Electron Microscope Observations  
of the Membrane Surrounding Polyhedral Inclusion Bodies  
of Insects  
Brief Report  

By  
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With 4 Figures  
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Summary  
Paracrystalline inclusion bodies (polyhedra) which occlude insect viruses are  
bounded by two membrane units which are closely appressed. The membranes  
appear to be similar to those of cisternae found in the nucleus. These cisternae  
were observed associated with polyhedra in various stages of condensation and  
appression.  

Paracrystalline inclusion bodies of a proteinaceous nature have been reported  
associated with a variety of plant (7) and animal viruses (5). These differ from  
the paracrystalline inclusion bodies of insects in that they do not occlude virus,  
whereas in insects viruses are occluded (3). One exception is apparently adenovirus  
type 5 (6, 9), where occasionally virus is found within paracrystalline bodies  
associated with virus formation. In addition COUCH (1) has reported that shrimp  
have a baculovirus which is occluded within a paracrystalline inclusion body.  
Insect viruses found occluded in paracrystalline proteinaceous bodies are the  
nuclear polyhedrosis and granulosis viruses, the cytoplasmic polyhedrosis viruses  
and the entomopox viruses.  
The function of paracrystalline inclusion bodies in which viruses are occluded  
has been suggested to be one of virus protection. In insects the inclusion bodies  
or polyhedra are liberated into the environment until they are ingested by another  
host. The paracrystalline structure surrounding the viruses would serve to protect  
the virus from environmental factors, and insure that a sufficient quantity of  
the virus would be ingested with one polyhedra to produce infection.
In baculovirus infections protection of the virus seems to be provided by a membranous structure surrounding the polyhedra. Possible stages in the formation of this membrane were observed in electron microscope studies of two baculovirus within *Spodoptera exigua* (2). The mechanism was the same for both viruses.

Fourth instar larvae of *Spodoptera exigua* were inoculated with one of two nuclear polyhedrosis baculoviruses (NPV). One virus was the baculovirus isolated from *S. exigua*, the second virus was that originally isolated from *Autographa californica*. Control larvae were inoculated with sterile distilled water. After 4 to 6 days, tissues were excised and processed for electron microscopy as previously described (2). In addition field collected *S. exigua* NPV was passed through *S. exigua* larvae. Tissues were homogenated and centrifuged (2000 × g, 5 minutes). The polyhedra obtained were stored in phosphate buffer (pH 7.0, 0.5 M) at 5°C until ready for use. These polyhedra were then centrifuged at 3000 rpm for 15 minutes. The pellet was resuspended in 1 ml of 0.02 M Na2CO3 + 0.9 per cent w/w NaCl for 15—30 minutes. One milliliter of 8 per cent glutaraldehyde was added, and the suspension recentrifuged (3000 rpm, 15 minutes). The pellet obtained was rinsed in buffer, postfixed in 1 per cent OsO4 in 0.1 M phosphate buffer, and processed for electron microscopy in the same manner as the tissue specimens. Observations were made on a Philips EM300 electron microscope.

Proteinaceous paracrystalline structures (polyhedra) appear in the nucleus after virus production and virus enveloping has started. At this stage there are no membranes associated with the polyhedra. Virus particles are occluded and membranes appear associated with some faces of the polyhedra (Fig. 1 E). In the finished polyhedra, membranes completely surround the proteinaceous paracrystalline inclusion body (Fig. 2 B).

The following events were observed in the nucleus along with polyhedra membrane formation. Within the nucleus diffuse, closed cisternae or flattened sacs bounded by a faint membrane on all sides were seen using serial sections (Fig. 1 A, t). These cisternae were elongate and of varying sizes; the maximum width between membranes observed was approximately 140 nm. The bounding unit membrane had an approximate thickness of 50 to 80 Å (Fig. 1 B). Within the cisternae was a diffuse material of a greater density than the nucleoplasm (Fig. 1 A, d). The cisternae were observed in apparent stages of condensation and appression with increasing density of the membranes (arrow, Fig. 1 A) and the appearance of electron lucid areas on either side of the membranes (Figs. 1 E, 2 A, a). The electron lucid area is bounded by a protein interface that is not easily visible on these micrographs. (They were underdeveloped to show membrane structure.) This interface is visible both around the condensing cisternal structures (Fig. 2 A, i) and the completed polyhedra (Fig. 2 B, i). The increasing density of the membranes could possibly result from the incorporation of the material contained within the cisternae as condensation occurs. The membranes at this stage appear globular but still measure around 80 Å in width (Fig. 1 C).

The condensing cisternae were associated with developing polyhedra in an apparently random fashion, since multiple layers of cisternae appear to be associated with any one face of the polyhedra (Figs. 1 A, 1 E). These condensing cisternal membranes were observed in close association with small areas of