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ELECTRON MICROSCOPY OF DNA-CORES IN NUCLEAR POLYHEDRAL VIRUSES[1]

Elektronenmicroscopie van deoxyribonucleïnezuur in kernpolyëdervirussen

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Electron micrographs show the presence of DNA-cores in the virus rods isolated from nuclear polyhedral bodies from *Barathra brassicae*, *Adoxophyes reticulana* and *Orgyia antiqua*. The DNA-cores in the virus rods are enclosed by virus protein.

INTRODUCTION

In the nuclear polyhedral bodies in several lepidopterous species the rod-like virus particles occur both singly and in bundles. Several investigators, summarized by BERGOLD (1963), have shown by means of chemical analyses that the virus rods contain desoxyribonucleic acid (DNA).

In the present study the occurrence of DNA-cores in alkali treated preparations of polyhedral bodies from three species of Lepidoptera viz. *Barathra brassicae* (L.), *Adoxophyes reticulana* (Hb.) and *Orgyia antiqua* (L.) was observed with the electron microscope.

MATERIALS AND METHODS

The nuclear polyhedral bodies from diseased larvae of *B. brassicae*, *A. reticulana* and *O. antiqua* were obtained by differential centrifugation. To separate the virus particles from the polyhedral protein in which they were embedded, a thin layer of the polyhedral suspension in distilled water on a formvar-covered copper grid was treated with a solution of 0.1 N sodium carbonate for 5 and 10 minutes, and then washed three times with distilled water. The preparations were shadowcasted with palladium or negatively stained with phosphotungstic acid adjusted to pH 5.5. The electron micrographs were taken with a Siemens Elmiskop 1 electron microscope at 80 KV.

RESULTS

By treating nuclear polyhedra with sodium carbonate the virus particles or virus bundles are liberated from the polyhedral protein. The virus particles were found to occur individually (*A. reticulana*, Fig. 1A, and *O. antiqua*, Fig. 1F) or as bundles (*B. brassicae*, Fig. 1C) in the preparations.

Treatment with sodium carbonate also disintegrated the membranes enclosing the virus particles (Fig. 1F) and finally the virus protein was dissolved resulting in the liberation of fibrillar or rod-shaped structures, presumably the DNA-cores of the virus particles (Fig. 1B, D, E, G, H).

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DISCUSSION

It is known (BERGOLD, 1950) that single virus rods from nuclear polyhedra are enclosed within two membranes, namely the inner (intimate) and the outer (developmental) membranes. The virus rods which occur in bundles have an inner membrane just like the single virus rods, whereas the outer membrane encloses the whole bundle.

By subjecting the virus rods to alkali treatment they begin to disintegrate into spherical or disc-shaped subunits (BERGOLD, 1953; TOMLIN & MONRO, 1955; KRIEG, 1957). KRIEG (1961) showed that these subunits have a hole in the centre, which could form a central channel in the virus rods. This central channel can be observed in the virus rod of *O. antiqua* depicted in Fig. 1F; it has a diameter of about 17 mμ and a length of about 267 mμ. BERGOLD (1953) and BIRD (1957) reported that the virus rods on alkali treatment showed occasionally a protrusion at one end with a diameter of about 10 mμ. KRIEG (1961) supposed that this protrusion was a part of the central channel being probably the DNA-core of the virus particle. A similar protrusion can be seen in Fig. 1G.

The dimensions of the DNA-cores of the virus particles from the nuclear polyhedra of *O. antiqua* as measured in the present investigation were 17×267 mμ and those from *B. brassicae* 15×280 mμ. The diameters of these DNA-cores correspond approximately with that of the central holes in the subunits of the virus rods present in the nuclear polyhedra from *Aporia crataegi* (L.), viz. 10–20 mμ (KRIEG, 1961). The lengths of the DNA-cores are smaller than those of the respective virus rods which may indicate that the DNA-core in the virus rod is enclosed at both ends by the coiled virus protein (PONSEN *et al.*, 1964).

The DNA-cores observed in the preparations from *A. reticulana* were more flexuous (Fig. 1B) than those from *B. brassicae* (Fig. 1D, E) and *O. antiqua* (Fig. 1G, H). The DNA-cores in Fig. 1B appear similar to the picture detained from salmon sperms, where it is reported to be of the double-stranded type (KLEINSCHMIDT & ZAHN, 1960; LANG *et al.*, 1960).

Electron micrographs of ultra-thin sections from all the three nuclear polyhedra studied above did not reveal a DNA-core in the virus rods, as also reported by BERGOLD (1963) for other nuclear polyhedral viruses. However, DAY *et al.* (1958) reported that some virus rods causing nuclear polyhedrosis in *Pterolocera amplicornis* Wlk., show an indication of osmiophilic central cores.

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SAMENVATTING

Na behandeling van kernpolyëders van *Barathra brassicae*, *Adoxophyes reticulana* en *Orgyia antiqua* met 0,1 N natriumcarbonaat werden met de elektronenmicroscoop de afzonderlijke virusstaafjes (fig. 1A, C, F), alsmede dunnere draad- en staafvormige deeltjes waargenomen (fig. 1B, D, E, G, H). Deze deeltjes stellen het deoxyribonucleïnezuur voor, dat als strengen in het virusstaafje aanwezig is.

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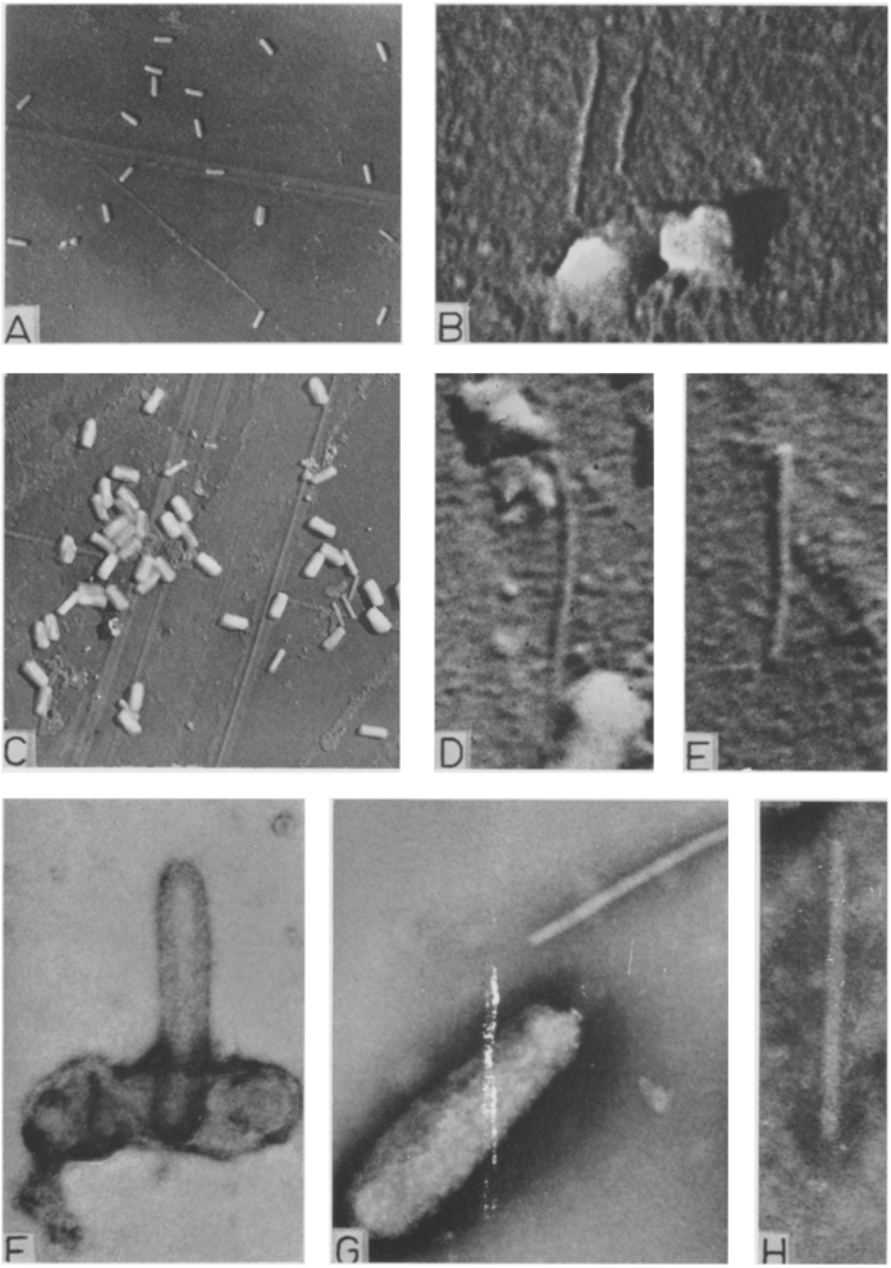
Fig. 1. Nuclear polyhedra treated with sodium carbonate and shadowed with palladium (A-E), or negatively stained with phosphotungstic acid (F-H). A. Single virus particles of *Adoxophyes reticulana.* ×10,000. B. Two virus particles of which the virus protein is partly dissolved showing the DNA-cores; they can also be seen in the photograph reproduced in A. ×100,000. C. Virus bundles of *Barathra brassicae.* ×10,000. D-E. Two DNA-cores occurring in the specimen reproduced in C. ×100,000. F. A single virus particle of *Orgyia antiqua* showing the shedded outer membrane; note the DNA-core in the virus particle. ×120,000. G. A single virus particle with a protrusion and a DNA-core from *O. antiqua.* ×120,000. H. A DNA-core of *O. antiqua.* ×160,000.

Kernpolyëders behandeld met natriumcarbonaat en bestraald met palladium (A-E), of behandeld met fosforwolframzuur (F-H). A. Afzonderlijke virusdeeltjes van Adoxophyes reticulana. *10.000×. B. Twee virusdeeltjes waarvan het nucleïnezuurdeel vrij is gekomen door gedeeltelijke oplossing van het viruseiwit; deze deeltjes komen ook voor in A. 100.000×. C. Bundels van virusdeeltjes van* Barathra brassicae. *10.000×. D-E. Twee nucleïnezuurstrengen voorkomend in hetzelfde preparaat als afgebeeld in C. 100.000×. F. Een afzonderlijk virusdeeltje van* Orgyia antiqua *dat uit het buitenste membraan steekt; in het virusdeeltje is een kern van nucleïnezuur zichtbaar. 120.000×. G. Een aan een uiteinde uitgestulpt virusdeeltje van* O. antiqua *benevens een vrijgekomen nucleïnezuurkern. 120.000×. H. Een nucleïnezuurkern van* O. antiqua. *160.000×.*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