DEMONSTRATION OF THE POLYHEDRAL VIRUS IN BLOOD CELLS OF SILKWORMS*

by

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The polyhedral virus of the silkworm (*Bombyx mori* L.) has been isolated from the so-called polyhedral bodies. These polyhedral bodies, containing the virus particles inside (about 5% by weight), develop in great numbers within the cell nuclei. The virus particles can be liberated from the polyhedral bodies by alkaline treatment and purified by centrifugation[1,2,3]. A life-cycle for this insect virus has been described[4]. It was suggested that the virus develops within a membrane from a small sphere to a rod-shaped particle (Fig. 1). These rods might consist of and disintegrate into small spherical sub-units.

So far, the only polyhedral viruses that have been illustrated have originated from polyhedral bodies. The purpose of this contribution is to demonstrate free virus particles in the cell nuclei before they are enclosed in the polyhedral bodies. They cannot be recognized in the light microscope, yet the different stages of their effect on the cells can easily be followed in the dark field. Blood cells of silkworms are particularly suitable for such observations. It was found that, about four days after injection of a lethal dose of virus particles, the nuclei of most blood cells show the blue "ringzone" characteristic of the early stage of the virus disease.

Two methods of preparing the samples for observation in the electron microscope have been used: 1. diseased blood cells were selected in the dark field of a light microscope at a magnification of about 800 with a pipette attached to a micro-manipulator and transferred on to the screens; 2. The diseased blood cells were separated by centrifugation and the concentrated cell suspension was pipetted on to the screens.

The second method is much simpler and was more frequently used in these experiments. In both methods it is advisable to remove the cytoplasm first and transfer naked nuclei to the screens. This can be done easily by suspending the sedimented blood cells in 0.05 *M* NaCl. (1.3 ml serum from six sixth-instar silkworms were used in this experiment). After about 10 min the cell plasm disintegrates, and the nuclei can be separated and concentrated by several short centrifugations at different speeds. The final sediment was taken up in 0.1 ml 0.05 *M* NaCl and pipetted on to the screens, 25 min after bleeding the silkworms. The small droplets on the screens were fixed by exposure to vapours of a 2% OsO_4 solution for three minutes. Many of the naked nuclei burst on the screen and their contents flow out.

About 80 nuclei or parts of them were found on 30 screens. In about 20 of these virus particles were observed and photographed.

The nucleus shown in Figs. 2 and 3, was prepared by the centrifugation method, the one shown in Fig. 4 was selected with the micromanipulator. Comparing Fig. 2, 3,

(*Text continued p. 400*)

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References p. 400.

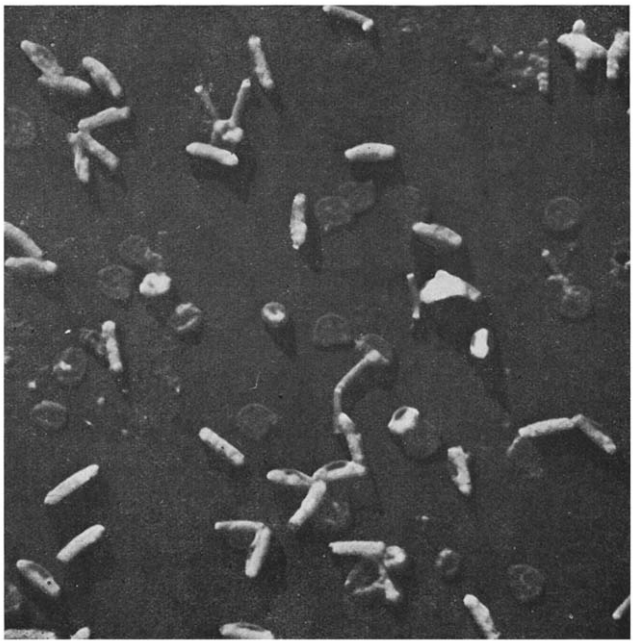

Fig. 1. Purified virus preparation isolated from polyhedral bodies of silkworms. Spherical developing stages, rods within and without membranes, empty round and tubular membranes. Magnification 25,000 ×. Uranium shadowing 6 mg. 12 cm. 1:4. Negative print.

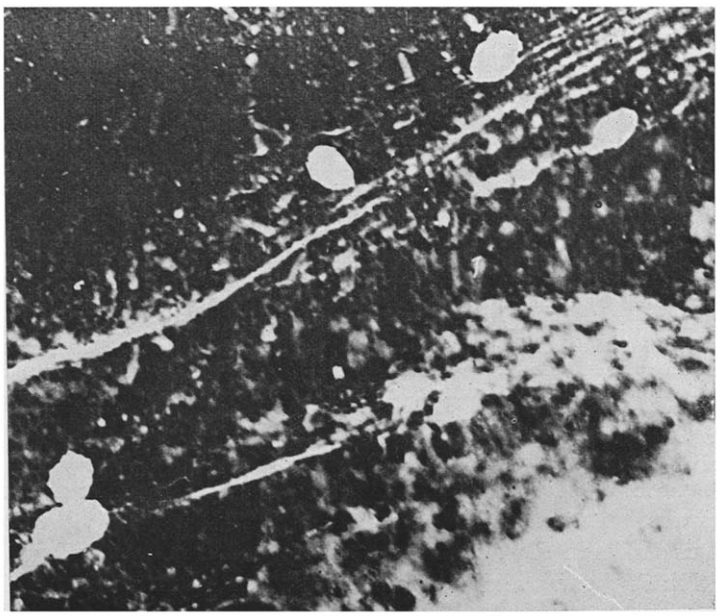

Fig. 2. Part of virus infected blood cell nucleus. Rods and spherical developing stages and an empty tube shaped membrane (top left) can be seen. Magnification about 25,000 ×. Negative prints.

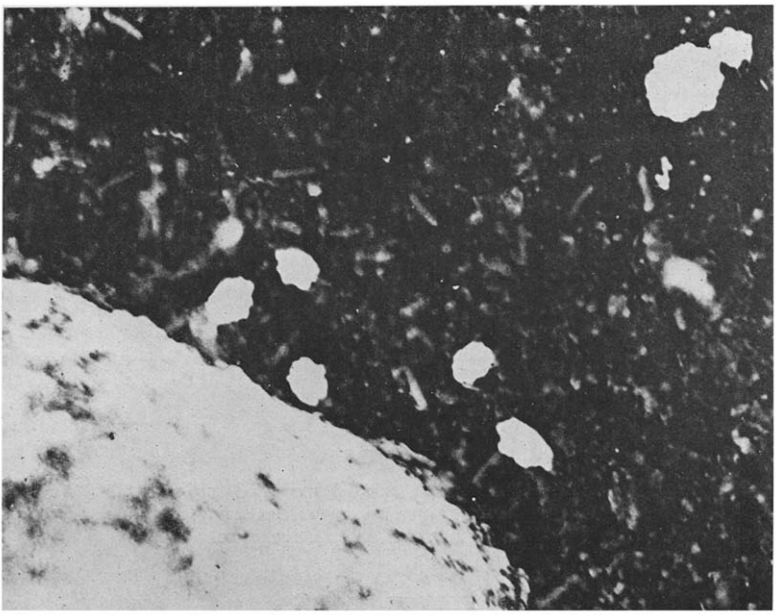

Fig. 3. Part of virus infected blood cell nucleus. Rods and spherical developing stages can be seen. Magnification about 25,000 ×. Negative prints.

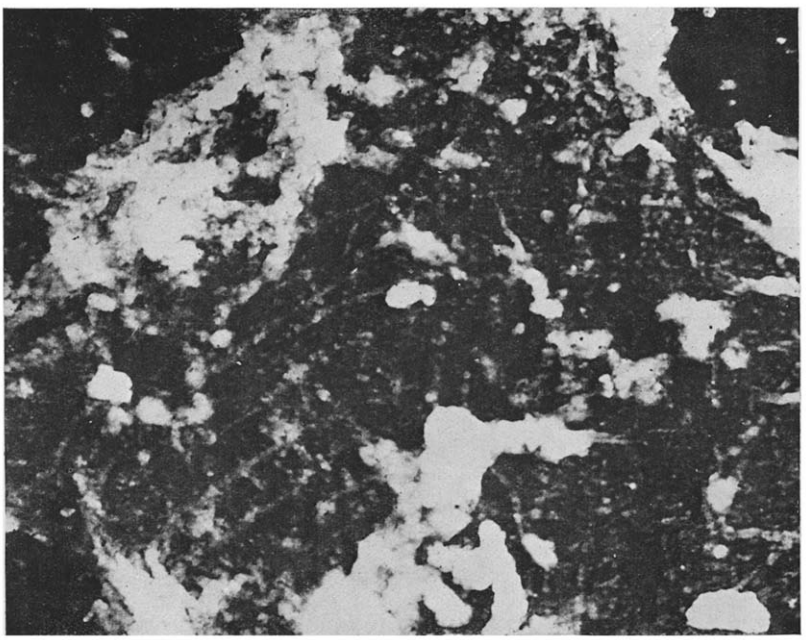

Fig. 4. Part of virus infected blood cell nucleus. Spherical developing stages and rods can be seen. Magnification about 25,000 ×. Negative print.

and 4 with Fig. 1, which shows a purified suspension of virus particles at different stages of development isolated from polyhedral bodies, one can observe particles of similar shape and size*. The spherical developing stages, rods within membranes and one empty tubular membrane (Fig. 2 top left) can be seen. The nucleus shown in Fig. 4, represents an early stage of the disease and contains mostly spherical particles.

F. T. BIRD of this laboratory arrived at similar conclusions from a study of thin sections of virus infected sawflies (Hymenoptera)[5].

SUMMARY

A comparison of purified virus preparations isolated from polyhedral bodies of silkworms with preparations from diseased blood cells indicates that similar rods, spherical developing stages, and tube-shaped virus membranes are present in both.

RÉSUMÉ

La comparaison d une préparation purifiée isolée de polyhèdres de vers à soie avec des préparations provenant de cellules de sang infectées indique que des baguettes, des stages sphériques et des membranes tubulaires de virus sont présents dans tous les deux.

ZUSAMMENFASSUNG

Ein Vergleich von gereinigten Virus Präparaten, isoliert aus Polyedern von Seidenraupen, mit Präparaten von erkrankten Blutzellen, lässt erkennen, dass ähnliche Stäbchen, kugelige Entwicklungsstadien und schlauchförmigen Membranen in beiden Präparaten vorhanden sind.

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* The slender projections that can be seen at the end of some of the virus rods in Fig. 1, and the isolation and chemical nature of the membranes, will be discussed in forthcoming papers.