Comparative Pathology of Isolates of Spodoptera frugiperda Nuclear Polyhedrosis Virus in S. frugiperda and S. exigua

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SUMMARY

Spodoptera frugiperda nuclear polyhedrosis virus was highly pathogenic to both S. frugiperda and S. exigua. Plaque-purified variants from two of the original isolates showed much greater differences in pathogenicity to the two insect species than the original isolates. Plaque-purified variants from one of the isolates (D) nearly lost pathogenicity for S. exigua while remaining pathogenic for S. frugiperda. Some of the plaque-purified variants produced atypical symptoms, even in S. frugiperda. These variants did not liquefy larvae and release polyhedra when the larvae died as is typical for nuclear polyhedrosis infections in Lepidoptera. These variants also produced fewer polyhedra per g larval weight and often had fewer virions per polyhedron. Light and electron microscopical studies of S. frugiperda and S. exigua infected with one of the original isolates (A) and two of the plaque-purified variants (B2 and D7) indicated that only S. frugiperda infected with isolate A had the highly productive infection and viral morphogenesis typical of nuclear polyhedrosis viruses. Exposure of S. frugiperda to isolate B2 or D7 resulted in a slightly delayed infection characterized by increased amounts of abnormal viral morphogenesis and polyhedra of decreased size. S. exigua infected with isolate A or B2 had greatly reduced and delayed infections that were accompanied by highly variable abnormal viral morphogenesis; virtually no normal polyhedra were produced in these instances. Isolate D7 produced neither nucleocapsids nor polyhedra in S. exigua. Large paracrystalline aggregates of nucleocapsids were common in S. frugiperda infected with isolates B2 or D7 and in S. exigua infected by isolate A. Infection of S. exigua by isolate A or B2 was typified by the accumulation of large amounts of excess envelope membrane in the form of strands and vesicles of various sizes. Normal virogenic stromata were characteristics only of S. frugiperda infected with isolate A or B2. Polyhedra produced in S. exigua by even the most pathogenic isolates (A and B2) contained few if any normal virions and were not infective for either S. frugiperda or S. exigua.

INTRODUCTION

Nuclear polyhedrosis (NPV) and granulosis viruses (GV) are baculovirus pathogens primarily of insects and contain circular, double-stranded DNA genomes (David, 1975; Smith & Summers, 1978). Differences in pathology and/or in the morphology of the virus or the polyhedral inclusion bodies (PIB) can occur during propagation in different hosts (Hunter et al., 1973; Vail & Jay, 1973; Tompkins et al., 1981). Continuous propagation of baculoviruses in tissue culture can also result in morphological changes in the virus and/or in pathogenicity (Hink & Vail, 1973; Ramoska & Hink, 1974; Mackinnon et al., 1974; Knudson & Harrap, 1976; Potter et al., 1976, 1978). Andrews et al. (1980) reported no significant differences in virulence of five different genotypic variants of Autographa californica NPV. Vail et al. (1982) reported one plaque-purified isolate of Trichoplusia ni multiple nucleocapsid (M) NPV which was more virulent to T. ni larvae than the wild-type but was less virulent to Heliothis virescens.
The purpose of this study was to compare the pathology and virulence of some plaque-purified variants of the *S. frugiperda* NPV (Sf M NPV) first described by Knell & Summers (1981).

**METHODS**

*Virus isolates.* Sf M NPV isolates A, B, C, D and plaque-purified isolates B1, B2, D5 and D7 were obtained from Dr M. D. Summers (Department of Entomology, Texas A & M University, College Station, Tx., U.S.A.). The *S. frugiperda* NPV variants were derived from a pool of purified polyhedra prepared from infected *S. frugiperda* larvae collected in Tifton, Georgia, in 1962 (Hamm, 1968; Knell & Summers, 1981). Isolate A, which had never been produced *in vitro*, was from the collection of J. J. H. in the form of purified polyhedra. Isolate B was received as extracellular virus from Dr R. H. Goodwin (USDA-ARS, Beltsville, Md., U.S.A.); this isolate was derived from polyhedra obtained directly from Tifton in 1963. Isolate C was received as purified polyhedra from Dr K. A. Harrap (formerly of N.E.R.C. Unit of Invertebrate Virology, Oxford, U.K.) and was derived from material sent to Oxford from Beltsville prior to 1973. Isolate D was derived as extracellular virus from Dr J. M. Viak (Mededelingen Landbouwhogeschool, Wageningen, The Netherlands); it was derived from material sent from Oxford in 1976. Preparation of the plaque isolates is described elsewhere (Knell & Summers, 1981). Knell & Summers (1981), using restriction endonuclease analysis, could distinguish all four isolates (A, B, C and D) on the basis of minor differences in EcoRI patterns. Submolar DNA restriction fragments were seen in all isolates, indicating that each isolate consisted of a mixture of different genotypes. The EcoRI fragment B was present in submolar concentrations in wild isolates A, B and C and in molar concentrations in clones B1 and B2. It was not observed in any clones from D. Based on the sums of the mol. wt. of EcoRI restriction fragments, Sf M NPV-B1 had a mol. wt. of 83.2 × 10^6 compared to 78.6 × 10^6 for Sf M NPV-D7. Fresh inocula of all virus isolates were produced by growing the isolates in *S. frugiperda* larvae. Samples of the fresh inocula were returned to Dr M. D. Summers, who reconfirmed the identity of the isolates by restriction endonuclease analysis.

**Bioassays.** Bioassays were conducted on *S. frugiperda* and *S. exigua* because preliminary tests indicated that Sf M NPV collected in Georgia, Mississippi, North Carolina and Ohio were all pathogenic to both *S. frugiperda* and *S. exigua.* The virus was mixed into bean diet, without formalin, at rates of 10^5 and 10^6 polyhedral inclusion bodies (PIB)/ml diet. Neonates were allowed to feed on treated diet for approx. 24 h, then isolated on bean diet with formalin (Burton, 1969). Larvae were observed daily until death or pupation. Assays consisted of two replicates per concentration with 36 larvae per replicate.

**Microscopy.** *S. frugiperda* and *S. exigua* larvae 4 days old were inoculated with Sf M NPV isolates A, B2 and D7 as described above. These isolates were chosen as being representative of wild-type field isolates of normal virulence (A), plaque isolates of normal virulence (B2), and plaque isolates of reduced virulence (D7) when used to infect *S. frugiperda* larvae. Tissues from *S. frugiperda* larvae were collected 4, 6 and 7 days post-treatment (PT). Tissues from *S. exigua* larvae were collected 7 and 10 days PT because of the slower development of Sf M NPV in this species. Larvae were dissected in phosphate-buffered 2.5% glutaraldehyde. Fat body and pieces of integument with attached tissues were removed, diced, and stored in fixative for 24 to 48 h. Specimens were then rinsed in buffer, post-fixed in phosphate-buffered 1% OsO_4 for 1 h, dehydrated, and embedded in Spurr's low viscosity resin (Spurr, 1969). Sections were cut with an LKB Ultratome V. One μm sections were mounted on glass slides, stained with borate-buffered toluidine blue, and examined with a light microscope (Chandler & Schoenwolf, 1983). Areas of interest were selected and located on the corresponding tissue blocks. Ultrathin sections 60 to 90 nm thick were cut from these areas, mounted on uncoated 200-mesh grids, and double-stained with 3% uranyl acetate in absolute methanol and Reynolds' lead citrate (Reynolds, 1963). Ultrathin sections were examined with an RCA EMU-4 electron microscope.
RESULTS

Bioassays

Isolates A, B and D were highly pathogenic to neonates of both *S. frugiperda* and *S. exigua*. Although mortality generally occurred a few days later in *S. exigua* than in *S. frugiperda*, the total percent mortality at any given concentration of PIB was generally as high or higher in *S. exigua* than in *S. frugiperda* (Fig. 1, 2 and 3). The plaque-purified variants of isolate B retained virulence for both *S. frugiperda* and *S. exigua*, although B1 appeared to be slightly less virulent than B or B2 (Fig. 2). The plaque-purified variants D5 and D7 showed an almost total loss of virulence for *S. exigua* while maintaining virulence for *S. frugiperda* at approximately the level of the original D isolate (Fig. 3).

When 5-day-old *S. frugiperda* larvae were treated with the four plaque-purified variants and the two original isolates A and C, A was more virulent (P < 0.05) than the other isolates (Table 1). Isolate A also released the highest percentage of PIB (Table 2), with the typical liquefaction of dead larvae, although not significantly more than C. The D5 and D7 plaque-purified variants released the lowest percentage of PIB, although not significantly less than B1 and B2. D5 and D7 resulted in significantly less liquefaction of dead larvae than A and C.

When productivity *in vivo* of the virus isolates in *S. frugiperda* was compared as PIB/g larval weight, significant differences were detected (Table 3).

When the most virulent isolates, A and B2, were produced in *S. frugiperda* and *S. exigua* and the PIB produced in the two hosts were compared for virulence (Table 4), the polyhedra produced in *S. exigua* produced no mortality in either host.

Microscopy

In larvae of *S. frugiperda* infected by isolate A, virtually all cells of the hypodermis and fat body were infected by 3 to 4 days PT, as were large numbers of cells in other tissues, such as muscle, tracheal matrix, blood and nerve tissue. Even at 3 to 4 days PT, most cells were in late stages of infection, their nuclei filled with PIB. A few cells, primarily those of the fat body, were in a variety of earlier stages. Characteristically, infected cells had a roughly spherical, greatly enlarged nucleus surrounded by a narrow band of cytoplasm (Fig. 4a). The cytoplasm frequently contained large masses of fibrous material which was often associated with membrane profiles. Occasionally, elongate fascicles of microtubules were situated immediately adjacent to the nucleus. Only very rarely did empty PIB occur in the cytoplasm. Infected nuclei passed through a series of stages. Initial nuclear enlargement was followed by concentration of heterochromatin at the periphery of the nucleus and clearing of the central region. Subsequently, scattered electron-dense areas appeared within the cleared central region and developed into reticulate virogenic stromata. Concomitantly with development of the virogenic stromata, nucleocapsids (NC) approximately 40 × 220 nm began to accumulate within the nucleus. NC could be seen budding through the nuclear envelope in a small number of cells at 3 to 4 days PT, but not at 6 to 7 days PT. More commonly, groups of three to seven or more NC were surrounded by a single-membrane envelope and the enveloped NC (ENC) occluded by developing PIB. PIB averaged 191 nm [95% confidence interval (CI) = 201 to 181 nm] in diameter (n = 101), were roughly polygonal (Fig. 4b), and contained large numbers of ENC. Nuclei of infected cells also contained small to large masses of fibrous material. Membrane profiles were frequently associated with the fibrous material (Fig. 4c). Nuclei of a very small percentage of infected cells contained PIB of unusual morphology, NC of indeterminate length, large masses of vesicles of various sizes, strands of membrane, and occasionally straight to sinuous microtubules. Except in these rare cells, excess envelope membrane did not accumulate in nuclei as vesicles or strands. Unless noted otherwise, the following host–virus combinations are similar to the foregoing.

Tissues of *S. frugiperda* larvae infected with isolate B2 were only lightly infected 3 to 4 days PT. Hypodermis and fat body became relatively heavily infected by 7 days PT. Infection of fat body lagged slightly behind that of the hypodermis and infection of other tissues lagged behind both of these. Large numbers of NC accumulated in infected nuclei in paracrystalline aggregates (Fig. 5a). When viewed in longitudinal sections, paracrystalline aggregates appeared as single or multiple tiers of NC arranged in parallel, each tier containing 20 to 50 or more NC. The number
Fig. 1. Cumulative percent mortality, from first to last day of mortality, as a function of time for *S. frugiperda* (---) and *S. exigua* (-----) neonates treated with (a) ●, 3.5 × 10⁶ or ○, 3.5 × 10⁵ PIB/ml diet Sf M NPV isolate A or (b) ●, 1.9 × 10⁶ or ○, 1.9 × 10⁵ PIB/ml diet Sf M NPV isolate C.

Fig. 2. Cumulative percent mortality, from first to last day of mortality, as a function of time for *S. frugiperda* (---) and *S. exigua* (-----) neonates treated with (a) ●, 1.5 × 10⁶ or ○, 1.5 × 10⁵ PIB/ml diet Sf M NPV isolate B or (b) ●, 4.9 × 10⁶ or ○, 4.9 × 10⁵ PIB/ml diet Sf M NPV plaque-purified variant B2 or (c) ●, 4.7 × 10⁶ or ○, 4.7 × 10⁵ PIB/ml diet Sf M NPV plaque-purified variant B1.

Fig. 3. Cumulative percent mortality, from first to last day of mortality, as a function of time for *S. frugiperda* (---) and *S. exigua* (-----) neonates treated with (a) ●, 1.6 × 10⁶ or ○, 1.6 × 10⁵ PIB/ml diet Sf M NPV isolate D or (b) ●, 3.6 × 10⁶ or ○, 3.6 × 10⁵ PIB/ml diet Sf M NPV plaque-purified variant D5 or (c) ●, 1.6 × 10⁶ or ○, 1.6 × 10⁵ PIB/ml diet Sf M NPV plaque-purified variant D7.
Table 1. *Mean percent mortality of S. frugiperda larvae* produced by six isolates of Sf M NPV

<table>
<thead>
<tr>
<th>Isolate</th>
<th>% Mortality†</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>70.3 a</td>
</tr>
<tr>
<td>B2</td>
<td>55.4 b</td>
</tr>
<tr>
<td>C</td>
<td>43.8 bc</td>
</tr>
<tr>
<td>D7</td>
<td>38.0 cd</td>
</tr>
<tr>
<td>B1</td>
<td>26.7 d</td>
</tr>
<tr>
<td>D5</td>
<td>24.4 d</td>
</tr>
</tbody>
</table>

* Five-day-old larvae held for 24 h on diet surface-treated with 0.1 ml of 10⁶ PIB/ml.
† Duncan's multiple range test. Numbers followed by the same letter are not significantly different, P > 0.05.

Table 2. *Mean percent release of polyhedral inclusion bodies by liquefaction produced by six isolates of Sf M NPV in S. frugiperda larvae*

<table>
<thead>
<tr>
<th>Isolate</th>
<th>% PIB released*</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>60.1 a</td>
</tr>
<tr>
<td>C</td>
<td>41.0 ab</td>
</tr>
<tr>
<td>B2</td>
<td>15.5 bc</td>
</tr>
<tr>
<td>B1</td>
<td>10.7 bc</td>
</tr>
<tr>
<td>D5</td>
<td>0.6 c</td>
</tr>
<tr>
<td>D7</td>
<td>0.5 c</td>
</tr>
</tbody>
</table>

* Duncan's multiple range test. Numbers followed by the same letter are not significantly different, P > 0.05.

Table 3. *Mean number of polyhedral inclusion bodies produced in S. frugiperda larvae by six isolates of Sf M NPV*

<table>
<thead>
<tr>
<th>Isolate</th>
<th>Mean no. PIB × 10⁸ per g body wt*</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>207.5 a</td>
</tr>
<tr>
<td>C</td>
<td>189.0 ab</td>
</tr>
<tr>
<td>B2</td>
<td>100.6 bc</td>
</tr>
<tr>
<td>D5</td>
<td>37.6 c</td>
</tr>
<tr>
<td>D7</td>
<td>35.5 c</td>
</tr>
<tr>
<td>B1</td>
<td>33.7 c</td>
</tr>
</tbody>
</table>

* Duncan's multiple range test. Numbers followed by the same letter are not significantly different, P > 0.05.

Table 4. *Percent mortality of S. frugiperda and S. exigua larvae* treated with two concentrations of two isolates of Sf M NPV produced in S. frugiperda and S. exigua

<table>
<thead>
<tr>
<th>Inoculum produced in S. frugiperda</th>
<th>Inoculum produced in S. exigua</th>
</tr>
</thead>
<tbody>
<tr>
<td>Isolate A</td>
<td>Isolate B2</td>
</tr>
<tr>
<td>Test species</td>
<td>10⁵</td>
</tr>
<tr>
<td>S. frugiperda</td>
<td>100</td>
</tr>
<tr>
<td>S. exigua</td>
<td>94.2</td>
</tr>
<tr>
<td>S. exigua</td>
<td>0</td>
</tr>
</tbody>
</table>

* Neonate larvae held 24 h on diet containing 4 × 10⁵ or 10⁶ PIB/ml, 23 to 72 larvae per treatment. (No mortality in controls.)
Fig. 4. Electron micrographs of *S. frugiperda* infected with Sf M NPV isolate A. (a) Fat body cell 4 days PT showing enlarged nucleus containing numerous polyhedral inclusion bodies (asterisk), virogenic stromata (VS) and enveloped nucleocapsids (arrowheads); (b) polyhedral inclusion body containing enveloped nucleocapsids (arrowheads); (c) fibrous material (FM) and associated membrane profiles (arrowheads). Bar markers represent (a) 1 μm, (b) 100 nm and (c) 500 nm.
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Fig. 5. Electron micrographs of S. frugiperda infected with Sf M NPV isolate B2. (a) Hypodermal cell 7 days PT showing nucleocapsids in paracrystalline aggregates (arrowheads); (b) polyhedral inclusion bodies with intimately associated fibrous material; (c) polyhedral inclusion bodies containing nucleocapsids enveloped in groups of one to three. Bar markers represent 1 μm.
of NC in these aggregates increased between 3 and 7 days PT. NC were enveloped singly or in groups of up to 10 or more particles and the ENC occluded by developing PIB. Larger groups of NC were only partially enveloped. Paracrystalline aggregates were frequently associated with envelope along one edge only. The tiers of an aggregate were occasionally separated by a row of small vesicles. Some cells contained few to many empty NC. Relatively large numbers of PIB were produced. PIB averaged 171 nm (95% CI = 181 to 161 nm) in diameter (n = 105) and contained numerous NC, mainly enveloped in groups of three to seven NC. Small numbers of cells with unusual PIB were encountered. PIB in these cells were similar to the abnormal PIB seen in S. frugiperda infected with isolate A and to that illustrated in Fig. 5(b). Nuclei with PIB containing enveloped groups of only one to three NC were relatively common (Fig. 5c), even when NC were produced in large enough numbers to form paracrystalline aggregates.

Large numbers of cells of larval S. frugiperda were infected with isolate D7 by 7 days PT. However, the nature of the infection varied greatly from cell to cell. Most of the infected cells were less enlarged than was typical for cells of S. frugiperda infected with isolate A and tended to have rather small PIB, NC aggregated into paracrystalline arrays, and abnormal virogenic stromata. The virogenic stromata generally took the form of small scattered electron-dense patches or a faint reticulum. Only very rarely did normal, fully developed virogenic stromata occur. NC were generally normal in length. However, some cells contained many empty NC (Fig. 6a) as well as numerous NC of indeterminate length. In cells having large numbers of NC, the NC formed numerous single and multiply tiered paracrystalline arrays (Fig. 6b). Occasionally, NC aggregated to form cylindrical structures (Fig. 6d). NC were enveloped in groups of one to 12, larger aggregates remained incompletely enveloped. Rarely, enveloped and partially occluded groups of up to 40 NC were observed. Envelope material was usually associated with NC. However, some nuclei contained very few NC and large amounts of excess membrane material in the form of strands and vesicles of various sizes. Other nuclei had relatively normal numbers of NC, but were devoid of envelope material. PIB averaged 127 nm (95% CI = 137 to 117 nm) in diameter (n = 106), although PIB in the few cells having normal virogenic stromata were larger, about 191 nm in diameter. PIB generally contained large numbers of ENC, with enveloped groups of three to seven NC predominating. Regardless of the number of NC or the amount of envelope material present, some cells contained only empty PIB or PIB with enveloped groups of one to three NC. Other abnormal PIB similar to those present in S. frugiperda infected with isolate A occurred, as did PIB similar to the one illustrated in Fig. 6(c). In a number of infected cells, the nuclear membrane was completely or partially disintegrated, resulting in the presence of virus-containing PIB in the cytoplasm and isolated mitochondria and lipid droplets in the nucleus. Membrane profiles and fibrous material were uncommon. When present, membrane profiles were scattered throughout the nucleus and infrequently associated with fibrous material.

In larvae of S. exigua infected with isolate A, only a few cells became infected, even at 7 days PT. At this time, scattered infected cells or small groups of no more than six infected cells occurred in various tissues, including hypodermis, fat body, muscle, tracheal matrix and Malpighian tubules. The infected cells often appeared to be in very early stages of infection. They were characterized by enlarged nuclei with heterochromatin concentrated at the periphery, cleared central regions, and a few scattered NC. Virogenic stromata were either absent or consisted of scattered, electron-dense patches or a diffuse reticulum. Only six cells with normal virogenic stromata were seen and these were all located in the hypodermis. NC were usually produced in small numbers and were highly variable in form. They were frequently of indeterminate length, variable diameter, and were often empty. The only cells that produced large numbers of normal NC were the few hypodermal cells having normal virogenic stromata. NC in these cells tended to accumulate in large paracrystalline arrays similar to those seen in S. frugiperda infected with isolates B2 and D7. Other infected nuclei often contained excessive amounts of envelope membrane in the form of vesicles of various sizes. Vesicles 30 to 40 nm in diameter predominated and were frequently occluded by developing PIB even though they did not contain NC. Except for the hypodermal cells with normal virogenic stromata, infected nuclei produced only small numbers of PIB. These PIB were commonly abnormal in
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Fig. 6. Electron micrographs of S. frugiperda infected with Sf M NPV isolate D7. (a) Apparently empty nucleocapsids (arrowheads); (b) paracrystalline arrays of nucleocapsids; (c) abnormal 'scalloped' polyhedral inclusion bodies; (d) nucleocapsids aggregated to form cylindrical structures. Bar markers represent (a to c) 500 nm and (d) 50 nm.

morphology (Fig. 7a, b) and contained occluded vesicles, enveloped groups of only one or two NC, or were devoid of occluded material. Fibrous material and membrane profiles, both nuclear and cytoplasmic, were much less common than in S. frugiperda infected with isolate A, again excepting the few hypodermal cells having normal virogenic stromata. Several infected nuclei contained inclusions that appeared as swirls or fingerprint-like configurations (Fig. 7c). Cross-sections showed these inclusions were composed of flexuous tubules somewhat smaller than microtubules (16 to 20 nm in diameter) interconnected by fine electron-dense strands and occasionally interspersed with solid electron-dense bodies about 8 nm in diameter (Fig. 7d).
Fig. 7. Electron micrographs of *S. exigua* infected with Sf M NPV isolate A. (a) Fat body cell 6 days PT showing abnormal polyhedral inclusion bodies (asterisk), membrane vesicles and tubules of various diameters (arrowheads); (b) enlargement of the polyhedral inclusion body marked by an asterisk in (a) showing vesicles being occluded (arrowheads) and occluded and non-occluded vesicles; (c) swirl or fingerprint-like inclusion; (d) cross-section through fingerprint-like inclusion. Bar markers represent (a to c) 1 μm and (d) 100 nm.

In *S. exigua* infected with isolate B2, occasional infected cells occurred in the hypodermis, fat body, muscle and tracheal matrix. There was a slight increase in the number of infected cells from 6 to 10 days PT. Infected cells usually lacked virogenic stromata. Instead, nuclei of infected cells were merely enlarged, with heterochromatin concentrated at the periphery and cleared central areas containing a few scattered NC. More virogenic stromata were observed at 10 days PT than at 6 to 7 days PT, with very few relatively normal virogenic stromata present at any time. NC were produced in very small numbers although rare cells did have larger amounts. Most NC were of indeterminate length and did not become enveloped (Fig. 8a). The few NC of normal dimensions tended to be enveloped in groups of one to three NC. Infected nuclei accumulated large amounts of membranous material, often becoming entirely filled with membranous strands and vesicles. Small to moderate numbers of PIB occurred in infected cells. Only rarely were infected cells found that had nuclei filled with PIB. PIB contained enveloped
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Fig. 8. Electron micrographs of S. exigua infected with Sf M NPV isolate B2. (a) Nucleocapsids of indeterminate length (arrowheads); (b) abnormal polyhedral inclusion bodies lacking enveloped nucleocapsids; (c) tubules of various diameters (arrowheads). Bar markers represent 500 nm.

groups of one to three NC and completely or partially occluded membranous strands and vesicles. PIB were frequently abnormal, with morphologies similar to the unusual PIB present in S. frugiperda and S. exigua infected with isolate A and to those shown in Fig. 8(b). Straight and sinuous tubules of various diameters occurred in some nuclei (Fig. 8c), as did fingerprint-like inclusions similar to those observed in S. exigua infected with isolate A.

Tissues of larvae of S. exigua exposed to isolate D7 were virtually uninfected, even at 10 days PT. Although a variety of tissues were examined, including hypodermis, fat body, tracheal matrix, muscle, nerve tissue and Malpighian tubules, only a single cell was observed that had obvious indications of infection. The nucleus of this cell contained a mass of fibrous material and had a vague virogenic stroma. Neither NC nor PIB were identifiable.
DISCUSSION

Knell & Summers (1981) considered the differences in restriction enzyme patterns of the wild isolates to be a reflection of the genotypic changes which have occurred in the virus since its original isolation. These changes involve a loss of the population of variants characterized by the presence of fragment \textit{EcoRI}b, as in Sf \textit{M} NPV-B2, and the dominance of variant Sf \textit{M} NPV-D7. This indicates that Sf \textit{M} NPV-D7 may have a selective advantage in the laboratory related to passage \textit{in vitro}.

The phenotypic differences expressed in comparative pathology and morphogenesis support the above interpretation. Isolate A, the ‘wild-type,’ showed the typical response for NPV with the production of large numbers of PIB and ‘melting’ or ‘wilting’ of dead larvae. It ranked highest in virulence to \textit{S. frugiperda}, as well as in the number of PIB produced and percent release of PIB by liquefaction. The other isolates showed various degrees of deviation from the standard of isolate A. Light and electron microscopical observations corresponded with the results of the bioassay tests. Isolate A in \textit{S. frugiperda} showed viral morphogenesis and associated pathology characteristic of NPV infection in a homologous host (Summers & Arnott, 1969). In comparison, isolate D7 caused a delayed infection of a reduced number of cells in \textit{S. frugiperda}. This delayed infection was accompanied by an increased incidence of morphogenetic abnormalities, including the absence of well-developed virogenic stromata and the production of PIB about 33\% smaller than those produced by isolate A in \textit{S. frugiperda}.

Reduced numbers of PIB and diminished numbers of occluded ENC have often been reported as a result of prolonged passage \textit{in vitro} in homologous host cells (MacKinnon \textit{et al.}, 1974; Andrews \textit{et al.}, 1980) and in heterologous host cells (Hirumi \textit{et al.}, 1975). Prolonged passage \textit{in vitro} can also result in abnormal viral morphogenesis. MacKinnon \textit{et al.} (1974) reported extremely long cylindrical profiles probably composed of capsid protein, unusual profiles in the form of sheets and whorls, extremely variable capsid diameters, and virogenic stromata and membranes but no virions. Such aberrant morphogenesis was associated with the absence of virions in some PIB and PIB containing aberrant shortened virions. Hirumi \textit{et al.} (1975) reported an increase in long tubular profiles after several passages of \textit{A. californica} NPV in a \textit{T. ni} cell line. They also reported PIB with very few intact virus particles, many ‘empty’ spaces and small vesicles apparently being occluded to form the ‘empty’ spaces. Knudson & Harrap (1976) observed electron-lucent tubular structures of variable lengths and the accumulation of what appeared to be two polyhedral membranes fused together, back-to-back, in \textit{S. frugiperda} cells infected with Sf \textit{M} NPV after repeated passage in those cells.

The high degree of mortality in \textit{S. exigua} resulting from aberrant and non-productive infections with Sf \textit{M} NPV was unusual. The aberrant development of \textit{A. californica} \textit{M} NPV in \textit{H. virescens} (Vail & Jay, 1973; Vail \textit{et al.}, 1978). On the other hand, an isolate of \textit{H. armiger} \textit{M} NPV produces normal symptoms and infective polyhedra in \textit{S. frugiperda} even though the LC\textsubscript{50} for \textit{S. frugiperda} is about 420 times as great as the LC\textsubscript{50} for \textit{H. zea} (Hamm, 1982). It is particularly interesting that Sf \textit{M} NPV-D7 failed to produce even an aberrant infection in \textit{S. exigua} but was still infective to \textit{S. frugiperda}.

The suggestion by Summers & Arnott (1969) and Chung \textit{et al.} (1980) that PIB are formed from fibrous material (FM) was not supported by the results of this study. Although FM was common in both the nucleus and cytoplasm of \textit{S. frugiperda} cells infected with the wild-type isolate, A, it was intimately associated with PIB only in several cells of \textit{S. frugiperda} infected with isolate B2. If PIB are formed directly from FM, more examples of close association of FM and PIB would be expected, especially in \textit{S. frugiperda} infected with isolate A. There was, however, an apparent correlation between decreasing incidence of FM and decreasing size of PIB in \textit{S. frugiperda} infected with isolates A, B2 and D7, respectively.

REFERENCES


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