

## Isolation and Purification of a Granulosis Virus from Infected Larvae of the Indian Meal Moth, *Plodia interpunctella*†

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A procedure was developed for purification of a granulosis virus inclusion body produced in vivo in the Indian meal moth, *Plodia interpunctella* (Hübner). Purification was accomplished by differential centrifugation, treatment with sodium deoxycholate, and velocity sedimentation in sucrose gradients. The adequacy of the procedure was confirmed by mixing experiments in which uninfected, radioactively labeled larvae were mixed with infected, unlabeled larvae. After purification, the virus was shown to be free of host tissue, to retain its physical integrity, and to be highly infectious per os. Preparations of purified virus consisted of homogeneous populations of intact inclusion bodies (210 by 380 nm) whose buoyant density was 1.271 g/cm<sup>3</sup> when centrifuged to equilibrium in sucrose gradients. Electron microscopy of thin-sectioned virus or of virus sequentially disrupted on electron microscope grids demonstrated three components: protein matrix, envelope, and nucleocapsid.

Studies on characterization of granulosis (GV) and nuclear polyhedrosis viruses are increasing because these viruses are being considered for use as biological insecticides. The use of virus for insect control is especially important in the case of the Indian meal moth, *Plodia interpunctella*. This insect, a serious pest of stored products, is difficult to control because it is becoming resistant to chemical insecticides such as malathion and synergized pyrethrins (25). Laboratory studies have demonstrated that a GV of the Indian meal moth can effectively control populations of this moth on stored nuts (11), corn, and wheat (18). Although the efficacy of the virus has been established, little information has been gathered on the biology of the virus. In our laboratory, we are concerned with the insecticidal properties of the GV and are trying to determine the biochemical basis of its insect infectivity. Highly purified virus preparations are required for such a study and, as a first step, we have developed procedures for isolating and purifying GV from infected larvae of the Indian meal moth.

Several methods have been described for purification of baculovirus (nuclear polyhedrosis virus and GV) inclusion bodies from their insect hosts (7, 9, 13). Typically, these procedures involve differential centrifugation followed by velocity sedimentation in sucrose gradients. However, the efficiency of purification has not been

examined critically. In two instances, virus purity was assessed only by electron microscopy (7, 14). The quality of purification of the GV of the Indian meal moth described in the present report is based on three criteria: (i) host contamination, (ii) structural integrity of the virus, and (iii) infectivity of purified virus.

### MATERIALS AND METHODS

**Rearing of insects.** A laboratory colony of Indian meal moth was reared on an artificial bran diet in 1-quart (about 0.9-liter) mason jars. The diet consisted of a mixture of coarse-ground wheat, 500 g; shorts, 500 g; wheat germ, 50 g; brewers' yeast, 40 g; sorbic acid, 2 g; and methyl-*p*-hydroxybenzoate, 2 g. This mixture was combined with honey, 120 ml; glycerine, 120 ml; and distilled water, 60 ml (R. Kinsinger, M.S. thesis, Kansas State University, Manhattan, 1975). Environmental conditions for the colony were 27°C, 70% relative humidity, and 15:9 photoperiod.

**In vivo radioactive labeling of uninfected larvae.** Early fourth-instar larvae of Indian meal moth were injected daily for 3 days with 1  $\mu$ l (0.5  $\mu$ Ci) of [<sup>3</sup>H]valine (23 Ci/mmol; Schwarz/Mann, Orangeburg, N.Y.). Injections were made with a microneedle (OD, 25  $\mu$ m) formed by use of a Narishige capillary puller. Microneedles were connected to a 10- $\mu$ l syringe so that speed and quantity of injection could be controlled. Injected larvae were maintained on diet until they reached wandering stage. At this time, they were removed from the diet and used in the mixing experiments described below.

**In vitro radioactive labeling of uninfected larvae.** Homogenates of uninfected larval tissue were labeled in vitro with <sup>14</sup>C by the method of McMillen

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and Consigli (19). One hundred late fourth-instar larvae were disrupted in 20 ml of water in a Pyrex hand homogenizer. Large debris was removed by centrifugation at  $1,000 \times g$  for 30 min. The supernatant mixture was treated with sodium borohydride and [ $^{14}C$ ]formaldehyde for 12 h at room temperature. Unbound isotope was removed by pelleting the larval tissue through a 3-ml shelf of 20% sucrose by centrifugation at 25,000 rpm (SW27 rotor) for 3 h at 10°C. The labeled tissue was suspended in water and frozen at -20°C until used.

**Production of GV.** Indian meal moth diet was seeded with GV at a concentration of 1 mg of virus per kg of diet. Early third-instar larvae were added to the infected diet (100 larvae/100 g of diet) and allowed to feed on the diet until extensive virus production was observed. Infection was determined by a change in the color of the larvae from tan to white. Infected larvae were then removed from the diet and stored at -20°C until used.

**Preparation of  $^3H$ -labeled GV.** At 72, 96, and 120 h after being placed on diet containing GV, larvae were injected with 1  $\mu$ l (0.5  $\mu$ Ci) of the desired isotope (either [ $^3H$ ]valine [23 Ci/mmol] or [ $^3H$ ]thymidine [71 Ci/mmol; Schwarz/Mann]). Injections before this time resulted in reduced incorporation of label into virus. Two to three days after the final injection, larvae were removed from the diet and viral inclusion bodies were purified by the procedure described below.

**Purification of GV inclusion body from infected larvae.** Infected larvae (20 insects) were suspended in 20 ml of water and disrupted in a hand homogenizer until the cuticle was extensively broken. Cells in the crude homogenate were disrupted by homogenization in a Servall Omni-Mixer at a rheostat setting of 70 for 2.5 min. The homogenate was then centrifuged at  $1,000 \times g$  for 30 min. The supernatant was further centrifuged at  $16,000 \times g$  for 30 min. The pellet was gently resuspended in 5 ml of water and filtered through two layers of Whatman no. 1 paper. The filtered preparation was treated with sodium deoxycholate at a final concentration of 1% (vol/vol) for 15 min at room temperature. The detergent was removed by pelleting the virus through a 4-ml shelf of 50% (wt/wt) sucrose by centrifugation at 25,000 rpm (SW27 rotor) for 2 h. The pellet was resuspended in 2 ml of water and banded in a 45 to 60% (wt/wt) sucrose gradient by centrifugation at 20,000 rpm (SW27 rotor) for 2 h. The virus band was recovered from the gradient by collecting 0.5-ml fractions. Absorbance of fractions was determined by using a M4 QIII Zeiss spectrophotometer, and radioactivity was measured in Triton scintillation mixture, using an LS-233 Beckman scintillation counter. Sucrose was removed by diluting the virus preparation 10-fold with water and pelleting it at  $16,000 \times g$  for 30 min. Purified GV was resuspended in water and stored at -20°C.

**Mixing experiments.** Mixing experiments were performed to determine the degree of in vivo host contamination. These experiments consisted of mixing 100 uninfected, radioactively labeled ([ $^3H$ ]valine) larvae with 20 infected, unlabeled larvae and subjecting the mixture to the purification procedure. At each step of the procedure, a portion of prepara-

tion was treated with cold trichloroacetic acid at a final volume of 10%. Radioactivity of acid-insoluble material was measured, and protein was determined according to Lowry et al. (16). Bovine serum albumin was used as standard in protein determinations.

**Infectivity and recovery of inclusion bodies after purification.** The infectivity of a crude homogenate of insects infected with GV was determined by adding 50 Indian meal moth eggs to 25-g samples of diet that had been inoculated with five serial dilutions of the virus preparation. The assays were done twice, and data were corrected by Abbott's formula (1) and analyzed by the minimum logit chi-square method of Berkson (5). The lowest dose needed to kill 50% of the insects ( $LD_{50}$ , expressed as milligrams of virus preparation per kilogram of diet) was determined and was designated as one infectious unit. In this manner the infectivity ( $LD_{50}$ ) of purified GV was compared with that of nonpurified preparations. The total number of infectious units in both the crude homogenate and the purified virus preparation was determined as the ratio of total protein (in milligrams) to the  $LD_{50}$  value. Recovery of infectivity after purification was then calculated as (total infectious units in purified GV/total infectious units in crude homogenate)  $\times$  100.

**Density and size of purified inclusion bodies.** The purified GV was centrifuged to equilibrium in a sucrose gradient (45 to 60%, wt/wt, in water) at 35,000 rpm (SW50.1 rotor) for 24 h at 10°C. Fractions (20 drops) were collected, and the virus was located by absorbance at 260 nm. Density was determined by weighing 50  $\mu$ l of the peak fraction containing the virus.

Inclusion body dimensions were determined by measurements of electron micrographs of purified virus. Bacteriophage T7 was included in the preparations as a size marker. Measurements were made of 100 GV inclusion bodies and 50 T7 virions.

**Electron microscopy of purified inclusion bodies.** For examination of intact virus, drops of purified virus suspension were placed on Formvar-coated grids and stained with 0.5% phosphotungstic acid, pH 7.0. For degradation studies, purified GV was air dried onto Formvar-coated grids. The grids were incubated for various periods in droplets of 0.05 M sodium carbonate, pH 10.5. Grids were then rinsed in distilled water and stained with 3% uranyl acetate. For thin sectioning, purified virus was collected by vacuum on a 25-nm-pore membrane filter (Millipore Corp.) (see Fig. 4a). The filter was then fixed in cold 3% glutaraldehyde and postfixed in 1% osmium tetroxide. Both fixatives were in 0.1 M cacodylate buffer, pH 7.4, and fixation in each was for 1 h at 4°C. The filter was dehydrated in ethanol, washed in toluene, and embedded in Epon 812. Sections were cut with a no. 322 516 Reickert ultramicrotome and were stained with 5% uranyl acetate and then with 0.2% lead citrate. All grids were examined with a Philips EM 201 at 60 kV.

## RESULTS

**Purification of GV.** The results of the mixing experiment in which uninfected, radioac-

tively labeled larvae were mixed with infected, unlabeled larvae are shown in Table 1. The differential centrifugation steps removed substantial amounts (95%) of host material from the virus. The low-speed centrifugation (Table 1, step 3) removed 37% of the labeled host protein from the virus preparation. The high-speed centrifugation (Table 1, step 4) pelleted the virus and eliminated all but 5% of the host contamination. Cuticular fragments which pelleted with the viral inclusion body were removed by filtration. After deoxycholate treatment, only 2% of the labeled larval protein remained (Table 1, step 6). This residual contamination was removed by sedimentation in the sucrose gradient. No host radioactivity could be detected in the resulting band of virus (Table 1, step 7; Fig. 1). If the detergent was omitted, 3% of the contaminating radioactive host material was found in the virus preparation after sedimentation in the sucrose gradient (Table 1).

To eliminate the possibility that the purification procedure failed to remove host protein not labeled after injection of live insects with radioisotope, purified tritium-labeled GV was mixed with larval homogenate labeled in vitro with  $^{14}\text{C}$ . The majority of proteins in this homogenate should have been made radioactive by the in vitro labeling technique because attachment of the label is to any  $\epsilon$ -amino groups on lysine residues. The mixture of [ $^3\text{H}$ ]valine-labeled virus and  $^{14}\text{C}$ -labeled larval tissue or of [ $^3\text{H}$ ]thymidine-labeled virus and  $^{14}\text{C}$ -labeled larval tissue was treated with detergent and then was sedimented in a 45 to 60% sucrose gradient. The resulting band of radioactive virus contained no detectable host radioactivity (Fig. 2).

Quality and recovery of purified GV. The

purified virus preparation was confirmed to consist of granulosis inclusion bodies by electron microscopy and infectivity studies. Micrographs (Fig. 3) of the material banding in the

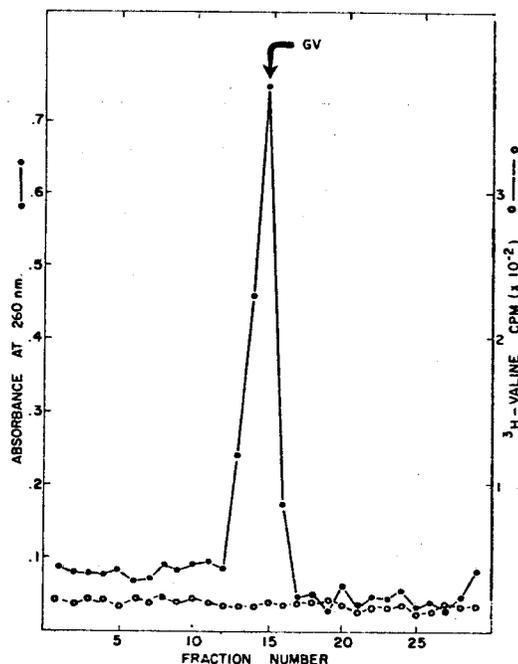


FIG. 1. Velocity sedimentation of virus preparation in sucrose gradient (Table 1, step 7). Uninfected, [ $^3\text{H}$ ]valine-labeled larvae were mixed with unlabeled, infected larvae. The mixture was subjected to step 1 through step 6 of the purification as described in Table 1. The preparation was then layered on the sucrose gradient (45 to 60%, wt/wt) and centrifuged at 20,000 rpm (SW27 rotor) for 2 h. Fractions (0.5 ml) were collected, the virus was located by absorbance at 260 nm ( $\bullet$ ), and the cpm of  $^3\text{H}$  host radioactivity in each fraction was measured ( $\circ$ ).

TABLE 1. Determination of purity of isolated GV from the mixing of [ $^3\text{H}$ ]valine-labeled, uninfected larvae with unlabeled, infected larvae

Step in viral purification procedure	Acid-insoluble material (protein) retained				
	Larval cpm	% Initial cpm	Protein (mg)	% Initial protein	cpm/mg of protein
1. Homogenize (manually)	$3.42 \times 10^6$	100.0	37.20	100.0	$9.19 \times 10^4$
2. Homogenize (Omni-Mixer)	$3.42 \times 10^6$	100.0	37.20	100.0	$9.19 \times 10^4$
3. Supernatant from low-speed centrifugation ( $1,000 \times g$ )	$2.16 \times 10^6$	63.1	27.40	73.6	$7.88 \times 10^4$
4. Pellet resuspended after high-speed centrifugation ( $16,000 \times g$ )	$1.98 \times 10^5$	5.8	4.00	10.7	$4.95 \times 10^4$
5. Filtration	$1.37 \times 10^5$	4.0	3.00	8.0	$4.57 \times 10^4$
6. Deoxycholate treatment	$6.16 \times 10^3$	1.8	0.64	1.7	$9.62 \times 10^3$
7. Velocity sedimentation in sucrose gradient ( $60,000 \times g$ )	0.0	0.0	0.56	1.5	0.0
Velocity sedimentation when deoxycholate is omitted	$1.02 \times 10^5$	3.0	2.23	6.0	$1.70 \times 10^4$

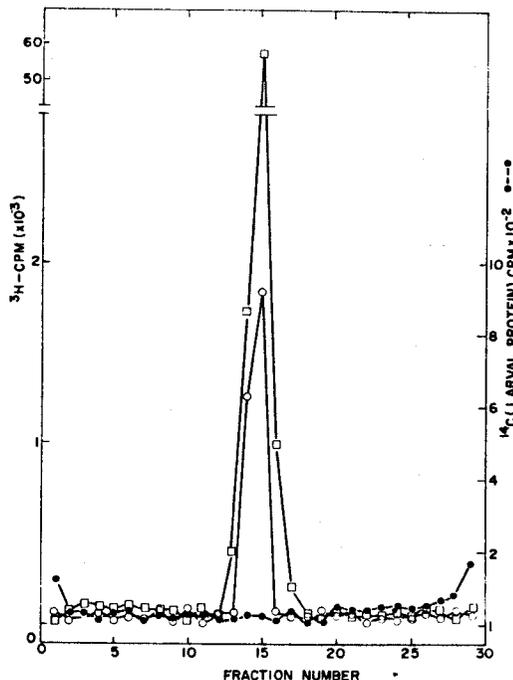


FIG. 2. Co-sedimentation of  $^3\text{H}$ -labeled GV and  $^{14}\text{C}$ -labeled larval tissue in sucrose velocity gradient. Either purified [ $^3\text{H}$ ]valine-labeled GV or purified [ $^3\text{H}$ ]thymidine-labeled GV was mixed with *in vitro*  $^{14}\text{C}$ -labeled larval homogenate. The mixture was treated with deoxycholate, layered on the sucrose gradient, and centrifuged at 20,000 rpm (SW27 rotor) for 2 h. Symbols: (○) [ $^3\text{H}$ ]valine-labeled GV; (□) [ $^3\text{H}$ ]thymidine-labeled GV; (●)  $^{14}\text{C}$ -labeled larval homogenate.

sucrose gradient (Fig. 1) demonstrated a homogeneous population of intact inclusion bodies averaging 210 by 380 nm in size. Equilibrium centrifugation of purified virus in a sucrose gradient revealed a single band with a density of 1.271 g/cm<sup>3</sup>.

Larvae fed on diet inoculated with the purified GV became infected, and GV was recovered from them. The LD<sub>50</sub> of the purified virus preparation was 0.0024 mg of virus per kg of diet (95% limits, 0.0015 to 0.0037). This value was 7.5 times smaller than the infectivity of the crude homogenate of infected larvae, which was 0.018 mg per kg of diet (95% limits, 0.012 to 0.027) (Table 2). The crude homogenate contained a total of 6,700 LD<sub>50</sub> infectious units, whereas the total number of LD<sub>50</sub> units in the purified virus preparation was 2,460 (Table 2). Based on these values, the recovery of infectious GV after the purification procedure was calculated as 37%.

**Structural integrity of purified GV.** When thin sections of the purified GV were examined

by electron microscopy, all structural components were shown to be intact. The outer protein matrix with its regular lattice spacing, the viral envelope, and the nucleocapsid were observable in the thin sections (Fig. 4b and c). The presence and integrity of the structural components of purified GV were also verified by sequentially degrading GV through exposure to carbonate for increasing periods of time. Degradation of the protein matrix was seen after short (10 s) exposure to carbonate. The matrix was extensively degraded after longer exposure (30 s), and the enveloped nucleocapsid was released from the matrix (Fig. 5a). Prolonged (2 min) exposure to carbonate caused disruption of the envelope and release of the nucleocapsid (Fig. 5b and c).

### DISCUSSION

The procedure we have developed for purification of GV from infected Indian meal moth larvae: (i) frees the viral inclusion body of detectable host contamination (Table 1; Fig. 1 and 2), (ii) preserves the structural integrity of the virus (Fig. 3-5), and (iii) results in the isolation of highly infectious virus (Table 2). Several reports in the literature describe the isolation and purification of other baculoviruses, using only differential centrifugation and velocity sedimentation in sucrose gradients (7, 8, 13, 15). In these reports, sufficient criteria were not presented to demonstrate that the "purified" viruses were free from host contamination. Our present report not only describes the purification of the Indian meal moth GV but also establishes criteria for purity of the virus isolated from whole insects by the use of radioisotopes (Table 1; Fig. 1 and 2).

To date, radioisotopes have had limited use in investigations on characterization of insect viruses (6, 20-22, 24). Due to a lack of appropriate tissue culture systems, many insect viruses are grown in whole insects. In these *in vivo* systems, difficulties have been encountered in obtaining radiolabeled host tissue or radiolabeled virus (23). The microinjection procedure described in this paper allows for incorporation of radioactive precursors by Indian meal moth larvae and by GV while it is replicating *in vivo*. The ability to radiolabel host and viral constituents allowed us to use mixing experiments (Table 1; Fig. 1 and 2) to determine the removal of host contaminants and the purity of the virus preparation.

Our purification procedure includes the use of deoxycholate (2, 12, 17, 23), and the mixing experiments demonstrated that this is an essential step because 3% of the initial host contamination remained with the virus (Table 1)

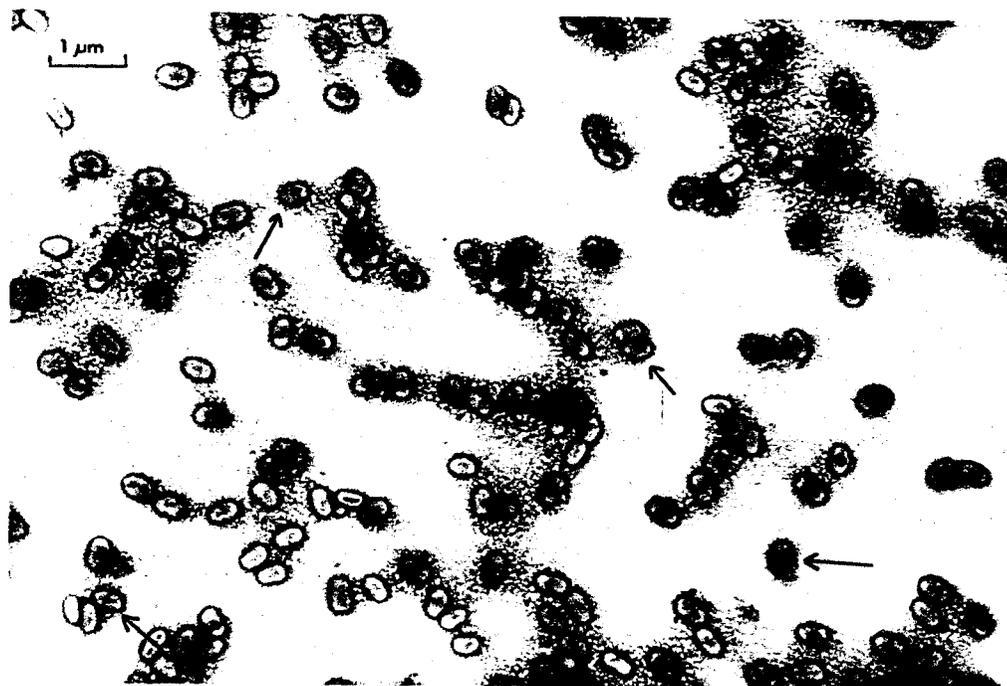


FIG. 3. Electron microscopy of purified GV inclusion bodies (arrows).

TABLE 2. Infectivity and recovery of GV after purification

Virus prepn	Total protein (mg)	Infectious unit <sup>a</sup>	Total infectious units <sup>b</sup>	% Initial infectious units <sup>c</sup>
Crude homogenate	120.00	0.018	6,700	100
Purified virus	5.9	0.0024	2,460	37

<sup>a</sup> One infectious unit defined as the LD<sub>50</sub> value (milligrams of virus/kilogram of diet).

<sup>b</sup> Ratio of total protein to the LD<sub>50</sub> value.

<sup>c</sup> (Total infectious units/total infectious units in the crude homogenate) × 100.

when the detergent treatment was omitted. The procedure developed for the purification of GV was capable of removing all detectable host contamination and resulted in the recovery of 37% of the infectivity originally present in the crude homogenate (Tables 1 and 2).

The integrity of GV was not altered during purification because electron microscopy of purified virus demonstrated that the structural components of the virus were intact (Fig. 3-5). The protein matrix, viral envelope, and nucleocapsid observed in thin sections of purified virus (Fig. 4b and c) are those characteristic of the baculoviruses (4, 8, 10) and closely resemble those seen by other investigators (3) in thin sections of Indian meal moth larvae infected with GV. The disruption of purified GV by

carbonate treatment causing the sequential degradation of the protein matrix, exposure of the enveloped nucleocapsid, and release of the nucleocapsid from the envelope (Fig. 5) confirms earlier studies of the morphogenesis of GV during its replication in Indian meal moth larvae (3). In these studies, Arnott and Smith (3) found that free nucleocapsids appeared in the cytoplasm, were then inserted into the envelope, and were finally encapsulated by the protein matrix. Our *in vitro* carbonate degradation studies demonstrate, in reverse, this assembly of virus components observed *in vivo*. The enveloped virus and nucleocapsid exposed by carbonate treatment (Fig. 5) closely resemble those seen in infected larval tissue. They are also morphologically similar to the viral forms isolated on sucrose gradients after incubation of baculovirus inclusion bodies in carbonate (4, 8, 13). The biological integrity of GV was also retained after purification, because the resulting inclusion bodies were highly infectious *per os* (Table 2).

Now that the purification of biologically active GV from larvae of the Indian meal moth is established, biochemical and biophysical characterization of the viral nucleic acid and structural proteins, as well as determination of the possible insecticidal properties of these viral components, can be accomplished.

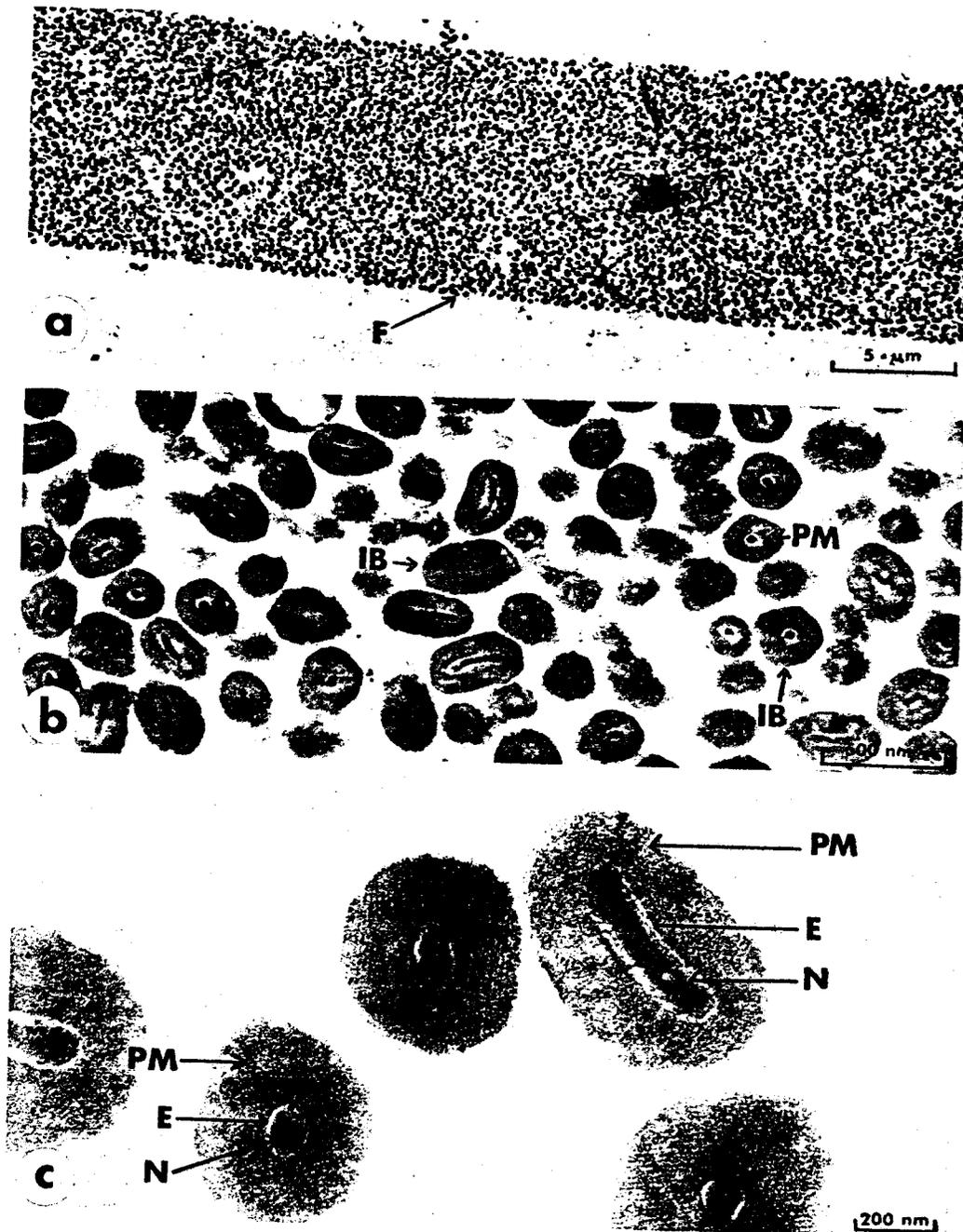


FIG. 4. Electron microscopy of thin-sectioned purified GV. (a) Thin section of purified GV showing the virus particles on top of the membrane filter (F). (b) Thin section of purified GV inclusion bodies (IB) demonstrating GV both in transverse and in longitudinal section. (c) Thin section of purified GV demonstrating the periodicity of the protein matrix (PM), the viral envelope (E), and nucleocapsid (N).

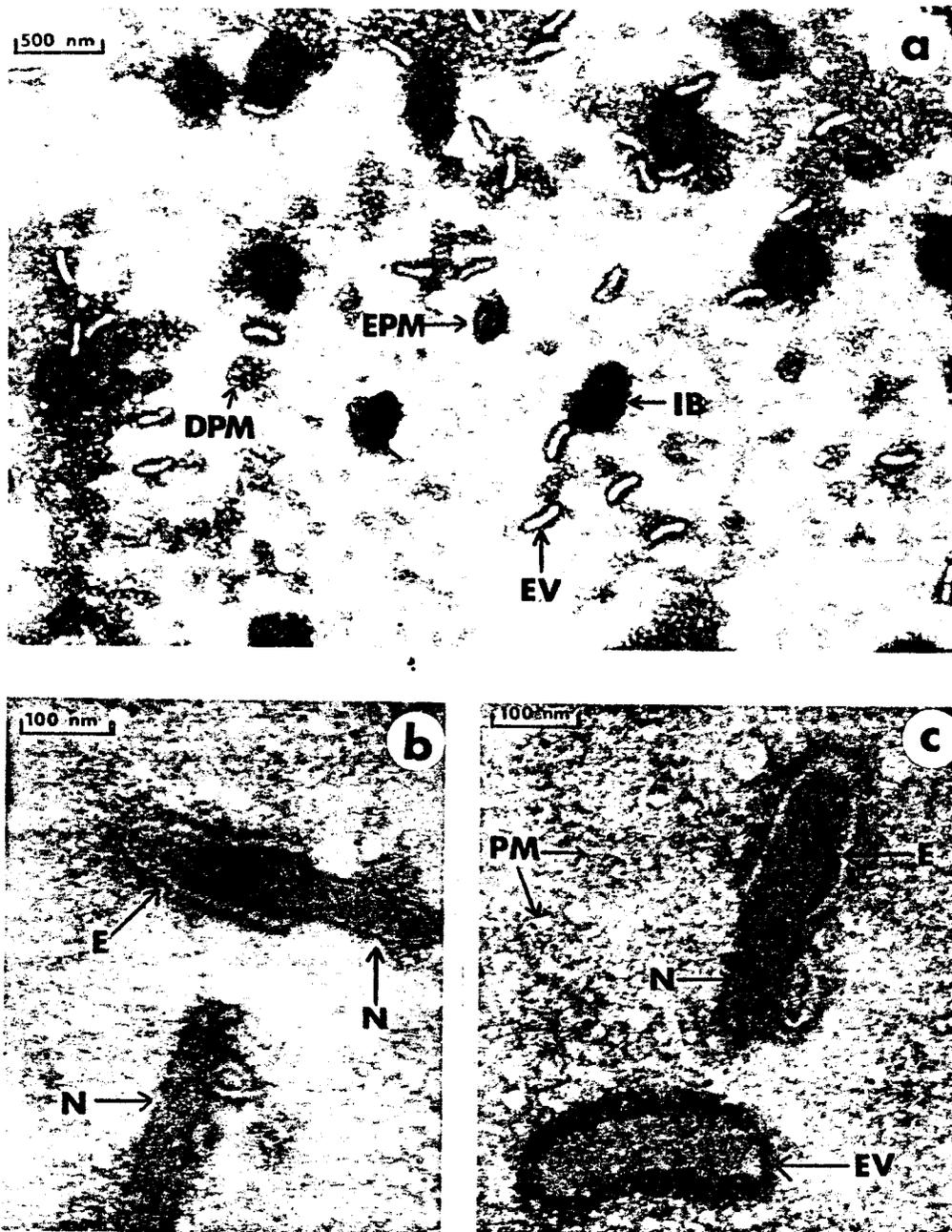


FIG. 5. Electron microscopy of purified GV sequentially degraded on electron microscope grids by carbonate. (a) Exposure of purified GV inclusion bodies (IB) to 0.05 M sodium carbonate for 30 s resulted in degradation of the protein matrix and release of the enveloped nucleocapsid (EV). Note the empty protein matrix (EPM), which has been partially disrupted at one end, causing the release of the enveloped nucleocapsid and the more extensively degraded protein matrix (DPM). (b and c) Prolonged exposure (2 min) of inclusion bodies to 0.05 M sodium carbonate resulted in disruption of the viral envelope (E) and subsequent release of the nucleocapsid (N). Extensively degraded protein matrix (PM) was also observed.

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## LITERATURE CITED

1. Abbott, W. S. 1925. A method of computing the effectiveness of an insecticide. *J. Econ. Entomol.* 18:265-267.
2. Arif, B. M., and K. W. Brown. 1975. Purification and properties of a nuclear polyhedrosis virus from *Choristoneura fumiferana*. *Can. J. Microbiol.* 21:1224-1231.
3. Arnott, H. J., and K. M. Smith. 1968. An ultrastructural study of the development of a granulosis virus in the cells of the moth *Plodia interpunctella* (Hbn.). *J. Ultrastruct. Res.* 21:251-268.
4. Beaton, C. D., and B. K. Filshie. 1976. Comparative ultrastructural studies of insect granulosis and nuclear polyhedrosis viruses. *J. Gen. Virol.* 31:151-161.
5. Berkson, J. 1953. A statistically precise and relatively simple method of estimating the bioassay with quantal response, based on the logistic function. *J. Am. Stat. Assoc.* 48:565-569.
6. Carnegie, J. W., and G. S. Beaudreau. 1969. Deoxyribonucleic acid synthesis in insect larvae after inoculation with nuclear polyhedrosis virus. *J. Virol.* 4:311-312.
7. Harrap, K. A. 1972. The structure of nuclear polyhedrosis viruses. I. The inclusion body. *Virology* 50:114-123.
8. Harrap, K. A. 1972. The structure of nuclear polyhedrosis viruses. II. The virus particle. *Virlogy* 50:124-132.
9. Harrap, K. A., and J. F. Longworth. 1974. An evaluation of purification methods for baculoviruses. *J. Invertebr. Pathol.* 24:55-62.
10. Hughes, K. M. 1972. Fine structure and development of two polyhedrosis viruses. *J. Invertebr. Pathol.* 19:198-207.
11. Hunter, D. K., S. J. Collier, and D. F. Hoffmann. 1973. Effectiveness of a granulosis virus of the Indian meal moth as a protectant for stored inshell nuts: preliminary observations. *J. Invertebr. Pathol.* 22:481.
12. Kawase, S., F. Kawamoto, and K. Yamaguchi. 1973. Studies on the polyhedrosis virus forming polyhedra in the midgut-cell nucleus of the silkworm, *Bombyx mori*. I. Purification procedure and form of the virion. *J. Invertebr. Pathol.* 22:266-272.
13. Khosaka, T., M. Himeno, and K. Onodera. 1971. Separation and structure of components of nuclear polyhedrosis virus of the silkworm. *J. Virol.* 7:267-273.
14. Kok, I. P., A. V. Chistyakova-Ryndich, and A. P. Gud'z'-Gorban'. 1972. Macromolecular structure of the DNA of the *Bombyx* nuclear polyhedrosis virus. *Mol. Biol. (USSR)* 6:323-331.
15. Longworth, J. F., J. S. Robertson, and C. C. Payne. 1973. The purification and properties of the inclusion body protein of the granulosis virus of *Pieris brassicae*. *J. Invertebr. Pathol.* 19:42-50.
16. Lowry, O. H., N. J. Rosebrough, A. L. Farr, and R. J. Randall. 1951. Protein measurement with the Folin phenol reagent. *J. Biol. Chem.* 193:265-275.
17. McCarthy, W. J., and S. Liu. 1976. Electrophoretic and serological characterization of *Porthetria dispar* polyhedron protein. *J. Invertebr. Pathol.* 28:57-65.
18. McGaughey, W. H. 1975. A granulosis virus for Indian meal moth control in stored wheat and corn. *J. Econ. Entomol.* 68:346-348.
19. McMillen, J., and R. A. Consigli. 1974. *In vitro* radioisotopic labeling of proteins associated with purified polyoma virus. *J. Virol.* 14:1627-1629.
20. Summers, M. D. 1975. Biophysical and biochemical properties of baculoviruses, p. 17-32. *In* M. Summers, R. Engler, L. A. Falcon, and P. Vail (ed.), *Baculoviruses for insect pest control: safety considerations*. American Society for Microbiology, Washington, D.C.
21. Summers, M. D., and D. L. Anderson. 1972. Characterization of deoxyribonucleic acid isolated from the granulosis viruses of the cabbage looper, *Trichoplusia ni* and the fall armyworm, *Spodoptera frugiperda*. *Virology* 50:459-471.
22. Summers, M. D., and D. L. Anderson. 1972. Granulosis virus deoxyribonucleic acid: a closed, double-stranded molecule. *J. Virol.* 9:710-713.
23. Summers, M. D., and K. Egawa. 1973. Physical and chemical properties of *Trichoplusia ni* granulosis virus granulin. *J. Virol.* 12:1092-1103.
24. Summers, M. D., and G. E. Smith. 1975. *Trichoplusia ni* granulosis virus granulin: a phenol-soluble, phosphorylated protein. *J. Virol.* 16:1108-1116.
25. Zettler, J. L., L. L. McDonald, L. M. Redlinger, and R. D. Jones. 1973. *Plodia interpunctella* and *Cadra cautella* resistance in strains to malathion and synergized pyrethrins. *J. Econ. Entomol.* 66:1049-1050.