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Electron Microscopy of Measles Virus Replication

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Replication of measles virus in HeLa cells was examined by electron microscopy with ultrathin sectioning and phosphotungstic acid negative staining methods. The cytoplasmic inclusion bodies consisted of masses of helical nucleocapsid which was similar in structure to the nucleocapsid found in measles virions. The cytoplasmic helical nucleocapsid appeared to align near the HeLa cell membrane, and the membrane differentiated into the internal membrane of the viral envelope and the outer layer of the short projections. The viral particles were released by a budding process involving incorporation into the viral envelope of membrane which was contiguous to but morphologically altered from the membrane of the HeLa cells. The intranuclear inclusion bodies were composed of tubular structures similar to those found in the cytoplasmic inclusion bodies. These structures aggregated to crystalline arrangement. The relationship between nuclear inclusion body and replication of measles virus was not clear.

In 1954, Enders and Peebles originally described the appearance of cytoplasmic and intranuclear inclusion bodies in cells infected in vitro with measles virus (6). Kallman et al. (8) and Tawara et al. (16) described the filamentous nature of the intranuclear and cytoplasmic inclusion bodies in the measles-infected HeLa cells. These filamentous fibers appeared to be tubular structures of about 15 to 20 nm in outer diameter; they seemed to occur randomly at the early stage of cellular infection and to aggregate to crystalline arrangement in the later stage (14, 15). Baker et al. (2) reported the existence of crystallites in the nucleus of measles-infected, human amnion cells, while Nishi et al. (11) observed electron-dense, virus-like particles of 100 to 150 nm in diameter in the intranuclear vesicles of the measles-infected KB cells. Baker et al. (2) and Ruckel-Enders (13) described the presence of measles virions on infected cell membranes which suggested the replication of the virus at the cell surface.

Electron micrographs of measles virions showed diameters of 120 to 250 nm; the virions possessed a well-defined envelope which was approximately 10 nm thick (17, 18). The envelope, which possessed short projections, enclosed the helical nucleocapsid and the capsid measured about 15 to 19 nm in diameter (12, 17, 18). Although extensive information is available concerning the morphological structure of measles virus and the filamentous nature of its inclusion bodies, the ultrastructural knowledge of the replication of this virus is still scant.

This paper describes the electron microscopic observations of the various stages of measles virus replication and the morphological changes induced in HeLa cells.

MATERIALS AND METHODS

Virus. The Edmonston strain of measles virus (6), propagated in HeLa cells, was used in this study.

Preparation and inoculation of HeLa cells. HeLa cells were grown at 37 C in Earle's solution supplemented with 10% calf serum. Monolayers of HeLa cells were grown in 200-ml milk-dilution bottles by seeding with approximately 8×10^5 cells. Fully grown cell sheets were washed twice with phosphate-buffered saline (PBS) and inoculated with measles virus at high-input multiplicity. After an adsorption period of 90 min at 37 C, Earle's solution with 3% calf serum was added and incubation continued.

Preparation of specimen for electron microscopy. At various times after infection, cells were removed from the glass surface by scraping them into PBS; this mixture was centrifuged for 5 min at $43 \times g$. The cells were washed in two changes of PBS, and the final pellet was fixed in 1% osmium tetroxide in a veronal-acetate buffer (*p*H 7.2) at 4 C for 60 min. The material was dehydrated in graded dilutions of ethyl alcohol, embedded in Epon, and sectioned. The preparations were stained with a saturated solution of uranyl acetate in ethyl alcohol for 60 min and stained further with lead hydroxide for 15 min. Control preparations

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of uninoculated HeLa cells were examined in a similar manner.

For negative staining, cells infected for 72 hr were scraped from three bottles, washed once with PBS, and disrupted by freezing and thawing six times. The material was clarified by three centrifugations at $43 \times g$ for 5 min, $1,300 \times g$ for 15 min, and $8,200 \times g$ for 30 min. Each time, the sediment was discarded. The final supernatant fluid was then ultracentrifuged at $80,000 \times g$ for 90 min. The sediment was resuspended in distilled water, and drops were mixed in equal volume with 2% potassium phosphotungstic acid adjusted to *p*H 7.2 and placed on carbon-coated grids for observation. All specimens were examined with a Hitachi HU-11A electron microscope.

RESULTS

Morphology of measles virions by negative staining. As previously reported (12, 17, 18), measles particles after negative staining with phosphotungstic acid resemble the paramyxovirus group, which includes mumps, Newcastle disease, and parainfluenza viruses. Measles virions possess an envelope with surface projections which are approximately 10 to 20 nm in length. This envelope encloses a helical internal nucleocapsid which measures about 17 to 18 nm in diameter (Fig. 1). Some virions were partially disrupted, and fragments of the hollow nucleocapsid were lying free near the disrupted particle (Fig. 2).

Viral morphology by thin section. In thin sec-

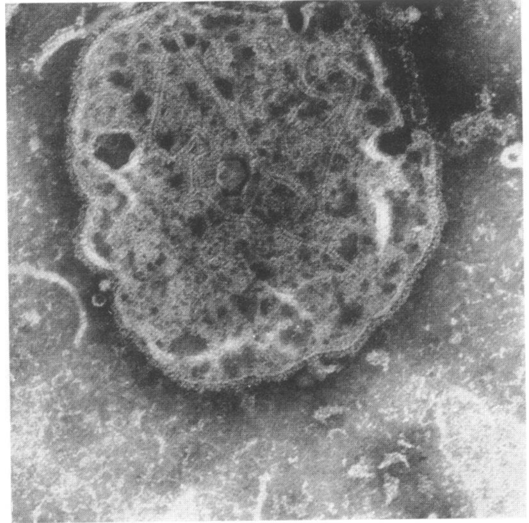

FIG. 1. *Measles particle negatively stained with phosphotungstic acid. The internal components (nucleocapsid) and the external surface projections are evident.* $\times$ *70,000.*

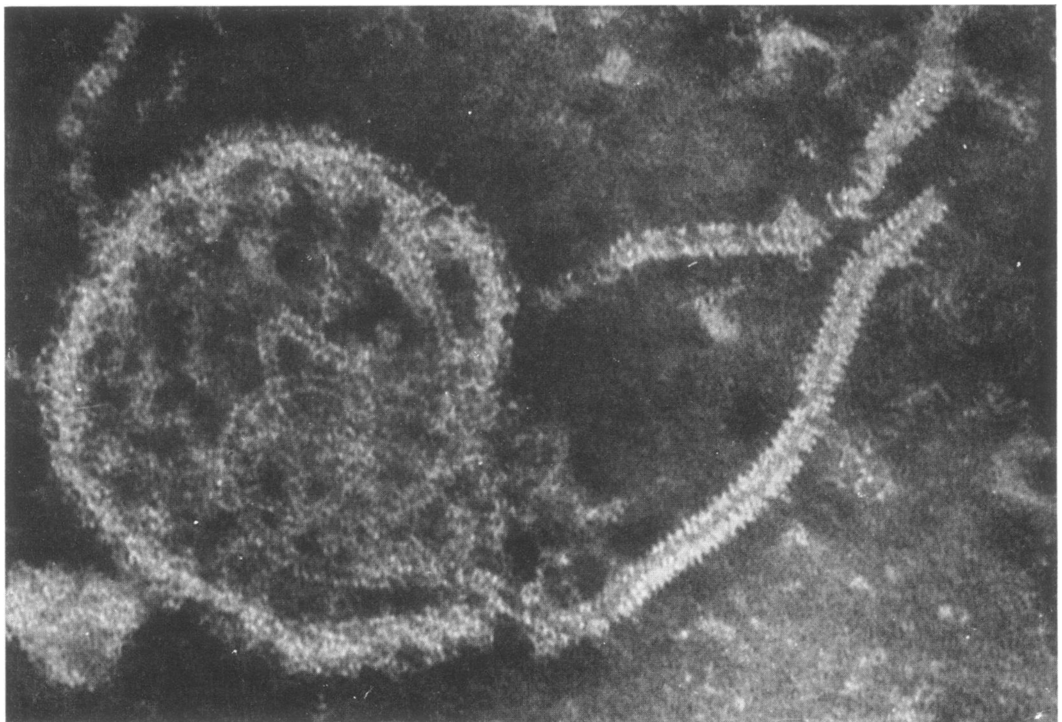

FIG. 2. *Disrupted measles particle stained with phosphotungstic acid. The helical nucleocapsid is released partially from the viral particle.* $\times$ *222,100.*

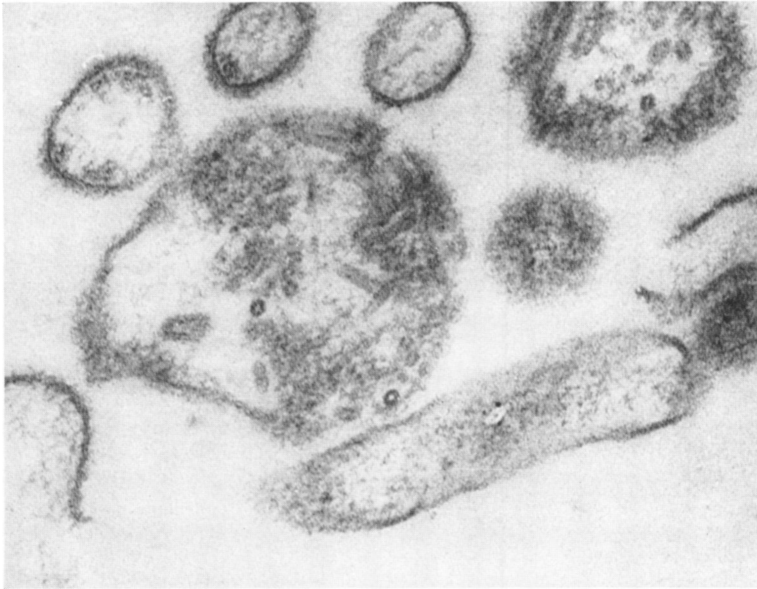

FIG. 3. *Thin section of spherical and filamentous particles. Cross and vertical sections of the nucleocapsid are shown.* × *119,000.*

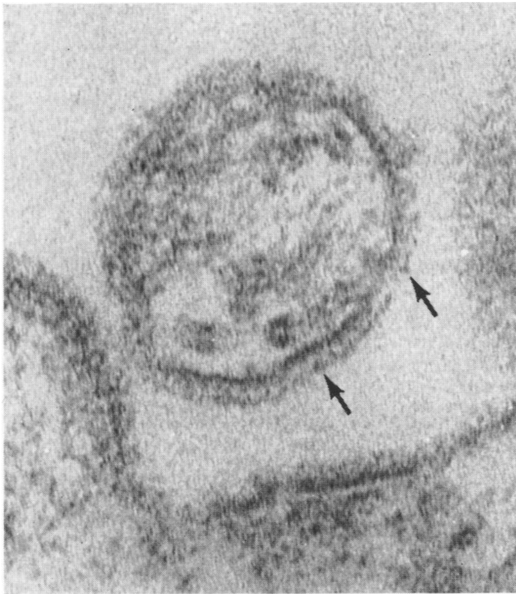

FIG. 4. *Thin section of a spherical particle, showing viral envelope with surface projections or spikes.* × *184,000.*

tions, the virions are pleomorphic and their sizes range from 180 to 600 nm (Fig. 3, 4). The filamentous forms are observed occasionally during the examination of ultrathin sections. The viral envelope consists of membrane with short, surface projections. The internal nucleocapsid of the virion consists of interwoven tubular strands, 15 to 17 nm in diameter, and these tubular strands are seen as circular or oval rings in cross and tangental sections of the tubules (Fig. 3, 4).

Fine structures of infected HeLa cells. The earliest microscopic change is the occurrence of cytoplasmic inclusion bodies which are detected 18 to 20 hr after viral infection. These inclusion bodies are composed of filamentous and granular structures (Fig. 5). Since the diameter of the granular structures corresponds to the width of the filaments, the granules probably represent cross sections of the filaments. The cytoplasmic aggregate of filamentous structures stained negatively with phosphotungstic acid is shown in Fig. 6. Helical structures of 17 to 18 nm in width are observed in the aggregate. At higher magnification, as in Fig. 7, the helical strands are indistinguishable from the helical nucleocapsid released from measles virion (Fig. 2). Fragments of nucleoprotein of varying lengths are seen, some of which are in the form of rings, representing cross sections of the nucleocapsid.

The changes observed after 30 to 42 hr are characterized by alteration of the cell membrane with an increase in electron density (Fig. 8). The altered cell membrane has similar thickness to that of the viral envelope. Collections of nucleocapsid in tubular, oval, and circular forms are observed just beneath the cell membrane (Fig. 9–11). Since some of the circular and oval forms are continuous with the tubular forms

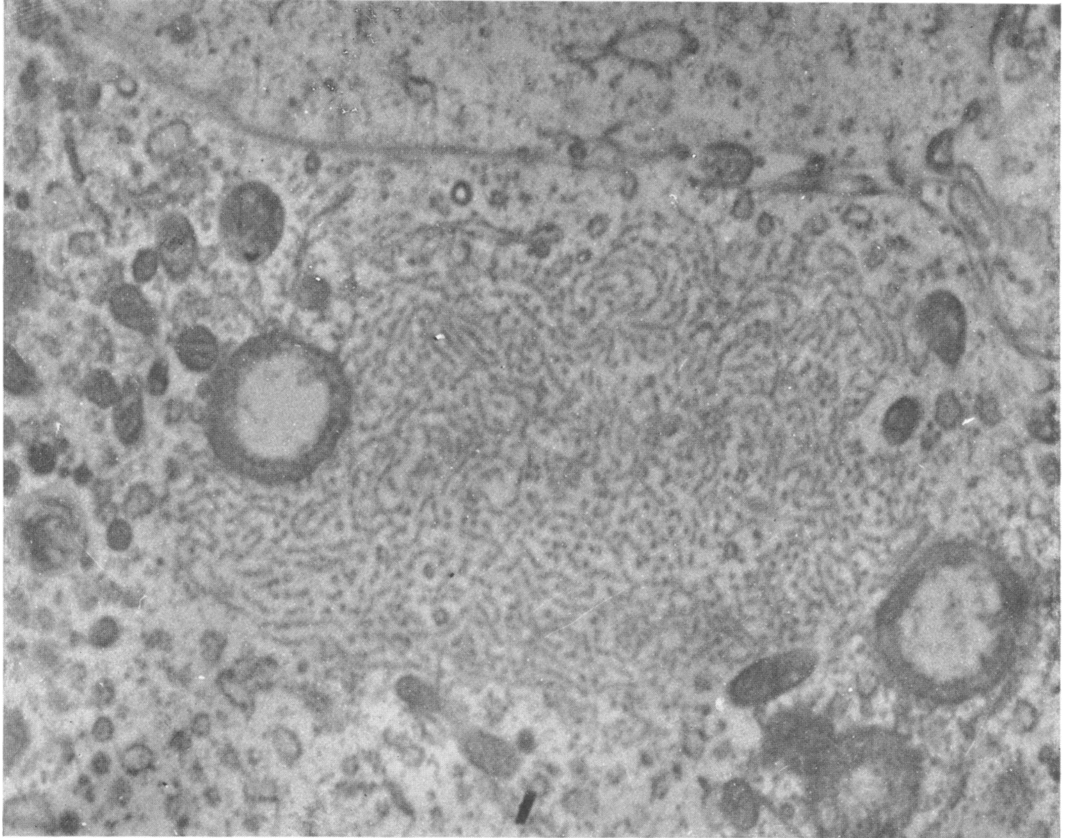

FIG. 5. *Cytoplasm of a HeLa cell 20 hr after measles inoculation. The cytoplasmic inclusion body is composed of filamentous and granular structures.* × *41,500.*

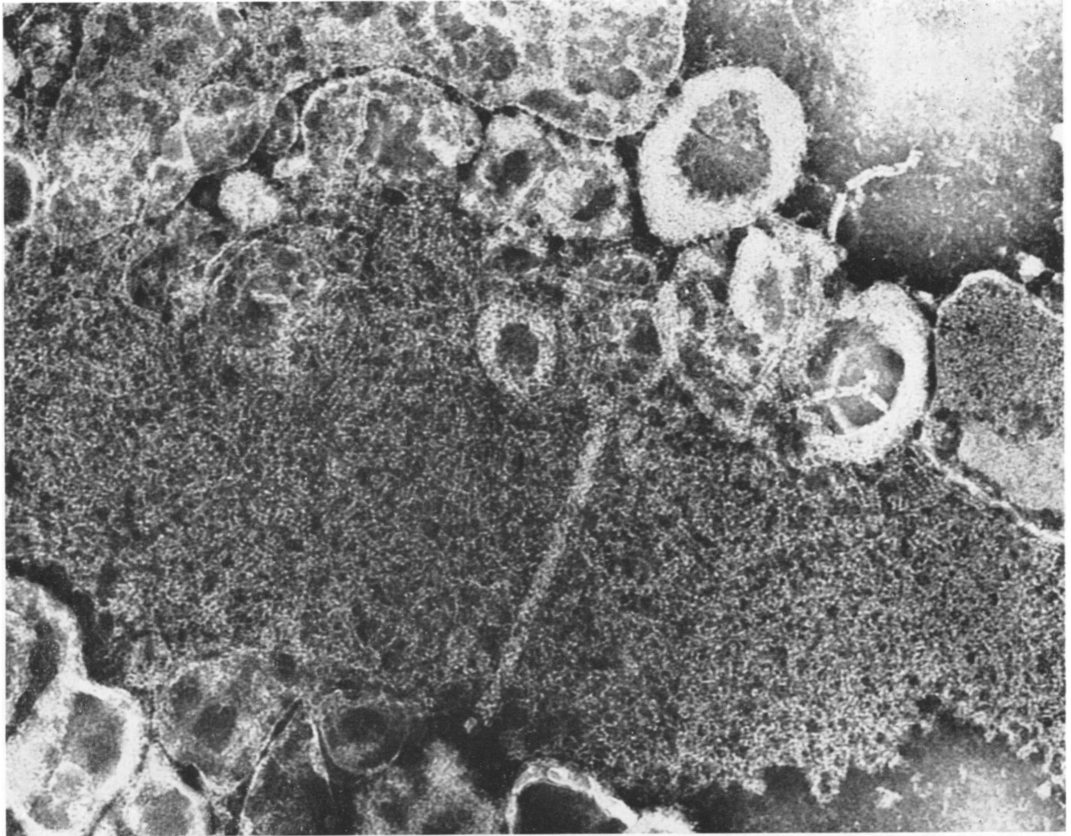

FIG. 6. *Large aggregate of nucleocapsid filaments from the cytoplasm of infected HeLa cell negatively stained with phosphotungstic acid.* $\times$ *75,600.*

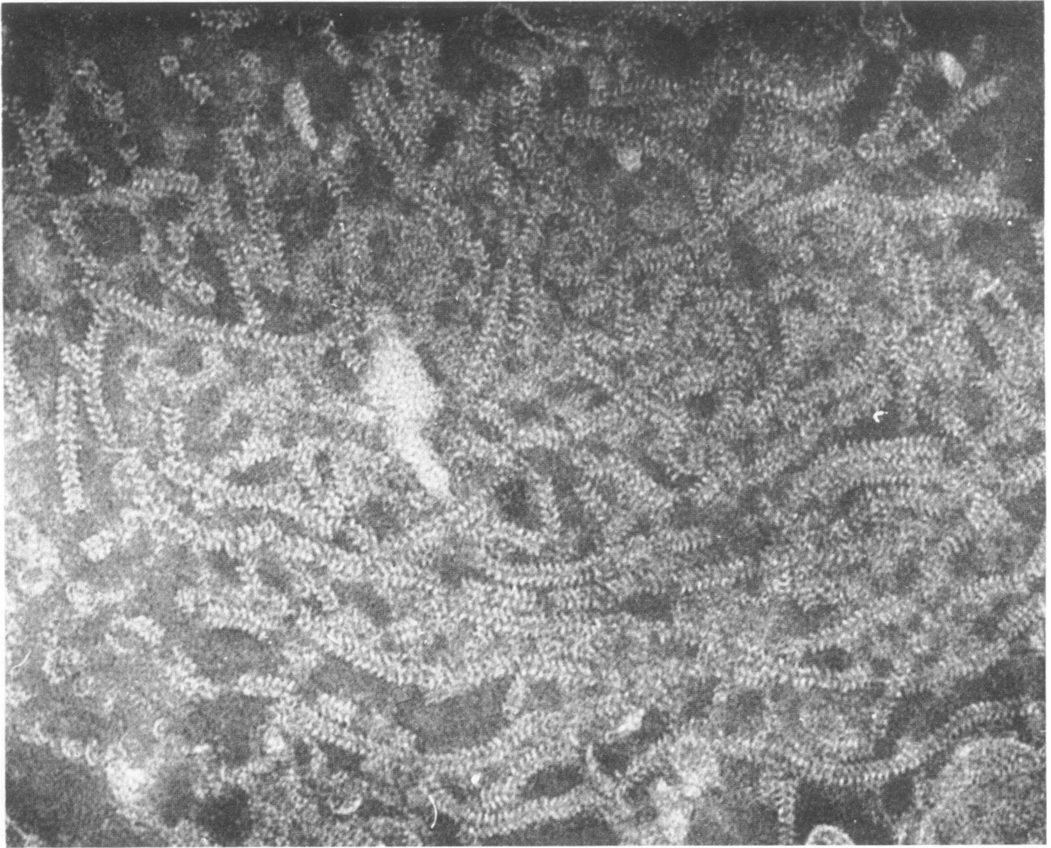

FIG. 7. *Higher magnification of Fig. 6, showing helical structure of the nucleocapsid. The diameter of the nucleocapsid is approximately 17 to 18 nm. The ring forms are the cross sections of the nucleocapsid.* × 158,000.

(Fig. 10), these forms may represent cross and tangental sections of the tubular forms. These similar structures, when stained with phosphotungstic acid, show helical strands similar to the nucleocapsid of the virion (Fig. 12). In Fig. 10, two pleomorphic particles can be seen in which the arrangement of the internal components appear helical and are arranged in a spiral extending the width of the particles.

Within 96 to 120 hr after infection, intranuclear inclusion bodies are observed in a large number of cells. These intranuclear inclusion bodies are composed of tubular structures similar to those found in the cytoplasmic inclusion bodies (Fig. 13, 14). The inner diameter of the tubular structures is approximately 15 to 17 nm. These structures aggregate to form a crystalline arrangement. The nuclear membrane remains intact and shows no damage in spite of the presence of the inclusion bodies.

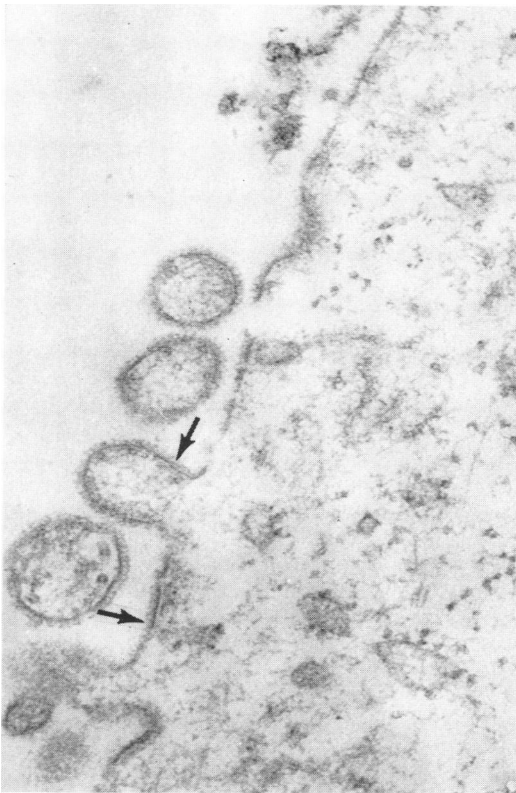

FIG. 8. *Thin section of the surface of an infected HeLa cell, showing increased electron density (arrows) and sperical particles budding from the cell surface.* × *70,800.*

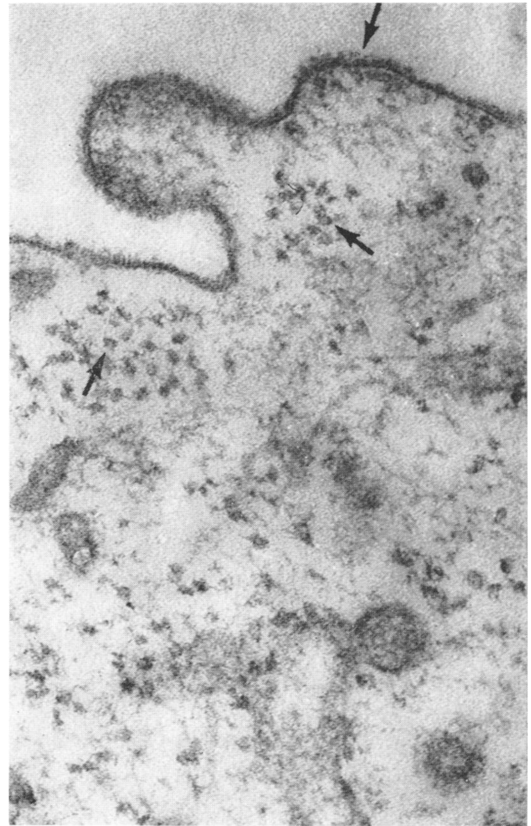

FIG. 9. *Section showing newly formed layer of surface projections on the outer edge of the cell membrane (arrow) and a budding virus particle. Cross sections of nucleocapsid strands are present under the cell surface (arrows).* × *117,400.*

DISCUSSION

Electron micrographs of tissues infected with measles virus have shown many fibrous filaments which occur in both the cytoplasmic and intranuclear inclusion bodies (2, 8, 10, 13–16). In our study, inclusion bodies with filamentous fibers were observed in the cytoplasm of HeLa cells 18 to 20 hr after infection with measles virus. Inclusion bodies of similar structure were seen in the nucleus 96 to 120 hr after infection. With negative staining and high magnification, the cytoplasm of cells infected 18 to 20 hr was shown to be composed of aggregates of helical tubular structures 17 to 18 nm in width. These helical

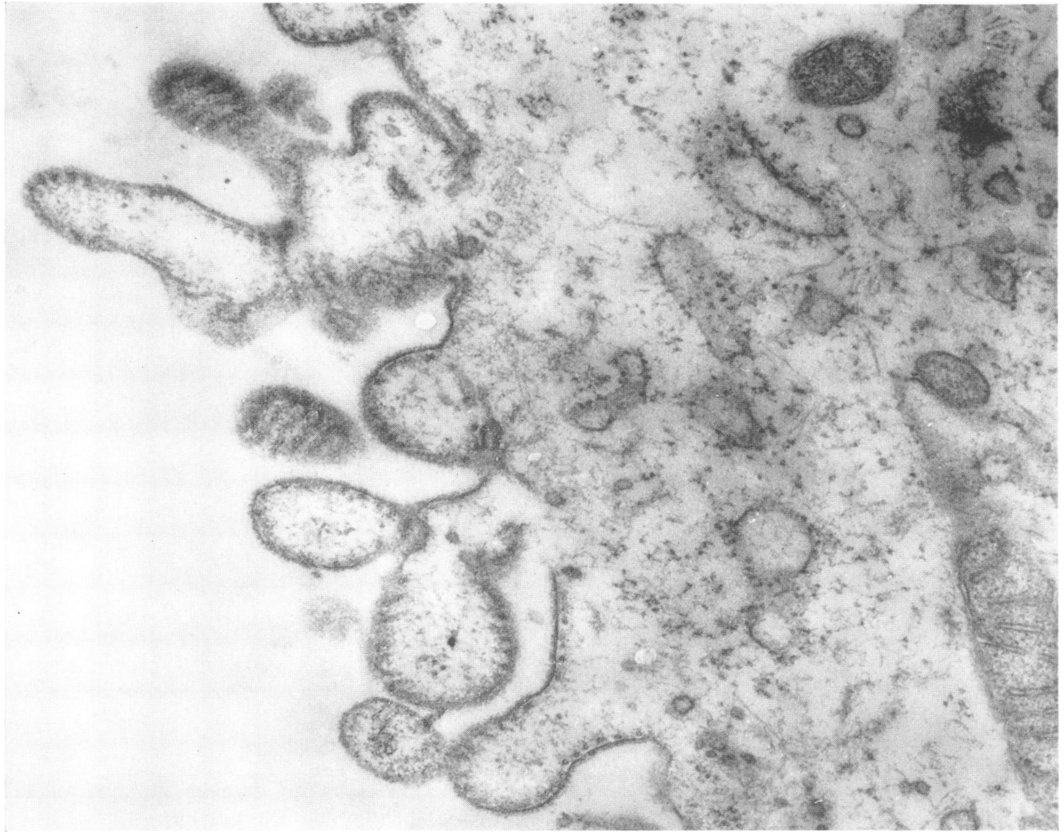

FIG. 10. *Pleomorphic particles in the process of budding. Cross sections (ring forms) or tangental sections of the nucleocapsid are evident. These are aligned adjacent to the cell surface. Two of the particles contain nucleocapsid arranged in a regular spiral extending the width of the particles.* × *47,800.*

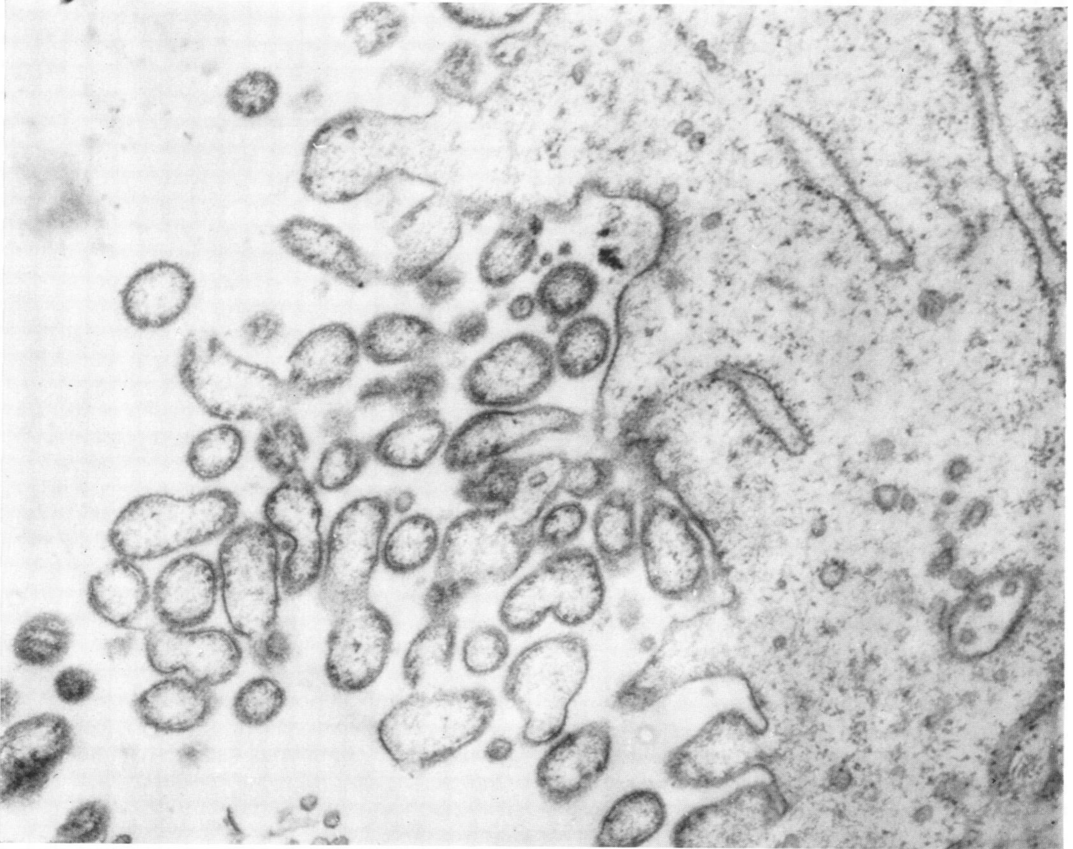

FIG. 11. *Spherical and pleomorphic particles at the free surface of the infected cell. Nucleocapsid stands can be seen in the particles.* $\times$ *44,600.*

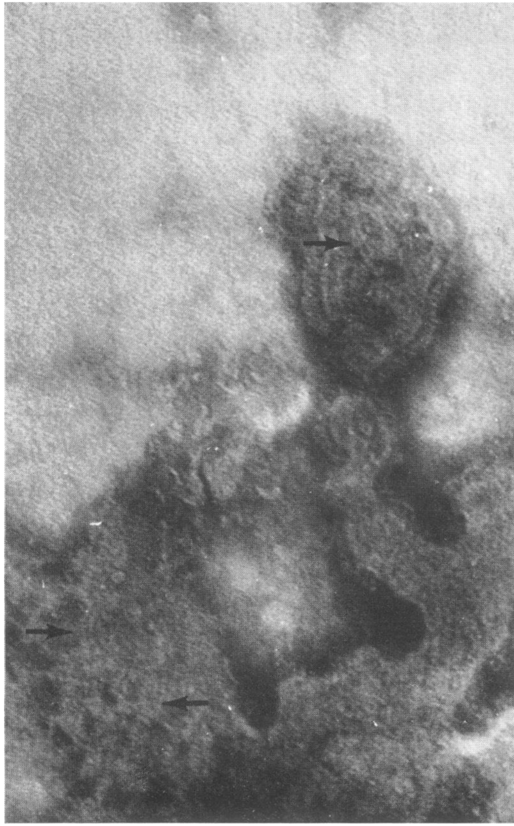

FIG. 12. *Fragments of an infected HeLa cell negatively stained with phosphotungstate. The projections on the surface of the virus particle and the cell are evident. The nucleocapsid arranged under the cell surface is characteristic (arrow).* × *83,300.*

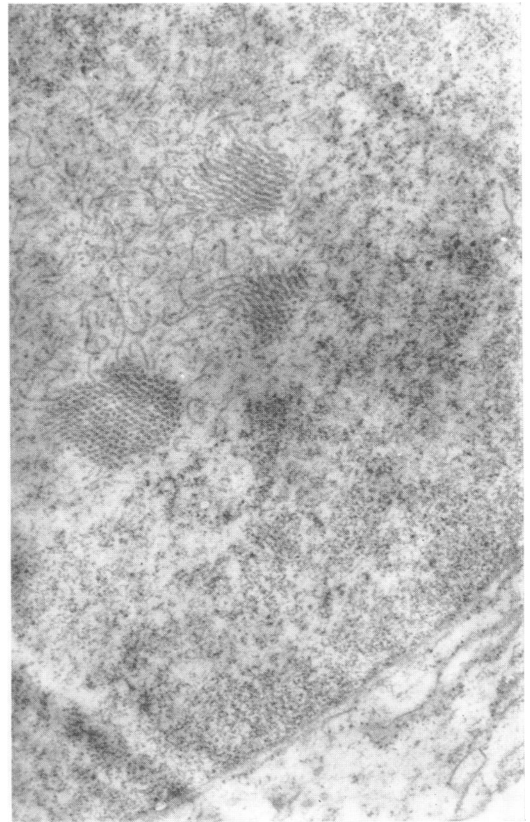

FIG. 13. *Intranuclear fibrous filaments present in HeLa cell 96 hr after inoculation. Aggregation of the fibrils is seen.* × *32,200.*

strands were indistinguishable from the nucleocapsid released from disrupted measles virions. It is speculated that the cytoplasmic inclusion bodies are associated with the formation of the nucleocapsid. Further proof of this would require examination of similar preparations with the use of specific antiserum labeled with ferritin.

Parallel arrangement of nucleocapsid was observed beneath the cell membrane about 42 hr after infection. In this area, the cell membrane showed morphological alteration consisting of increased electron density. Adjacent to the collection of nucleocapsid, the cell membrane differentiated into the internal membrane of the envelope and outer layer of short projections. The newly formed electron-dense cell membrane and the viral envelope had similar thicknesses and were morphologically indistinguishable. Virus maturation was accomplished at the cell surface, and the virions were released by budding. The release of mature virions from infected cells seemed to be continuous and slow. Similar electron microscopic observations have been made with replication of parainfluenza and mumps viruses (3–5, 7).

The structure of intranuclear inclusion bodies closely resembled cytoplasmic inclusion bodies. The intranuclear bodies could not be associated with the formation of virions since, at this stage, large numbers of mature or budding virions could be observed on the cell surface and the nuclear membrane was still intact.

The relationship between the nuclear inclusion body and the replication of measles virus is not clear. Actinomycin D has not been shown to inhibit measles virus replication but, instead, has some enhancing effect (1, 9). This suggests that the multiplication of measles virus is independent of the synthesis of deoxyribonucleic acid-dependent ribonucleic acid by the host cell.

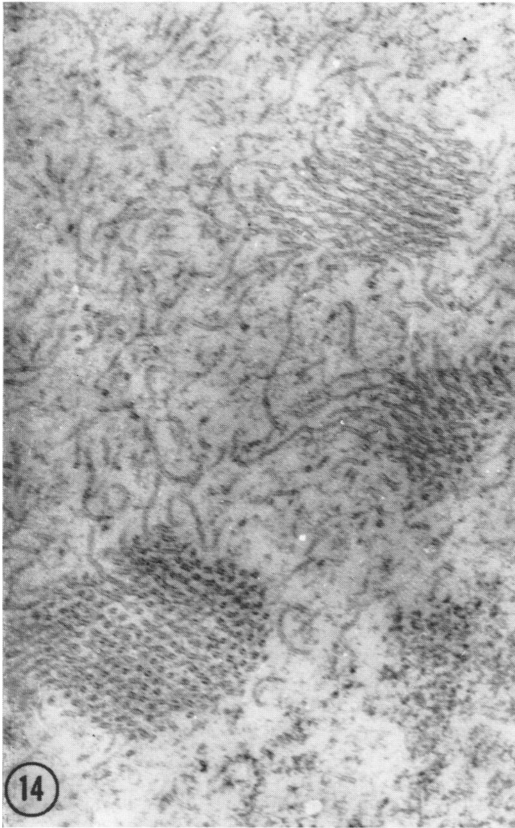

FIG. 14. *Higher magnification of Fig. 13, showing tubular structures of the fibrils with diameter of approximately 15 to 17 nm.* × *49,600.*

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ADDENDUM IN PROOF

After submission of this paper for publication, two additional articles came to our attention. Mannweiler (Arch. Ges. Virusforsch. **16:**89, 1965) and Anisimová et al. (Acta Virol. **12:**289, 1968) described the filamentous tubular structures in the cytoplasm of cells infected with measles virus. These results correspond very closely to those we have observed.

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