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BRIEF NOTE

CONNECTION BETWEEN INCLUSION MEMBRANE AND RETICULATE BODIES OF *Chlamydia psittaci* : FURTHER ELECTRON MICROSCOPIC OBSERVATIONS OF INCLUSIONS ISOLATED FROM THE HOST CELLS WITHOUT USE OF ALBUMIN

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Recently a procedure for isolation of the intracytoplasmic inclusions of *Chlamydia psittaci* was established (1). The electron microscopic observations of the isolated inclusions revealed that the reticulate bodies (RB) of the organisms were connected with the inner surface of the inclusion membrane by means of the projections. The isolated inclusions were, however, very unstable even in 0.25M sucrose which is widely used for isolation of organelles from various types of cells and tissues, but stabilized in the presence of 5% albumin in the suspension. To examine the connection between the RB and the inclusion membrane in the inclusions prepared without use of albumin, further observations were carried out.

L-cells in suspension cultures were infected with the meningopneumonitis strain of *Chlamydia psittaci* at 10 inclusion forming units per cell and harvested at 20 hours after infection. After several washings with phosphate buffer saline (2), the cells were suspended in 0.2M sucrose-ET buffer (1 mM EDTA-3Na in 10 mM Tris-HCl buffer, pH 7.3) and homogenized for 20 strokes in a Dounce tissue grinder 15 ml in size. The homogenate was transferred into a syringe 10 ml in size and filtrated through a stainless-steel, 2,000 mesh screen set in a holder of Millipore filter 2.5 cm in diameter. The filtrate was directly dropped into cold 5% glutaraldehyde in 0.2M cacodylate buffer (pH 7.3) to make a final concentration of 2.5% glutaraldehyde. The mixture was kept in ice for 30 minutes followed by centrifugation at 1,500×g for 10 minutes to collect the inclusions into a pellet. To enhance the specimen opacity, 4% tannic acid in 0.1M cacodylate buffer was overlaid on the pellet for 3 hours by the method previously reported (3). After overnight washing, the sample was fiixed again with 1% osmium tetroxide in 0.1M cacodylate buffer for 2 hours in ice, dehydrated in an ethanol series and then embedded in the low viscosity epoxy resin described by Spurr (4). Thin sections cut on a Porter-Blum microtome were mounted on a copper grid without film and then stained doubly with uranyl acetate and lead citrate solutions. The specimens were examined with a Hitachi H-500 electron microscope with a 20 μm objective aperture at 75

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or 100 kV. When the specimen was examined three-dimensionally, sections with purple in interference color were cut and stereo pairs of micrographs were taken at tilting angles of ±8° with a Hitachi HU-12A electron microscope at 100 kV.

The isolated inclusions retained nearly normal in their morphology by the fixation immediately after filtration, while the inclusion membrane was broken at several places (Fig. 1). RB in each inclusion appeared to be normal in their shape, but the opacity of some RB were remarkably higher than the others, suggesting the different permeability of the RB envelopes against tannic acid

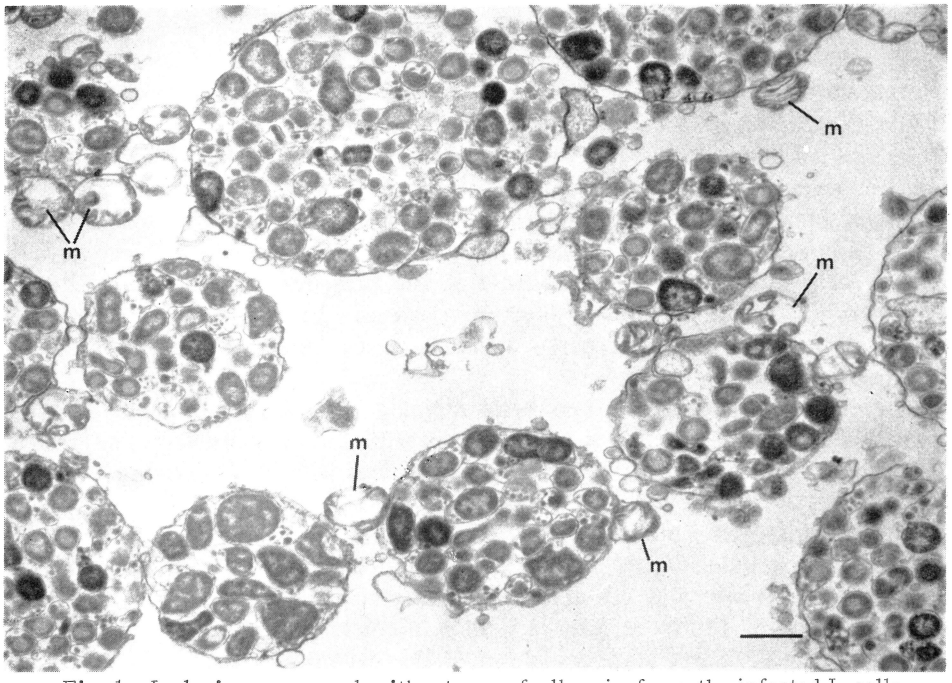

Fig. 1. Inclusions prepared without use of albumin from the infected L-cells at 20 hours postinoculation. Inclusion and RB appeared to be normal in the morphology, while the inclusion membrane is broken at some places. m : mitochondria. Bar indicates 1 μm.

after glutaraldehyde fixation. The association of mitochondria on the outer surface of the inclusion was frequently observed. At higher magnification, the connection between RB and inclusion membrane by means of the projections was clearly seen (Fig. 2a and b). The RB in Fig. 2a is cut almost perpendicularly against the projections, with which the RB appears to be tightly connected with the inclusion membrane. It is clear that the projections on RB located within a limited area of the envelope and that the projections protrude from cytoplasmic membrane of the RB and pass through the gaps between the

cytoplasmic membrane and the cell wall and between the cell wall and the inclusion membrane continuously. In a stereo pair shown in Fig. 2b, the projections which pierce the inclusion membrane and protrude outside of the inclusion are clearly seen.

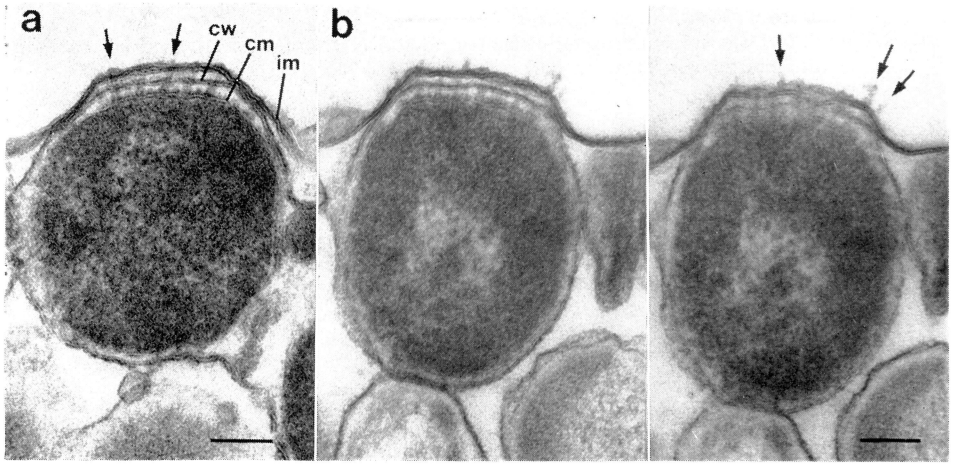

Fig. 2. Connection sites between RB and inclusion membrane by means of the projections. (a) : the projections cylindrical in shape are clearly seen (arrows). cm : cytoplasmic membrane of the RB. cw : cell wall of the RB. im : inclusion membrane. (b) : a stereo pair of the connection site. The projections pointed with arrows pierce the inclusion membrane. Bars indicate 100 nm.

These results confirm the fact, as previously reported (1), that the RB are connected by means of the projections with the inner surface of the inclusion membrane which is derived from the host plasma membrane as the infectious elementary bodies are phagocytized (5) and show that the connection between the RB and the inclusion membrane is well preserved in spite of the absence of albumin which was used to stabilize the inclusion membrane in the native form in the previous experiment (1).

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