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High-Voltage Electron Microscope Study of the Release of Vaccinia Virus from Whole Cells

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High-voltage (1,000-kV) electron microscope examination of whole BSC-1 cells infected with vaccinia virus at different times after infection revealed the presence of increasing numbers of virions no longer confined to factories but situated along the cell periphery of monolayer cells. Stereoscopic images showed each virus enclosed within a membrane-like component of the host cell cytoplasm. Viruses within factories appeared to lack similar enclosures. Cytochalasin B, but not vinblastine, caused the enclosures to disrupt. Vaccinia viruses were observed to escape the host cell individually from the tips of microvilli and within packets of cytoplasm. Observations suggest that the intracellular movement and release of vaccinia virus utilize a host cell cytoplasmic network that involves microfilaments for stability.

Poxvirus maturation occurs within localized regions of the cell cytoplasm called factories. Electron microscope studies utilizing sectioned specimen (2, 3, 7), freeze-fracture (5), and antibody labeling techniques (10, 13) have revealed various aspects of the complex assembly process and virus-host cell interactions. Mature viruses migrate from the factories and are released along the cell surface by a process not understood but which resembles budding. The previously mentioned techniques are limited in examining this aspect of vaccinia maturation due to the restrictions imposed by specimen thickness. Virus-cell interactions that occur within large complex structures, at low frequency, or last for very short durations, could easily escape detection.

The present investigation was designed to study the involvement of host cell organelles with vaccinia virus intracellular movement and release as visualized in unsectioned whole-cell preparations. This was accomplished by use of a high-voltage (1,000-kV) electron microscope (HVEM) and critical point-dried infected monolayer cells. Fonte and Porter (6) first used the technique to visualize herpes simplex virus-infected whole cells.

The results show that, in cells infected with vaccinia virus, immature viruses form independently of membrane-like interconnections with the host cell, but maturing and releasing virus particles appear to be enclosed by a membrane-like host cell organelle. Viruses are released from infected cells within small packets of cytoplasm and individually through the cores of specialized microvilli located along the cell periphery. The composition of the above-mentioned membrane-like structure is discussed.

MATERIALS AND METHODS

All experiments were done with African green monkey kidney (BSC-1) cells (American Type Culture Collection) grown in F-12 nutrient medium (Grand Island Biological Co., Grand Island, N.Y.) containing 10% fetal calf serum, 0.1% sodium bicarbonate, and 50 U of both penicillin and streptomycin per ml.

Vaccinia virus (strain WR) stocks were obtained from David M. Prescott (University of Colorado, Boulder). Cells were infected routinely with vaccinia virus by adding a 0.5-ml suspension of virions (5×10^6 PFU/ml) into petri dishes (35 by 10 mm) containing 2×10^5 cells growing on glass cover slips. Each cover slip contained gold electron microscope grids covered by a monolayer of 0.7% Formvar and a layer of evaporated carbon. Cultures were swirled and incubated for 30 min at 37 C. Cells were washed three times in culture medium and incubated in 2.0 ml of fresh medium.

At various times after infection, cells were washed several times in Puck saline G (PSG), fixed in 2% gluteraldehyde in PSG for 1 h at 37 C, washed with PSG, and postfixed for 15 min with 1% OsO_4 in 0.1 M cacodylate buffer containing 0.1 M sucrose (pH 7.4). Grids were carefully removed from the cover slips, washed in distilled water, stained with 0.05% uranyl acetate in a 15% acetone-water solution for 2 min, dehydrated rapidly through acetone to 100%, and critical point-dried through CO_2 (12). Carbon was evaporated on both sides of the grids, which were then stored under vacuum until used.

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BSC-1 cells infected with vaccinia virus for 20 h were exposed to either cytochalasin B (10 μg/ml; Aldrich Chemical Co., Inc., Milwaukee, Wis.) or vinblastine sulfate (10 μg/ml; Sigma Chemical Co., St. Louis, Mo.) dissolved in F-12 medium, at 37 C for 1 h before fixation in 2% gluteraldehyde.

Specimens were examined with a JEM-1000 electron microscope (HVEM) operating at 1,000 kV. (The HVEM is located in the Laboratory for High Voltage Electron Microscopy, in the Department of Molecular, Cellular and Developmental Biology, University of Colorado, Boulder.) Stereoscopic images were obtained by taking photographs of selected areas from different tilt angles which varied, as indicated in the figures, with magnification.

RESULTS

The perinuclear region of an uninfected BSC-1 cell (Fig. 1) showed the presence of numerous fibrous structures and mitochondria of varying shapes. Microvilli appeared more clearly along the cell periphery than over the cell nucleus. Cells infected with vaccinia virus for 5 h (Fig. 2) displayed electron-dense regions and smaller bodies measuring approximately 150 by 250 nm not seen in uninfected cells. Most of the large amorphous regions were located in the perinuclear regions. These electron-dense areas were interpreted as representing vaccinia factories. Very few individual virus particles were distinguishable along the cell periphery at 5 h after infection.

A cell infected with vaccinia for 20 h (Fig. 3) contained an abundance of virus particles. Individual viruses were no longer confined to factories, which were still present, but appeared dispersed throughout the cytoplasm. Numerous packets of cytoplasm containing several viruses were observed along the cell periphery. This represents one release mechanism not previously observed in vaccinia virus maturation.

Factories viewed stereoscopically 5 h after infection (Fig. 4) showed empty viral envelope material and immature viral forms containing electron-dense material. Each immature particle appeared not to be connected to any cytoplasmic membrane-like structure. Assembling virions were gathered into small clusters, which shared a central, electron-dense mass believed to represent viral nucleoproteins. Each factory had no apparent limiting membrane separating it from the remaining cytoplasm.

Virions within cells infected for 20 h (Fig. 5) were present along the cell periphery at high concentrations and varying stages of core condensation. Each virion was enclosed by an extension of the cytoplasmic network. Particles appeared to align themselves with cytoplasmic

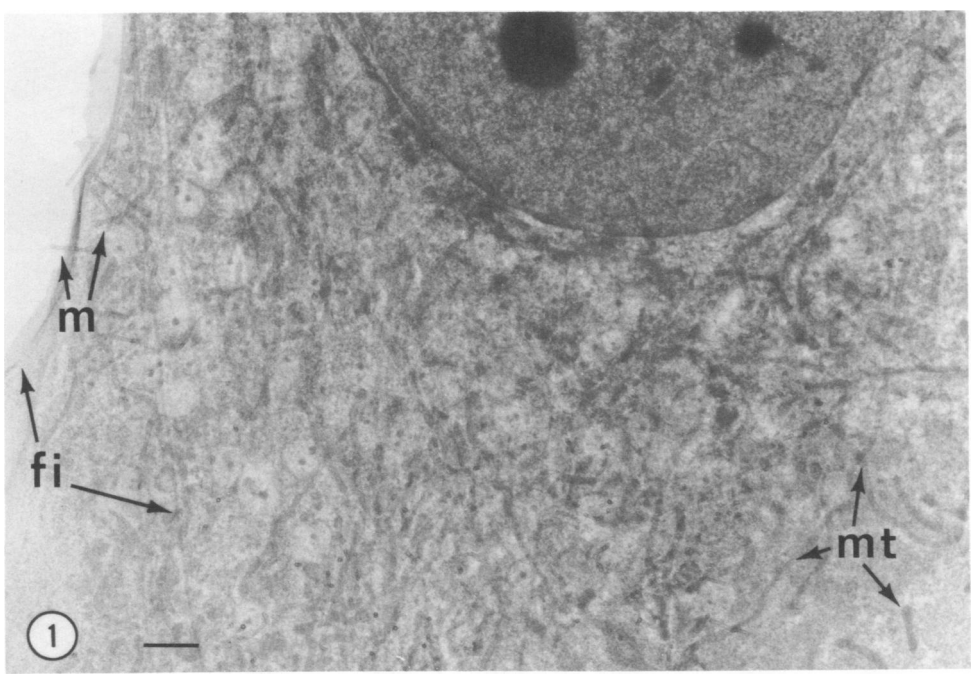

FIG. 1. *High-voltage electron micrograph of a whole, uninfected BSC-1 cell. Mitochondria (mt) appear as slender structures throughout the cytoplasm. Filament bundles (fi) align paralled to the cell periphery and interior. Small microvilli (m) are shown along the cell periphery and surface.* ×5,100. *Scale line, 1.5 μm.*

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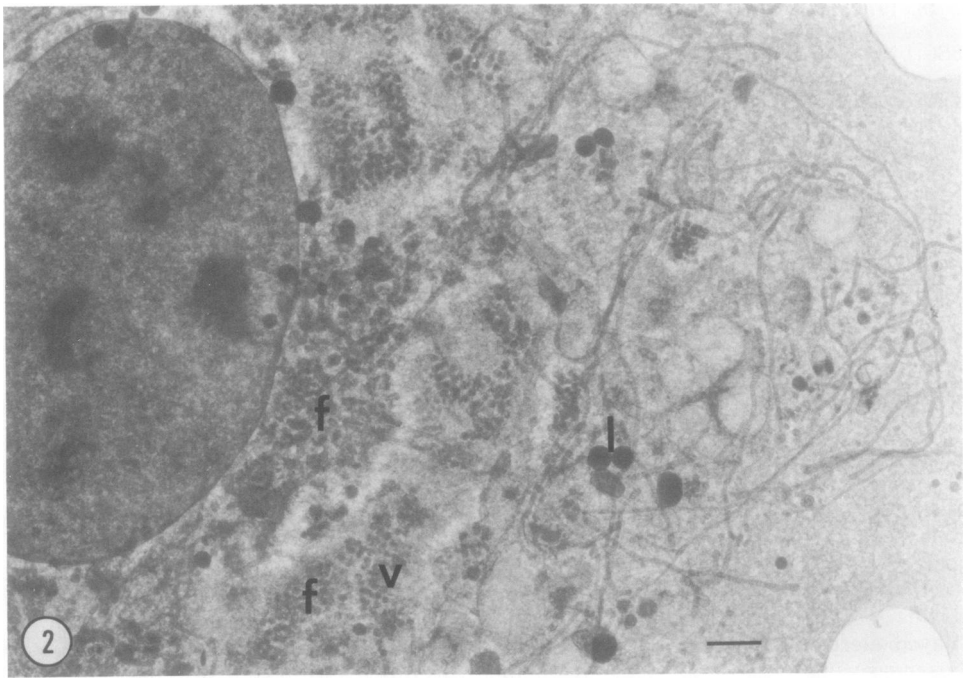

FIG. 2. *High-voltage electron micrograph of a whole BSC-1 cell infected with vaccinia virus for 5 h. Small factories of assembling viruses (f) are located in discrete patches within the cytoplasm. The individual virions (v) appear smaller and less electron dense than lipid globules (1). Note the absence of viral particles along the cell periphery. ×5,100. Scale line, 1.5 μm.*

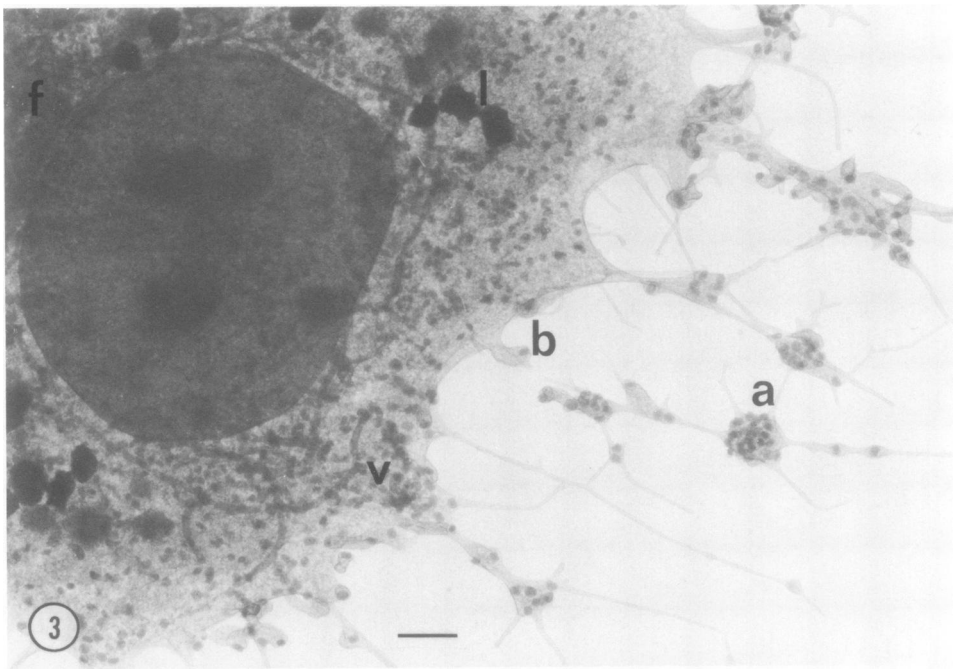

FIG. 3. *High-voltage electron micrograph of a whole BSC-1 cell infected for 20 h. Viruses (v) are abundant throughout the cytoplasm. Note the large electron-dense factory (f) of assembling vaccinia and lipid globules (1). Note the two virus-release mechanisms: (a) cytoplasmic packets along the cell periphery and (b) release individually from the tips of microvilli. The latter is more obvious in Fig. 6 and 8. ×5,100. Scale line, 1.5 μm.*

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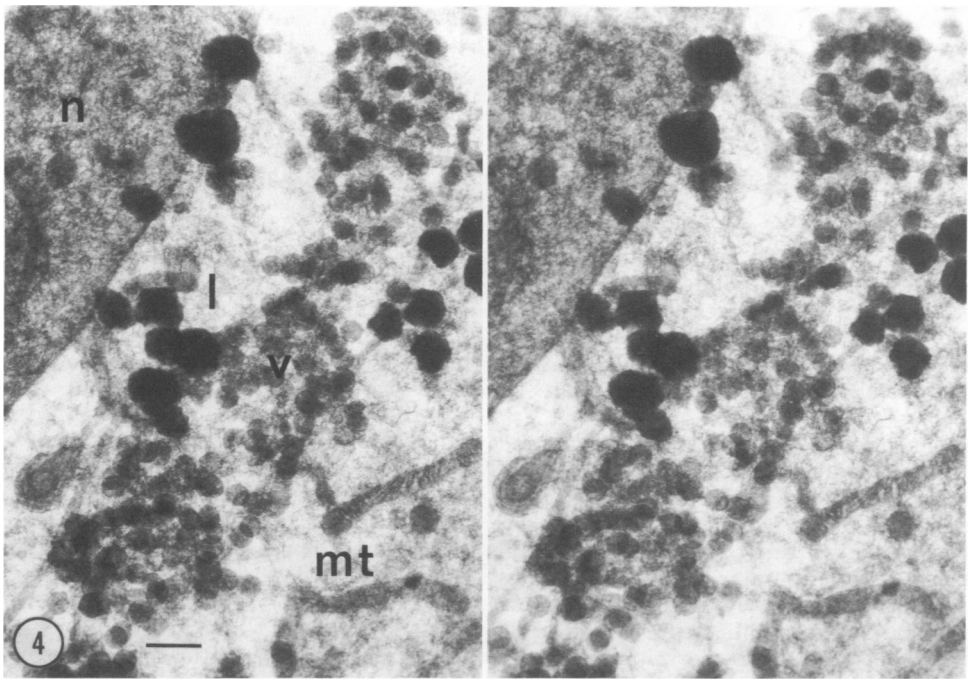

FIG. 4. *Stereoscopic high-voltage electron micrograph of vaccinia virus factories within a whole BSC-1 cell 5 h after infection. Assembling viruses (v) form in small clusters near the cell nucleus (n). Lipid globules (l) and mitochondria (mt) are shown near factories. No virus-host cell connections are apparent. The specimen was tilted ±5°. ×15,000. Scale line, 500 nm.*

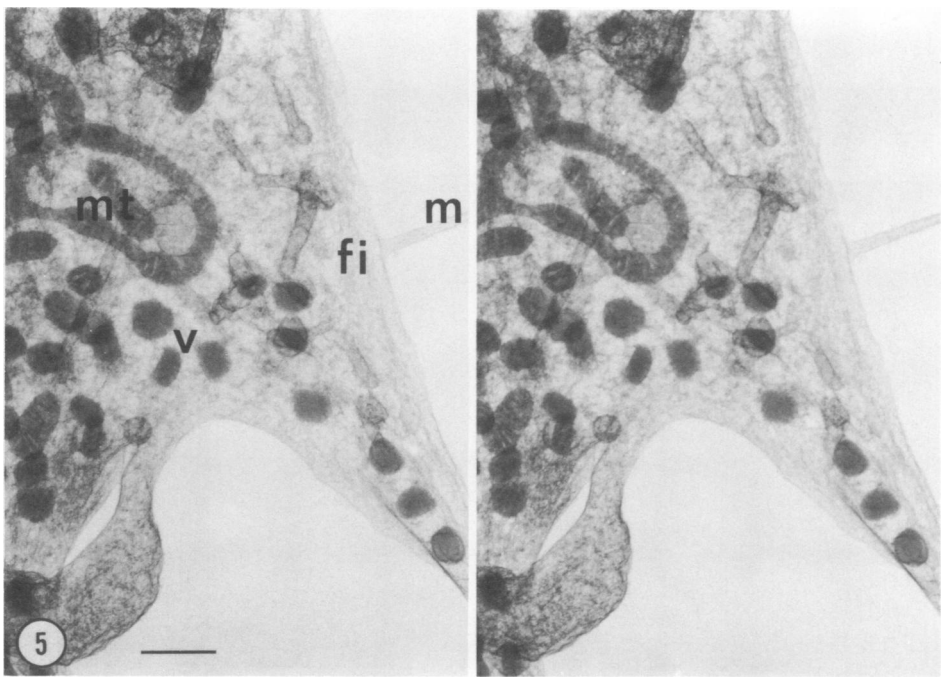

FIG. 5. *Stereoscopic high-voltage electron micrograph of the cell periphery of a whole BSC-1 cell infected with vaccinia virus for 20 h. Note the cytoplasmic connections between each virion (v) and the cytoplasmic network. Viruses appear channeled into a cytoplasmic extension parallel to filaments (fi). Mitochondria (mt) and microvilli (m) are also shown. The specimen was tilted ±5°. ×20,000. Scale line, 500 nm.*

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fibers, which extended into the cores of microvilli. Inspection of infected cells after 20 h revealed no gross disruption of the cytoplasmic organization. The orientation of fibers and membranes appeared similar to that in uninfected cells.

Figures 6 and 8 show the second novel release mechanism of vaccinia virus maturation not observed previously. Individual viruses appeared to penetrate the cores of microvilli situated predominantly along the cell periphery. Microvilli containing viral particles were larger than those not containing virions (Fig. 8B).

The membrane-like structure (Fig. 7A and 8B) that surrounds each viral particle is believed to be associated with the ability of the viruses to be displaced from one intracellular location to another and to penetrate microvilli at release points. Cellular contractile proteins such as microfilaments and microtubules might be present. This possibility was examined by testing agents that depolymerize microtubules and microfilaments, vinblastine and cytochalasin B, respectively. Cells infected with vaccinia virus for 20 h (Fig. 7) were exposed to either depolymerizing agent 1 h before fixation. The membrane-like sheath surrounding each virion (Fig. 7A) was not affected by the addition of vinblastine (Fig. 7B), but cytochalasin B (Fig. 7C) caused the enclosing structure to disrupt. Both agents prevent the movement of vaccinia virions from factories into the surrounding cytoplasm (G. V. Stokes, unpublished data), but only cytochalasin B affected the host membrane-like structure surrounding each virion. These results suggest that polymerized microfilaments, but not microtubules, may constitute a stabilizing component of the membrane-like sheath enclosing each mature vaccinia virus particle.

Occasionally virions at the tips of microvilli showed an enlarged membrane-like covering (Fig. 8B) which could have been acquired within the host cell cytoplasm. The intact enclosure was not present on all releasing virions (Fig. 8A). It appeared to be very fragile and easily disrupted.

DISCUSSION

The present investigation of vaccinia virus in whole-cell preparations has revealed a host-cell interaction and virus release mechanism not observed previously in sectioned preparations. The technique utilizes the high penetration ability of the 1,000-kV transmission electron

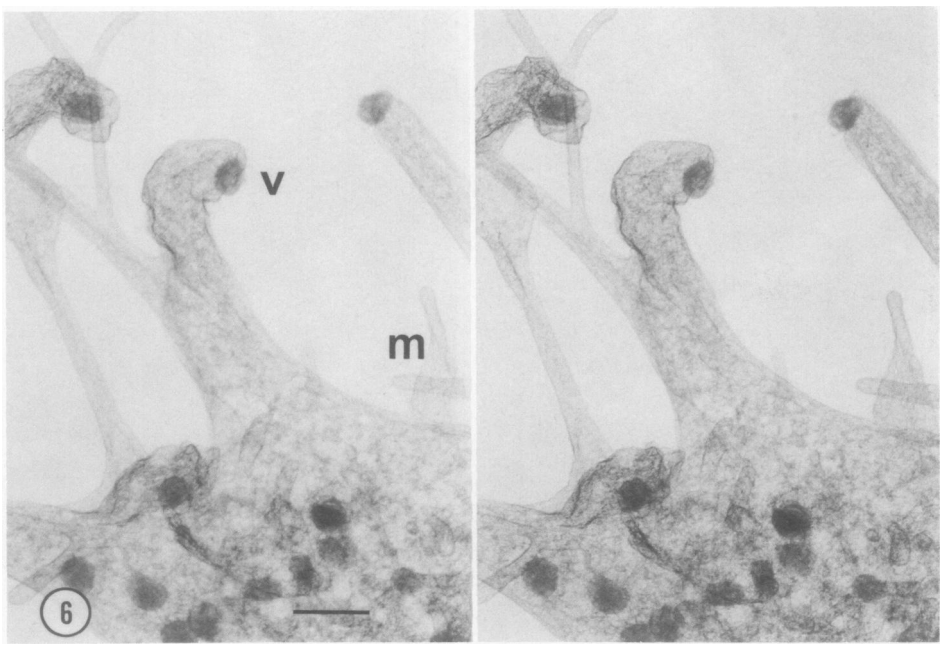

FIG. 6. *Stereoscopic high-voltage electron micrograph of vaccinia virus releasing from a whole BSC-1 cell 20 h after infection. Vaccinia virus (v) can be seen at three stages of penetration along microvilli. Note the larger dimensions of microvilli containing virions compared with those lacking viruses (m). The specimen was tilted ±5°. ×20,000. Scale line, 500 nm.*

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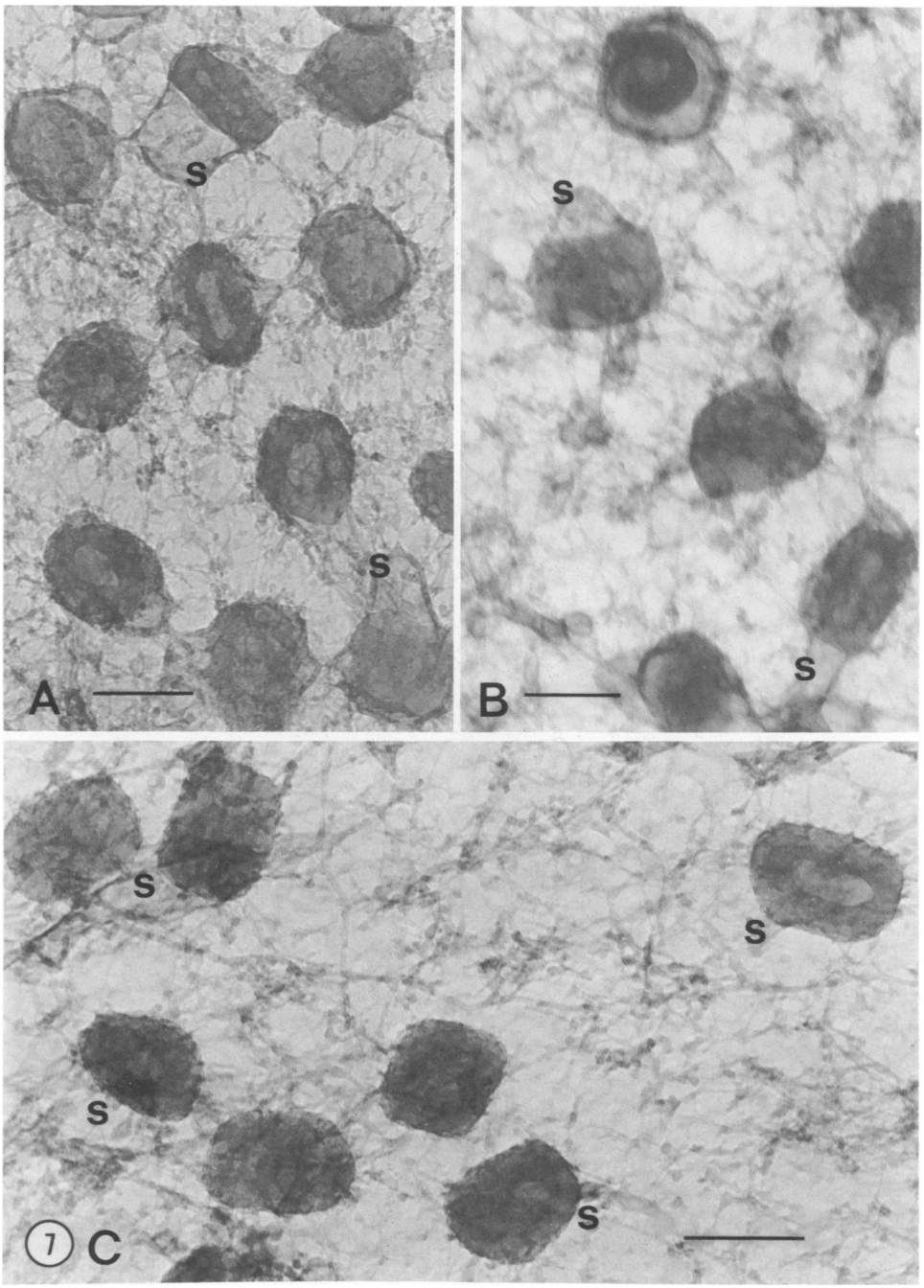

FIG. 7. *High-voltage electron micrograph of vaccinia virus located near the cell periphery in whole BSC-1 cells 20 h after infection. (A) Untreated cells show the membrane-like sheath (s) surrounding each virion. ×68,800. Scale line, 200 nm. (B) Cells treated with vinblastine sulfate (10 μg/ml) for 1 h before fixation. Note the change in cytoplasm structure and the presence of the sheath surrounding each virion. ×68,800. Scale line, 200 nm. (C) Cells treated with cytochalasin B (10 μg/ml) for 1 h before fixation. Note the disruption of the membranous sheath (s) surrounding each virion. ×82,500. Scale line, 200 nm.*

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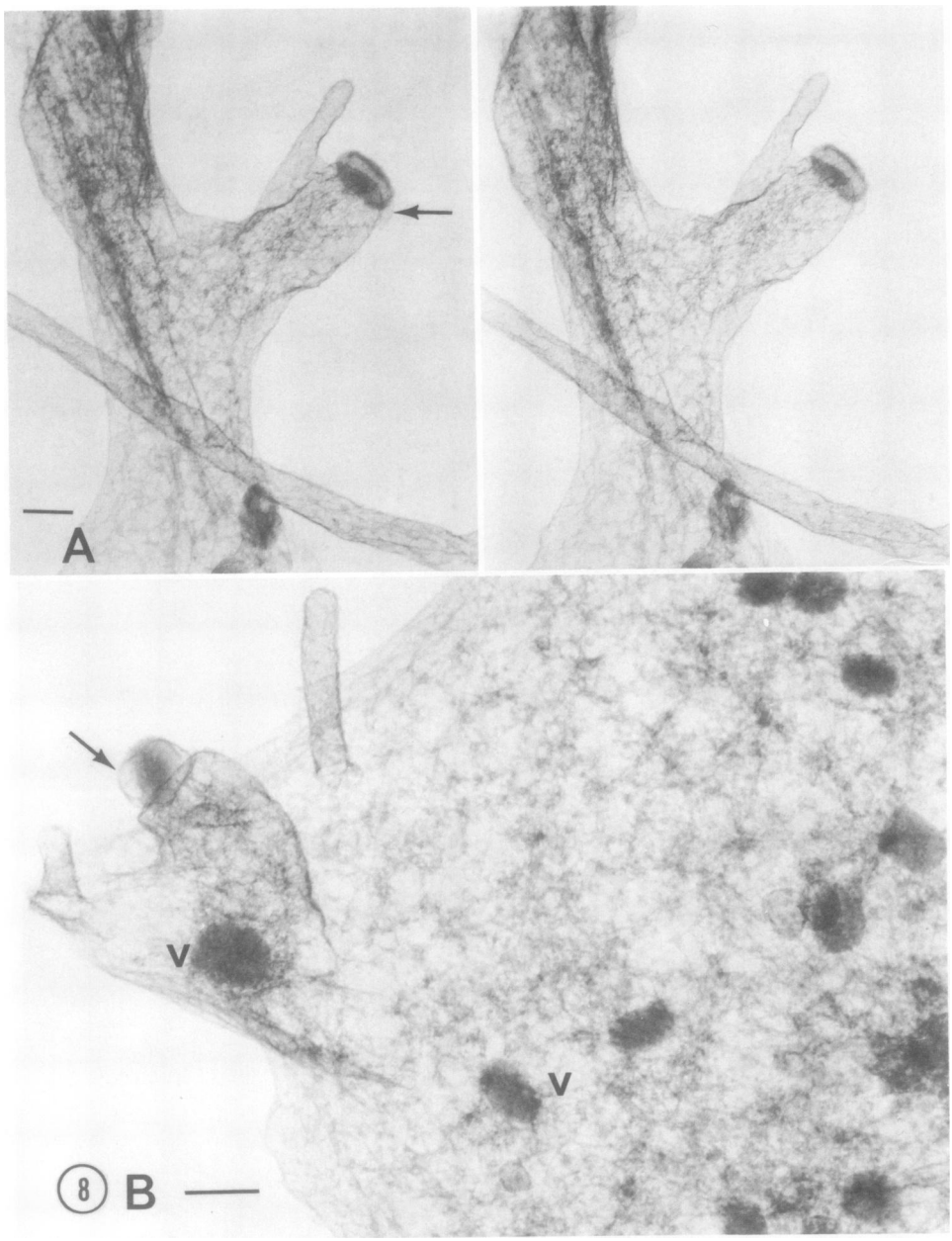

FIG. 8. *High-voltage electron micrograph of vaccinia virus releasing from the tips of microvilli. (A) Stereoscopic pair showing the ruptured membrane-like sheath (arrow) near the base of the virion. The specimen was tilted ±5°. ×30,000. Scale line, 200 nm. (B) Releasing virion with a complete membranous sheath (arrow). Note the slight depression in which rests the virion at the microvilli tip. ×40,000. Scale line, 250 nm.*

microscope and critical point-dried preparations of monolayer cells. The dimensions and morphological appearance of viruses compared closely with those obtained from sectioned (2, 3) and negatively stained (11) preparations.

The means by which vaccinia virions migrate from one intracellular location to another was studied. Vaccinia virions are assembled in discrete regions called factories, located in the perinuclear region of the host cell cytoplasm. Later in infection viruses can be found dispersed throughout the cytoplasm. A membrane-like sheath was observed surrounding mature vaccinia virions and absent from immature

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viral particles within factories. The sensitivity of the membrane-like sheath to the drug cytochalasin B, but not to vinblastine, suggests the presence of polymerized microfilament, but not microtubule, proteins which stabilize the structure. Both drugs, added separately, prevent the dispersal of vaccinia virus from factories into the cell periphery (Stokes, unpublished data). Releasing virions at the tips of microvilli sometimes appeared to be enclosed by a structure believed to be obtained from the cell cytoplasm and not the plasma membrane. The enclosing structure may represent the membrane-like sheath observed around viral particles near the cell periphery. The intracellular movement of vaccinia virus may require enclosure of each particle by the host membrane-like sheath. This interpretation agrees with the observation by Buckley (1), who believes that the basis of subcellular motility is a host cell cytoplasmic network, also called the subplasmalemmal network. (Characterization of the cytoplasmic network is the work of J. J. Wolosewick and K. R. Porter [personal communication].)

The occurrence of an intracellular structure that enables a virus to migrate from one intracellular location to another may be unique to vaccinia virus and other poxviruses. Fonte and Porter (6), studying herpes simplex virus, did not observe a similar structure. Studies with adenovirus (4, 8) and barley stripe mosaic virus (9) report an association between these viruses and microtubules.

Additional studies with whole-cell preparations may supply more information on vaccinia virus maturation. An HVEM technique for labeling specific vaccinia virus antigens using monospecific antibody and whole-cell preparations is currently under study. Such a technique would permit the identification of host cell and viral antigens in situ.

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