A COMPARATIVE ELECTRON MICROSCOPIC STUDY ON
THE MORPHOGENESIS OF CANINE DISTEMPER
AND RINDERPEST VIRUSES

Masanori Tajima, Tsunemasa Motohashi, Shigeru Kishi
and Junji Nakamura

Nippon Institute for Biological Science, Tachikawa, Tokyo

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The morphology of canine distemper and rinderpest viruses has been studied with
the electron microscope by negative staining\textsuperscript{7,18,19}. Virions of both viruses demonstrated
in these studies were structurally similar to each other and resembled those of measles
virus as well as other paramyxoviruses. Although they varied both in shape and size,
most of them were circular or oval and measured between 110 and 300 nm in diameter.
They had three distinct morphological components, an internal helical component, an
outer membrane, and a layer of surface projections. On the other hand, ultrastructural
knowledge on the intracellular development of these viruses is still lacking. \textit{Tajima et al.} \textsuperscript{24} described that the cytoplasmic inclusions associated with rinderpest virus-infection
were composed of numerous filaments with a helical structure similar to the internal
component of rinderpest virion. In preparations examined by them, however, no par-
ticles resembling mature virus were identified, so that the sequence of events in the
development of enveloped viral particles remained to be determined.

In the present study, the morphogenesis of canine distemper and rinderpest viruses
grown in cultured cells was examined in thin sections with the electron microscope. The
negative staining technique was also applied to cells infected with both viruses so that
the interpretation of findings obtained by thin-sectioning might be facilitated. The
results of the study are described as follows.

MATERIALS AND METHODS

Viruses and cells: Avianized strain of Nakamura III lapinized rinderpest virus (LA
strain) at its 393 to 465 passage level in chick embryo, was used in the present work.
Infected chick embryo spleens collected 5 days after infection were used for inoculation
of cell cultures.

Hk strain of canine distemper virus, isolated at the authors’ institute from a milk,
passed 4 times in dog kidney cell cultures and 21 to 24 more times in chick embryo cell
cultures, was employed for inoculation of cell cultures.

Chick embryo cell cultures were made with trypsinized cells obtained from 11-day-old
embryos. The cells were grown in Hanks’ balanced salt solution containing 0.1%, yeast
extract, 0.5% lactalbumin hydrolysate, and 2% calf serum. Antibiotics were added giving
a final concentration of 200 units of penicillin G potassium and 20 ng of dihydro-
streptomycin sulfate per ml of medium. For maintenance, the serum was omitted from
the medium. An established line of African green monkey kidney (Vero) cells\textsuperscript{29} was
grown and maintained in modified Earle’s balanced salt solution containing 0.5%
lactalbumin hydrolysate and 1% calf serum with the antibiotics of the same concentration as used for chick embryo cells.

Tube cultures were inoculated with 0.1 ml of rinderpest virus at input multiplicity of about 0.1 TCID₅₀ per cell. After being adsorbed for 1 hour at 37°C, the cultures were fed with 0.9 ml of medium and rolled at 37°C.

In experiments with canine distemper virus, the medium in each culture tube with developing monolayer cell sheet was renewed with 1.0 ml of fresh medium in which 1 to 10 TCID₅₀ of virus per cell had been suspended.

Electron microscopy: Samples of cells were harvested 2, 3, and 4 days after inoculation with either canine distemper virus or rinderpest virus. Cultured cell monolayers were washed with Hanks' solution, scraped and centrifuged at a low speed for 5 minutes. The resultant pellet was fixed in glutaraldehyde, postfixed in buffered osmium tetroxide, dehydrated in an ethanol series and embedded in epoxy resin 812. Sections were cut with glass knives and stained with uranyl acetate followed by lead citrate. All sections were examined with a JEM-6S electron microscope.

The procedure for negative staining used in the present study has been previously described²⁴. This was applied to cultured cells harvested 3 and 4 days after virus-inoculation.

Light microscopy: Cultured cells infected with both viruses were also examined with the light microscope so that the interpretation of electron micrographs might be facilitated. For this purpose, a coverslip, 6 by 18 mm, was placed in a tube before seeding with the cells. At appropriate intervals after infection, the coverslips were removed, placed in Bouin's solution to fix the adhering cells and then stained with hematoxylin and eosin.

Uninoculated cultures were similarly harvested and processed for both light and electron microscopy, and used as controls for possible nonspecific cellular changes.

RESULTS

Canine distemper virus in chick embryo cells and Vero cells

The results obtained from experiments with the two cell systems used, chick embryo cells and Vero cells, were essentially the same with the exception that in the latter, the progress of a cytopathic effect appeared to be more rapid than that in the former.

In stained preparations, the cytopathic effect was characterized by the syncytium formation, the appearance of eosinophilic, cytoplasmic and intranuclear inclusions. Cells containing droplet-like, cytoplasmic inclusions stained homogeneously with eosin and small syncytia with 2 to 4 nuclei began to be observed in scattered foci of the cell sheets harvested 24 hours after inoculation. The intranuclear inclusions were detectable 48 hours or more after infection, mostly in cells with the cytoplasmic inclusions. With the lapse of time, both the cytoplasmic and intranuclear inclusions increased gradually in size and number, and became irregular in shape (Fig. 1). More than 50 per cent of the cells in the cell sheets harvested on the 3rd day contained cytoplasmic inclusions. The number of syncytia and nuclei contained in a syncytium also increased with the advancement of infection. During the earlier stages of infection, the cytoplasm of cells containing the inclusions appeared to be extended and thin, but the cells were soon vacuolated, showing a retraction of both the cytoplasm and the nucleus, with increasing basophilia. They remained attached to their neighbors with thin cytoplasmic processes. The cells were eventually detached from the glass surface, gaps being left in cell sheets.

Viral particles identified in thin sections with the electron microscope were similar,
in size and structure, they are pleomorphic, most of them being circular or oval. They varied in size from 160 to 900 m\(\mu\) at their maximum diameters, including surface projections, although the majority had diameters ranging from 200 to 500 m\(\mu\). The particles were bounded by an enveloping membrane, on the outside of which was a layer of surface projections approximately 16 m\(\mu\) in length. Various amounts of internal tubular strands or nucleocapsids were enclosed by the viral envelope (Figs. 3, 9, 10, and 11). The tubular strands had a uniform average width of 16 m\(\mu\); when cut in cross section, they appeared as electron-dense circular ring with an inner diameter of about 6 m\(\mu\). Most viral particles were loosely packed with the tubules arranged either in random fashion or concentric; some of them were filled with electron-dense fine granular and fibrillar material, and the tubules were hardly discernible; still some others appeared to be empty. It was worthy of note, however, that in almost all these particles, a single layer of the tubular strands was seen immediately beneath the envelope. No filamentous forms were seen in the present cell systems.

Distinctive features, morphologically related to the replication of canine distemper virus in infected cells, were an accumulation of tubular strands in the cytoplasm and the nucleus, and the presence of viral buds at various stages of development at the cell surface. The tubular strands seen in the cytoplasm were morphologically identical to the nucleocapsids observed within viral particles. Since the cytoplasmic tubules were usually associated with electron-dense material, their fine structure was obscured (Fig. 7). When sectioned longitudinally, striations with a periodicity of about 5.5 m\(\mu\) were sometimes resolved along their long axis, suggesting the turns of the helix (Fig. 4). The cytoplasmic tubules frequently formed a large aggregate in which they oriented either randomly or parallel (Fig. 4). Ribosomes and other cytoplasmic organelles were displaced to the periphery of the aggregates. These large aggregates of tubules appeared to correspond, in size, shape, and distribution, to the cytoplasmic inclusions seen in stained preparations.

When infected cells were examined by the negative staining technique, nucleocapsid strands were easily demonstrated (Figs. 5 and 6). The nucleocapsids resembled a herring bone pattern (Fig. 6) characteristic of internal nucleoprotein component of distemper virions as has been described by previous workers\(^7\).\(^{18}\). They had almost the same dimensions as the tubular strands observed in thin sections of infected cells; their outer and inner diameters measured approximately 19 m\(\mu\) and 5 m\(\mu\), respectively, and the periodicity of regular serrations along their edges averaged approximately 6 m\(\mu\). A systematic determination of nucleocapsid length was not carried out in the present work but isolated nucleocapsids having a length of more than 2 m\(\mu\) were not infrequently encountered. An example measured over 4 m\(\mu\) is given in Fig. 5.

As has been described with other paramyxoviruses\(^2\),\(^3\),\(^6\),\(^9\),\(^11\),\(^15\),\(^16\),\(^20\)\(^-\)\(^22\), maturation of canine distemper virus appeared to occur by a process of budding at the cell surface. The tubular strands formed in the cytoplasm were arranged in a single row, at a regular distance, beneath and along some portions of the cell membrane (Fig. 7). Such a peripheral alignment of the tubules could clearly be seen when cells were cut tangentially to the surface or cytoplasmic extensions sectioned obliquely (Figs. 7 and 8). In these portions, the cell membrane increased in electron-density and was covered by a layer of surface projections approximately 16 m\(\mu\) in length (Figs. 7 and 9). Thus, the outer zone of the cells consisting of a newly formed layer of the surface projections, the plasma membrane, and a single layer of tubules became morphologically indistinguishable from the peripheral zone of viral particles (Fig. 9). At the next stage, the outfolding of the modified cell membrane occurred, and finally, viral buds detached from the cell surface.
(Figs. 10 and 11). On rare occasions, viral particles appeared to originate as a bud on the tip of another bud (Fig. 11). During the budding process, some of the tubules lying free in the cytoplasmic matrix also appeared to be incorporated into the viral particles. This was confirmed by the observation that the continuity between the free cytoplasmic tubules and the tubules within viral bud was repeatedly seen (Figs. 10 and 11).

Intranuclear accumulation of tubular strands was detected in all samples taken from 2 to 4 days after virus inoculation, but large aggregates of tubules apparently corresponding to the intranuclear inclusions seen with the light microscope were most frequently encountered in the 4-day sarle (Fig. 12). Intranuclear tubules were morphologically similar to the nucleocapsids contained in the cytoplasm and viral particles, having outer and inner diameters of approximately 17 m and 7 m, respectively. In them, striations with a periodicity of about 6 m were clearly visible, because unlike cytoplasmic tubules, no electron-dense material was present around the intranuclear tubules (Fig. 13). The intranuclear tubules were observed to be disposed at random in clear areas within the nucleus. No crystalline arrangement of the tubules was detected in the present cell systems. In severely degenerated cells at the advanced stages of infection, some intranuclear tubules occasionally appeared to be in the process of escaping from the nucleus into the cytoplasm through a break of the nuclear membrane (Fig. 12).

Rinderpest virus in chick embryo cells and Vero cells

Eosinophilic, cytoplasmic inclusions and small syncytia first clearly appeared at the same time, and thereafter, progressed in the same manner as those described above for cells infected with canine distemper virus. Fig. 2 shows multinucleated giant cells in the culture of Vero cells infected 3 days previously. Numerous cytoplasmic and intranuclear inclusions of irregular shape can be seen in the cells.

The fine structure of the cytoplasmic inclusions in various cell types infected with rinderpest virus has been described in detail[24], and is illustrated in Fig. 14.

Large intranuclear aggregates of tubular strands arranged in a haphazard fashion which appeared to correspond to the intranuclear inclusions seen with the light microscope were frequently observed in cells sampled at later stages of infection (Fig. 15).

Viral particles showed considerable pleomorphism, although most of them were roughly circular in outline with the greatest diameters ranging from 250 to 500 m, and indistinguishable from distemper virions. As was described above for distemper virions, three morphological components—internal nucleocapsids, an envelope, and surface projections—were recognized in rinderpest virions (Figs. 16 and 17). The nucleocapsid strands contained in the cytoplasm, nucleus and virion were identical in diameter and in the periodicity between the striations.

As was found and described above with distemper virus, viral nucleocapsids formed in the cytoplasmic matrix were incorporated into virions by a budding process (Figs. 16 and 17).

DISCUSSION

The present comparative study of canine distemper and rinderpest viruses has indicated that the findings with both viruses are strikingly similar in many respects: with regard to the sequential development of cytopathic effects, ultrastructure of virions, and mode of virus replication in infected cells. These findings are also in agreement with those described by previous workers using measles virus[15,16,21]. On the basis of their immunological relationship, biological and physical similarities[8,12,23,28], viruses of
measles, canine distemper, and rinderpest have been placed in a subgroup of the para-
myxoviruses (14). This classification is further justified by the present results.

As has been reported with measles virus15,16,21 and other paramyxoviruses2,3,6,9,11,20,22
particles of canine distemper and rinderpest viruses were observed to form by a process of
budding from the cell membrane. Tubular strands identical in dimensions and structure
to the internal nucleoprotein component or nucleocapsid of canine distemper7,18 and
rinderpest virions19 first appeared in the cytoplasm. They became aligned immediately
beneath some portions of the cell membrane. In these portions, the cell membrane
increased in electron density and was covered with an outer layer of surface projections.
The tubular strands associated with the differentiated portion of the cell membrane and
some other strands free in the cytoplasmic matrix were incorporated into a virion by a
budding process. The latter appeared to form nucleocapsids scattered and oriented
randomly within the virion.

There have been some controversial opinions as to whether tubular strands are first
aligned under the cell membrane or surface projections are first formed on the outer
surface of the membrane. Colobert and Berkaloff3, in examining KB cells infected
with para-influenza virus 1, described that the cell membrane transforms into the viral
envelope only in the presence of viral nucleoprotein. Their opinion was supported by
Duc-Nguyen and Rosenblum9 in a study of mumps virus grown in chick embryo fibro-
blasts, stating that the parallel arrangement of nucleoprotein strands at the cellular
periphery has a determining role in the formation of the envelope of infectious particles.
On the contrary, Howe et al.11, in their study with type 2 para-influenza virus propagated
in FL and HeLa cells, observed that subjacent cytoplasmic nucleocapsid was not invariably
present at the differentiated portion of the cell membrane. In this regard, no conclu-
sive evidence could be obtained in the present systems, since the nucleoprotein strands
lying beneath the cell membrane were always associated with the differentiated portions
of the membrane. It is noteworthy, however, that the presence of incomplete or defec-
tive particles in cells infected with measles virus and with type 2 para-influenza virus has
been suggested by several investigators11,16,21. Each of these particles had an envelop-
ing membrane bearing characteristic projections on the outer surface but lacked recog-
nizable nucleoprotein strands. It therefore seems likely that the morphological altera-
tion of the cell membrane and the peripheral alignment of nucleoprotein strands beneath
the membrane are independent processes.

Filamentous forms described by previous workers using the negative staining tech-
nique for canine distemper16 and rinderpest viruses19 were not observed in the present
work. Since cytoplasmic extensions packed with nucleoprotein strands were repeatedly
seen in the present systems, the detachment of such extensions from the cell might result
in the appearance of the filamentous forms previously described. It is also possible that
filamentous forms seen in negatively stained preparations could result from a distortion
of spherical forms during concentration and negative staining. In almost all particles of
canine distemper and rinderpest viruses examined in the present work, the internal
nucleocapsid could be recognized but the amount of nucleocapsid present apparently
varied with particles. Some particles which contained only a small amount of nucleocapsid
just beneath the envelope and which appeared to be an empty sack might correspond to
the doughnut-shaped particle described by Norrby et al.18) for canine distemper virus.

Only a limited amount of nucleoprotein strands formed in the cytoplasm was
incorporated into virions by a budding process. The greater part of them remained in
the cytoplasm as an aggregate which undoubtedly represents the cytoplasmic inclusion
seen by light microscopy. It was suggested by Compans et al.8, examining MK and
BHK21-F cells infected with the parainfluenza virus SV5, that if a balance exists between synthesis of nucleoprotein and its continuous release within virions, large aggregates of nucleoprotein do not accumulate in the cytoplasm. Accordingly, the cytoplasmic inclusions associated with infections of canine distemper and rinderpest viruses might represent a cytoplasmic accumulation of overproduced nucleoprotein strands.

Distribution of the length of nucleocapsids in negatively stained preparations has been investigated with other paramyxoviruses by several workers. The results obtained in these studies showed a single, sharp peak centered between 1.0 and 1.1 μ with the mean length of 1.06 μ for Newcastle disease virus, 0.9 and 1.1 μ with a mean of 1.02 μ for parainfluenza virus SV5, 1.4 and 1.6 μ with the mean value of 1.54 μ for measles virus, and a sharp peak at 1.0 μ for HVJ, a member of the parainfluenza 1 group. In the present work with canine distemper and rinderpest viruses, although a systematic determination of nucleocapsid length was not done, nucleocapsids with a length of over 2 μ were sometimes seen, and the maximum length rarely exceeded 4 μ. As suggested by previous authors, however, the possibility that these longer pieces of nucleocapsid might result from end-to-end aggregation of some nucleocapsids and stretching of the nucleocapsid helix, or both, cannot be excluded.

Intranuclear aggregates of nucleoprotein strands observed in cells infected with canine distemper and rinderpest viruses resembled closely those that have been described with measles virus. However, intranuclear strands arranged in paracrystalline arrays which were frequently found in measles virus-infected cells were not observed in the present cell systems. From present observations and those of previous workers, there is little doubt that these aggregates of nucleocapsids correspond to the intranuclear inclusions observed by light microscopy. Although the intranuclear nucleocapsids were morphologically identical to those within virions and in the cytoplasm, it is unlikely that the intranuclear nucleocapsid plays a significant role in the replication of canine distemper and rinderpest viruses. Because the liberation of intranuclear nucleocapsids into the cytoplasm occurred only rarely in severely degenerated cells at the later stages of infection, and most of them were retained within the intact nuclear membrane throughout the course of infection. This assumption is supported by a recent study with fluorescent antibody demonstrating that the antigen synthesis of rinderpest virus occurred initially and mainly in the cytoplasm, and that cytoplasmic fluorescence was more closely related to the rise in infective titer than intranuclear one. However, further work is necessary to determine the significance of intranuclear inclusions in the infectious process of canine distemper and rinderpest viruses.

SUMMARY

Replication of canine distemper and rinderpest viruses was morphologically studied in primary chick embryo cells and in a line of African green monkey kidney (Vero) cells. Both viruses closely resembled each other in all respects with regard to cytopathic effect seen in stained preparations, ultrastructure of virions and mode of viral particle formation as observed by electron microscopy.

In cells infected with either canine distemper virus or rinderpest virus, eosinophilic, cytoplasmic inclusions and syncytia were observed as the first manifestation of infection, and about 24 hours later, eosinophilic, intranuclear inclusions began to appear. Under the electron microscope, both the cytoplasmic and intranuclear inclusions were observed to be composed of numerous tubular strands identical to the internal nucleoprotein
component or nucleocapsid within virions. The incorporation of nucleoprotein strands formed in the cytoplasm into the viral envelope occurred by a process of budding at the cell surface in a similar manner to that described with other paramyxoviruses. Only a limited amount of nucleoprotein strands synthesized in the cytoplasm was utilized for assembling virions, and most of them accumulated in the cytoplasm as an aggregate, undoubtedly corresponding to the cytoplasmic inclusion seen by light microscopy. Filamentous budding particles frequently found in cells infected with WB virus, parainfluenza virus SV5, type 2 parainfluenza virus, and mumps virus were not seen in the present work. Virions of canine distemper and rinderpest were pleomorphic and various in size, but most of them were spherical in outline, with sizes ranging from 200 to 500 μm in canine distemper and from 250 to 500 μm in rinderpest. No evidence suggested that the nucleocapsids accumulated in the nucleus play some role in the replication of both viruses.

The results obtained with canine distemper and rinderpest viruses are quite consistent with those described for measles virus except that intranuclear nucleocapsids arranged in paracrystalline arrays have often been found in measles virus-infected cells whereas they were arranged only in a haphazard fashion in the present systems.

REFERENCES

ジステンバー及び牛痘ウイルスの増殖に関する比較電子顕微鏡的研究

田島正典・本橋常正・岸 茂・中村修治
日本生物科学研究所
（昭和45年4月28日受付）

ジステンバーおよび牛痘ウイルスの増殖を、初代培養鶏胚細胞および Vero 細胞において、形態学的に研究した。両種ウイルスは、染色標本において認められる細胞変性効果、電子顕微鏡的に見られるウイルス粒子の微細構造およびウイルス粒子形成様式のすべての点において、相互に良く類似していた。

両種ウイルスに感染した細胞において、好酸性細胞質核内体と合胞体が、感染の最初の表現として観察され、約24時間経過して、好酸性核内染色体が出現を始めた。電顕下で、細胞質および核染色体は、ウイルス粒子の内部成分である nucleocapsid と同一構造をもつ。無数の管状構造からなると見られた。細胞質で形成された nucleocapsid のウイルス粒子への取り込みは、細胞表面において発芽様式によって行なわれた。細胞質で合成された nucleocapsid の一部のみがウイルス粒子の組立てに利用され、大部分の nucleocapsid は光学顕微鏡で観察される細胞質核内体に対応する集団として、細胞質に集積した。フィラメント状の芽生芽粒は、本研究では見られなかった。両ウイルスの粒子は多角形を示し、大きさも様々であるが、大部分は球形を呈し直線は牛痘ウイルスでは 250〜500μμ、ジステンバーウイルスでは 200〜500μμ であった。核内に集積した nucleocapsid が、両種ウイルスの増殖において、何等かの役割を演じることを示唆する証拠は得られなかった。

ジステンバーおよび牛痘ウイルスの増殖に関する形態学的研究によって得られた成績は、麻疹ウイルスに関して得られた成績に極めて良く一致していた。ただし、麻疹ウイルス感染細胞では結晶状配列を示す核内 nucleocapsid が観察されているが、本研究ではそのような規則正しい配列は認められなかった。
EXPLANATION OF PLATES

PLATE I

Figures 1 and 2 are light micrographs taken from monolayer cultures grown on coverslips, fixed in Bouin's solution and stained with hematoxylin and eosin.

Fig. 1. Chick embryo cells 5 days after inoculation with canine distemper virus showing a portion of a large syncytium with large, irregularly shaped cytoplasmic (thick arrows) and intranuclear inclusions (thin arrows). ×400.

Fig. 2. Vero cells 3 days after inoculation with rinderpest virus. Syncytia with numerous cytoplasmic (thick arrows) and intranuclear inclusions (thin arrows) are shown. ×400.

Figures 3 through 15 are electron micrographs taken from chick embryo cells infected with canine distemper virus.

Fig. 3. Three days postinoculation. Extracellular viral particles seen in sections of infected cells. Three morphological components, an outer layer of surface projections, an envelope, and nucleocapsids (arrows) are evident in two particles. The others are filled with electron-dense, fine granular and fibrillar material, and the nucleocapsids are hardly discernible. ×100,000. Inset shows a large, extracellular particle in which nucleocapsids are arranged in concentric layers. This particle might not be identified as a virion with certainty, because of the absence of surface projections. ×60,000.

PLATE II

Fig. 4. Four days postinoculation. A well-defined, cytoplasmic aggregation of tubular strands, apparently corresponding to a cytoplasmic inclusion seen with the light microscope, is present in the upper center of the field. Part of the nucleus is seen on the lower border of the figure. ×13,000. Inset shows cytoplasmic tubular strands at higher magnification. Striations are clearly visible on the tubules suggesting the turns of the helix. ×160,000.

Fig. 5. Three days postinoculation. An electron micrograph taken from a negatively stained preparation. Part of a large aggregate of nucleoprotein strands which probably represents a cytoplasmic inclusion. ×24,000. Inset shows an isolated nucleocapsid measured over 4 μ in length. ×32,000.

PLATE III

Fig. 6. Three days postinoculation. High-power magnification of cytoplasmic nucleocapsids negatively stained. Each strand has a hollow central region and serrations with regular periodicity. ×200,000.

Fig. 7. Two days postinoculation. A portion of the cell surface apparently in the bulging process. Peripheral alignment of tubular strands beneath the cell membrane is evident. Some tubules have been cut transversely and the others obliquely. A layer of surface projections can be seen on the outer surface of the membrane. The rest of the cytoplasm is filled with scattered tubular strands. Arrows indicate an electron-dense material surrounding the tubules. ×100,000.

PLATE IV

Fig. 8. Three days postinoculation. Peripheral portion of a cell showing cytoplasmic extensions sectioned obliquely. The nucleocapsid strands lying beneath and along the plasma membrane are clearly visible. ×120,000.

Fig. 9. Two days postinoculation. A spherical extracellular viral particle containing a single layer of nucleocapsid beneath the envelope and scattered nucleocapsid in the central area is present in the upper left corner of the field. Portions of two cells occupy the
lower half and the upper right corner of the figure, respectively. The outer portion of the cytoplasm consisting of a newly formed layer of surface projections, the plasma membrane, and underlying nucleocapsid is indistinguishable from the peripheral zone of the viral particle, suggesting that some regions of the plasma membrane differentiate into viral envelope. ×100,000.

PLATE V

Fig. 10. Two days postinoculation. One particle in the process of budding from the cell surface is seen on the left. In this bud, a single layer of internal nucleocapsids associated with the viral envelope, and some other nucleocapsids scattered within the envelope and continuous with those in the cytoplasm (arrow) are visible. In two extracellular particles on the right, the nucleocapsids sectioned transversely or longitudinally are recognizable. ×120,000.

Fig. 11. Four days postinoculation. Part of a cell occupies the lower left corner of the figure, showing viral particles in process of budding from the cell surface. Two particles in the lower right corner are connected with each other by a narrow stalk, suggesting the presence of a dual budding process. In them, the continuity between the cytoplasmic nucleocapsid and the internal nucleocapsid within the bud is evident (arrows). ×60,000.

PLATE VI

Fig. 12. Four days postinoculation. Part of the nucleus showing a large aggregate of nucleocapsids which undoubtedly corresponds to the intranuclear inclusion seen by light microscopy. The cell is at an advanced stage of degeneration, and some nucleocapsids appear to be in the process of being released from the nucleus into the cytoplasm through a break of the nuclear membrane (arrows). ×26,000.

Fig. 13. A portion of Fig. 12 is shown at higher magnification. Periodic striations are visible along their long axis, suggesting the helical structure. The electron-dense rings are the nucleocapsids cut in cross section. ×120,000.

PLATE VII

Figures 14 through 17 are electron micrographs taken from Vero cells infected with rinderpest virus.

Fig. 14. Four days postinoculation. A large cytoplasmic aggregate of tubular strands cut longitudinally or transversely. Part of the nucleus is in the lower left corner of the figure. ×18,000.

Fig. 15. Four days postinoculation. A portion of the nucleus showing an intranuclear accumulation of tubular strands. ×48,000. Inset shows intranuclear tubular strands with regular striations at higher magnification. ×120,000.

PLATE VIII

Fig. 16. Four days postinoculation. In a part of the cytoplasm occupying the lower right corner of the figure, the plasma membrane bulges outward at several points to form buds. In the bulging portions, the membrane increases in electron density and is covered with a layer of surface projections. Tubular strands are visible in parallel with the bulging portion of the plasma membrane and lying free in the cytoplasmic matrix. Three pleomorphic viral particles, in which an envelope bearing surface projections and internal tubular strands are recognizable, are seen extracellularly. ×80,000.

Fig. 17. Four days postinoculation. Several extracellular viral particles and a budding viral particle are shown. It is difficult to discern nucleocapsids in them, because of the increased electron density of their contents. ×80,000. Inset shows a spherical extracellular particle and a budding particle. Nucleocapsids sectioned transversely and longitudinally can be seen in them. ×125,000.