *R. prowazekii* were fixed either in buffered saline containing 5% Formalin and then Caulfield's fixative, or in glutaraldehyde and then osmium tetroxide (D. D. Sabatini, K. Besch, and R. J. Barnett, *J. Cell Biol.* 17:19, 1963), were dehydrated in graded dilutions of ethyl alcohol, and were embedded either in an Epon-Araldite mixture (H. H. Mollenhauer, *Stain Tech.* 39:111, 1964) or in Epon (J. H. Luft, *J. Biophys. Biochem. Cytol.* 9:409, 1961). Sections were stained with aqueous uranyl acetate or lead citrate, or both. For the second preparation, infected yolk sacs were mixed in a blender with buffered saline containing 4% Formalin, and the rickettsiae were then partially purified by differential centrifugation and ether extraction before fixation and embedment in Vestopal (E. Kellenberger and A. Ryter, *J. Biophys. Biochem. Cytol.* 4:323, 1958).

In all thin sections of typhus rickettsiae in yolk sacs, regardless of the fixation, embedding, or staining procedure employed, we found a faintly staining, rather amorphous layer which surrounded the sharply defined cell wall (Fig. 1).

This capsule-like layer was absent or much reduced in sections of partially purified rickettsiae (Fig. 2). As yet, the nature of the external layer is unknown, but perhaps this material is the "soluble antigen" which is readily removed from rickettsiae by differential centrifugation and ether extraction (N. H. Topping and M. J. Shear, *National Institute of Health Bulletin* 183, p. 13, 1945). Our observations are consistent with the conclusion by Balayeva, Zubok, and Nikolskaya (*Acta Virol.* 10:161, 1966) that the soluble antigen of typhus rickettsiae "presumably is weakly bound to the surface of rickettsiae and can be easily released into the suspending medium."

A second feature which has not been described previously is the five-layered cell wall. At low magnification, only three layers were observed, the outermost dark layers and a middle light layer. However, at higher magnification, three dark layers were seen, the outermost layer and two inner layers which were separated by a very thin light layer. This type of cell-wall architecture resembles that reported for *Escherichia coli* (S. de Petris, *J. Ultrastruct. Res.* 12:247, 1965; R. G.

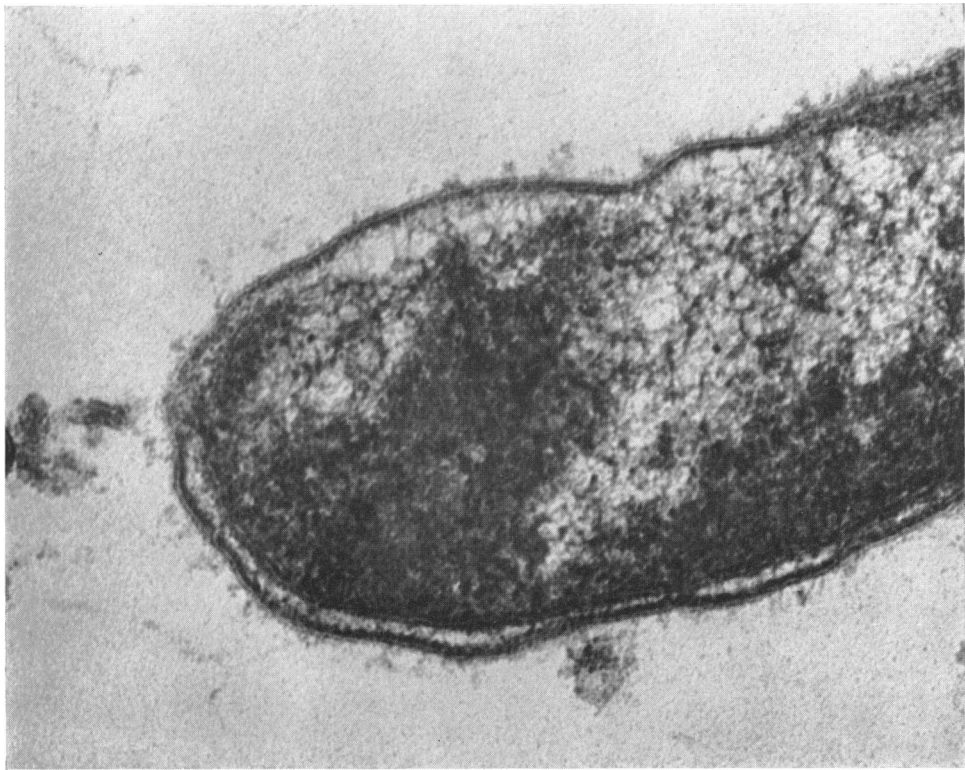


FIG. 2. Thin section of *Rickettsia prowazekii* from suspension partially purified by differential centrifugation and ether extraction. This organism has much less material in the capsule-like layer than does the organism shown in Fig. 1. $\times 147,000$.

E. Murray, P. Steed, and H. E. Elson, *Can. J. Microbiol.* **12**:547, 1965).

A micrograph showing simple invagination of the cytoplasmic membrane of *R. quintana* has been published by S. Ito and J. W. Vinson (*J. Bacteriol.* **89**:481, 1965), and mention of the infolding of the cytoplasmic membrane of *R. prowazekii* was made by Anderson et al. (*J. Bacteriol.* **90**:1387, 1965); however, the presence of an intracytoplasmic membranous organelle, such as that shown in Fig. 1, has not been reported

for rickettsiae. This organelle was found infrequently in *R. prowazekii*, but it has been observed on a number of occasions. Perhaps, this membrane is an extension of the cytoplasmic membrane, but, as yet, this connection has not been demonstrated. Recently, E. H. Cota-Robles (*J. Ultrastruct. Res.* **16**:626, 1966) described similar intracytoplasmic membranous structures in magnesium-deficient *E. coli* and showed in addition that this cytoplasmic structure was indeed derived from the cytoplasmic membrane.