ABORTIVE CELL CULTURE INFECTIONS OF NUCLEAR POLYHEDROSIS VIRUSES AS MODEL SYSTEMS FOR HOST SPECIFICITY

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ABSTRACT – We describe virus growth and cytopathic effect (CPE) in an abortive infection involving *Autographa californica* nuclear polyhedrosis virus (AcMNPV) and a *Bombyx mori* line. Our results show that AcMNPV causes complex and very unusual CPE in *B. mori* cells. This involves the formation of sacs and protrusions in addition to the nuclear hypertrophy and rounding normally observed in productive infections. Infection of *B. mori* cells with UV-inactivated AcMNPV induces only sacs, indicating that viral gene expression is not required for this effect. No infectious virus is produced in *B. mori* cells. Electron microscopy show that virogenic stroma, but no virions or polyhedral inclusim bodies, are formed in *B. mori* cells. Nucleocapsids are detected in 20 percent of the infected cells, but are defective. Our results suggest that a virion component of an NPV causes cytopathic effect. This principle has important implications in the identification of viral genes for engineering transgenic crop plants resistant to pest insects.

Index terms: *Autographa californica, Bombyx mori,* virogenic stroma.

INFECÇÕES ABORTIVAS EM CULTURA DE CÉLULAS PELO VÍRUS DA POLIEDROSE NUCLEAR COMO SISTEMAS-MODELO PARA ESPECIFICIDADE DO HOSPEDEIRO

RESUMO - Descreve-se a proliferação do vírus e o efeito citopático numa infecção abortiva que envolve o vírus de poliedrose nuclear de *Autographa californica* (AcMNPV) e uma linhagem de *Bombyx mori*. Os resultados indicam que o AcMNPV acarreta efeitos citopáticos (CPE) complexos e raros nas células do *B. mori*. Tal fato envolve a formação de sacos e protusões, além da hipertrofia e arredondamento do núcleo, normalmente observados em infecções produtivas. A infecção de células de *B. mori* com AcMNPV inativado por raios ultravioleta induz a formação somente de sacos, o que indica que a expressão gênica virial não é necessária para este efeito. Nas células de *B. mori* nenhum vírus infeccioso é produzido. A microscopia eletrônica mostra que somente o estroma virogênico é formado nas células de *B. mori* e não os vírions ou PIBs (corpos de inclusão poliédricos). Detectam-se nucleocapsídeos em 20% de células infectadas, porém estes são defectivos. Os resultados sugerem que um componente virial de Vírus de Poliedrose Nuclear (NPV), causa efeito citopático. Este princípio tem importantes implicações na identificação de genes virais visando a produção de plantas transgênicas resistentes a insetos-praga.

Termos para indexação: *Autographa californica, Bombyx mori,* estroma virogênico.

INTRODUCTION

Nuclear polyhedrosis viruses (NPVs) are members of the family Baculoviridae and consist of rod-shaped (50 x 250 nm) enveloped particles (Bilimoria 1986). They contain a circular, supercoiled, double-stranded DNA molecule which has a molecular weight of approximately 85 x 10^6

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daltons (Miller & Dawes 1979; Smith & Summers 1979). Following assembly in the nucleus, viral nucleocapsids enter the cytoplasm and bud through the plasma membrane, producing the non-occluded, budded virus (BV). Alternatively, nucleocapsids may acquire a membrane while in the nucleus and then become embedded within a polyhedral inclusion body (PIB) composed primarily of polyhedrin, a polypeptide with a molecular weight of approximately 30,000 (Summers et al. 1980). Occluded virions (OV) are responsible for transmitting the virus from host-to-host, whereas cell-to-cell transmission in infected insects and in cultured cells is mediated by non-occluded virus (NOV) (Granados & Lawlor 1981). For a recent revious of the NPVs, the reader is referied to Bilimoria (1991).

While most NPVs are not species specific, they do have a narrow host range. Generally, NPVs infect only members of the genus or, in some cases, the family of the original host. Furthermore, there is evidence that the SNPVs are more specific than the MNPVs (Bilimoria 1986).

An understanding of the mechanisms of NPV host specificity is of interest from the perspective of differential expression of viral genes; such understanding is also important for developing viral pesticides having broad target specificity and increased virulence. Moreover, this line of research could yield information on the molecular basis of viral pathogenicity, and this, in turn, would be useful in the development of transgenic plants resistant to insect pests. However, it is a curious fact of baculovirus research that good host range mutants have not been isolated, and this has necessitated the use of semipermissive and nonpermissive cell lines in the study of host specificity (Carpenter & Bilimoria 1983; Bilimoria et al 1986; Bilimoria, 1991).

Studies with vertebrate cell lines have shown that while uptake of virus may occur, there is no convincing evidence of NPV replication in these cells (Tjia et al 1983). A report (Himeno et al. 1967) that DNA from *Bombyx mori* NPV induces production of PIBs in mammalian amnion cell cultures remains unconfirmed, and at least two laboratories have shown that, contrary to a previous report, AcMNPV does not replicate in the hamster ovary cell line CHO-K1. Tjia et al. (1983) studied the replication of AcMNPV in five human cell lines and a mammalian cell line and showed that even at multiplicities of 100, AcMNPV did not replicate in them. Tests for the replication or transcription of viral DNA were negative, and there was no evidence for the persistence of viral DNA or fragments of viral DNA in these mammalian cells. AcMNPV appears to enter the nucleus of cell lines derived from frog, turtle, and trout, but there is no synthesis of viral RNA or DNA.

NPV infections of semipermissive and nonpermissive insect cell lines show that restriction of infection in alternate hosts can occur at a number of different stages in the replication cycle of the virus.

The first infection of this type was described by Carpenter & Bilimoria (1983) who studied the infection of *Trichoplusia ni* (TN-368) cells with SfMNPV. In this infection, the virus induces early cytopathic effect (CPE), including nuclear hypertrophy and the formation of a virogenic stroma characteristic of SfMNPV infection in the permissive *Spodoptera frugiperda* (IPLB-SF-21) cells but does not produce complete virions. While earlier experiments had suggested that some progeny infectious virus was produced, more recent work strongly suggests that infectious virus is not produced and that the infection is nonpermissive (Liu 1987). In other nonpermissive infection systems, restriction seems to be occurring at a somewhat later stage of the NPV growth cycle. McClintock et al. (1986) showed that in infections of nonpermissive gypsy moth cells, IPLB-LD-652Y, with AcMNPV, early but not late ICSPs were synthesized. The virus produced classic CPE in these cells but failed

to produce infectious progeny. Viral DNA replication was initiated from eight to 12 hr p.i. and continued at a maximum rate up to 20 hr p.i. The rate of DNA synthesis approximated that observed in permissive TN-368 cells.

Due to its broad host range in vivo and its infectivity in many species of cultured insect cells, AcMNPV has great potential as a biological pesticide for controlling agriculturally important insect pests (Summers & Smith 1985). This and the recent use of this virus as a gene expression vector (Carbonell et al 1985; Pennock et al 1984; Smith et al. 1983) has prompted investigation of the molecular basis of host range for AcMNPV. In the model system we describe, AcMNPV replicates permissively in *Spodoptera frugiperda* (SF) cells but semipermissively in *Bombyx mori* (BM) cells. Earlier work by Summers et al. (1978) utilizing an immunoperoxidase staining technique, showed that 90 percent of AcMNPV-infected BM cells produced enveloped nucleocapsid antigens and infectious virus, but less than 1 percent of the cells produced polyhedra. In addition, polyhedrin antiserum failed to detect any polyhedrin in these cells. These observations raise important questions of how the BM cell is able to block some function(s) required for polyhedrin synthesis and subsequent PIB formation. In this paper, we describe the cytopathic effect of AcMNPV in BM cells and show that infected BM cells develop a distinct virogenic stroma but no virions. We also show that the CPE detectable by light microscopy is very distinct from that observed in any other baculovirus infections thus far and that one of the two unusual types of CPE can be induced by UV inactivated virus.

MATERIALS AND METHODS

Cell growth and maintenance

Continuous cell lines of SF cells, *Spodoptera frugiperda* (SF-IPLB-21) (Vaughn et al. 1977) and BM cells, *Bombyx mori* (BM-5) (Grace 1967) were grown in Hink's TNMFH medium (Hink et al. 1977), supplemented with 10 percent fetal bovine serum. Both cell lines were grown at 28° C. Routine subculture of both SF and BM cells were done at 6-day intervals but at a 1:10 and 1:15 ratio respectively.

Virus stock production

Virus stocks were grown in vitro in permissive SF cells (Hink et al. 1977). Budded virus (BV) was harvested by centrifugation of media at 2000 x g for 10 minutes to remove any cell debris. The virus in the supernatant was titrated using the tissue culture infectious dose 50 ($TCID_{50}$) assay (Brown et al. 1975). A stock suspension of virus (titer 1.4×10^8 IU/ml) was used.

Virus growth curve

Both of the above cell lines were grown in Corning 6-well trays, infected with virus, and washed twice with PBS. At specific times following infection, supernatant samples were collected and titrated for budded virus. In order to obtain the total virus titer, cell monolayers were disrupted and collected with the supernatant; then they were sonicated briefly to release intracellular virus. $TCID_{50}$ assays for extracellular and total virus were performed in triplicate using SF cells as the indicator line.

UV inactivation of virus

The stock suspension of the virus (titer 1.4×10^8 IU/ml) was diluted 1:100 in PBS. This suspension was placed in a watch-glass over ice and irradiated with UV light for 75 seconds. A 6-watt, short-wave (254 nm) UV source placed 10 cm above the watch-glass was used.

Cytopathic effect

Both SF and BM cells were grown in Costar 6-Well tissue culture trays. The cells were seeded at a density of 1×10^6 cells/well and allowed to attach overnight. They were then infected with AcMNPV (both normal and

UV-inactivated virus) at a multiplicity of infection (MOI) of 20 plaque-forming units (PFUs) per cell. The trays were rocked gently on a Bellco rocker for 2 h at room temperature to allow virus adsorption. Control cells were mock infected with fresh medium only. Following adsorption, the virus inoculum was removed and the cells incubated with fresh medium at 28° C. At specific times post-infection, the cells were observed for cytopathic effect by phase contrast microscopy.

Cell and virus preparation for electron microscopy

Corning tissue-culture flasks (75 cm^2) were seeded with 9 x 10^6 SF and BM cells and allowed to attach overnight. The cultures were inoculated with AcMNPV at an MOI of 50. Mock-infected flasks did not contain any virus. Virus was allowed to adsorb for two h at room temperature on a Bellco rocker platform. The cells were then washed and 10 ml complete TNM-FH medium was added to each flask. Incubation was at 28° C. At specific times post-infection (6, 24, and 60 h), cells were washed and fixed for electron microscopy.

Electron microscopy

Infected and control SF and BM cells were detached from tissue culture flasks at specific times post-infection with a rubber policeman and were fixed in 0.1 M phosphate-buffered 2.5 percent glutaraldehyde (pH 7, 380 milliOsmoles) for 1 h at room temperature (3). Cells were post fixed in 2 percent OsO_4 in phosphate buffer, dehydrated in a graded ethanol series and then embedded in Spurr's embedding medium (Carpenter & Bilimoria 1983). Cells were centrifuged and resuspended during each step of fixation and embedded in plastic beam capsules. The cells were sectioned with an LKB ultramicrotome. Silver sections were collected on 200-mesh copper grids and stained for 5 min with uranyl acetate and 15 min with lead citrate. Sections were examined with a Hitachi HS-9 transmission electron microscope.

RESULTS

Cytopathic effect detected by phase contrast microscopy

Reinisch (1989) had shown that *B. mori* cells infected with AcMNPV manifested unusual CPE. In order to determine if viral gene expression is necessary for the cytopathic effect observed in *B. mori* cells and to precisely determine the time of appearance of the various types of CPE, we initiated infections of SF (*S. frugiperda*) and BM (*B. mori*) cells with normal AcMNPV as well as AcMNPV that was irradiated with UV.

Figure 1 shows that mock-infected cells were devoid of any CPE that could be interpreted as being of viral origin. The first CPE observed in SF cells infected with normal AcMNPV was the appearance of nuclear hypertrophy at 6 h p.i. (Figure 2A). From 6 h onwards, nuclear hypertrophy was observed in 100 percent of the cells. At 24 h p.i., the nucleus was distinctly granular and by 36 h p.i., 10 percent of SF cells manifested polyhedral inclusion bodies (PIBs) within the enlarged nuclei (data not shown). By 96 h p.i. 100 percent of the cells contained PIBs and 20 percent of the cells had lysed (Figure 3A). SF cells infected with UV-inactivated AcMNPV showed no CPE, and contrary to the typical pattern in cells infected with normal AcMNPV, these cells continued to divide throughout the course of the incubation period.

Figure 2B shows that BM cells infected with normal AcMNPV displayed the first signs of CPE by 6 h p.i. These cells were rounded and contained dark granules within enlarged nuclei. Although some rounding and nuclear hypertrophy were noticeable in mock-infected BM cells, the CPE in virus-infected cells was much more pronounced and characterized by the presence of distinct granules. Nuclear hypertrophy was

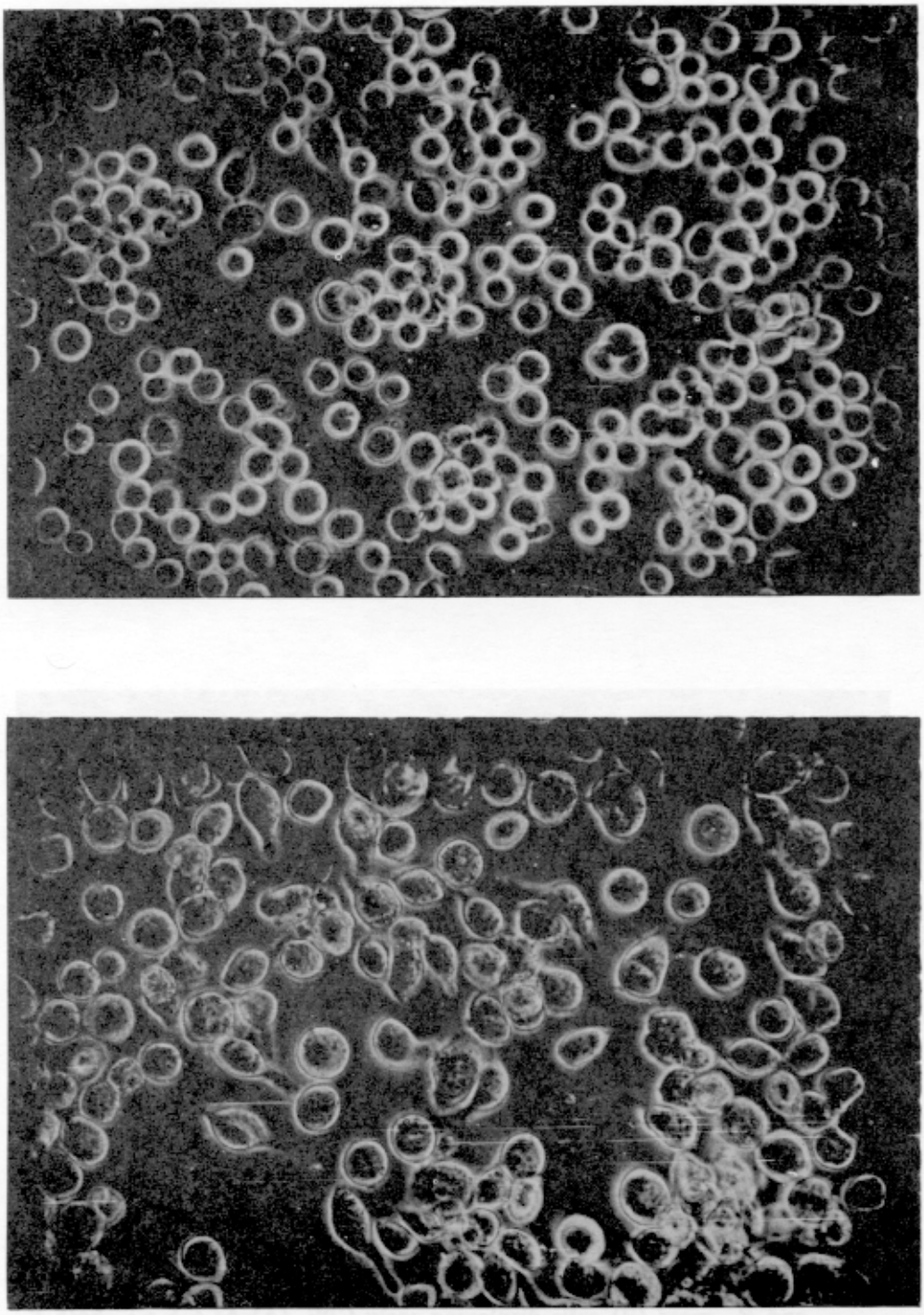

FIG. 1. Phase contrast micrographs of mock-infected *Spodoptera frugiperda* (A) and *Bombyx mori* cells (B). The cells are normal except for minor morphological changes due to the washing steps in the mock infection procedure.

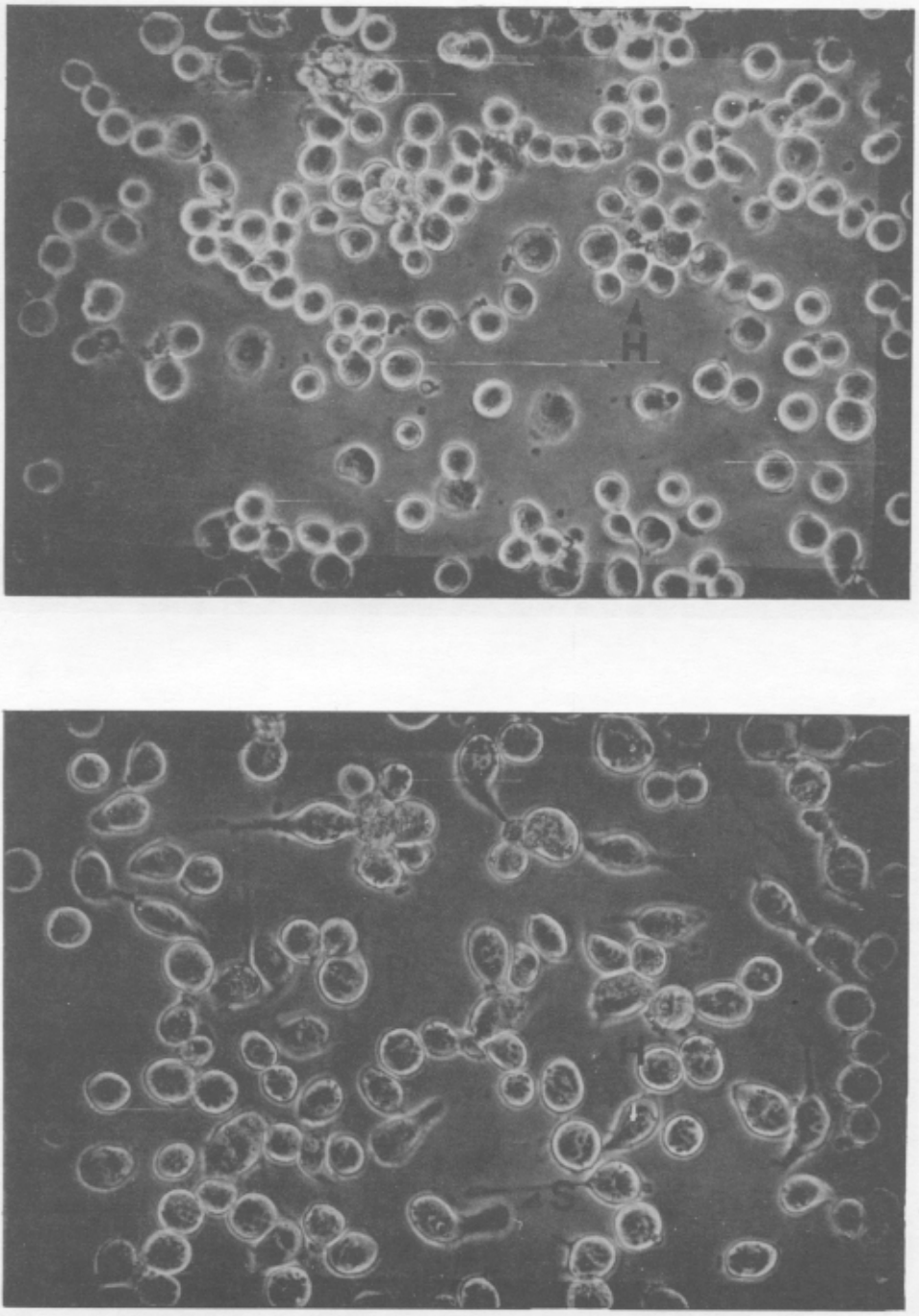

FIG. 2. Phase contrast micrographs of AcMNPV-infected *S. frugiperda* (A) and *B. mori* (B) cells displaying early CPE. (A) At 6 h p.i., both *S. frugiperda* and *B. mori* cells undergo nuclear hypertrophy (H). Some cell rounding (R) and the formation of sac-like bodies (S) are also observed at this time.

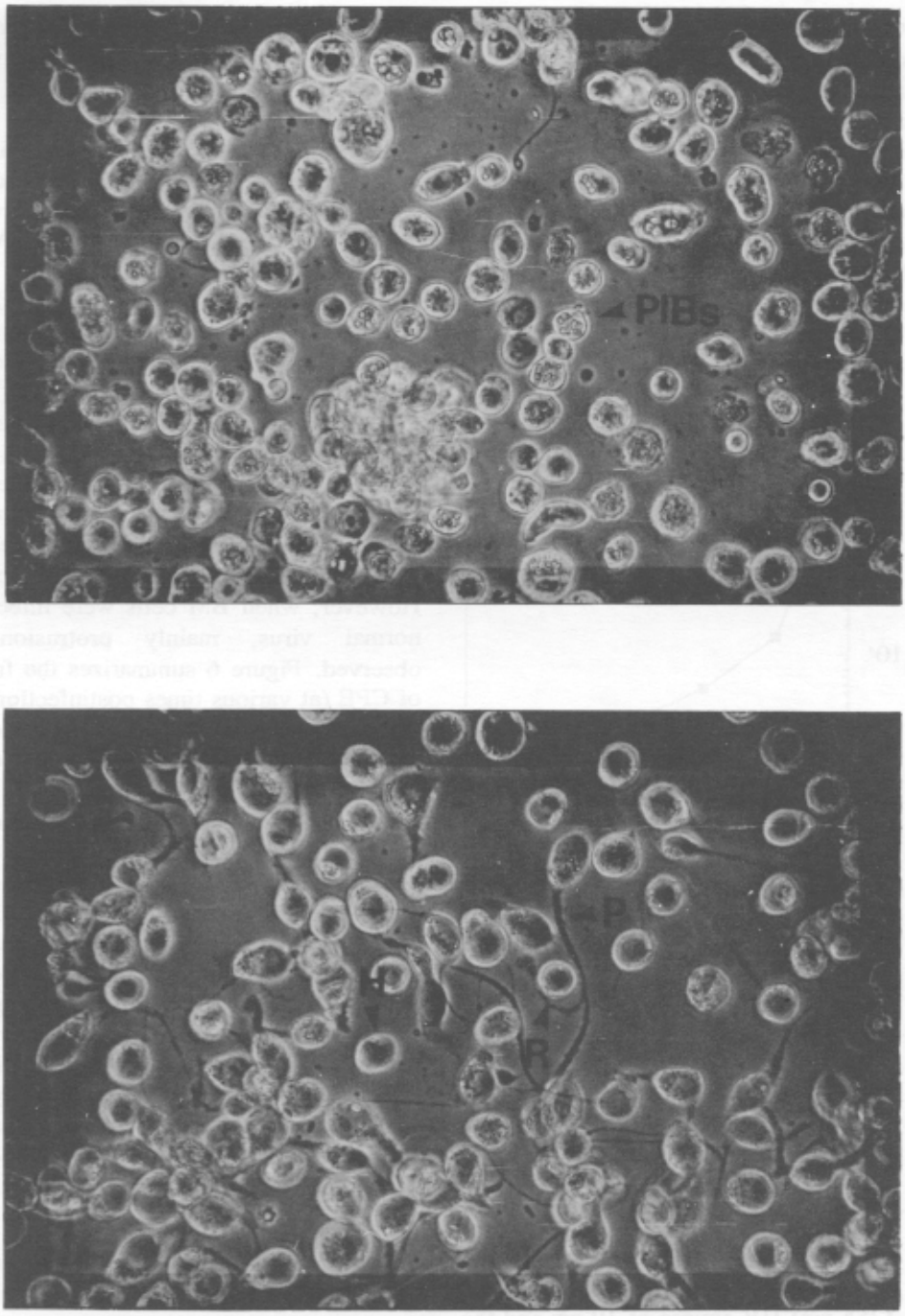

FIG. 3. Phase contrast micrograph of AcMNPV-infected *S. frugiperda* and *B. mori* cells at very late stages of the infection (96 h p.i.). (A) *S. frugiperda* cells have numerous polyhedral inclusion bodies (PIBs) within the nucleus, while some cells show lysis. (B) *B. mori* cells are rounded (R) and have protrusions (P).

observed in 100 percent of the cells and rounding in 90 percent of the cells throughout the course of the infection. By this time in the infection, 10 percent of the cells had formed sac-like bodies at their periphery. Figure 6 shows that by 18 h p.i., the number of cells with sac-like bodies had decreased to less than 5 percent, while 10 percent of the cells had formed protrusions. At 48 h p.i., none of the cells had sac-like bodies, but the appearance of protrusions had increased to 40 percent of the population. The percent of the cell population with protrusions increased to 60 by 96 h p.i.

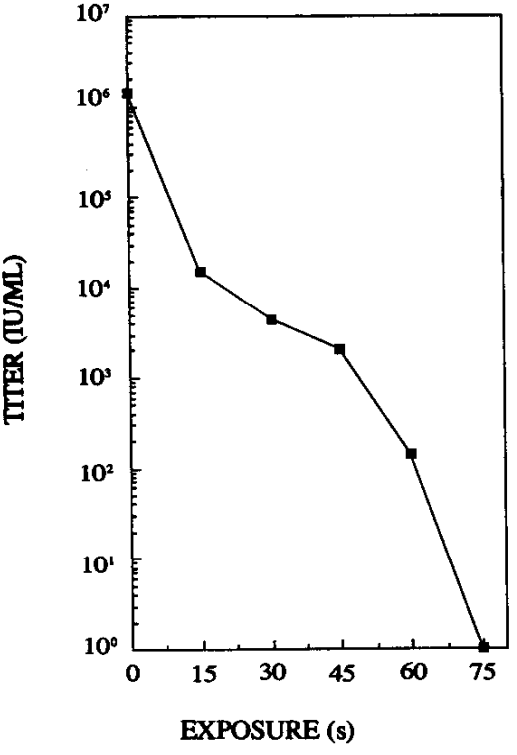

FIG. 4. UV-inactivation of AcMNPV. A stock suspension of AcMNPV diluted in PBS to a titer 1.4 x 10^6 IU/ml was irradiated with a 6-watt shortwave (254 nm) UV source at 10 cm and the titer was determined from $TCID_{50}$ infectivity assays in the permissive *S. frugiperda* cells. The virus was completely inactivated after a 75 s exposure.

In order to determine whether viral gene expression was required for any of the CPE, it was necessary to use UV inactivated virus. To ensure that the UV treatment of AcMNPV completely inactivated the virus, we exposed virus preparations to increasing doses of radiation. Figure 4 shows that under our conditions, a 75 s exposure was needed to completely inactivate AcMNPV, and this was the exposure used throughout this study. Furthermore, all UV-inactivated virus was tested in SF cells in parallel with their use in BM cells. Figure 5 shows that at 48 h p.i., the CPE in the infection of BM cells with UV-inactivated virus consisted of sacs. However, when BM cells were infected with normal virus, mainly protrusions were observed. Figure 6 summarizes the frequency of CPE (at various times postinfection) in BM cells infected with UV-inactivated AcMNPV. Comparison against mock-infected cells showed that the earliest CPE was at 6 h p.i., when sac-like bodies were observed in 5 percent of the cells. The number of sacs peaked to 25 percent at 18 h p.i. By 48 h p.i. the frequency of sacs decreased to 10 percent of the population, and by 96 h p.i. the cells appeared to be normal and were devoid of sacs. Hypertrophy and rounding were observed in approximately 25 percent of the cells, but there was no granularity, and therefore these effects were probably due to stress caused by overcrowding. They were also observed at these levels in mock-infected BM cells.

Infectious virus yields in permissive and semipermissive cells

In order to determine if the different CPE in SF and BM cells was associated with differences in the pattern of infectious virus production, the kinetics of AcMNPV infection

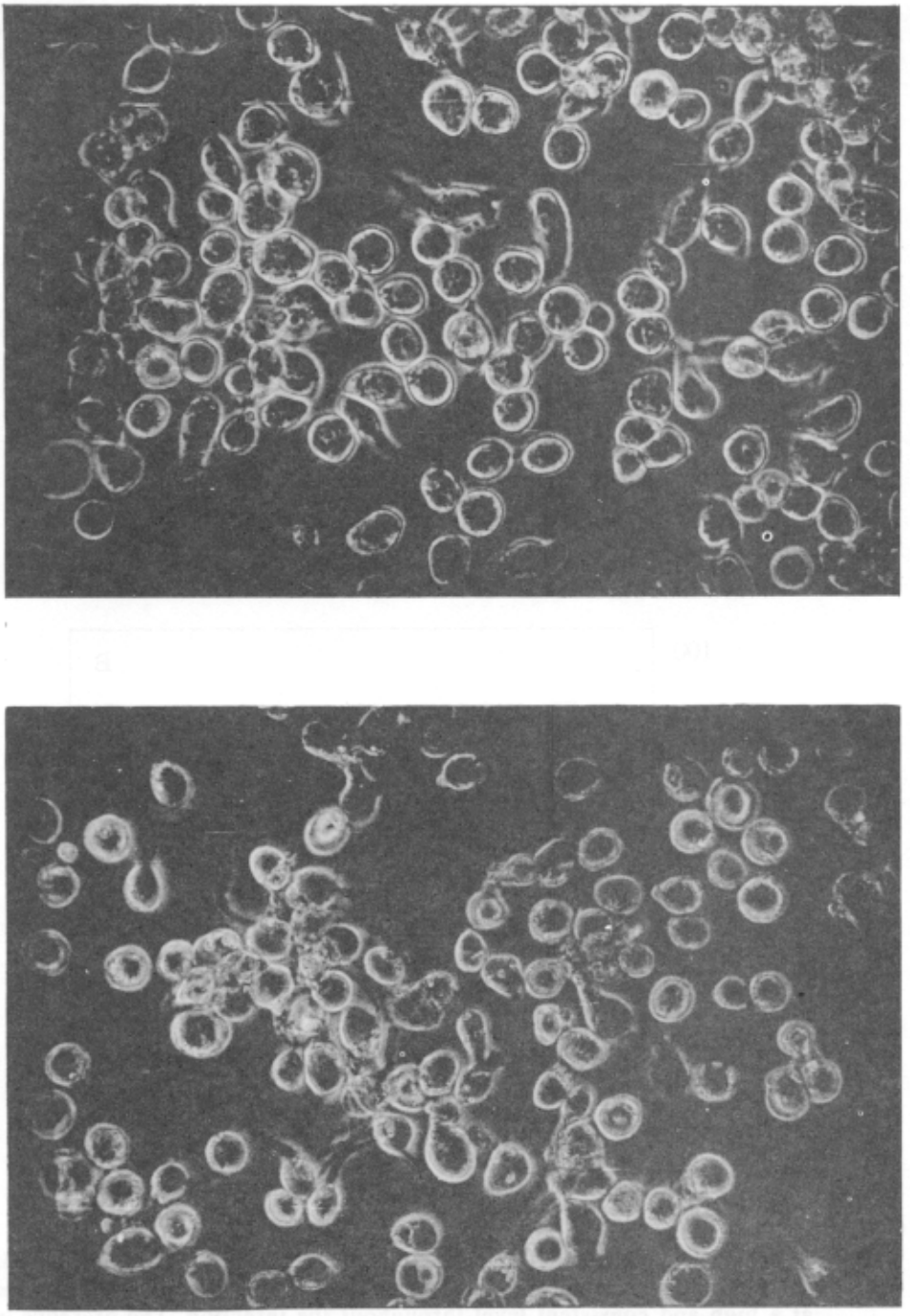

FIG. 5. Phase contrast micrograph of AcMNPV-infected *B. mori* cells. (A) At 48 h p.i., *B.mori* cells infected with UV-inactivated AcMNPV displayed characteristic sac-like bodies (S) and rounding (R) of cells. (B) In *B. mori* cells infected with normal AcMNPV, cells appear rounded (R) and many of these cells display protrusions (P).

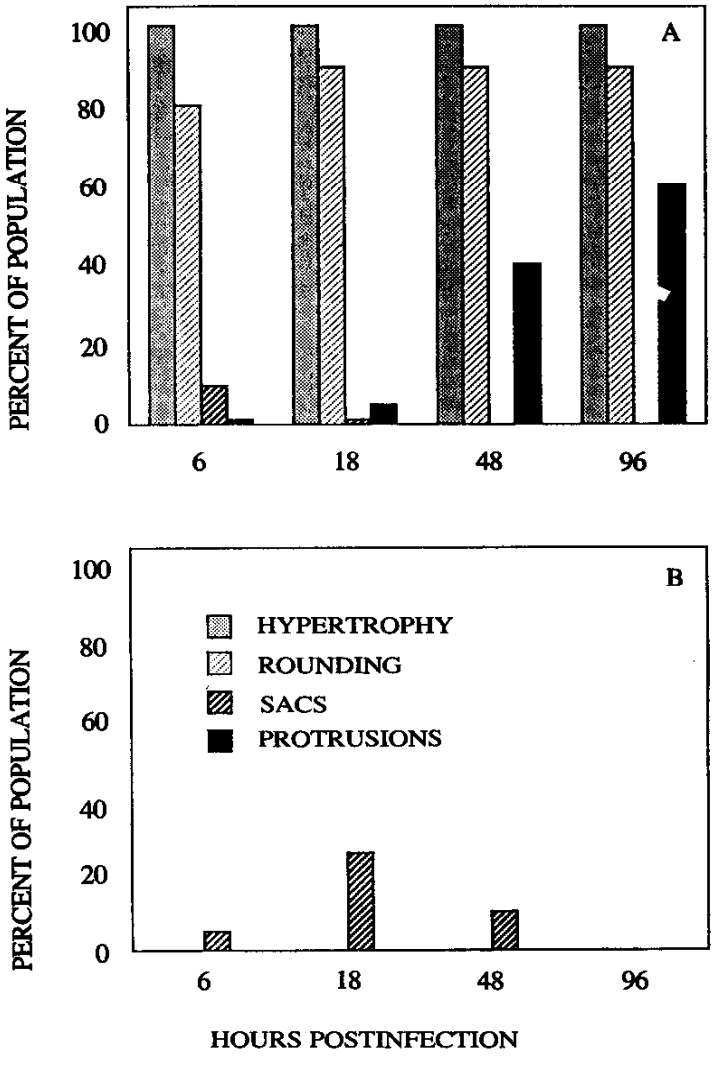

FIG. 6. (A) Percentage of the cell population showing the various types of CPE in *B. mori* cells infected with AcMNPV. Hypertrophy and rounding appear at a high and consistent level throughout the infection. The presence of sacs decreases after its appearance at 6 h p.i. The number of cells displaying protrusions increases to a maximum of 60 percent of the cell population by 96 h p.i. (B) *B. mori* cells infected with UV-irradiated AcMNPV. Compared to mock-infected cells, the degree of hypertrophy and rounding was very low. The appearance of sacs peaks at 18 h p.i.

in these cell lines were compared. Figure 7 shows that the total virus titers produced in SF cells showed greater than a 3-log (2000-fold) increase from time 0 to 40 h p.i. In BM cells, there was no significant production of infectious virus even up to 60 h p.i. Considering the accuracy of the $TCID_{50}$ assay (± 20 percent), these data suggest that the increase in infectious virus in SF cells is significant, but the apparent rise and fall of virus in BM cells is not.

In order to assure that there was no amplification of virus levels at all in BM cells, a series of blind passages of AcMNPV in BM cells was performed and the level of virus in the culture medium was measured by the $TCID_{50}$ assay after each passage. The results are shown in Figure 8. It is clear that while virus levels accumulate with passage in SF cells, there is no accumulation in BM cells. These data strongly support the conclusion from Figure 7 that no infectious virus is produced in BM cells.

Cytopathic effect detected by electron microscopy

Earlier work by Summers et al. (1978) utilizing an immunoperoxidase staining technique showed that 90 percent of AcMNPV

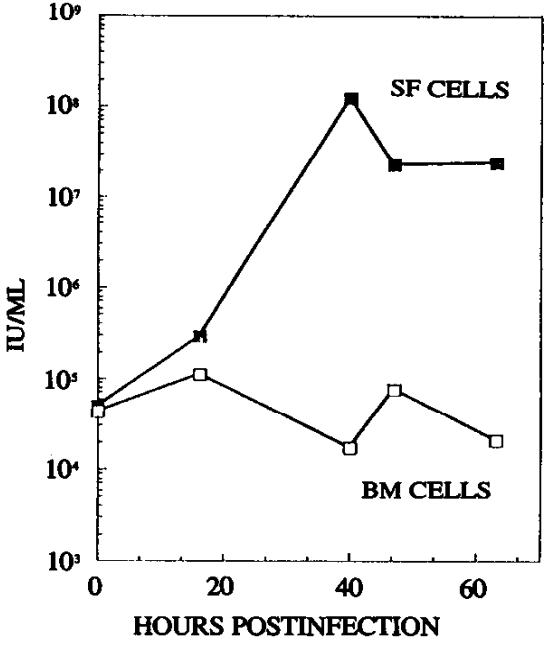

FIG. 7. Virus growth kinetics in AcMNPV infected *S. frugiperda* and *B. mori* cells. The curves represent total virus titers. At specific times postinfection, cell monolayers were disrupted and collected with supernatants. Samples were then sonicated to release intracellular virus, and $TCID_{50}$ assays were coducted to determine the levels of total virus.

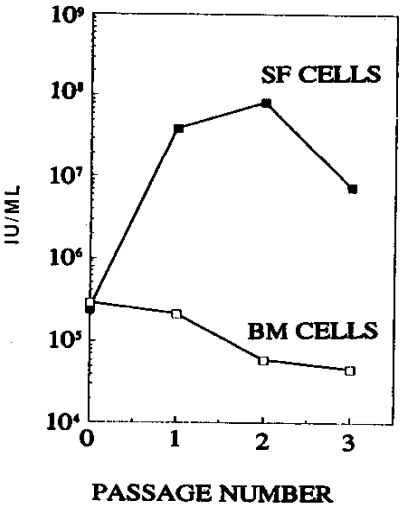

FIG. 8. Blind passage of AcMNPV in *S. frugiperda* and *B. mori* cells. At 48 h after the initial infection (passage 1), a sample of the monolayer was diluted 1:2 in fresh medium and used to infect a monolayer of cells (passage 2). After a 2 h adsorption period, the inoculum was removed and fresh medium was added. Following 48 h incubation, the supernatant was diluted as before and used to infect yet another batch of cells (passage 3). Prior to dilution, a sample of supernatant was titered for virus at each passage in the series.

infected BM cells produced enveloped nucleocapsid (i.e. virion) antigens and infectious virus, but less than 1 percent of the cells produced polyhedra. Recently, Reinisch (1989) showed that polyhedrin and many other ICSPs are not synthesized in infected BM cells and that infectious virus is not produced in these cells. These observations raise important questions about the morphogenesis of infection in BM cells. Specifically, are virions or nucleocapsids synthesized in BM cells, and if they are, are they of normal appearance? Therefore, we carried out a series of electron microscopic studies on this abortive infection. Infected SF cells served as positive controls.

The data show that while mock-infected cells were perfectly normal (Figure 9), the permissive infection develops a virogenic stroma (VS) and manifests nucleocapsids by at least 6 h p.i. (Figure 10A). On the other hand, VS formation is observed by 6 h p.i. in AcMNPV infected BM cells (Figure 10B), but in the vast majority of the samples examined, the VS in BM cells was not as condensed as in the SF cells, and no nucleocapsid formation was observed. Between 6 to 24 h p.i., the number of nucleocapsids in SF cells increased (not shown). At 24 hour p.i. most of the nucleocapsids gained envelopes and became virions, resulting in a decrease in the number of naked nucleocapsids (Figure 11A). From 6 to 24 h p.i., the VS in infected BM cells became more condensed (Figure 11B) and appeared to be very similar in its structure to mature VS in the permissive SF cells. Nucleocapsid-like structures were observed, but in only 20 percent of the infected BM cells and not earlier than 24 h p.i. (Figure 11C). These structures are similar to the early stage of NCs produced in the SF cells but appear to be somewhat defective. At 60 h p.i., polyhedral inclusion bodies (PIBs) along with continued virion formation was observed in SF cells (Figure 12A). No virions or PIBs were seen in BM cells at these late times. Furthermore, the nucleocapsids detected at 24 h p.i. were not seen and virogenic stroma was present but in a degenerated form (Figure 12B).

DISCUSSION

We have observed distinct differences in CPE and differential production of infectious virus in permissive versus semipermissive AcMNPV infections. In permissive *S. frugiperda* cells, cytopathology was characterized by greatly enlarged nuclei accompanied by PIBs and the eventual lysis of the majority of infected cells. Although *B. mori* cells exhibited swollen nuclei similar to that of SF cells early in the infection, subsequent stages of CPE were very different. The BM cells were characterized by the total absence of PIBs, which is the most dramatic phenotypic expression of an underlying host range restriction in this system. The appearance of sac-like bodies, protrusions, and the virtual absence of cell lysis were also distinguishing features of the CPE in BM cells. While protrusions continue to increase in frequency at very late times in the infection, sacs appear relatively early and are not observed at later times. On the other hand, when UV-treated virus is used, sacs are the only type of CPE observed in BM cells. Alternative explanations are possible, but these observations do not preclude the possibility that sac production is triggered by a structural component of the virion (perhaps a surface protein) whereas protrusion formation requires viral gene expression. Thus, the AcMNPV-BM infection may provide a good opportunity to identify a virion protein that may be responsible for CPE and, possibly, pathogenicity.

It is clear from our results that no infectious virus is produced in this abortive infection. Carpenter & Bilimoria (1983) thought that some infectious virus was produced in their SfMNPV-*T. ni* cells infection but subsequent work has shown that no infectious virus is produced in this abortive infection either. In semipermissive gypsy moth cell line studies

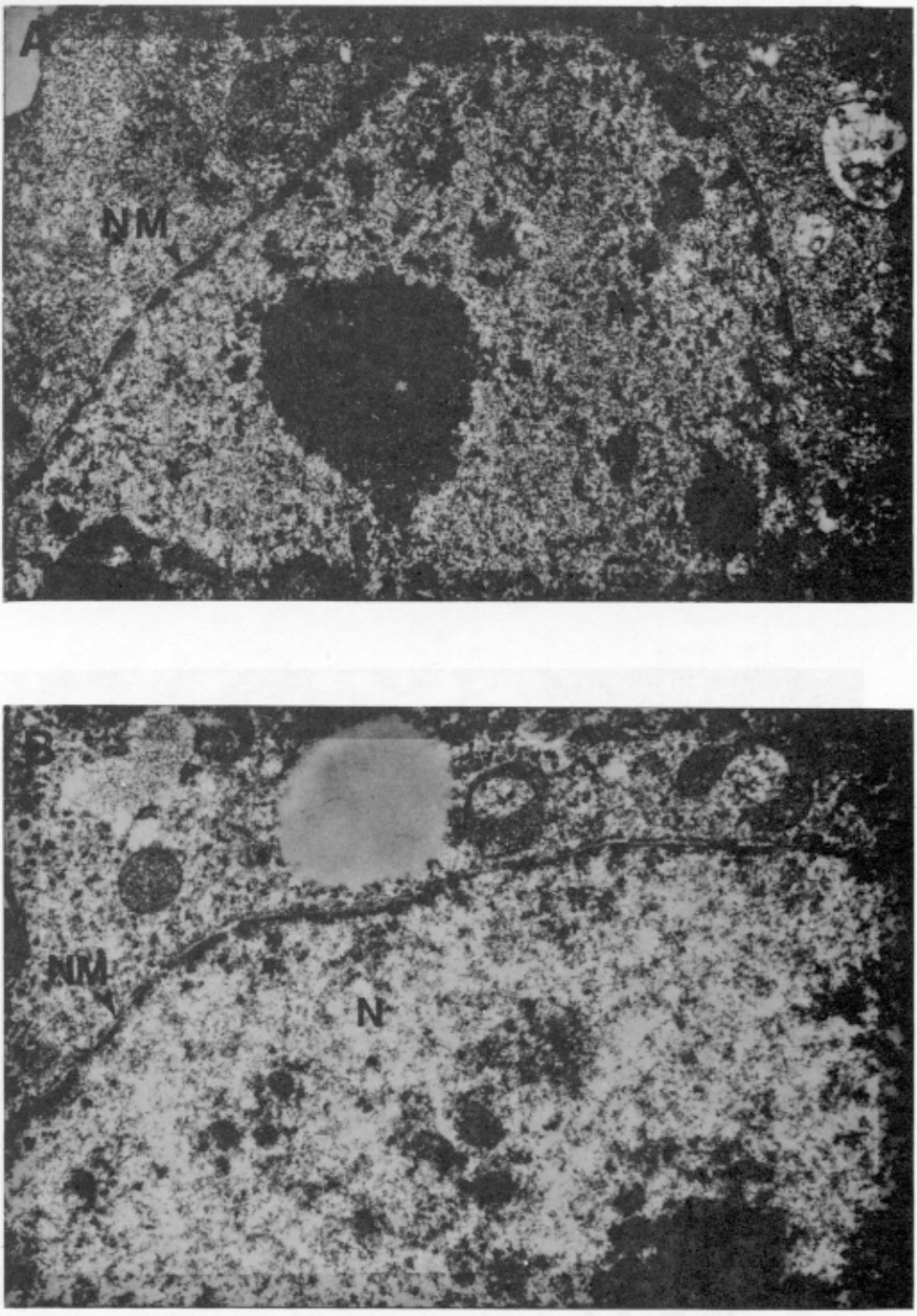

FIG. 9. Electron micrograph of mock-infected *S. frugiperda* cells (A; 21, 000X) and *B. mori* cell (B; 19, 000X). N: nucleus; NU: nucleolus; NM: nuclear membrane.

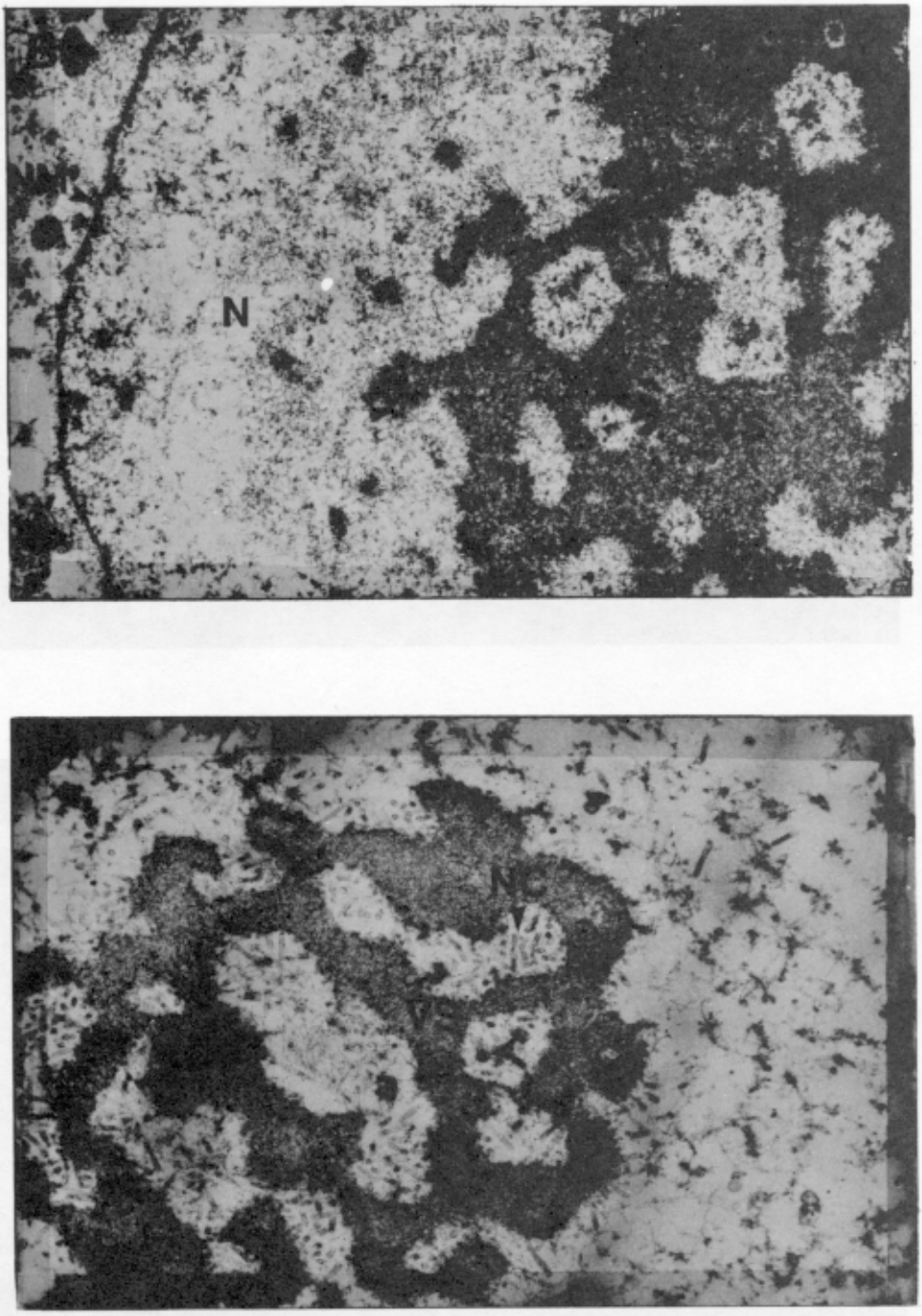

FIG. 10. Electron micrograph of *S. frugiperda* cells (A; 24, 000X) and *B. mori* cells (B; 20, 000X) inoculated with AcMNPV at 6 h post infection (MOI = 50). The *S. frugiperda* cell nucleus (N) contains distinct virogenic stroma (VS) and numerous nucleocapsids (NC). Virogenic stroma is also observed in *B. mori* cells, but no nucleocapsids are seen at this stage.

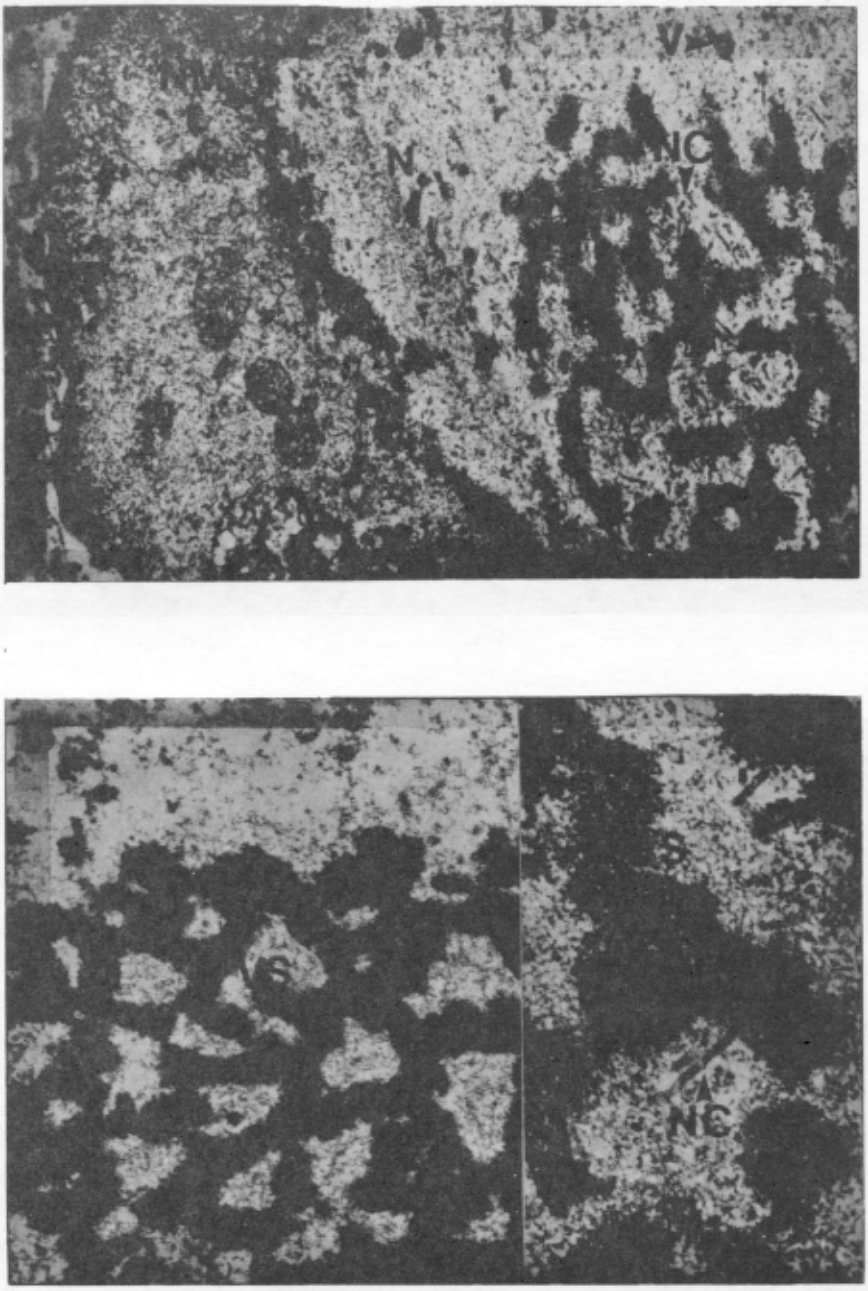

FIG. 11. Electron micrograph of AcMNPV-infected (MOI = 50) *S. frugiperda* cells (A; 15, 000X) and *B. mori* cells (B; 19, 000X) at 24 h postinfection. The *S. frugiperda* nucleus (N) displays distinct virogenic stroma (VS) with numerous progeny virions (V) and nucleocapsids (NC). In *B. mori* cells, the nucleus (N) shows a distinct and mature virogenic stroma (VS) but only 20 percent of the cell population contain nucleocapsids, which appear to be defective (C; 34, 000X).

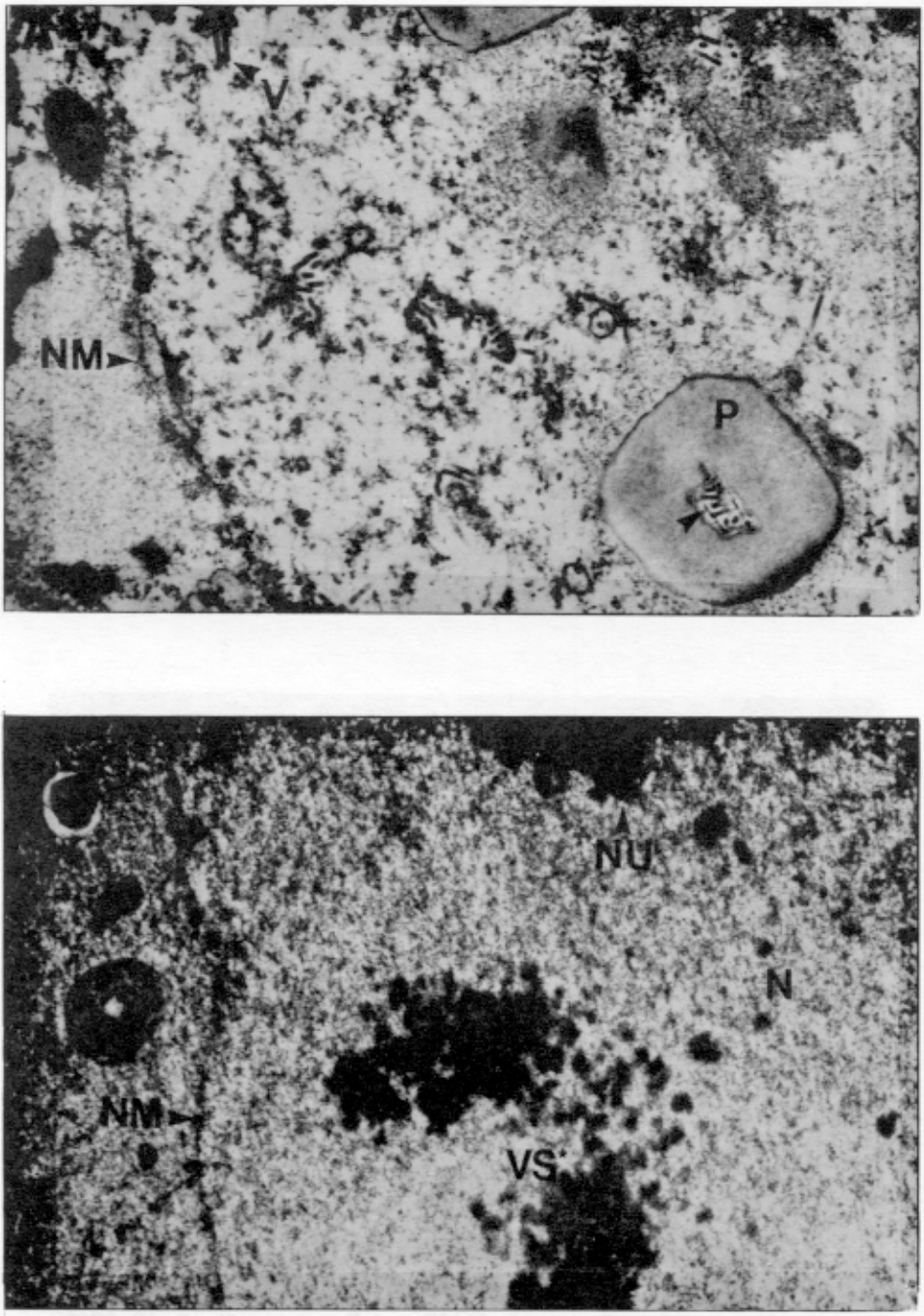

FIG. 12. Electron micrograph of AcMNPV-infected (MOI = 50) *S. frugiperda* cells (A; 21, 000X) and *B. mori* cells (B; 23, 000X) at 60 h postinfection. Mature PIBs (P) with occluded virus (arrow) and nonoccluded virions (V) are observed in the *S. frugiperda* cells. The virogenic stroma (VS*) in the *B. mori* cell nucleus (N) is apparently degenerating by this stage of the infection. NM: nuclear membrane; NU: nucleolus.

described by McClintock (1986), AcMNPV produced classic CPE but failed to yield progeny virus. Thus, it appears that none of the reasonably well-characterized abortive systems produce any infectious virus.

In the absence of infectious virus, it is still possible that virus particles might be produced. However, the electron microscopic data we present in this paper do not show any virions. The nucleocapsids detected in 20 percent of the BM cells appear to be defective and are not seen until at least 24 h after infection. The virogenic stroma in the AcMNPV-BM infection is quite strong, but it does not reach full form until 12 h after infection, at least 6 h later than the productive infection in SF cells. This suggests that viral DNA replication probably occurs in this infection but peaks late. (We are currently investigating this aspect of the infection by dot blot hybridization.) The AcMNPV-BM infection we describe here is somewhat similar to the SfMNPV-*T. ni* infection described by Carpenter & Bilimoria (1983) in that virogenic stroma is observed in all cells and defective nucleocapsids are observed in less than five percent of the cell population.

Our results clearly show that the AcMNPV-BM infection is abortive, but that enough viral activity is going on to merit further study of this system with the eventual aim of delineating the mechanism or mechanisms of virus restriction. Our laboratory has carried out some studies on the synthesis of polypeptides in this infection (Reinisch 1989). Pulse labeling at late times after infection (17 and 24 hr p.i.) with ^{35}S-methionine and ^{3}H-amino acid mixtures have indicated that polyhedrin, the 10K polypeptide, and several other ICSPs were not synthesized in *B. mori* cells but were detected in AcMNPV infections of permissive SF cells. An intriguing aspect of this research is that several ICSPs not found in the permissive cells were detected in *B. mori* cells. The meaning of these dramatic differences in CPE and ICSP synthesis between the permissive and abortive infections is not clear, and it is not known if there is a direct cause and effect relationship between the unusual CPE and unique ICSPs observed in the *B. mori* cells (Reinisch 1989). It should be of interest to determine if the block in the AcMNPV-BM infection is at the transcriptional or translational level. This would be done by probing Northern blots of poly A associated RNA in productive and restrictive infections with plasmids containing cDNA fragments of the polyhedrin and p10 genes, respectively. A clear demonstration of the lack of RNAs homologous to these probes would suggest that the restriction is at the transcriptional level. On the other hand, the presence of transcripts would suggest that restriction is at some post transcriptional level.

In another abortive infection (SfMNPV-*T. ni* cells), Bilimoria et al. (1986) and Liu & Bilimoria (1990) showed that only two infected cells specific polypeptides (ICSPs; 97K and 29K) were synthesized in the SfMNPV-*T. ni* nonpermissive infection. The effects of reversal of inhibition of ICSP synthesis with cycloheximide and the effect of cytosine arabinoside on the synthesis of these proteins in the permissive SF cell line indicates that the 97K ICSP is a delayed-early protein and the 29K ICSP is a late protein. Dot blot hybridization data show that a low level (<15 percent of permissive infection) of viral DNA replication does occur (Liu 1987). While the synthesis of delayed-early and late ICSPs in the absence of immediate-early proteins is somewhat perplexing, it is interesting that the 67K ICSP, which is one of the two immediate-early ICSPs in SfMNPV infections of permissive cells, is a structural protein. Thus, the possibility that the limited expression of a delayed-early and a late ICSP in nonpermissive cells is due to induction by the 67K protein in parental virus cannot be ruled out. However, since the synthesis of neither of the two immediate-early ICSPs detected in permissive infections occurs in nonpermissive infection, the primary restriction in this system must occur during

expression of the immediate-early genes. Rice & Miller (1986) have shown that specific AcMNPV transcripts were synthesized in nonpermissive *Drosophila melanogaster* cells, but late viral transcripts were not observed. Clearly, the above infections of nonpermissive cells with AcMNPV are restricted at apparently different steps of the temporal cascade of NPV gene expression.

It is clear from the above discussion that one or more distinct mechanisms of restriction might be operational in each of the above host specificity model systems. The role of enhancers, and the specific motifs within them, in the host specificity of simian virus 40 and other papovaviruses is well established (Maniatis et al 1987). As more information on the specific promoter classes, enhancer sequences, and host specific factors operational in the temporal and abundance cascade of the NPV replication cycle becomes available, it should be possible to test the role these elements play in regulating the specificity and tissue tropism of these viruses. At the same time, recent information on characterization of the major surface glycoprotein of AcMNPV (Stiles & Wood 1983; Charlton & Volkman 1986) should provide interesting opportunities for studying cell surface-level mechanisms of NPV specificity.

The study of abortive NPV infections has good potential in the delineation of the factors responsible for baculovirus CPE since such infections allow the study of pathological effects in the absence of a full-fledged viral infection. If these factors also are responsible for pathogenicity in the insect host, then their isolation and characterization could have important implications for the construction of transgenic crop plants that are resistant to a variety of insect pests.

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