JOURNAL OF VIROLOGY, June 1976, p. 1040-1050
Vol. 18, No. 3

Printed in U.S.A.

Strain Selection During Serial Passage of *Trichoplusia ni* Nuclear Polyhedrosis Virus

KATHLEEN N. POTTER, P. FAULKNER,* AND E. A. MACKINNON

Department of Microbiology and Immunology and Department of Anatomy, Queen's University, Kingston, Ontario, Canada*

Received for publication 30 December 1975

Two strains of a nuclear polyhedrosis virus (NPV) of *Trichoplusia ni* were isolated on the basis of plaque morphology. They are designated as MP (having greater than 30 polyhedra per nucleus) and FP (having fewer than 10 polyhedra per nucleus). Serial, undiluted passage of plaque-purified MP nonoccluded virus (NOV) in tissue culture led to the production of the FP phenotype detectable at passage 9. With continued serial, undiluted passage, FP became the predominant strain. Comparative growth curves showed that FP NOV are released faster than MP NOV. MP morphology was not observed after 14 serial, undiluted passages of plaque-purified FP. By the plaque neutralization assay, NOV from both strains of virus was neutralized by the homologous and heterologous antisera. The FP phenotype was observed when FP virus was grown in culture at 17, 22, and 27 C. Hence, the FP phenotype was not considered to be the result of temperature-inhibited crystallization of polyhedrin under standard tissue culture conditions. The NOV of both strains killed insects when injected directly into the hemocoele of *T. ni* larvae. Only MP inclusion bodies were virulent per os. The FP inclusion bodies fed to cabbage looper larvae did not kill, and no infectious agent could be detected in the hemolymph. Electron micrographs of MP polyhedra showed bundles of nucleocapsids of normal length within the polyhedra, whereas FP polyhedra contained heterogeneous, electron-dense material, which could account for their lack of pathogenicity.

The nuclear polyhedrosis viruses (NPV) of insects are members of the genus *Baculovirus* (22). Nuclei of infected cells contain polyhedral-shaped inclusion bodies (PIB), which consist of virions embedded in a noninfectious protein called polyhedrin (20). Under natural conditions of infection, the inclusion bodies dissolve in highly alkaline regions of the larval insect gut and release virions that initiate an infection in the gut epithelium (9).

Several NPV have been propagated in continuous insect cell lines. These are initially infected with virus by inoculating cultures with hemolymph (4, 8, 19) or with a cell extract from insects previously fed NPV PIB (21). Infected tissue cultures form PIB within their nuclei and also nonoccluded virions (NOV) at the cell membrane (16). NOV may be used to propagate the virus in tissue culture (4, 15).

Recently, a plaque titration technique was applied for assay of the NPV of *Autographa californica* in *Trichoplusia ni* cells (13). Two types of plaque morphology were observed: those with many PIB per cell nucleus (MP) and those containing few PIB per nucleus (FP). Electron microscope examination indicated that MP plaques contained inclusion bodies of the multiply embedded virus (MEV) type and FP plaques contained singly embedded virus (SEV) in inclusion bodies (17). The term MEV is used to describe PIB in which several nucleocapsids are surrounded by a single, outer envelope to form bundles; the term SEV indicates that the nucleocapsids are singly dispersed throughout the inclusion bodies.

Working with the NPV of *T. ni* propagated in a *T. ni* cell line, we have also observed two plaque morphologies and in this report show that they result from infection with separate strains of NPV. Under conditions of serial passage in vitro, the MP strain is replaced by an FP strain, probably due to mutation of the MP virus, followed by selection pressure in the tissue culture system which favors growth of FP virus.

MATERIALS AND METHODS

Tissue culture. An uncloned, continuous, insect cell line, TN-368, derived from *T. ni* (12) was grown as monolayers in either 25-cm^2 Falcon plastic flasks,

150-cm² Corning plastic flasks, or 35-mm diameter plastic petri dishes at 27 C. Tissue culture medium BML/TC10 (7) was sterilized by positive-pressure filtration through a series of membrane filters (Millipore Corp.) with pore sizes of 1.2, 0.8, 0.45, and 0.22 μm and stored at 4 C. Before use, the medium was supplemented with heat-inactivated fetal calf serum (10%, vol/vol) (Grand Island Biological Co.) and gentamicin sulfate (5 mg/100 ml of medium) (gift from Schering Corp., Montreal).

Virus. To provide virus suitable for infection of tissue culture, cabbage looper larvae were fed inclusion bodies of the MEV type (10). Hemolymph was collected and after dilution and filtration was used to infect the *T. ni* cell cultures (4). Progeny PIB found in tissue culture were examined using an electron microscope and were confirmed as being of the MEV type (16).

Plaque assay and plaque purification of viral strains. NOV was assayed by plaque titration on *T. ni* cells by the method of Hink and Vail (13). After 72 h at 27 C, plaques were observed and counted by using an inverted light microscope.

Plaques were of two morphological types. Some foci consisted of cells whose nuclei were packed with PIB (MP type) and others consisted of foci of cells exhibiting hypertrophy and contained <10 PIB/nucleus (FP type). MP and FP strains of virus were plaque purified three times on *T. ni* cells. Isolated plaques on plates containing fewer than five plaques were picked, using 20-μl Microcaps (Drummond Scientific Company), and expelled into 1 ml of medium. This suspension was used as inoculum for a second plaque assay. Isolated plaques were picked and replaqued. Isolated plaques were again picked, and this final plaque suspension was used to infect cells in a 25-cm² Falcon plastic flask. The resulting NOV suspension was called passage 1 and was used as an inoculum to grow larger virus stocks. All studies were done with virus derived from a single plaque that had not been passed more than three times in tissue culture.

End-point dilution assay for NOV. The method of Brown and Faulkner (1), in which cells were incubated in wells of a Falcon Microtest plate, was used for the end-point dilution assay of NOV. After incubation for 72 h at 27 C in a humid container to prevent evaporation, wells were observed under a microscope and were scored for the presence or absence of cytopathic effect. The 50% tissue culture infectious dose ($TCID_{50}$) was calculated by the statistical method derived by Reed and Muench (18). The relationship 1 $TCID_{50}$ = 0.7 infectious unit was applied for calculations of multiplicity of infection (MOI) (15).

Growth curves. Falcon plastic tissue culture flasks (25 cm²) seeded with 10^6 cells were infected at an MOI of 4 with MP or FP NOV. At the end of the virus adsorption period (1 h), unadsorbed virus was removed and the cells were washed twice with warm growth medium. The third wash was taken as time zero. Cultures were fed 5 ml of growth medium and, at regular time intervals thereafter, fluid from each flask was removed and centrifuged at 1,000 × *g* for 10 min to sediment floating cells (15). The supernatant was removed and stored at 4 C for assay of NOV. Pelleted cells were resuspended in 5 ml of fresh, warmed growth medium and returned to the culture flask. The flasks were incubated at 27 C until the next sampling period.

Serial undiluted passage of NOV. Falcon plastic petri dishes (35 mm) were seeded with 10^5 cells suspended in 2 ml of growth medium. The cells were allowed to settle for 1 h at 27 C. For the first passage, cells were infected at an MOI of approximately 100. The virus was left to adsorb for 1 h at 27 C, and the inoculating fluids were removed. The cells were fed 3 ml of growth medium and returned to the 27 C incubator for 48 h. At this time, 0.5 ml of supernatant containing NOV was used to initiate the next passage. The undiluted inoculum was added to 10^5 cells. After 1 h the inoculum was removed, replaced with 3 ml of growth medium, and incubated at 27 C for 48 h. The process of undiluted serial passage was repeated up to the levels described in the Results section. Fluids for assay were stored at 4 C and analyzed by plaque titration.

Quantitation of polyhedra per cell. Falcon plastic tissue culture flasks (25 cm²) seeded with 10^6 cells were maintained at 27 C for 1 h for cell attachment. Cells were infected at an MOI of 4 with plaque-purified NOV of the MP or FP strains, using virus from the second tissue culture passage. After 1 h the inoculum was removed and 5 ml of medium was added; 72 h later, cells were suspended in growth medium by shaking the flask and pelleted by centrifugation at 3,000 × *g* for 30 min. PIB were released by treatment of the pellet with 1 ml of 10% deoxycholate, 1 ml of Triton X-100, and 1 ml of deionized water for a minimum of 1 h at room temperature (4). The PIB were counted in a hemocytometer and, together with the cell number, the average number of polyhedra per cell was determined.

Effect of temperature on polyhedra production. Falcon plastic petri dishes (35 mm) were seeded with 5×10^5 cells per dish and infected at an MOI of 4 with either MP or FP NOV. After virus adsorption at 27 C, the inoculating fluids were removed and replaced with 2 ml of medium. Duplicate dishes were then incubated at 17, 22, and 27 C. Five days postinfection, polyhedra were released from infected cells, as described previously, and counted in a hemocytometer.

Preparation of immune sera. Female rabbits (approximately 2.5 kg) were injected intradermally with plaque-purified MP or FP NOV from the third tissue culture passage. NOV was concentrated by centrifuging virus-containing tissue culture fluids at 1,500 × *g* for 30 min to remove cell debris and was pelleted at 80,000 × *g* for 30 min in a Spinco SW27 rotor at 4 C. The virus was suspended overnight in 1 ml of growth medium and centrifuged through 29 ml of 20% sucrose at 73,000 × *g* for 1 h in a Spinco SW25 rotor at 4 C (11). Virus pellets were suspended overnight in 0.5 ml of Freund complete adjuvant (Difco Laboratories, Detroit, Mich.). An inoculum consisted of approximately 10^8 $TCID_{50}$/0.5 ml of adjuvant. The rabbits were injected intradermally and 4 weeks later were given three booster shots intradermally for two weeks at 3-day intervals. Ten days

after the final booster, the rabbits were exsanguinated by cardiac puncture, using Vacutainers (Beckton, Dickinson and Co., Canada Ltd.), and the serum was prepared. Serum was heated at 56 C for 30 min and stored in small aliquots at −20 C.

Plaque neutralization tests. Equal volumes (1 ml) of serum dilutions and a constant amount of virus (100 PFU) were mixed and incubated for 90 min at room temperature. Controls were (i) diluent and virus and (ii) normal rabbit serum and virus. The diluent was serum-free insect cell growth medium. Mixtures were analyzed for unneutralized virus by plaque assay.

Bioassays. PIB from cultured *T. ni* cells infected with second-passage MP and FP NOV were isolated as described previously and purified by centrifugation through 20 ml of a 40% sucrose solution at 6,700 × *g* for 30 min. The purified suspension of PIB was suspended in 1 ml of water, and the number of PIB was determined with a hemocytometer. Aliquots (5 μl) of dilutions were dispensed onto 0.83-cm^2 disks of collard leaf (previously washed in 1::100 Tween 20), and the deposit was allowed to dry. Disks were fed to 25 third-instar cabbage looper larvae, individually reared in shell vials (25 by 95 mm) plugged with corks, per dilution. After 24 h, larvae that had eaten at least 75% of the disk were fed a piece of diet and reared for an additional 10 days at 25 C after which time mortality by NPV was recorded (14). Dilutions of NOV strains were fed to larvae and scored in the same manner.

Aliquots (1 μl) of NOV of MP and FP variants were injected into 25 fourth-instar larvae per dilution. One hour later, live larvae were fed synthetic diet. After 24 h, larvae that had died because of injection trauma were removed from the assay. The insects were reared an additional 11 days at 25 C at which time mortality by NPV was recorded.

Hemolymph was collected from infected larvae 5 days postinfection by puncturing the insect at the base of the heart and withdrawing the hemolymph into a Pasteur pipette. The hemolymph from five insects was dispensed into 2 ml of growth medium and filtered through a Millipore-Swinnex apparatus containing a 0.3-μm filter. The virus strain content of the hemolymph suspensions was determined by the plaque assay method.

Electron microscopy. Infected cells that had been incubated for 72 h postinfection at 27 C were removed from the surface of Falcon plastic petri dishes and prepared for electron microscope examination as described previously (16). Purified PIB were prepared for electron microscopy after release from cells with Triton X-100 and deoxycholate (4).

RESULTS

Plaque morphology and titration of MP and FP virus strains. Typical plaque morphologies produced by the MP and FP virus strains are shown in Fig. 1. MP plaques (Fig. 1B) are composed of cells containing many PIB (greater than 30 per cell), whereas FP plaques (Fig. 1A) are foci of rounded cells containing 0 to 10 polyhedra per cell. Both MP and FP plaques are approximately 0.5 mm in diameter.

The number of plaques on several plates was determined on days 3, 4, and 5 postinfection with no change in plaque count. Thus, the overlay (0.6% methylcellulose) allowed only localized transmission of progeny virus to adjacent cells. A linear dose response obtained by plaque titration of the FP strain NOV is given in Fig. 2.

Plates containing FP plaques were observed microscopically on days 3, 4, and 5 postinfection. The FP plaques did not produce the quantity of PIB characteristic of MP plaques and, therefore, are not considered to be precursors of MP plaques.

Growth curves. Figure 3 shows the growth cycles of MP and FP NOV after infection of cell cultures at an MOI of 4. The titer of the released virus was determined at intervals and the accumulated titer was calculated. The latent period for both virus strains was 12 h followed by an exponential phase reaching the maximal titer in 2 days. The steeper logarithmic-rise period indicates that virions of the FP strain were released faster than those of the MP strain and, in two separate experiments, reached a maximal titer one-half log higher than MP virus. The virus yield was 30 and 90 infectious units per cell for MP and FP, respectively. If the DNA content of *T. ni* polyhedra is taken as 12.2 μg/mg (5), we calculated that there are approximately 330 nucleocapsids per PIB. Therefore, between 1×10^4 and 2×10^4 nucleocapsids are produced per MP-infected cell. In these experiments the intracellular NOV content was not measured. Titrations of the pooled, unadsorbed virus and two washings indicated that both MP and FP NOV adsorb to cells with equal efficiency (76 and 80%, respectively).

Plaque neutralization tests. Antisera made against plaque-purified MP and FP NOV were reacted with both homologous and heterologous virus. Figure 4 indicates that both MP and FP antisera neutralize both MP and FP NOV. Diluent and normal rabbit sera had no effect on the number of plaques observed for either strain. These observations indicate that the surface antigens of both MP and FP virions are very similar in antigenic composition.

Polyhedra production. PIB were released from cells infected with NOV at an MOI of 4. Cells infected with MP NOV consistently yielded an average of greater than 30 PIB/cell, whereas cells infected with FP NOV yielded less than 10.

Purified PIB from the third-passage level

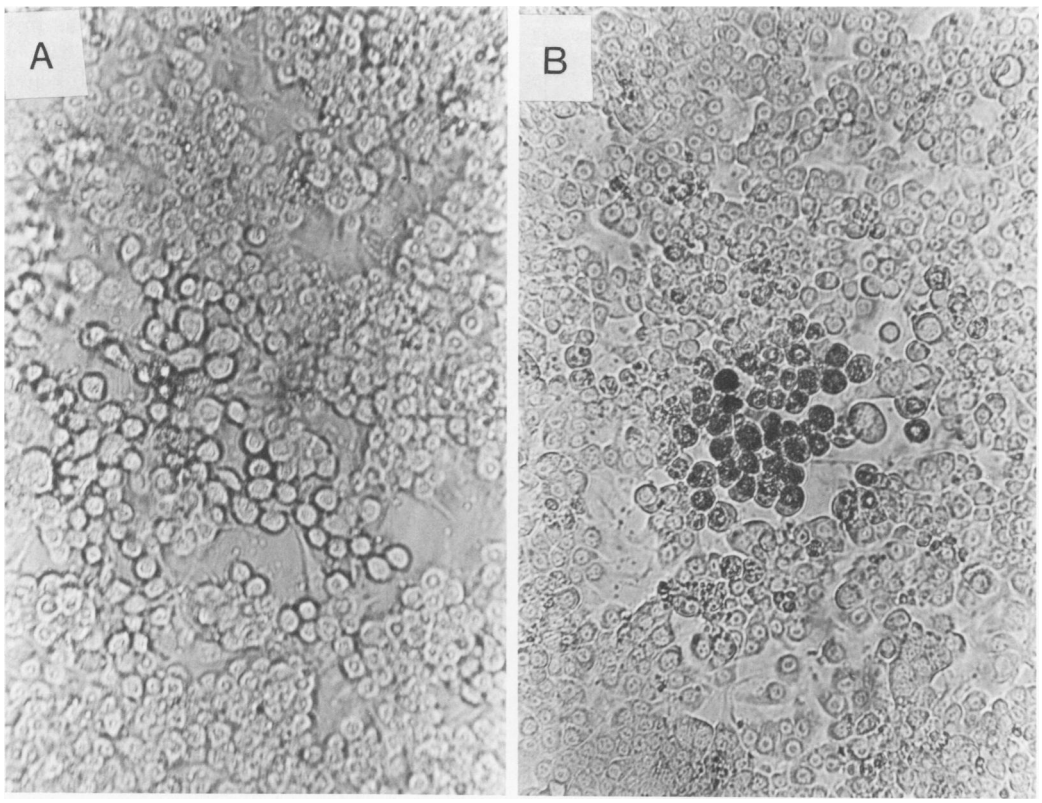

FIG. 1. *Appearance of FP (A) and MP (B) plaques on T. ni cells 72 h postinfection under an inverted light microscope, ×240. FP plaques exhibit rounded cells with hypertrophied nuclei with few PIB. MP plaques exhibit rounded cells and hypertrophied nuclei filled with PIB, giving infected cells an overall black appearance.*

were examined in an electron microscope. PIB from the MP virus strain were of the MEV type (Fig. 5). The virus bundles consisted of two to seven nucleocapsids. Although many nucleocapsids were occluded within the nucleus of MP-infected cells, a few nucleocapsids budded through the plasma membrane 72 h postinfection.

PIB from the FP strain did not contain rod-shaped nucleocapsids (Fig. 6). Sectioned inclusion bodies often appeared empty, but sometimes enveloped structures were seen. These contained fragments of electron-dense material, which did not fall into a particular size class and did not resemble viral nucleocapsids. Nucleocapsids were observed budding at plasma membranes of cells infected with the FP strain (Fig. 7). Virions were released in large numbers and were more loosely enveloped than those released from MP-infected cells.

NOV that budded from cells infected with FP and MP strains contained nucleocapsids of similar lengths (335 nm).

Production of polyhedra at reduced incubation temperatures. The possibility was considered that the genome of the FP strain may instruct a temperature-sensitive inclusion body protein that would not crystallize effectively at 27 C and, hence, would give rise to the FP phenotype characterized by few inclusions per cell.

Table 1 shows that the number of PIB produced by the FP strain is not influenced by these temperatures. PIB production by the MP strain was the same at 27 and 22 C but was reduced at 17 C. At 27 and 22 C, uninfected cell controls remained healthy, whereas at 17 C they began to deteriorate.

Infectivity of strains. The virulence of PIB and NOV was assessed in cabbage looper larvae (Table 2). Only MP strain PIB were pathogenic with a 50% lethal dose (LD_{50}) of 56 PIB per larva. MP NOV was detected in the hemolymph after feeding MP PIB. FP strain PIB neither killed the larvae nor yielded an infectious agent in the hemocoels.

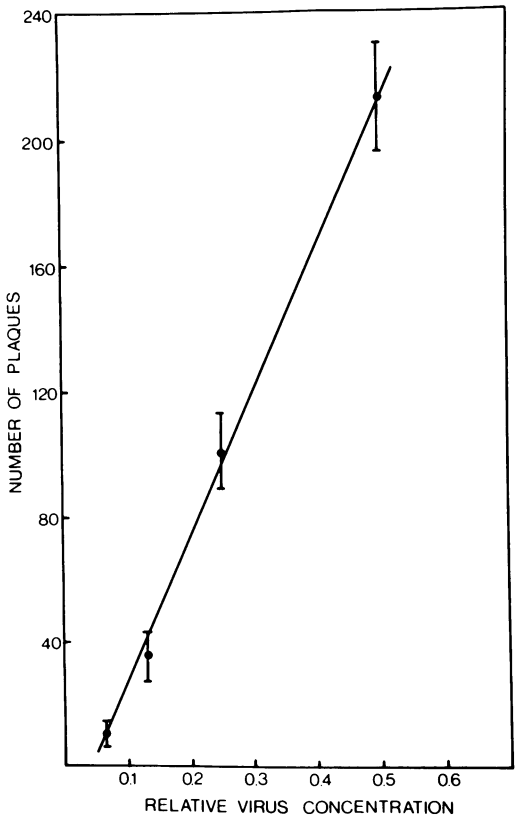

FIG. 2. *Proportionality of FP virus concentration to plaque count. Twofold dilutions of virus were analyzed by plaque assay in duplicate. Plaques were counted, unstained, 72 h postinfection under an inverted light microscope.*

NOV of both MP and FP strains were equally lethal for insects when injected directly into the hemocoel but did not kill after per os infection. Plaque assays on the hemolymph from insects injected with MP strain NOV indicated that a mixture of MP and FP strains was present (97% MP and 3% FP). Assays on hemolymph from insects injected with FP NOV remained homogeneous for the FP strain.

Changes in phenotype after serial passage of virus. MacKinnon et al. (16) reported that the average production of PIB per cell decreases as the NPV is serially passed in tissue culture. Subsequently, we investigated the distribution of MP and FP strains at up to 50 passages from the original isolation of the virus from hemolymph of insects fed PIB. The data in Fig. 8 show that the predominant strain of virus at passage level 2 was MP (approximately 99%) but that by passage level 5 the FP strain was already in excess and by passage level 25 had become the sole plaque-producing strain in the cultures.

A second series of serial passages was initiated with plaque-purified MP virus (Fig. 9). MP virus remained homogeneous with regard to phenotype for eight consecutive passages but, beyond this level, FP plaques appeared and displaced production of MP virus. FP virus remained homogeneous for 14 consecutive passages with no reversion to the MP phenotype.

DISCUSSION

A plaque assay technique (13) was used to assay the NPV of *T. ni* and to provide foci of infected cells for plaque purification of the virus. MP and FP foci (Fig. 1) were readily distinguished on plates inoculated with virus that had been grown for several passages in tissue culture before plaque assay. Three cycles of plaque purification were carried out to obtain virus strains that exclusively produced the MP and FP phenotypes.

The plaque assay technique used here is based on that of Dulbecco (2) for other animal

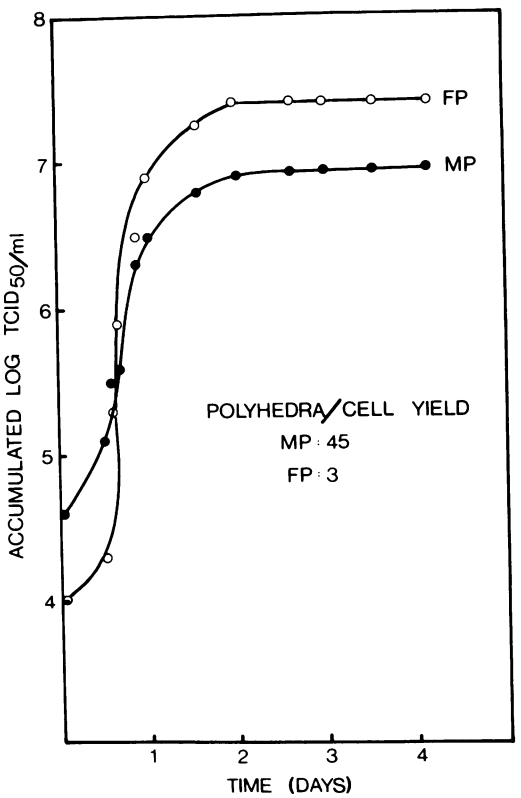

FIG. 3. *Growth curves of MP and FP NOV in tissue culture. Cultures were infected at an MOI of 4, and the extracellular infectivity was monitored at intervals. The accumulated log $TCID_{50}$ per milliliter was calculated at each interval. Symbols: ○, FP strain; ●, MP strain.*

viruses. The linear dose response obtained by plaque titration of the FP NOV strain shown in Fig. 2 indicates that a plaque is caused by a single infectious particle. If more than one virus was required to initiate a plaque, the curve would not be linear (3). By demonstrating that a single FP virion can initiate a plaque, it is concluded that FP virions, although having the ability to make only small amounts of polyhedrin, are not defective particles and do not require a "helper" virus for infection to proceed.

Both plaque-purified strains were used to raise antibodies, and each kind of antibody neutralized both MP and FP viral strains (Fig. 4). It remains to be resolved whether host antigens are an integral part of the virion or could have contaminated the antigen preparation. In either instance, cross-reaction between the viral strains would also be observed. However, the two strains of NOV appear to share many similar antigenic determinants, and it is possible that the altered plaque morphology is due to differences in the polyhedrin of inclusion bodies rather than to major differences in virion nucleocapsids and envelopes.

The FP strain shows a selective advantage in growth in *T. ni* cells cultured in vitro. Virus is released more rapidly and rises to a higher titer than is observed with the MP strain (Fig. 3). The production of inclusion bodies by the FP strain relative to the MP strain is not considered to be the result of incubation at a restrictive temperature (Table 1). It is not known whether the FP genome codes for more polyhedrin than is observed as crystallized PIB, or if large amounts of polyhedrin are synthesized but do not crystallize.

When NPV derived from insect hemolymph was serially passed in vitro there was a fall in the average yield of PIB/cell corresponding to a gradual selection of the FP strain (Fig. 8). Starting with the plaque-purified MP strain, there was a delay of eight passages before the FP phenotype appeared, but thereafter there was rapid replacement of the MP strain with FP virus (Fig. 9). In over 14 serial passages no MP virus arose after the series was initiated with FP virus. These results bear out the hypothesis that FP strains grow more readily in *the T. ni* cell line and, if they arise by mutation, have a selective advantage over MP strains.

Previous studies established that aberrant forms of virus are present in inclusion bodies in late-passage virus but not in early-passage virus (16). We examined the polyhedra and NOV in thin sections of cells infected with early-passage, plaque-purified MP and FP strains of virus. NOV consisting of enveloped nucleocapsids from both strains had similar morphology.

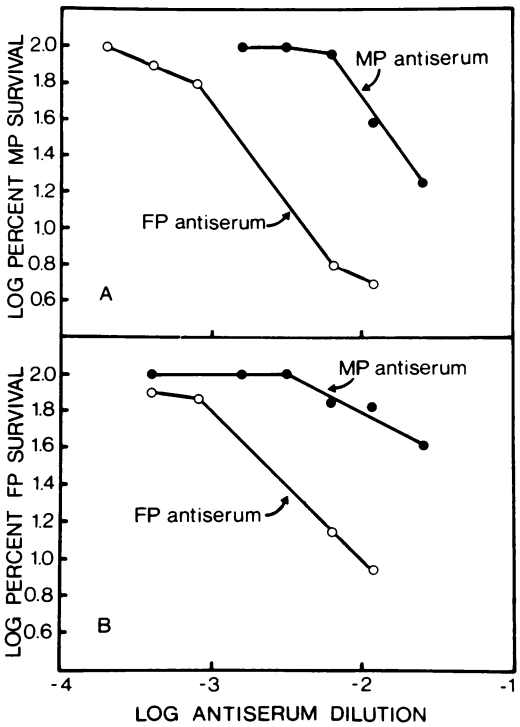

FIG. 4. *Plaque neutralization of MP and FP NOV. Equal volumes of serum dilutions (anti-MP or anti-FP) and approximately 100 PFU of virus were mixed and incubated for 90 min at room temperature. The mixtures were analyzed for unneutralized virus by plaque assay. The neutralization of MP virus (A) and of FP virus (B) with MP and FP antisera are shown. Symbols: ○, FP antiserum; ●, MP antiserum.*

Electron micrographs of NOV budding at the cell membrane reveal that occasionally more than one nucleocapsid is enclosed within a single envelope, i.e., polyploidy. This is not considered unusual since polyploidy is common among animal viruses that mature by budding from cell membranes (6). Major differences were seen between sections of MP and FP polyhedra. Sections of MP inclusions appeared to have typical MEV structure and were infectious per os (Fig. 5, Table 2). The polyhedra contained many bundles consisting of groups of nucleocapsids enclosed by a common membrane. The morphology is similar to the MEV *T. ni* NPV described by Heimpel and Adams (10). It is the predominant structure observed when the *T. ni* NPV is passed in insects. The fine structure of FP inclusion bodies was different in that the inclusions did not contain distinct rod-shaped nucleocapsids. These inclusions were sparsely populated with enveloped structures that contained some heavily stain-

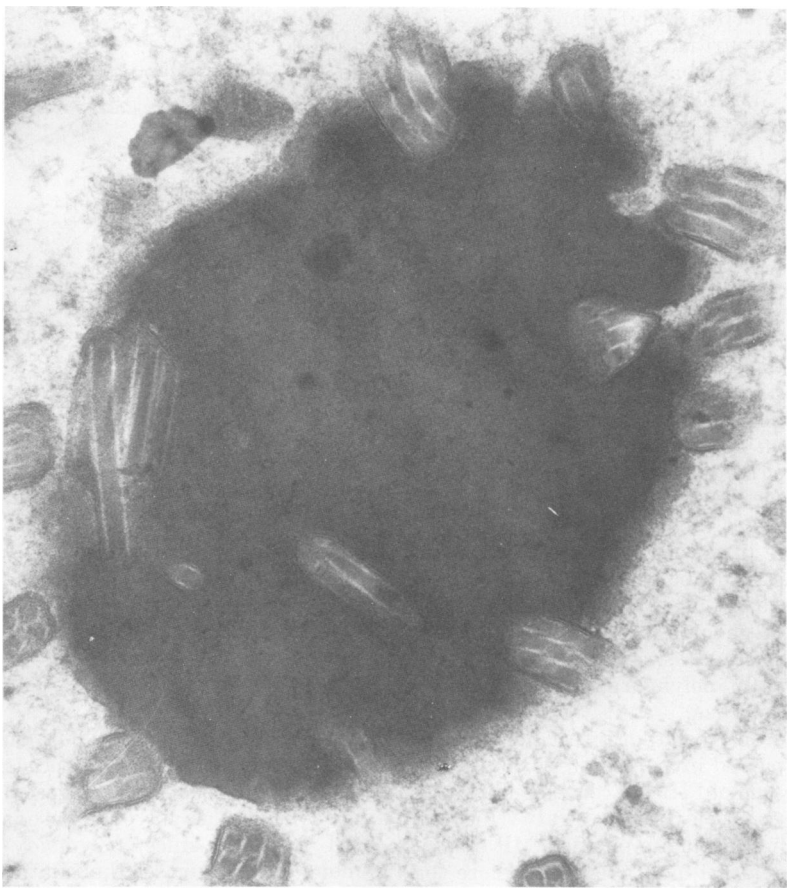

FIG. 5. *Thin section of MP PIB 96 h postinfection. Third-passage PIB, ×104,000.*

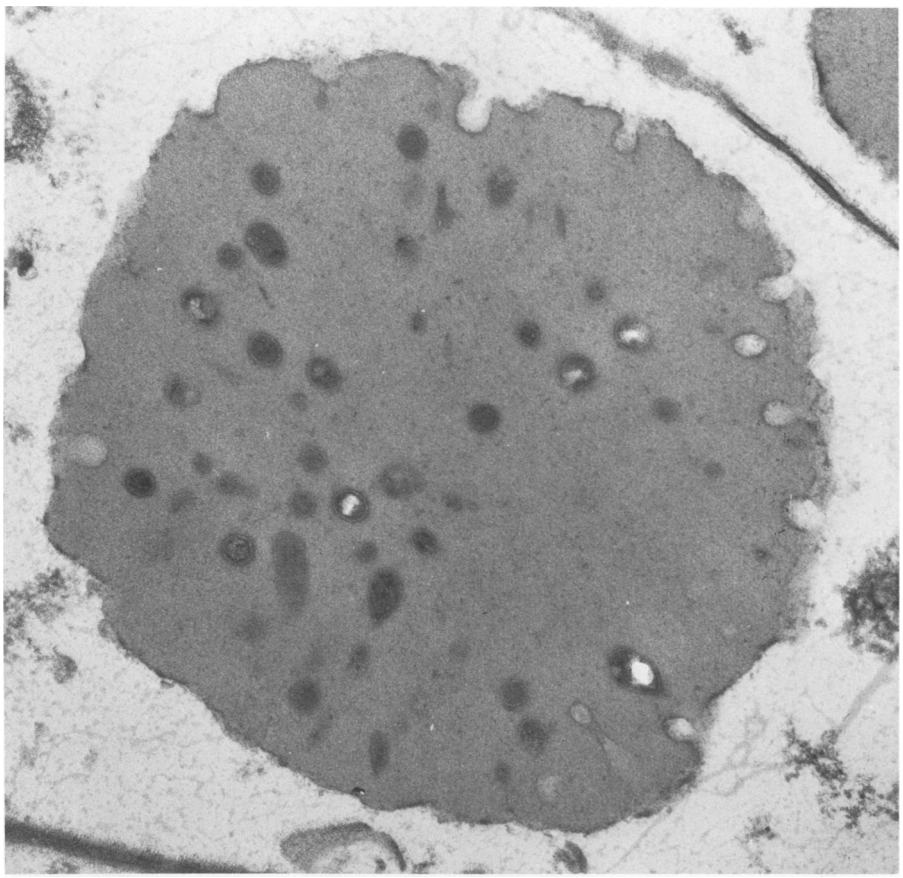

FIG. 6. *Thin section of FP PIB 96 h postinfection. Third-passage PIB, ×57,000.*

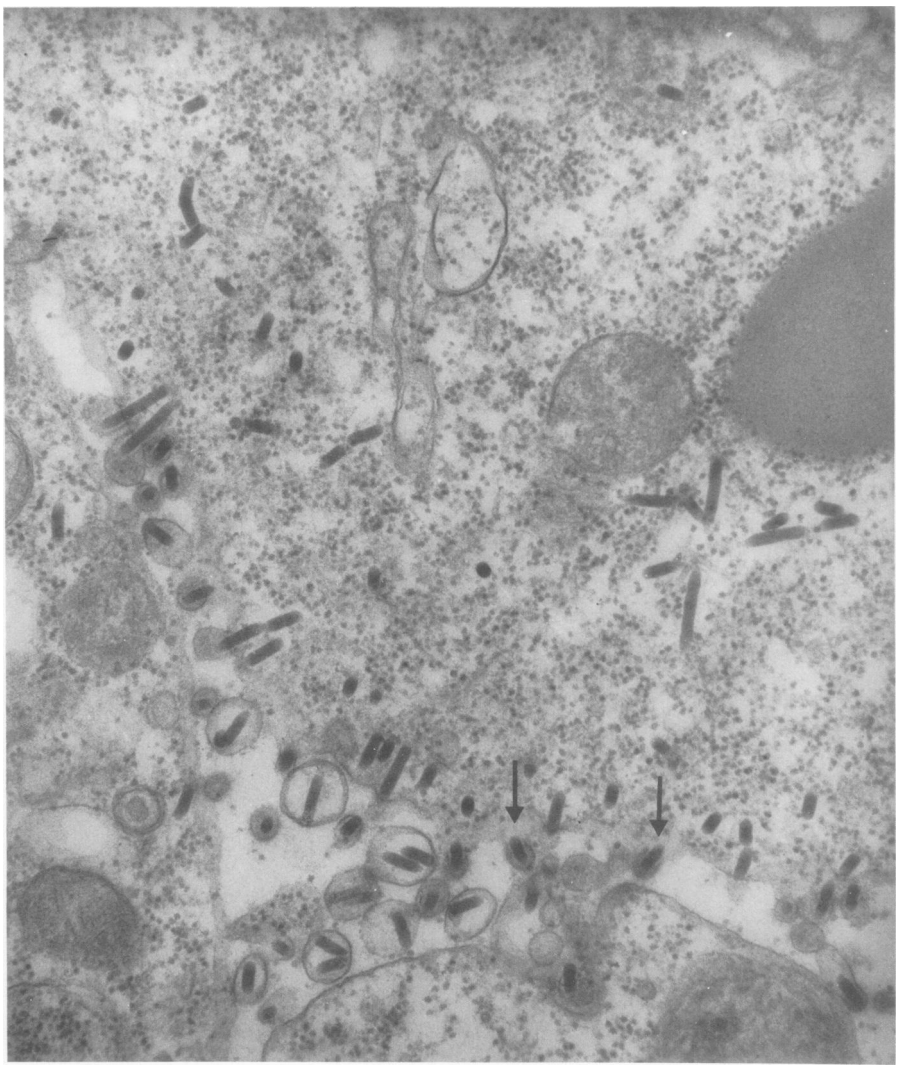

FIG. 7. *FP-infected cells. Infected cells were removed from the growth surface by shaking at 72 h postinfection. The cells were pelleted and prepared for electron microscopy. Nucleocapsids are seen budding at the cell membrane (arrows). ×13,000.*

TABLE 1. *Effect of temperature on polyhedra production*[a]

Incubation temp (C)	Polyhedra/cell	
	MP	FP
27	70	5
22	67	6
17	30	5

[a] Effect of temperature on polyhedra production. Falcon plastic petri dishes (35 mm) were seeded with 5×10^5 cells per dish and infected at an MOI of 4 with either MP or FP NOV. After 1 h at 27 C, inoculating fluids were removed and replaced with 2 ml of medium. Duplicate dishes were then distributed to 17, 22, and 27 C incubators. Five days postinfection, PIB were released from infected cells by treatment with 1 ml of 10% deoxycholate, 1 ml of Triton X-100, and 1 ml of deionized water for a minimum of 1 h at room temperature and were then counted in a hemocytometer.

TABLE 2. *Bioassays of cell culture products*[a]

Virus	LD_{50} fed (PIB/larva)	LD_{50} injected ($TCID_{50}$/insect)
NOV		
MP	0	16 (97% MP, 3% FP)
FP	0	29 (100% FP)
Polyhedra		
MP	56 (100% MP)	
FP	0 (no CPE in cell culture)	

[a] Bioassays of viral tissue culture products. Cabbage looper larvae were fed and injected with purified NOV and fed purified PIB. Control experiments showed that infectious NOV could be recovered from collard leaves on which virus had been deposited and dried as described in the text. Numbers in parentheses refer to the proportion of MP and FP NOV detected in hemolymph by the plaque assay.

ing material but of no distinct form (Fig. 6). The FP inclusion bodies were not infectious per os (Table 2) and did not resemble the SEV of *T. ni* NPV. Thus, FP polyhedra do not appear to encapsulate FP nucleocapsids. If nucleocapsid material is present in the FP inclusions, it may have been distorted by the crystallization process. An alternative is that cellular components have become trapped in viral material, and these pseudovirions may have become occluded.

Results given in Table 2 indicate that FP NOV arise in vivo. Although FP polyhedra are not infectious per os, injected FP NOV were lethal and the virus was propagated as the FP strain. When MP NOV were injected, the hemolymph of diseased insects contained both MP and FP NOV (Table 2). This finding suggests that new strains with an FP phenotype arise during systemic replication of MP NOV. Should FP polyhedra be formed in vivo as a consequence of the strain change they would not be replicated under natural conditions of virus transmission since FP polyhedra are not infectious per os (Table 2).

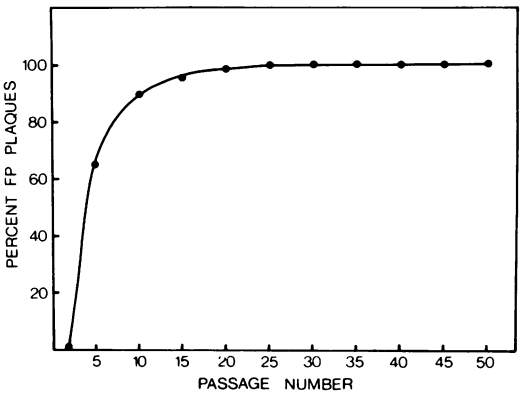

FIG. 8. *Serial, undiluted passage of uncloned virus. The virus was originally derived from infectious hemolymph as described previously (16). Samples were thawed and analyzed by plaque assay for the proportion of MP and FP plaques at the passage levels indicated.*

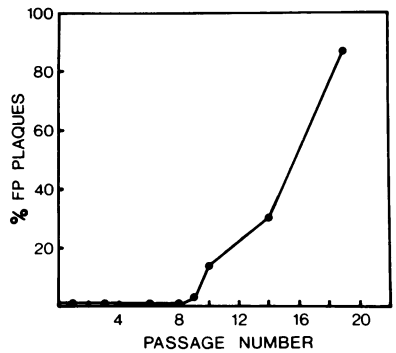

FIG. 9. *Serial, undiluted passage of plaque-purified MP NOV. Falcon plastic petri dishes (35 mm) were seeded with 10^5 cells per dish. After 1 h at 27 C, the cells were infected with MP virus at an MOI of 100 and incubated for 48 h at 27 C. An aliquot (0.5 ml) was used to initiate the subsequent passage. Successive passages were carried out in the same manner. Viral harvests were analyzed by plaque assay, and the proportion of MP and FP plaques was determined.*

ACKNOWLEDGMENTS

We gratefully acknowledge financial support of the Medical Research Council, Ottawa, Canada.

We thank R. P. Jaques, Canada Agriculture Research Station, Harrow, Ontario, for assistance in the in vivo bioassays and Sheila Leonard for technical assistance.

LITERATURE CITED

1. **Brown, M., and P. Faulkner.** 1975. Factors affecting the yield of virus in a cloned cell line of *Trichoplusia*

ni infected with a nuclear polyhedrosis virus. J. Invertebr. Pathol. 26:251-257.
2. **Dulbecco, R.** 1952. Production of plaques in monolayer tissue cultures by single particles of an animal virus. Proc. Natl. Acad. Sci. U.S.A. 38:747-752.
3. **Dulbecco, R., and M. Vogt.** 1954. Plaque formation and isolation of pure lines with poliomyelitis viruses. J. Exp. Med. 99:167-182.
4. **Faulkner, P., and J. F. Henderson.** 1972. Serial passage of a nuclear polyhedrosis disease virus of the cabbage looper *(Trichoplusia ni)* in a continuous tissue culture cell line. Virology 50:920-924.
5. **Faust, R. M., and Z. E. Estes.** 1965. Nucleic acid composition of the nuclear polyhedrosis virus from *Trichoplusia ni*. J. Invertebr. Pathol. 7:521-522.
6. **Fenner, F., B. R. McAuslan, C. A. Mims, J. Sambrook, and D. O. White.** 1974. The biology of animal viruses, 2nd ed. Academic Press Inc., New York.
7. **Gardiner, G. R., and H. Stockdale.** 1975. Two tissue culture media for production of lepidopteran cells and nuclear polyhedrosis viruses. J. Invertebr. Pathol. 25:363-370.
8. **Goodwin, K. H., J. F. Vaughn, J. R. Adams, and S. J. Louloudes.** 1970. Replication of a nuclear polyhedrosis virus in an established insect cell line. J. Invertebr. Pathol. 16:284-288.
9. **Harrap, K. A.** 1973. Virus infection in invertebrates, p. 271-299. *In* A. J. Gibbs (ed.), Viruses and invertebrates. North Holland Publishing Co., Amsterdam.
10. **Heimpel, A. M., and J. R. Adams.** 1966. A new nuclear polyhedrosis of the cabbage looper, *Trichoplusia ni*. J. Invertebr. Pathol. 8:340-346.
11. **Henderson, J. F., P. Faulkner, and E. A. MacKinnon.** 1974. Some biophysical properties of virus present in tissue cultures infected with the nuclear polyhedrosis virus of *Trichoplusia ni*. J. Gen. Virol. 22:143-146.
12. **Hink, W. F.** 1970. Established insect cell line from the cabbage looper, *Trichoplusia ni*. Nature (London) 226:466-467.
13. **Hink, W. F., and P. V. Vail.** 1973. A plaque assay for titration of alfalfa looper nuclear polyhedrosis virus in a cabbage looper (TN-368) cell line. J. Invertebr. Pathol. 22:168-174.
14. **Jaques, R. P.** 1967. The persistance of a nuclear polyhedrosis virus in the habitat of the host insect, *Trichoplusia ni*. I. Polyhedra deposited on foliage. Can. Entomol. 99:785-794.
15. **Knudson, D. L., and T. W. Tinsley.** 1974. Replication of a nuclear polyhedrosis virus in a continuous cell culture of *Spodoptera frugiperda*: publication, assay of infectivity, and growth characteristics of the virus. J. Virol. 14:934-944.
16. **MacKinnon, E. A., J. F. Henderson, D. B. Stoltz, and P. Faulkner.** 1974. Morphogenesis of nuclear polyhedrosis virus under conditions of prolonged passage *in vitro*. J. Ultrastruct. Res. 49:419-435.
17. **Ramoska, W. A., and W. F. Hink.** 1974. Electron microscope examination of two plaque variants from a nuclear polyhedrosis virus of the alfalfa looper, *Autographa californica*. J. Invertebr. Pathol. 23:197-201.
18. **Reed, L. J., and H. Muench.** 1938. A simple method of estimating fifty per cent end-points. Am. J. Hyg. 27:493-497.
19. **Sohi, S. S., and J. C. Cunningham.** 1972. Replication of a nuclear polyhedrosis virus in serially transferred insect hemocyte cultures. J. Invertebr. Pathol. 19:51-61.
20. **Summers, M. D., and K. Egawa.** 1973. Physical and chemical properties of *Trichoplusia ni* granulosis virus granulin. J. Virol. 12:1092-1103.
21. **Vail, P. V., D. L. Jay, and W. F. Hink.** 1973. Replication and infectivity of the nuclear polyhedrosis virus of the alfalfa looper, *Autographa californica*, produced in cells grown *in vitro*. J. Invertebr. Pathol. 22:231-237.
22. **Wildy, P.** 1971. Classification and nomenclature of viruses, p. 32. *In* Monographs in virology, no. 5. S. Karger, Basel.