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Antigenic and Morphological Similarities of Progressive Pneumonia Virus, a Recently Isolated "Slow Virus" of Sheep, to Visna and Maedi Viruses

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Progressive pneumonia virus, the causative agent of a slow, pulmonary disease of Montana sheep, was shown to be antigenically related to two other slow viruses of sheep, visna and maedi. Electron microscopic examination of infected cells revealed that the virus matures by a budding process and that the budding particles as well as the mature, extracellular virions bear striking resemblances to the oncogenic ribonucleic acid (RNA) viruses. Recent findings of an RNA-dependent deoxyribonucleic acid polymerase associated with the virions of this group of slow viruses lend further support to the notion that they may tentatively be classified with the oncogenic RNA tumor viruses.

Kennedy et al. (6) reported the isolation in tissue culture of a virus from the lungs of sheep in Montana affected with a chronic pulmonary disease. The disease closely resembled the slow viral infection known as maedi (10, 11), a pulmonary disease of Icelandic sheep. The progressive pneumonia virus (PPV) isolated by Kennedy et al. (6) produced cytopathic effects in tissue culture characterized by the formation of multinucleated giant cells.

PPV shared some properties with maedi virus, i.e., inactivation by heat (56 C) and ether, but definitive serological evidence concerning relationships of the two viruses was lacking. This paper provides data showing that PPV is indeed related to maedi and another closely related virus, visna, which causes a slow demyelinating disease in sheep (12, 13). Furthermore, electron microscopic observations revealed that PPV matures by a budding process and that budding viruses as well as mature particles closely resemble the oncogenic ribonucleic acid (RNA) viruses.

MATERIALS AND METHODS

Viruses. Progressive pneumonia virus was isolated from clarified 10% homogenate of lungs from an infected sheep. The virus was propagated in a sheep testes cell strain (ST cells) described below. Visna virus was received from Halldor Thormar and propagated in ST cells.

Quantitation of virus was by cytopathic end point calculated by the method of Reed and Muench (9).

Antiserum. PPV, visna, and maedi antisera were obtained from sheep which had been experimentally infected with these viruses. The latter two sera were kindly provided by Halldor Thormar.

Cell culture. Fragments of testicular tissue from a young lamb were received from the Rocky Mountain Laboratory, Hamilton, Mont. The tissue fragments were dispersed with trypsin and grown in Eagle's medium with 10% fetal bovine serum. The cell strain which was derived consisted of predominantly epithelial-like cells which could be propagated for approximately 15 passages before cessation of cell growth.

Electron microscopy. Medium was removed from cultures, washed once with phosphate-buffered saline (*p*H 7.2), covered with 2.5% glutaraldehyde in phosphate buffer (*p*H 7.3), and stored at 4 C for at least 24 hr. Cells were scraped from the dishes with a rubber policeman, centrifuged, postfixed in 1% osmium tetroxide in phosphate buffer, and embedded in epon-araldite. Sections were cut on a Porter-Blum microtome with a diamond knife, stained first with uranyl acetate and then lead citrate, and examined in an RCA EMU 3H electron microscope.

Fluorescent-antibody tests. For fluorescent-antibody (FA) testing, cells were grown on cover slips. Both infected and uninfected cover-slip cultures were air-dried and fixed in cold acetone for 10 min. The indirect procedure was employed with fluorescein-conjugated rabbit anti-sheep globulin.

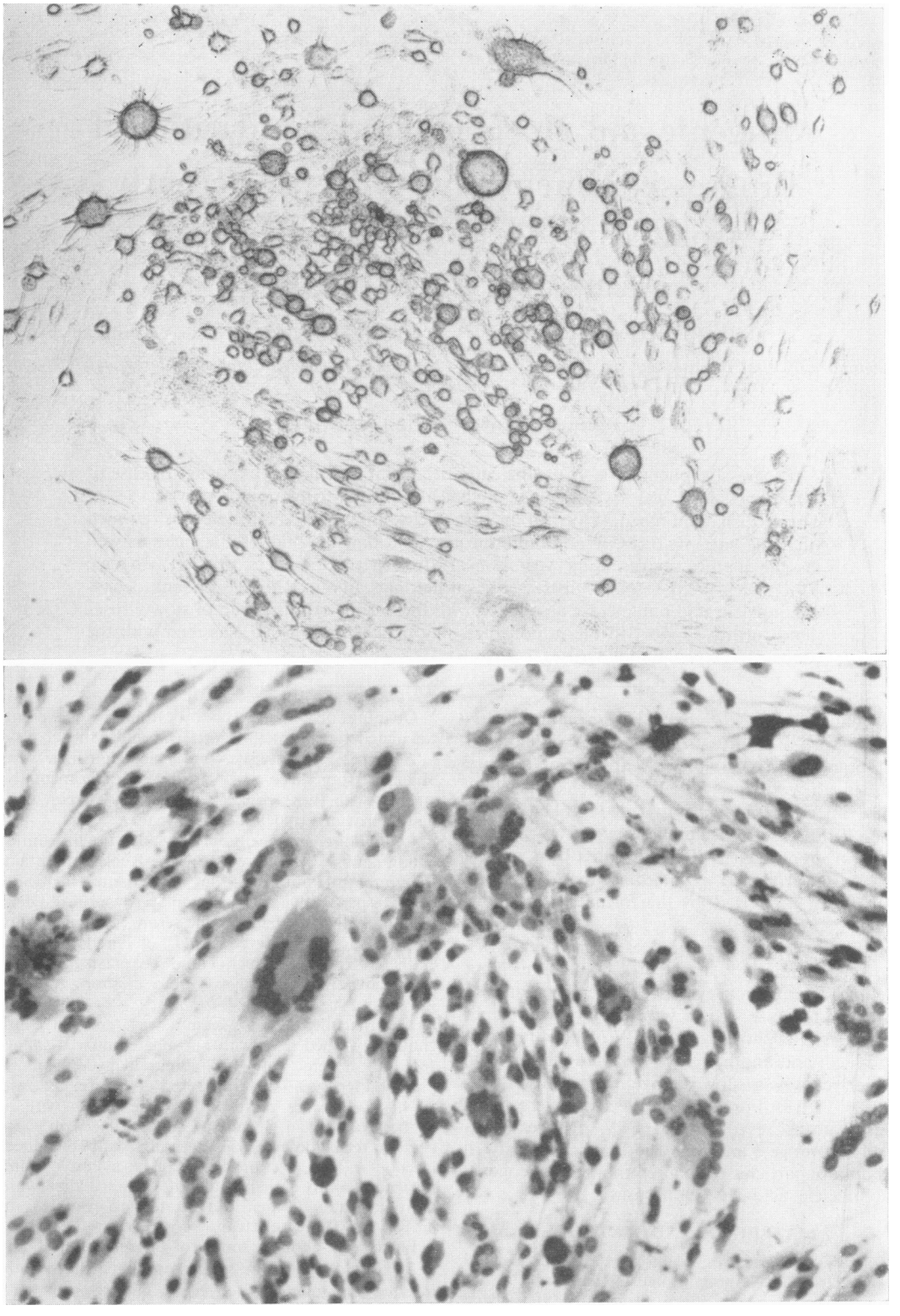

FIG. 1. *(Top) Cytopathic effects of progressive pneumonia virus (PPV) in ST cells 5 days after infection, showing numerous small and large, highly refractile cells. Unstained photograph. × 100. (Bottom) Giemsa-stained preparation of PPV-infected cells 5 days after infection showing multinucleated giant cells with nuclei in rosette-like arrangement. × 100.*

RESULTS

Growth of PPV in ST cells. At input multiplicities of infection greater than one 50% tissue culture infectious dose ($TCID_{50}$) per cell, early cytopathic effects of PPV were observed by 24 hr. The cells became round and highly refractile. By 5 days, numerous polykaryocytes and stellate cells were seen (Fig. 1, top and bottom). Eventually most of the infected cells fused into giant syncytial cells.

To determine the kinetics of virus production, ST cells were infected at a multiplicity of 2 $TCID_{50}$ per cell. The inoculum was removed after an adsorption period of 2 hr, and cultures were washed three times. Fresh medium was then added. At various times thereafter, supernatant fluids were removed and frozen at −70 C until assayed. Newly synthesized virus was first detected at 20 hr (Fig. 2) and increased exponentially up to approximately 50 hr.

The latent period of 20 hr for PPV is very similar to that of visna (17). Furthermore, it was shown in another experiment that there was more extracellular virus than cell-associated virus, as is the case with visna (17).

FA studies. Cover-slip cultures of ST cells infected for various periods of time with PPV or visna were tested by the indirect procedure using antiserum to PPV, visna, and maedi. The three different antisera reacted with both kinds of infected cells and clearly established that PPV shared common antigens with visna and maedi. Control, uninfected cover-slip cultures of ST cells gave no reaction with the three kinds of antisera, and normal sheep serum was also negative when reacted with infected cells.

PPV antigen was detected in the cytoplasm of infected cells at 20 hr. By 3 days, most of the cells showed bright cytoplasmic fluorescence (Fig. 3). The type of fluorescence was similar to that described for visna by Harter et al. (4) and was seen only in the cytoplasm of infected cells.

Electron microscopic observations. Cells infected for 5 days revealed numerous virus particles budding from the plasma membrane (Fig. 4). The outer diameter of the budding particles was between 140 and 150 nm; these particles consisted of an outer membrane and a densely staining, inner shell of 110 to 115 nm in diameter (Fig. 4 and 5). These forms were seen either attached to the cell membrane or immediately adjacent to cells but without visible connection between them. The more complete inner ring profile in the latter suggested that they were cut perpendicular to their budding axis and that many may have been still attached to the cell by stalks not seen in the plane of section. Densely staining granules, 15 to 20 nm in diameter, were sometimes seen within the incomplete inner shell (Fig. 5). These budding forms were indistinguishable from those of visna virus observed by Thormar (16) and Coward et al. (3) in sheep choroid plexus cells.

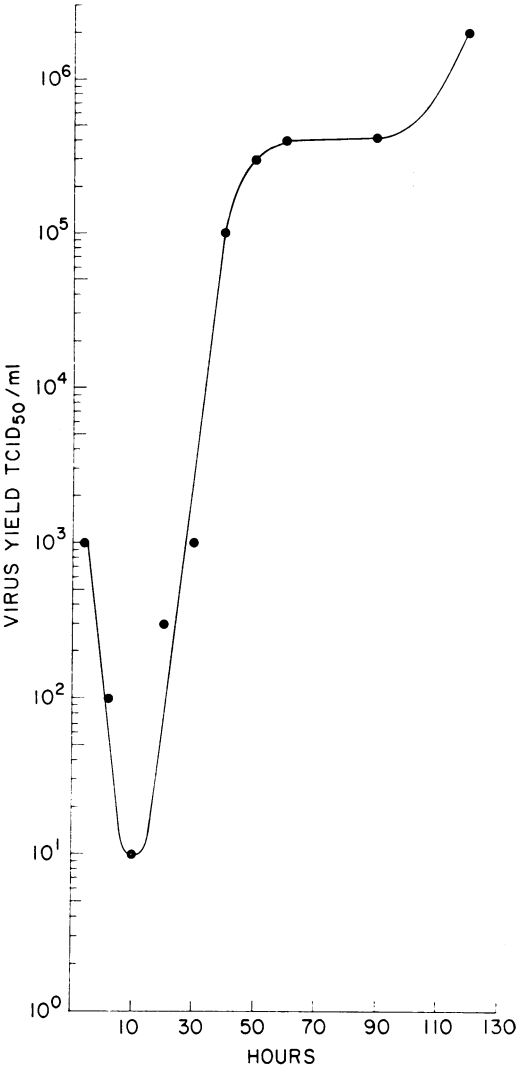

FIG. 2. *Growth curve of progressive pneumonia virus in ST cells. Cells were infected with a mulitplicity of 2, and supernatant fluids were assayed for released virus as described.*

Extracellular particles (Fig. 5) were variable in size and morphology, and the smaller particles with dense, eccentric nucleoids probably are the infectious particles. These measured between 90 and 110 nm in diameter.

Partially purified PPV was pelleted and pre-

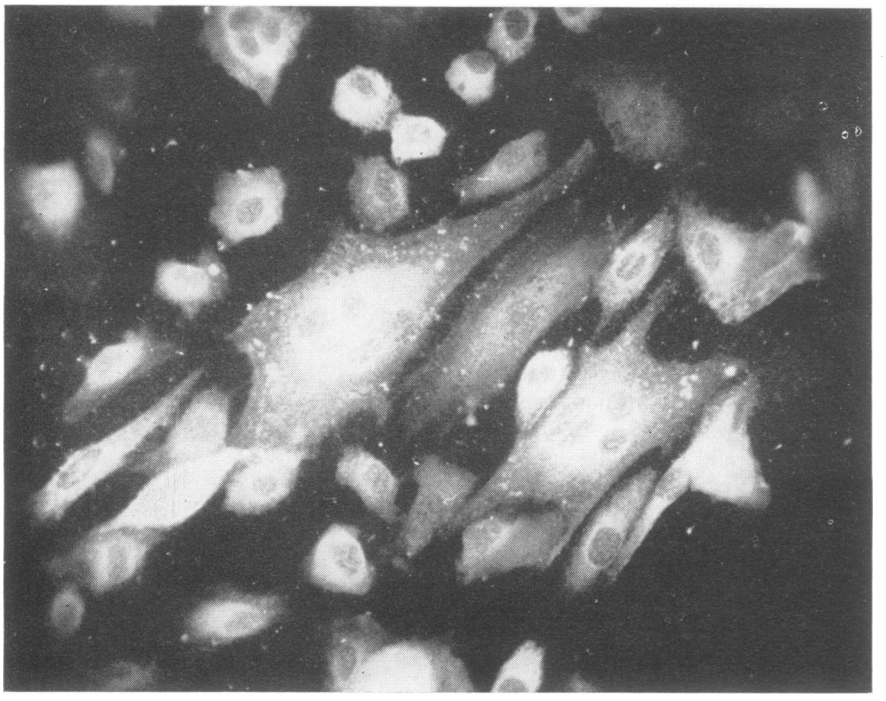

FIG. 3. *Cytoplasmic fluorescence of ST cells 3 days after infection with progressive pneumonia virus. The indirect fluorescent-antibody technique was used with antivisna antibody. Note the characteristic multinucleated giant cells with cytoplasmic fluorescence.* × *100.*

pared for thin sectioning. This preparation revealed particles of about 100 nm with densely staining, solid, eccentrically placed nucleoids about 30 nm in diameter. They were similar to the extracellular particles seen in sectioned cells (Fig. 5). Since these particles were associated with high infectivity (greater than 10^8 $TCID_{50}$/ml), they undoubtedly represent the mature virion.

Similar observations on visna virus have been reported by Thormar (16) and Coward et al. (3).

Unusual cytoplasmic structures (Fig. 4 and 6) were also seen in a number of infected ST cells. These structures were seen in large numbers in multinucleated cells late in the infectious cycle. They appear as approximately spherical structures consisting of from one to six or more complete or partial, densely staining rings, which were similar to the inner shell of the budding form. Occasionally the sectioned profile appeared as a continuous coil. The innermost shell or coil was somewhat variable in size. However, single closed rings were about 95 to 105 nm in diameter. A ring of ribosomes surrounded the outermost layer, and there was a region of moderate staining capacity between adjacent rings or coils which were spaced about 16 nm apart. Although these cytoplasmic structures have not been reported in visna-infected sheep choroid plexus cells by other investigators, we have also observed them in visna-infected ST cells.

DISCUSSION

Serological tests by FA clearly established the antigenic relationship of progressive pneumonia virus to visna and maedi, the viruses which cause two distinct types of slow infections in Icelandic sheep. Previous serological studies on the antigenic relationship between a number of strains of visna and maedi indicated that the two viruses share common antigens but are distinct (20).

Electron microscopic examination of cells infected with PPV showed numerous budding particles and free extracellular particles which contained an electron-dense nucleoid. Both types of particle have been observed in visna-infected cells (3, 16). However, the intracytoplasmic structures found with PPV-infected cells have not been previously observed. These complex bodies were also seen in visna-infected sheep testis cells and may be a developmental state in the morphogenesis of these viruses.

The budding forms seen with both PPV and visna bear a striking resemblance to the RNA tumor viruses, an observation made a number of years ago by Thormar (16). Similarities between

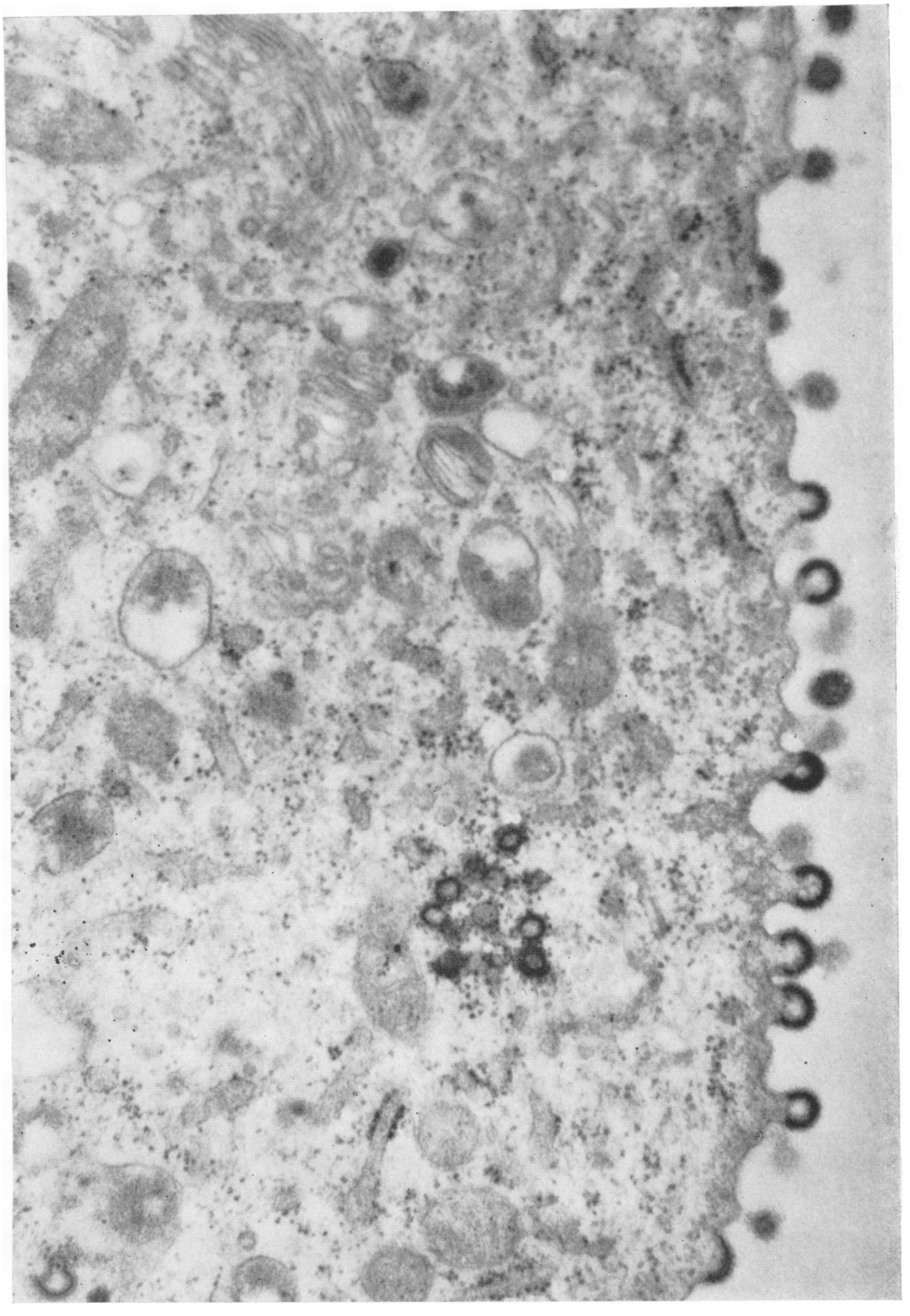

FIG. 4. *Budding particles of progressive pneumonia virus with a cluster of cytoplasmic particles.* × *53,000. This and subsequent electron micrographs are from a 5-day infected culture.*

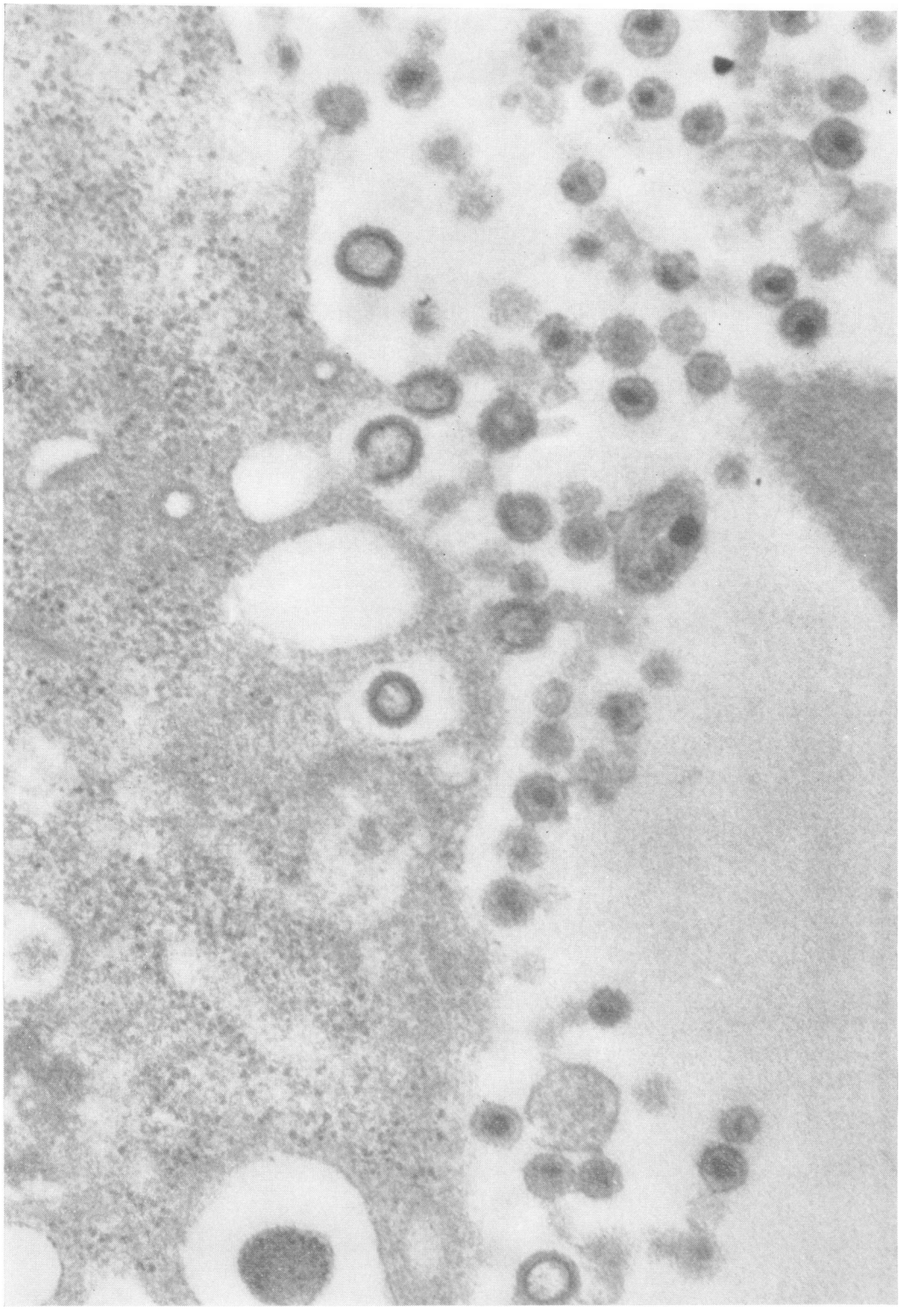

FIG. 5. *Extracellular and budding forms of progressive pneumonia virus. Free particles vary in size and show an electron-dense internal nucleoid, frequently eccentrically displaced.* × *68,000.*

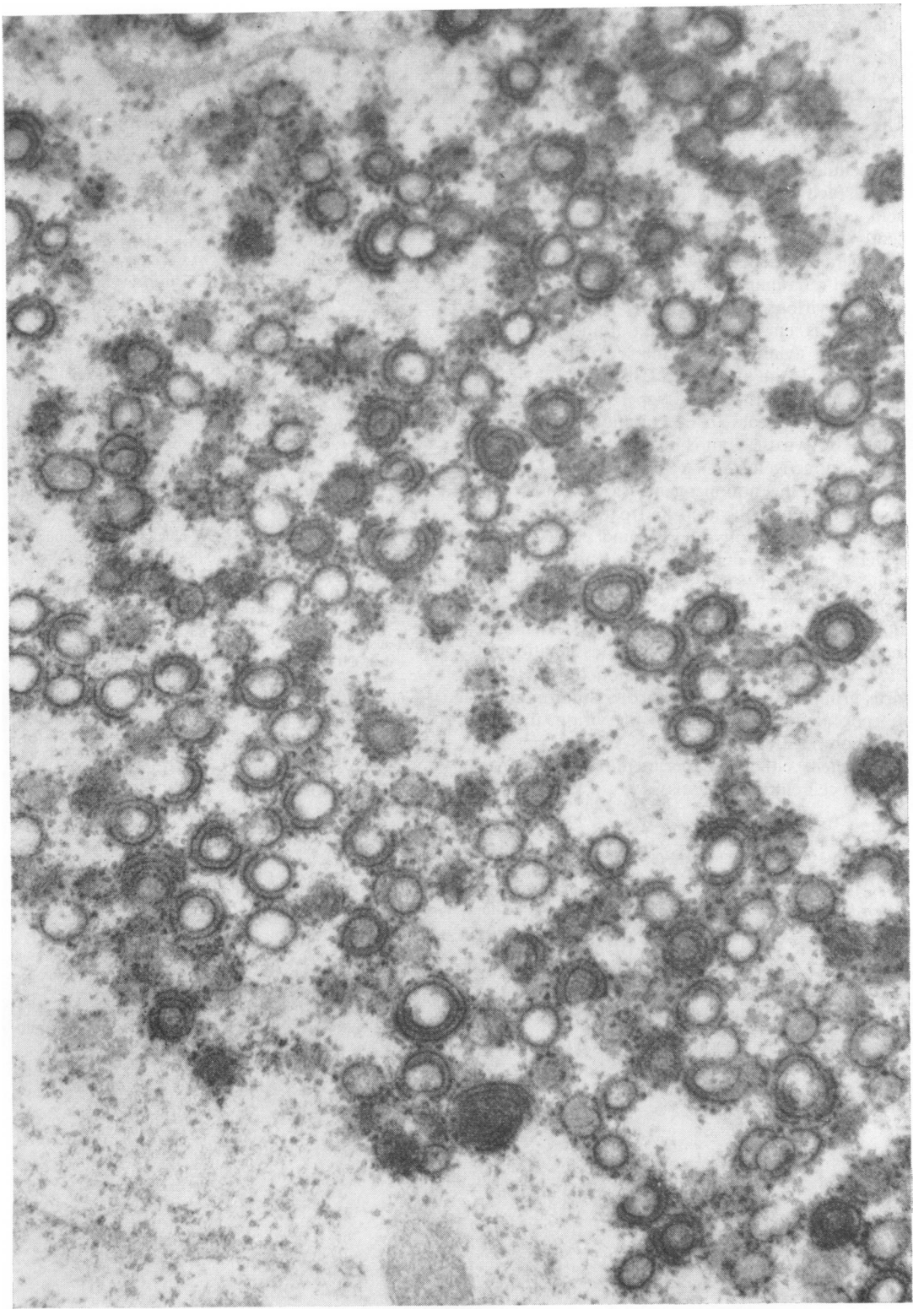

FIG. 6. *Cytoplasm of a progressive pneumonia virus-infected cell showing numerous multilaminated structures with clusters of ribosomes adjacent to them.* × *68,000.*

visna and maedi viruses and the oncogenic RNA viruses have been outlined by Thormar (18). These include size and structure of the mature particles, site of virus maturation, requirement for cellular deoxyribonucleic acid (DNA) synthesis (actinomycin D sensitivity), and nucleic acid composition. A further similarity has recently been reported: an RNA-dependent DNA polymerase has been found within visna virions (7, 14). Preliminary evidence indicates its presence in PPV virions also (Stone et al., *unpublished data*). This enzyme has been found in RNA-containing avian and murine leukemia-sarcoma viruses (2, 15) as well as in C-type RNA viruses of the cat, hamster, and viper (5). Thus, the polymerase appears to be common to all oncogenic RNA viruses, but is not generally associated with nononcogenic RNA viruses. Therefore, its presence in the nononcogenic slow viruses suggests a role in replication and pathogenesis.

The mature virions of PPV and visna contain a dense internal nucleoid, frequently eccentrically placed, and "spikes" are present on the external surfaces (19). These properties suggest close similarities with the virion of the mouse mammary tumor virus (1, 8). Classification of PPV, visna, and maedi with the oncogenic RNA virus group will depend on further studies on the morphology and structure of the virion, the size and composition of the RNA, and possible oncogenicity either in experimental animals or in in vitro cell culture systems.

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