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ELECTRON MICROSCOPE OBSERVATIONS OF NUCLEAR POLYHEDRA FROM *MALACOSOMA NEUSTRIA* (LEPIDOPTERA: LASIOCAMPIDAE[1]

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The nuclear polyhedral bodies from *Malacosoma neustria* are enclosed within a membrane. The diameter of the nuclear polyhedra varies from 0.9 to 2.8 μ with an average of 1.8 μ. In the nuclear polyhedra the rod-like virus particles occur both singly and in bundles. The single virus rods are enclosed within two membranes, namely the intimate membrane and the developmental membrane. The virus rods which occur in bundles have an intimate membrane just like the single virus rods, whereas the developmental membrane encloses the whole bundle. The virus rods are closely packed by the intimate membrane and between the intimate and the developmental membrane is a space. The diameter of the virus rods without membranes, determined from sectioned polyhedra, is about 25 mμ and the length 250 mμ.

INTRODUCTION

The presence of a nuclear polyhedrosis in *Malacosoma* (= *Bombyx*) *neustria* (Linnaeus) was reported by KOVAČEVIĆ as early as 1926 and has been regularly observed by several authors (HENZE, 1935; ARVY, 1953; BILIOTTI, 1955; GERSHENSON, 1955; KOVAČEVIĆ, 1956; GRISON, 1956; GÜNTHER, 1958; VAN DAMME & VAN DER LAAN, 1959; LAUX, 1962). According to BERGOLD (1953) the virus particles measure 39 × 333 mμ.

In the present study the structure of the nuclear polyhedra from *M. neustria* was investigated.

MATERIALS AND METHODS

The polyhedral bodies were obtained by differential centrifugation from a homogenate of diseased larvae kindly supplied by Dr. P. A. VAN DER LAAN (Laboratory for Applied Entomology, University of Amsterdam).

The pellet with the polyhedra was mixed with 2% gelatin. After cooling, the gelatin containing the polyhedral bodies was cut in small blocks and fixed in osmium tetroxide (1%) veronal buffer (pH 7.4) at 1°C for two hours. The blocks were dehydrated in graded ethanol concentrations ranging from 50 to 100%. The blocks were then put in a mixture of butyl and methyl methacrylate containing 2% benzoylperoxide as initiator. They were finally transferred into gelatin capsules containing a drop of a prepolymerized (at 80°C) mixture of butyl and methyl methacrylate to be polymerized in an incubator at 60°C. Sections about 250–500 Å thick were cut with a Porter-Blum ultramicrotome using a glass knife. The sections were mounted on formvar-covered copper grids and stained with 2% potassium permanganate (LAWN, 1960).

To separate the virus particles from the polyhedral body protein in which they are embedded, a thin layer of the polyhedral suspension in distilled water on a formvar-covered copper grid coated with carbon was treated with a

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solution of 0.1 N sodium carbonate for 5 and 10 minutes, and then washed three times with distilled water. These preparations were either shadowed with palladium or negatively stained with phosphotungstic acid at pH 5.5, according to BRENNER and HORNE (1959).

Most electron micrographs were taken with a Siemens Elmiskop 1 at 80 KV and some with a Philips electron microscope type EM 100 at 80 KV.

RESULTS AND CONCLUSIONS

The diameter of the nuclear polyhedral bodies of *Malacosoma neustria* as measured from shadowed preparations varies from 0.9 to 2.8 μ, with an average of 1.8 μ (Fig. 1A). According to BENZ (1963) the diameter of the nuclear polyhedra found in the related species *Malacosoma alpicola* (Staudinger) ranges from 0.6 to 4.5 μ.

Nuclear polyhedra of *M. neustria* treated with sodium carbonate show the presence of single virus particles and bundles (Figs. 2A–D, 3A). The single virus rods are enclosed within two membranes, namely the intimate membrane and the developmental membrane (Fig. 3C). The virus rods which occur in bundles have an intimate membrane just like the single virus rods, whereas the developmental membrane encloses the whole bundle (Figs. 3B, D, E). The virus rods are closely packed by the intimate membranes (Fig. 3D); as a result of dissolution of the intimate membranes the virus rods are curved (Figs. 3H, I). Figs. 3F, G show intimate membranes of which the virus rods are dissolved. These empty, flattened intimate membranes (tube-shaped membranes) were first described by BERGOLD (1950) and later on by many other investigators. The developmental membranes (Figs. 3C, D) show a structure quite different from the polyhedral (Figs. 2A–C) and the intimate membranes (Figs. 3F, G).

The polyhedral bodies are enclosed within a membrane (Figs. 2A–C). The liberation of the solubilized polyhedral protein showed that the membrane is not formed by the action of the sodium carbonate upon the protein (Fig. 2D). HUGHES (1950), BERGOLD (1951), YAMAFUJI *et al.* (1952), SMITH & XEROS (1953) and BENZ (1963) already showed polyhedral membranes in alkali-treated preparations of nuclear polyhedral bodies from other lepidopterous species. Electron micrographs of ultra-thin sections of nuclear polyhedral bodies (MORGAN *et al.*, 1955; BERGOLD, 1963) do not reveal these polyhedral membranes. BERGOLD (1958) mentioned, that the polyhedral membranes are probably artifacts produced by the alkali treatment that denatures the surface of the polyhedra. However, the polyhedral membranes (Figs. 2A–C, 3A) resemble those of the intimate membranes (Figs. 3F, G).

Electron micrographs of sectioned polyhedral bodies show that the rod-like virus particles occur both singly and in bundles randomly in the polyhedral protein (Figs. 1B, 4A–G). The single virus particles and virus bundles are clearly surrounded by the developmental membranes, which are about 5 mμ thick and much lesser dense than the surrounding protein. The intimate membranes, which are closely packed to the virus rods, are not to distinguish from the virus rods, just like the polyhedral membranes from the polyhedral protein. These membranes are probably too thin to be observed in ultra-thin sections. Only the developmental membranes show a good contrast after the treatment with potassium permanganate. BERGOLD (1963) mentioned a space of lesser

density between the developmental membrane and the intimate membrane, and between the intimate membrane and the virus rod. Numerous electron micrographs of our sectioned polyhedral bodies just show a space of lesser density surrounding the dark virus rods, bordered by the white developmental membrane.

Longitudinal and cross sections through virus rods show a dense and homogeneous mass. Spherical or disc-shaped subunits were never found, nor a central channel, protrusion at one end of the virus rod, or empty developmental or intimate membranes. This is in accordance with observations of BERGOLD (1963) for nuclear polyhedra of other Lepidoptera.

In many sections of polyhedral bodies the virus particles with their developmental membrane had disappeared from the polyhedral protein leaving an open space. Probably these virus particles together with their developmental membrane had been lost during sectioning of the polyhedral bodies. From this observation may be concluded that the virus particles with their developmental membrane are not completely integrated with the polyhedral protein. This accords with the fact that the structure of the polyhedral protein (Fig. 4) is different from that of the virus particles as has been pointed out by MORGEN *et al.* (1955, 1956), DAY *et al.* (1958), and BERGOLD (1963).

The bundles are more frequent than single virus rods. The maximum number of virus particles observed within one bundle was 7 (Fig. 4D). This is in contrast with what has been found by BENZ (1963) for nuclear polyhedral viruses in the related species *Malacosoma alpicola* and *M. disstria* Hübner. In those cases the polyhedral bodies were reported to contain usually two virus particles surrounded by a common developmental membrane.

The diameter of the virus rods in sectioned polyhedra of *M. neustria* is about 25 mμ and the length 250 mμ. In preparations treated with sodium carbonate the virus particles without the developmental membrane are 50 × 350 mμ. The diameter and length of virus particles treated with sodium carbonate are larger than those of particles in sectioned polyhedral bodies. This is probably due to the fact that the virus particles swell during the sodium carbonate treatment and are subsequently flattened by surface tension during drying. According to BERGOLD (1953) the virus particles without the developmental membrane from *Malacosoma neustria* are 39 × 333 mμ, from *M. americanum* (Fabricius) 45 × 316 mμ, and from *M. disstria* 46 × 324 mμ. STEINHAUS (1949) reported that the virus particles without the developmental membrane from *M. disstria* measure 40 × 315 mμ, from *M. pluviale* (Dyar) 40 × 350 mμ, and BENZ (1963) from *M. alpicola* 35–41 × 270–370 mμ.

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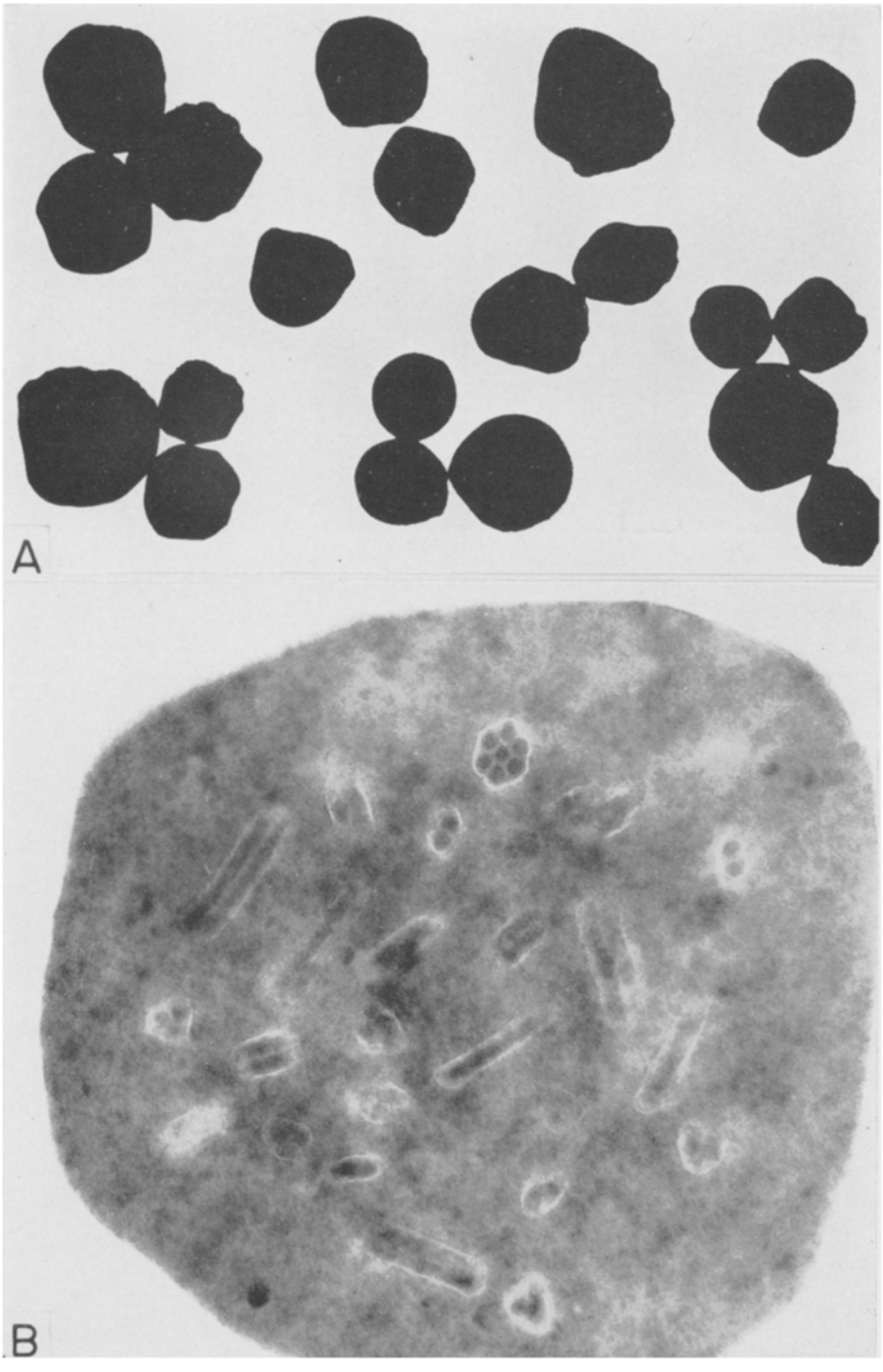

FIG. 1. A. Shapes of nuclear polyhedra from larvae of *Malacosoma neustria* as observed in shadowcast preparations. × 10,000.
B. Section of a polyhedral body. Cross and longitudinally sectioned virus bundles are embedded randomly. × 80,000.

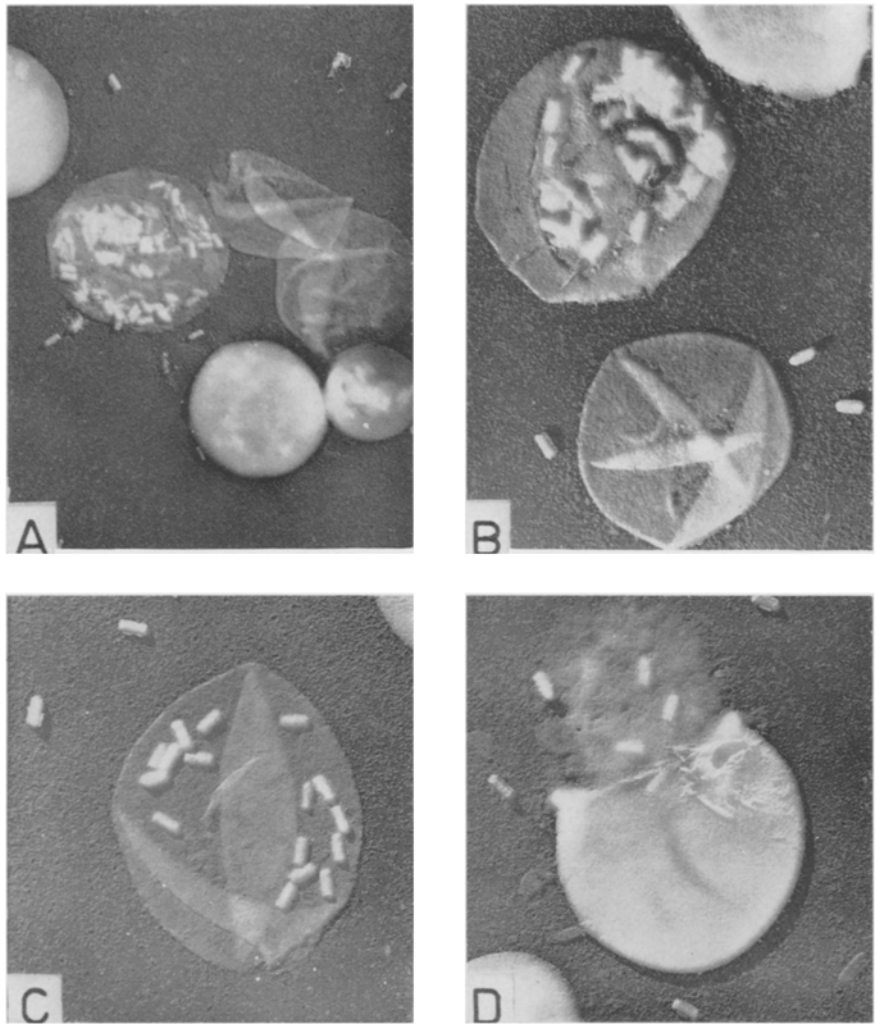

FIG. 2. Nuclear polyhedra treated with sodium carbonate and shadowed with palladium. A. Partly dissolved polyhedral bodies and empty polyhedral membranes containing single virus particles and bundles. × 6000. B–C. × 10,000. D. A polyhedral body of which the polyhedral protein flows out. × 10,000.

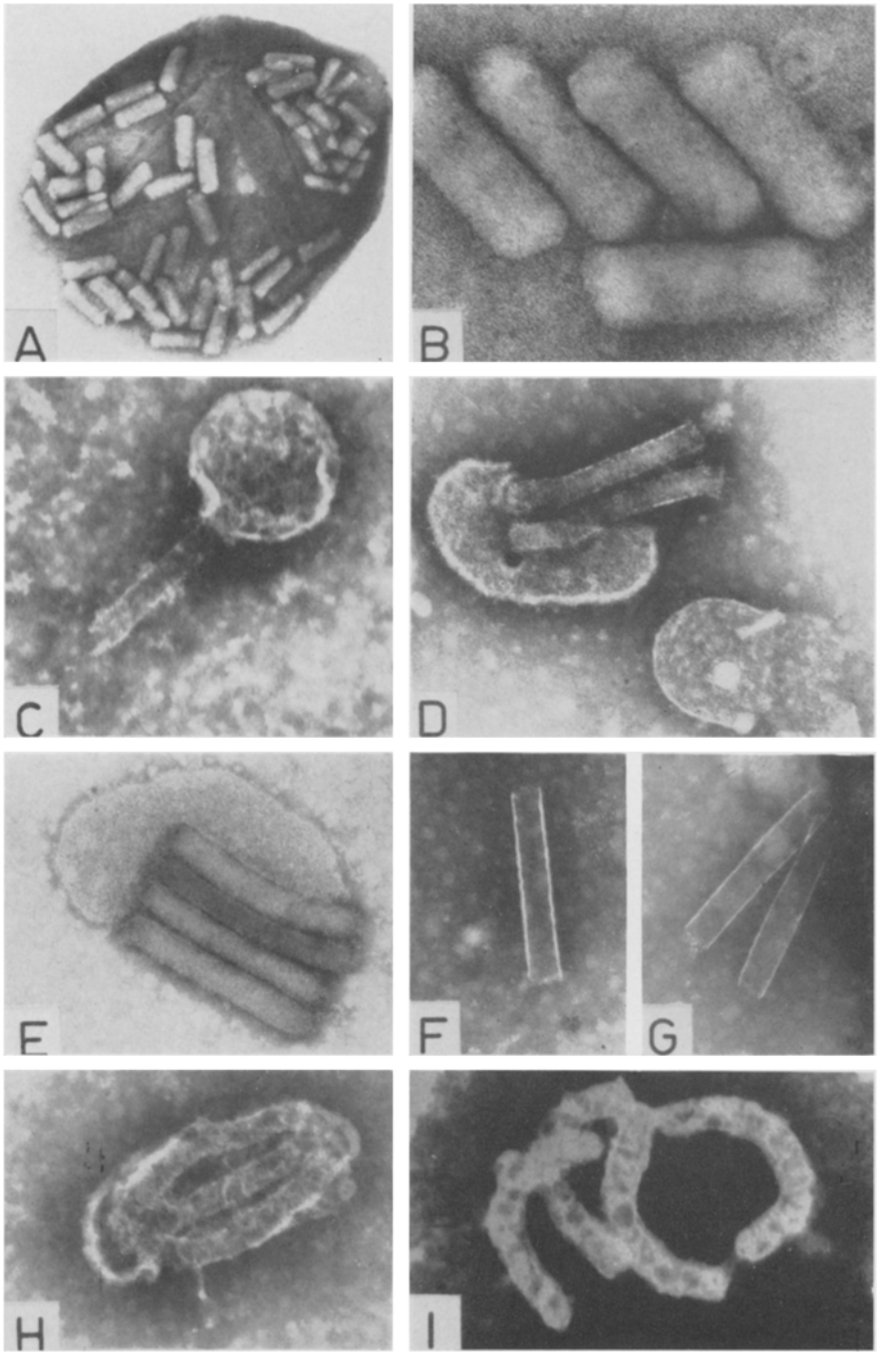

FIG. 3. Nuclear polyhedra treated with sodium carbonate using the negative staining technique. A. Polyhedral body showing the polyhedral membrane and the virus bundles. × 20,000. B. Entire virus bundles. × 80,000. C. A single virus particle of which the developmental membrane is partly dissolved. × 80,000. D. A virus bundle with two virus particles of which the developmental membrane is partly dissolved. × 80,000. E. A virus bundle with four virus particles of which the developmental membrane is dissolved. × 80,000. F–G. Three empty intimate membranes. × 80,000. H–I. Curved virus rods of which the intimate membrane has been dissolved. × 80,000.

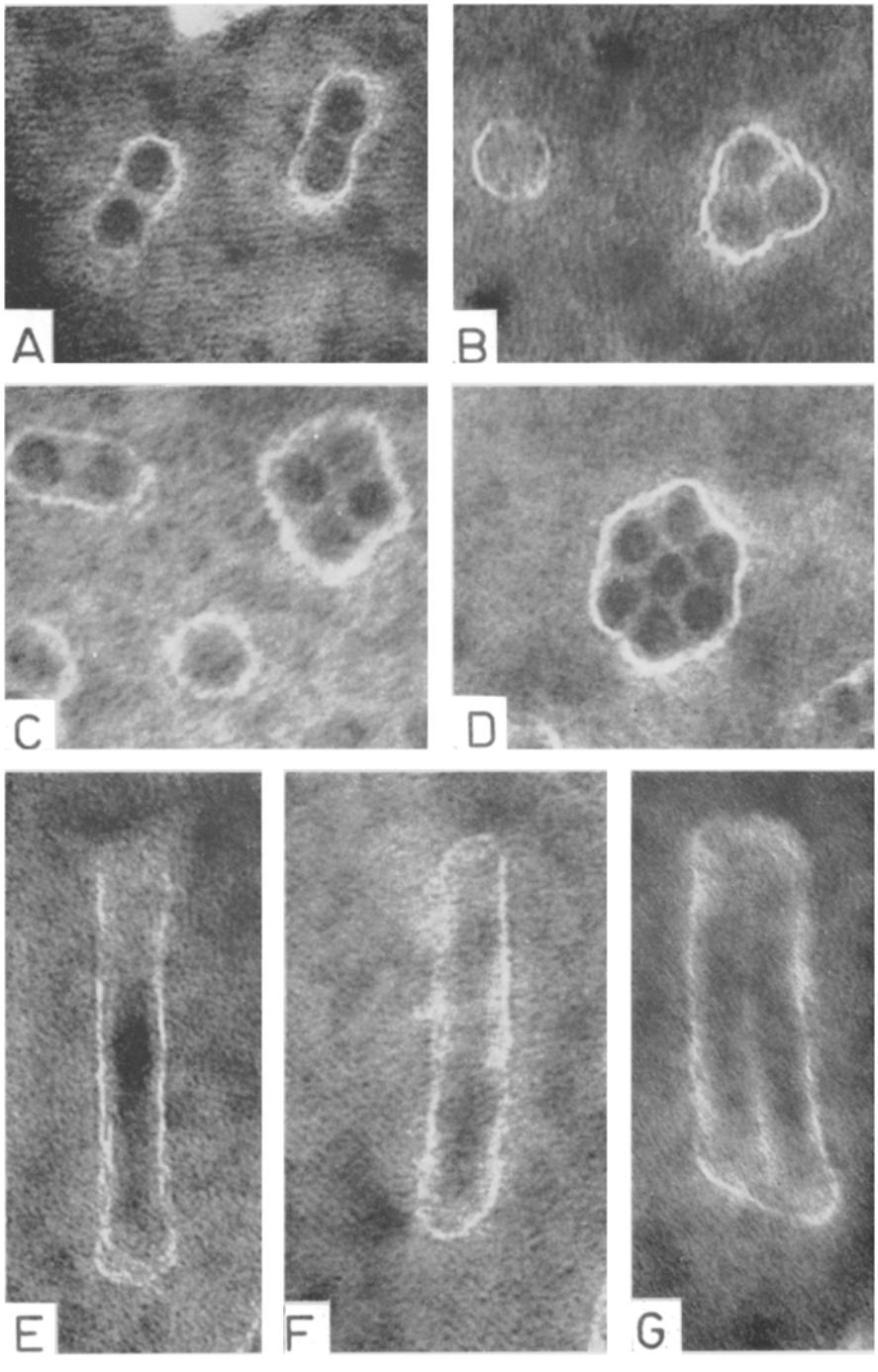

FIG. 4. A–D. Cross sections of virus particles occurring both singly and in bundles. × 200,000.
E–G. Longitudinal sections of virus particles. × 200,000.