

Ultrastructural Comparison of a Virus from a Rhesus-Monkey Mammary Carcinoma with Four Oncogenic RNA Viruses

(primate cancer/murine mammary tumor virus/murine leukemia virus/
L1210 leukemia-associated virus)

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ABSTRACT The ultrastructure and morphogenesis of Mason-Pfizer monkey virus, isolated from a mammary carcinoma in a Rhesus monkey, was compared with those of murine mammary tumor virus, murine leukemia virus, L1210 leukemia-associated virus, and avian myeloblastosis virus. The simian virus resembled murine mammary tumor virus and the L1210 virus in that it produced intracytoplasmic particles that were enveloped during budding. It resembled L1210 virus and murine leukemia virus in budding with smooth envelopes. It differed from all the others in being more fragile. These similarities, combined with biochemical characteristics reported elsewhere in this issue, suggest that the monkey virus is an oncogenic RNA virus.

A mammary carcinoma that arose spontaneously in a Rhesus monkey was found (1) to contain numerous virions with morphological characteristics of both the virions of murine leukemia (MuLV) and those of murine mammary tumor (MuMTV). This virus, designated (2) as the Mason-Pfizer monkey virus (M-PMV), was successfully transmitted to a number of monkey cell cultures as well as to early passaged human embryonic cell cultures and an established human cell line of lymphocytic origin (3, 4). In the present study, the M-PMV is compared with other oncogenic RNA viruses with respect to ultrastructure and cell-virus relationship.

MATERIALS AND METHODS

Cells

A mixed monkey embryo-monkey mammary tumor cell culture producing M-PMV was supplied to us by Robert Nowinski of the Sloan-Kettering Institute, New York, N.Y. after 10 subcultures. These cells were grown in monolayer cultures in RPMI medium 1640 with 20% newborn calf serum. NC-37, a cell line established at the John L. Smith Memorial Laboratories, Maywood, N.J., from a human lymphocyte culture and infected with M-PMV as an established cell line were grown as suspension in Hanks'-Eagle's minimal essential medium with 10% newborn calf serum.

Preparation for electron microscopy

Virus pellets were prepared for electron microscopy from tissue culture fluids by the procedure of Nowinski *et al.* (5). Some of the virus pellets were resuspended in a few drops of phosphate-buffered saline and negatively stained by the

method of Brenner and Horne (6). Other pellets were first fixed for 60 min in a solution of 2.5% glutaraldehyde, brought to pH 7.4 with phosphate buffer, washed for 18-24 hr in phosphate-buffered saline, fixed again for 60 min in Dalton's chrome-osmium (7) and postfixed in a 0.5% aqueous solution of uranyl acetate. The pellets were then dehydrated and embedded according to the method of Luft (8). The blocks were sectioned, stained with aqueous uranyl acetate, and counterstained with lead citrate. The cultured cells were fixed, dehydrated, embedded, sectioned, and stained by the method described for virus pellets. Other cell cultures were prepared for whole-cell-mount electron microscopy by the method of Kramarsky *et al.* (9).

RESULTS

Examination of virus pellets

Electron microscopic examination of thin sections of virus pellets revealed certain similarities between M-PMV and MuLV. The diameter of both virions is approximately 120 nm. Their envelopes are not coated with spikes. Their cores are usually nearly centric, but in the case of M-PMV, since the core boundaries were often poorly defined in these preparations, core localization could not be definitely established from thin sections of virus pellets. Occasionally, when nucleoprotein strands were oriented parallel with the plane of section, they could be resolved (Fig. 1).

M-PMV also resembled MuLV in negatively-stained preparations. Intact virions were tadpole-shaped; damaged ones were round. The envelopes were free of surface spikes. About one M-PMV particle in twenty had knobby protrusions on its envelope. These knobs were about 5 nm long and about 5 nm in diameter, and had a center-to-center spacing of about 10 nm. Similar knobs have been observed at about the same frequency in negatively-stained preparations of avian myeloblastosis virus (AMV) (10). The tails of the M-PMV often tended to be somewhat less straight and stubby than those of MuLV. They also often formed a bleb along their sides, which is seen rarely with MuLV but occasionally with MuMTV. In virions penetrated by the phosphotungstate, the helical nature of the nucleoprotein strand was frequently resolved. These strands have a diameter of 3 nm (Fig. 2).

Examination of infected cells

In the cytoplasm of infected cells, doughnut-shaped particles were often seen, either singly or in clusters. The clusters were usually found near the Golgi apparatus, while single particles

Abbreviations: M-PMV, Mason-Pfizer monkey virus; MuLV, murine leukemia virus; MuMTV, murine mammary tumor virus.

FIG. 1 (a-c, top). Thin-sectioned preparation of purified virions. (a) M-PMV $\times 67,500$ (b) high magnification of a M-PMV virion showing nucleoprotein strands in core (arrow) $\times 169,200$; (c) MuMTV $\times 90,000$.

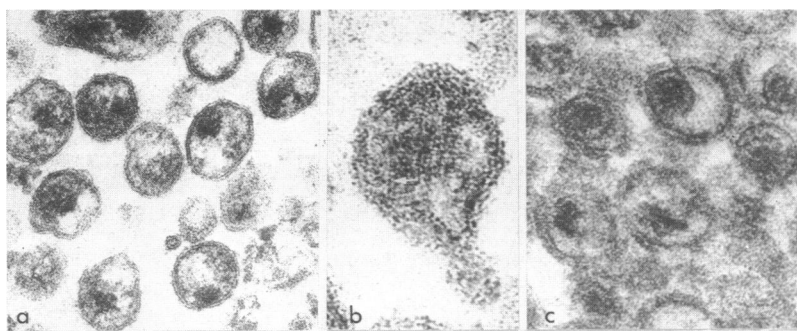


FIG. 2 (a-d, below). Negatively-stained preparations of purified virions. (a) M-PMV, intact virions (single arrows) and virions penetrated by phosphotungstate (double arrows) $\times 75,000$; (b) M-PMV, high magnification of a virion showing the surface knobs (arrows) $\times 225,000$; (c) M-PMV, high magnification of a virion penetrated by phosphotungstate, showing nucleoprotein strands (arrow) $\times 112,500$; (d) MuLV $\times 101,250$.

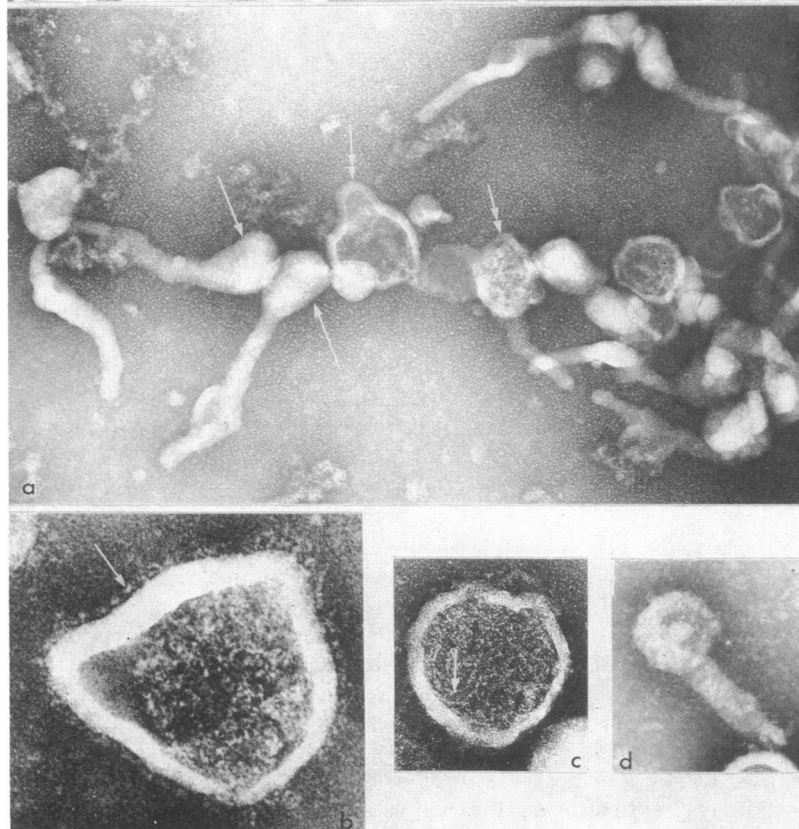


TABLE 1. Comparison of M-PMV with four oncogenic RNA viruses

Characteristics	M-PMV	MuMTV	L1210 leukemia-associated virus	MuLV	AMV
Presence of intracytoplasmic A particles in infected cells	+	+	+	-	-
Virions usually bud with preassembled cores	+	+	+	-	-
Virion envelope has "spikes"	-	+	-	-	-
"Knobs" on virion envelope	+	-	-	-	+
Diameter of mature virion in thin sections (nm)	125	130	110	110	110
Diameter of intracytoplasmic A particle					
(a) in thin sections (nm)	90	75	75	-	-
(b) in whole cell mounts (nm)	140	75	75	-	-
Position of core in mature virions	centric	eccentric	centric	centric	centric
Presence of core "membrane"	+	+	-	-	+
Budding process is rapid	+	-	-	+	+
Infects tissue-culture cells	+	-	-	+	+
Crosses species barrier	+	-	-	+	+
Virions fragile (see Discussion)	+	-	-	-	-

* Observed in about 5% of the virions.

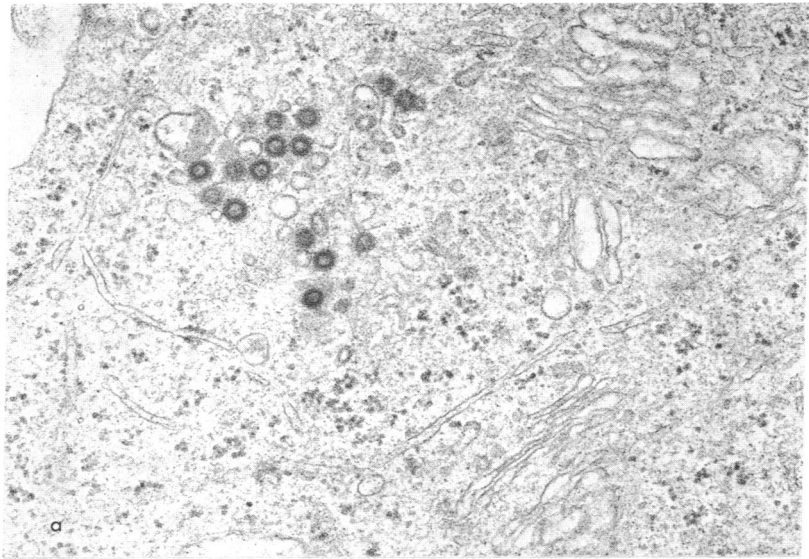


FIG. 3. Thin sections of virus-infected cells. (a) Low magnification of the Golgi region of an NC-37 cell infected with M-PMV, showing a cluster of intracytoplasmic particles $\times 32,500$. (b) Higher magnification of the above, showing the close association of Golgi vesicles with the particles (arrows) $\times 97,500$. (c) A cluster of intracytoplasmic A particles in a cell of a murine mammary tumor $\times 87,750$.

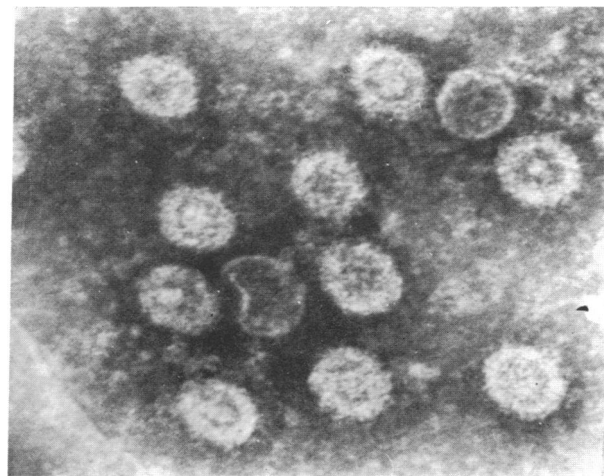
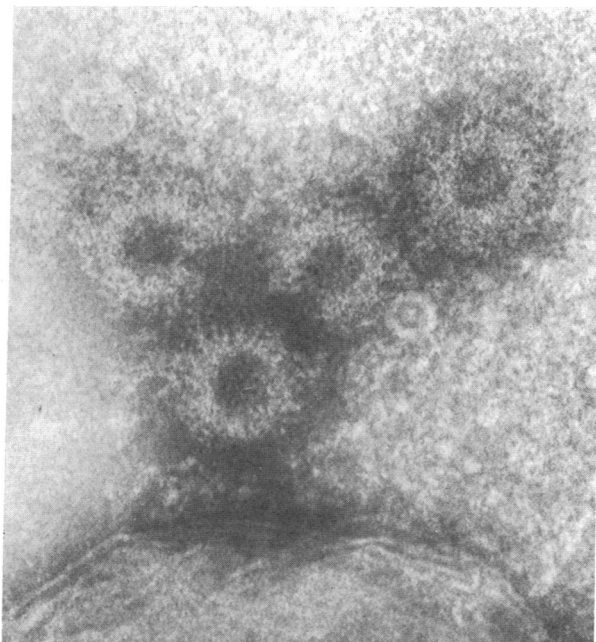
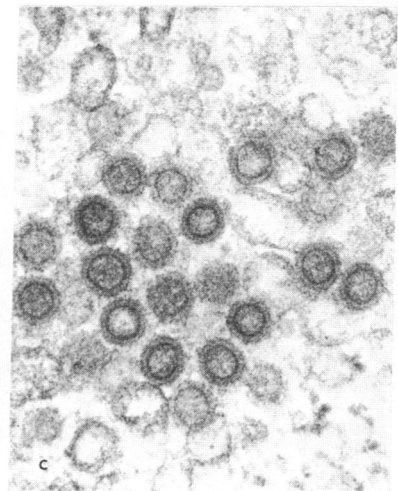
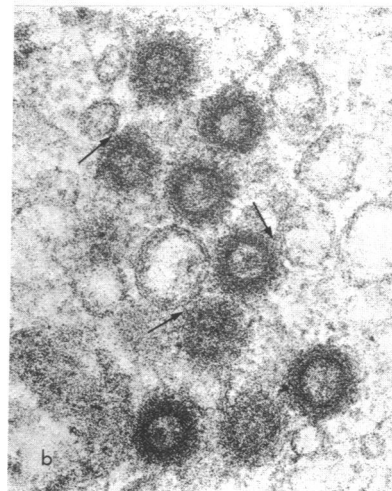


FIG. 4. Negatively stained whole-cell mounts of infected cells, showing intracytoplasmic particles. (left) M-PMV, $\times 125,000$; (right) MuMTV, $\times 120,000$.

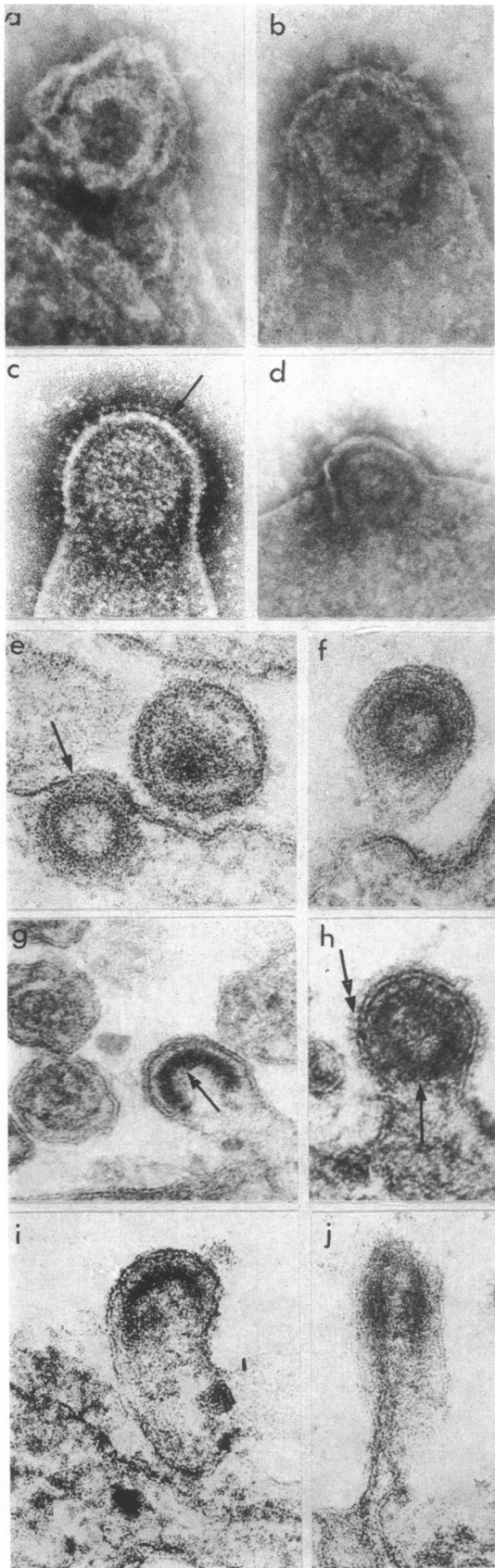


FIG. 5. Budding tumor virions: *a-d*, negatively-stained whole-cell mounts; *e-j*, thin sections. (*a*) and (*b*), NC-37 cells infected with M-PMV, $\times 125,000$. (*c*) MuMTV, showing the surface spikes (*arrow*), $\times 140,000$. (*d*) L1210 leukemia, $\times 120,000$.

were found throughout the cytoplasm. These particles were morphologically similar to the intracytoplasmic A particles found in MuMTV-infected cells, in Gardner's testicular tumor (11), and in L1210 leukemia cells (12). The intracytoplasmic particles of M-PMV-infected cells had an outer diameter of 90 nm in thin sections (Fig. 3*a*) and of 140 nm in negatively-stained whole-cell mounts (Fig. 4*a*). Type A particles in MuMTV-infected cells have a diameter of 75 nm in thin sections (Fig. 3*b*) and 75 nm in whole-cell mounts (Fig. 4*b*). The intracytoplasmic particles were usually closely associated with Golgi vesicles. The detailed fine structure of the simian intracytoplasmic particles showed some subtle difference from that of the murine A particle. The supercoiled helix that made up the annular structure of the particle was less tightly wound than that of the murine particle. In whole-cell mounts that were subjected to hypotonic conditions during preparation, an additional uncoiling was observed, resulting in a 50% increase in the diameter of the intracytoplasmic particle over that observed in thin sections. In MuMTV, the particles had the same diameter in both preparations.

Intracytoplasmic particles in contact with a cell membrane became enveloped by this membrane as they were extruded from the cytoplasm into extracellular space. Budding virions of M-PMV resembled MuMTV in having already assembled cores rather than cores that are assembled during the budding process, as is characteristic of MuLV. Budding M-PMV differed from budding MuMTV and resembled budding MuLV in that their envelopes were not covered by surface spikes. These two features are identical to those of virions associated with the murine L1210 leukemia (Chopra *et al.* (12)] (Fig. 5). Unusual forms of budding virions were observed in M-PMV-infected monkey embryo cells. These had either tubular cores of various lengths or crescent-shaped cores of greater than normal width (Fig. 5). Similar structures have been observed in cells infected with MuMTV (13) and MuLV (unpublished observation). These may be noninfectious particles and resemble the polyhead structures of defective phages. The cores of budding virions had the same dimensions as the intracytoplasmic particles, while the condensed cores of mature virions varied greatly in shape and size. Budding virions of M-PMV and MuMTV had an overall diameter of about 120 and 100 nm in thin sections and 190 and 120 nm, respectively, in whole-cell mounts. When the M-PMV virion separated from the cell surface, the viral core became condensed and the envelope distended, forming the mature virion. The diameters of mature virions of M-PMV and MuMTV were approximately 125 and 130 nm, respectively, in thin sections. Mature virions observed in sections of M-PMV-infected NC-37 cells had cores which varied greatly in morphology. The cores were more or less centric but often they appeared tubular or triangular (Fig. 6).

In preparations of NC-37 cells infected with M-PMV, both intracytoplasmic and mature extracytoplasmic particles were observed about ten times as frequently as budding virions.

(*e*) and (*f*), NC-37 cells infected with M-PMV ($\times 150,000$ and $\times 175,000$, respectively). Incipient budding is shown in *e* (*arrow*), while budding is nearly complete in *f*. Note the completed cores. (*g*) MuLV. Note the incomplete crescent-shaped core (*arrow*), $\times 120,000$. (*h*) MuMTV. Note the complete core (*single arrow*) and the surface spikes (*double arrow*), $\times 130,000$. (*i*) and (*j*), Monkey embryo cells infected with M-PMV, showing unusual forms of budding; $\times 150,000$.

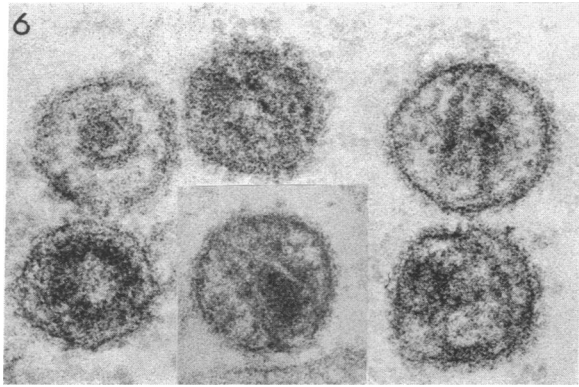


Fig. 6. Mature virions in the intercellular space between NC-37 cells. Note the tubular cores seen both in cross section at the upper left and longitudinal section at the upper right. A triangular core is seen at the lower center. $\times 150,000$.

This was usually not the case with MuMTV and suggests that the budding process was more rapid in the case of M-PMV than in that of MuMTV. MuLV also buds rapidly, whereas L1210 virus buds slowly.

In whole-cell mounts of NC-37 cells infected with M-PMV, budding virions occasionally burst open, releasing their cores (Fig. 7). This was not observed in MuMTV- or MuLV-infected cells or in L1210 cells. Table 1 summarizes the comparison of morphological and morphogenetic features of M-PMV with those of other oncogenic RNA viruses.

DISCUSSION

M-PMV was found to be morphologically and morphogenetically similar to MuMTV in some respects and to MuLV in several others. M-PMV therefore resembles the L1210 leukemia-associated virus, a virus produced by a murine tumor which contains antigens of both MuLV and MuMTV (14). One characteristic peculiar to M-PMV was the fragility of the virions, which manifested itself in the following ways: (a) The envelope occasionally burst open in the preparation of whole-cell mounts, releasing the core, a phenomenon not observed in any of the murine viruses. (b) Purified virus prepared for thin sections had poorly-defined cores, as if the core had been damaged during purification. (c) Prolonged centrifugation in a sucrose density gradient resulted in the formation of a band at a density of 1.23 g/ml, in addition to the virion band, which banded at 1.16 g/ml (15). This fragility is probably due to an inherent tendency of the supercoiled nucleocapsid to unwind, exerting a pressure upon the virion envelope.

The simian virions contain an RNA-instructed DNA polymerase (15) which further indicates their relationship to the oncogenic RNA viruses (16). This virus is of special importance because it is the first RNA virus found associated with a spontaneous tumor in a primate.

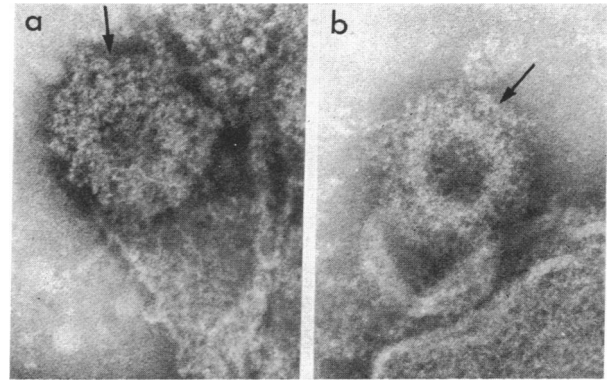


Fig. 7. Negatively stained whole-cell mounts of NC-37 cells infected with M-PMV. Two budding virions are bursting open, releasing their cores (arrows). $\times 175,000$.

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