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THE FINE STRUCTURE OF MAREK'S DISEASE VIRUS AND HERPESVIRUS OF TURKEY IN CELL CULTURE*¹

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Morphological changes of virus particles in cell cultures inoculated with various strains of Marek's disease virus (MDV) and herpesvirus of turkey (HVT) were studied by an electron microscope. Various types of immature particles were found in the infected cell nuclei, and the small nuclear particles and their crystalline structures were frequently found in the infected cells of HVT. The cross-shaped capsids were also frequently found in the infected cells of Type 2 plaque-producing agent (PPA) of MDV and HVT. Some of the immature particles were investigated in three-dimensional structures and their structural schemes were demonstrated. Enveloped particles of different sizes were found in the nuclear vesicles (ca. 140~170 m μ) and in the cytoplasm (ca. 190~230 m μ).

INTRODUCTION

Marek's disease (MD) of chickens is characterized by the proliferation of lymphoreticular cells in the neural, ocular and cutaneous tissues, and in the visceral organs^{12,13}. MDV can be propagated in chick kidney cell (CKC) cultures^{6,7}, in duck embryo fibroblast cultures^{24,37}, in quail fibroblast (QF) cultures³⁰, and in chicken embryo fibroblast cultures¹⁶. MD is considered to be caused by a highly cell-associated group B herpesvirus^{7,26}.

HVT was also isolated from apparently healthy turkeys^{15,41}. Many of the characteristics of HVT, including virological and serological properties, were similar to those of MDV. HVT was apathogenic for chickens.

MIKAMI & BANKOWSKI^{17~19} observed two distinct types of plaques on a serial passage of Cal-1 strain of MDV in CKC cultures. In 2 out of 5 trials, the smaller Type 1 plaque, normally observed on primary isolation of the agent from chickens infected with MD, was mainly replaced by the larger Type 2

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plaque by the 14th serial passage. The Type 2 plaques grew more rapidly and were characterized by pronounced cytoplasmic vacuolation of the affected cells, followed by lysis. Intranuclear inclusions were more frequent in cells of Type 2 plaques than in cells of Type 1 plaques. The Type 2 PPA was less virulent for chickens than was Type 1 PPA.

From the characteristics described above, it appears that the JM strain of MDV in low passages is considered to be similar to Type 1 PPA of the Cal-1 strain of MDV, and the FC 126 strain of HVT is similar to Type 2 PPA of the Cal-1 strain of MDV^{22,31,41}.

The present study was undertaken with the purpose of comparing morphological changes of various forms of virus particles in cell cultures infected with 4 different strains (Type 1 and Type 2 PPA of Cal-1 strain of MDV and JM strain of MDV, and FC 126 strain of HVT) of herpesvirus by an electron microscope. In particular, some types of immature particles were investigated in three-dimensional structures by the use of high angle tilting apparatus attached to an electron microscope.

MATERIALS AND METHODS

Viruses and cell cultures

Type 1 and Type 2 PPA derived from the Cal-1 strain of MDV were kindly supplied by Dr. BANKOWSKI (Univ. of California, Davis, Calif., U.S.A.). Type 1 PPA was obtained from infected CKC cultures of the 8th passages and Type 2 was the 28th passages of stock MDV. The characteristics of these agents *in vivo* and *in vitro* have been described elsewhere^{2,17,18}.

The JM strain of MDV and the FC 126 strain of HVT were obtained from Dr. SAZAWA (Nat. Vet. Assay Lab., Tokyo) and Dr. YUASA (Nat. Inst. of Anim. Hlth., Tokyo), respectively. These viruses were originally supplied by Dr. BURMESTER (Poultry Research Branch, U.S.D.A., East Lansing, Mich., U.S.A.). The JM strain of MDV, which was obtained from monolayer cultures of kidneys of inoculated birds, was used for CKC inoculation following passage 3 times in CKC cultures. The virus (3rd passage) used for QF inoculation was adapted in QF cultures as described by ONODA et al.³⁰ and additionally passed 4 times in QF cultures.

The FC 126 strain of HVT used for QF inoculation was passed 3 times in QF cultures. The virus was originally passed 11 times in duck embryo fibroblast cultures and 14 times in CKC cultures when they were obtained.

These 4 viruses containing the titer of 400 or more plaque forming units per 0.1 ml were inoculated in either the CKC or the QF culture, which were

grown in plastic petri dishes.

Cultures from the kidneys of 35 day old chickens (Line M) obtained from a resistance-inducing-factor-free and specific-pathogen-free flock (Nippon Inst. for Biol. Sci., Tokyo) were prepared by a method described previously¹⁷⁾. Cultures from decapitated 9 day old Japanese quail embryos obtained as hatching eggs from Dr. TAKAKU (Kanonji Inst. of Foundation for Microbial Dis. of Osaka Univ., Kanonji, Kagawa) were prepared as described by ONODA et al.³⁰⁾

Eagle's minimal essential medium (Nissui Seiyaku Co., Tokyo) supplemented with 10% tryptose phosphate broth, 0.15% sodium bicarbonate and 10% calf serum was used as the growth medium. The concentration of serum was reduced to 5% for the maintenance medium. To the medium was added 200 units of penicillin, 200 μ g of streptomycin and 2.5 μ g of fungizone per ml.

Preparation for electron microscopy

Monolayer cultures inoculated with either MDV or HVT showed maximum cytopathic effect from the 5th to the 11th day post inoculation. They were scraped from the petri dishes and centrifuged at 3,000 rpm for 10 minutes. The pellet was fixed for one hour with 6.25% glutaraldehyde in 0.1 M sodium cacodylate buffer and washed several times in the same buffer. The pellet was re-fixed for one hour in 1% osmium tetroxide in a 0.1 M phosphate buffer at pH 7.4, dehydrated through graded ethyl alcohol, and then embedded in Epon 812 (LUFT). Sections were cut with a Porter-Blum MT-1 ultramicrotome and stained with uranyl acetate and lead citrate. All preparations were examined with a JEM-7 type electron microscope.

Observations of three-dimensional structures

Thick sections (ca. 100~500 m μ) were examined with an HK-5 type high angle tilting apparatus attached to an HU-12 type electron microscope at 125 kV. These observations were made by the use of a point filament. Many photographs of the same sample were taken from different angles ($0^\circ \pm 40^\circ$). Then two of them were chosen and observed at the same time by a stereoscope.

Count of virus particles

In order to compare the distribution of the virus particles of 4 strains, electron micrographs of particles in the infected cells were photographed at random with the same enlargement ($\times 20,000$, ca. 3 μ^2) in different fields.

RESULTS

A Morphology in cell culture

The monolayer of CKC and QF cultures inoculated with either Type 1

PPA of Cal-1 strain or the JM strain of MDV showed the typical plaques at 4~5 days post inoculation. Plaque sizes varied between 0.3 and 0.8 mm in diameter. On the other hand, in the cultures (CKC & QF) inoculated with either Type 2 PPA of Cal-1 strain of MDV or the FC 126 strain of HVT, typical plaques were formed in 2~4 days post inoculation and their sizes varied between 1 and 2.5 mm in diameter.

By an inverted microscope, the rounded refractile cells could be seen to be concentrated along the periphery of the plaques. The affected monolayer cells stained with Giemsa showed polykaryocytosis, cytoplasmic vacuolation and intranuclear inclusion bodies. These changes in particular were more frequently observed in the monolayer cultures inoculated with either Type 2 PPA of Cal-1 strain of MDV or the FC 126 strain of HVT than in those inoculated with either Type 1 PPA of Cal-1 strain or the JM strain of MDV.

B Electron microscopy

a Nuclear changes and intranuclear forms of the virus

Infected CKC and QF cultures often had several nuclei (polykaryocytosis) and the nuclear contour was irregular (fig. 2). Most of the nuclei of the infected cells were pale and comparatively short of chromatin. Margination of nuclear chromatin was located at the marginal areas of the nuclear envelopes. One or more large nucleoli were present in the infected cells. Nuclear pockets or nuclear vesicles due to the cytoplasmic invagination of the nucleus were also observed. Some enveloped virus particles were present in these nuclear vesicles. Various types of immature particles were found in the nucleoplasm. Details are given below. These particles were usually found in the marginal areas of the nuclei.

Small nuclear particle (figs. 3, 12 & 13)

These small particles had a diameter of ca. 30 m μ , and were spherical (icosahedron) in shape. They were scattered in the nucleoplasm, and sometimes formed a crystalline structure.

Empty nucleocapsid (figs. 3 & 9)

These empty capsids measured ca. 90~100 m μ in diameter, were hexagonal in profile, and lacked a central core.

Cross-shaped capsid (figs. 3~5, 12 & 13)

The term "cross-shaped capsid" has already been used by NAZERIAN et al.²⁵⁾ These capsids contained several particles which had almost the same shape and size (ca. 30 m μ) as the small nuclear particles (figs. 3, 12 & 13). In thin sections,

the number of small particles found in these capsids varied between 1 and 5. Usually 4 particles could be seen within one capsid. As these particles were situated on the inner capsid wall, so the clear space between these particles delineated an electron lucent cross shape. The cross-shaped capsid appeared to obtain an envelope by budding through the inner nuclear membrane of the nuclear vesicle (fig. 5).

Nucleocapsid with horseshoe-shaped core (fig. 6)

These capsids contained a horseshoe-shaped core with one or two particles.

Nucleocapsid with peculiar-shaped core (figs. 7, 8, 19~23)

In these capsids, there were one or two parallel-arranged rods with some crossed structures with a low electron density. Three-dimensional structures of these above described cores will be shown later.

Double shell nucleocapsid (fig. 9)

These capsids contained an electron lucent inner core of ca. 50 m μ in diameter.

Nucleocapsid with dense core (fig. 9)

These capsids contained an electron dense core (i. e. central nucleoid) of ca. 50 m μ in diameter.

Incomplete enveloped particle (fig. 9)

These particles measured ca. 140~170 m μ in diameter and contained no materials or empty capsids.

Enveloped particle in the nuclear vesicle (figs. 5 & 9)

Envelopment appeared to occur by the immature particle budding out at a nuclear membrane and acquiring an additional envelope from this membrane as it passed through. This process was often found at the inner nuclear membrane, at membrane-bounded intranuclear vesicles, so that these enveloped particles were found only in the perinuclear space and within nuclear vesicles. These enveloped particles measured ca. 140~170 m μ in diameter and had nucleocapsids of ca. 90~100 m μ . The nucleocapsid had a dense core (i. e. a central nucleoid) of ca. 50 m μ . The central nucleoid was also diffuse and heavily stained.

b Changes in the cytoplasm and forms of the virus (fig. 2)

The cytoplasm of CKC and QF cells were well developed and had many cytoplasmic processes. There were many sizes of vacuoles in the smooth surfaced endoplasmic reticulum in the cytoplasm. These vacuoles phagocytized

various kinds of debris. A Golgi apparatus was well developed, but free ribosomes and rough-surfaced endoplasmic reticula were poorly developed. Many long and slender mitochondria were distributed in the cytoplasm. Aggregation of an electron dense matrix, i.e. cytoplasmic inclusion bodies, was found in the cytoplasm (fig. 10). The cytoplasmic inclusion bodies contained some small immature particles (ca. 80~90 m μ) and large enveloped particles (ca. 190~230 m μ) without peripheral spikes (fig. 10).

Enveloped particle in the cytoplasm (fig. 11)

These particles measured ca. 190~230 m μ in diameter and had a wide and electron-dense envelope, which was covered by a series of many peripheral spikes, and contained a nucleocapsid of 80~90 m μ with an electron lucent halo. These particles were found in the endoplasmic reticula and extracellular spaces.

C Three-dimensional structures of virus particles

Many photographs from different angles were taken of the same particle of cross-shaped capsid (figs. 12 & 13), nucleocapsid with horseshoe-shaped core (figs. 16~18) and nucleocapsid with peculiar-shaped core (figs. 19~21). From the results of the observation, the following changes were obtained: the cross-shaped capsid consequently contained six spherical (icosahedron) particles (fig. 14); the nucleocapsid with horseshoe-shaped core consisted of one doughnut-like ring which was placed between the two particles (fig. 15), the horseshoe-shaped core in thin section appeared to represent an image of tangential section of these structures (fig. 6); the nucleocapsid with a peculiar-shaped core had three-dimensional structures as shown in the scheme of figure 24. Namely, these cores consisted of two round plates which crossed each other. These plates had a doughnut-like, dense and tubular margin, and were lucent in the center; some of these particles occasionally looked like a ball with vertical stripes as a result of the widened dense margin of these plates (figs. 23 & 25).

D Incidence of various types of virus particles

Almost all of the types of particles described above were seen in each cell cultures. Incidence of these particles was illustrated in the table 1. The small nuclear particles and especially their crystalline structures were mostly found in the infected cells with HVT. The cross-shaped capsids were also demonstrated in almost all cell cultures, but more frequently in the cells inoculated with Type 2 PPA of Cal-1 strain of MDV (31.5%) and the FC 126 strain of HVT (44.1%). The nucleocapsid with horseshoe-shaped or peculiar-shaped cores could be found in only a small percentage of all the cell cultures. The enveloped particles in the nuclear vesicles were found more in the cells inoculated

with Type 1 PPA of Cal-1 (13.0%) or the JM strain (8.0%) of MDV and the FC 126 strain of HVT (8.7%) than in those inoculated with Type 2 PPA of Cal-1 strain of MDV (0.8%). The enveloped particles in the cytoplasm were found frequently in the cells inoculated with FC 126 strain of HVT (7.5%). However, they were also found in the cell cultures inoculated with Type 1 and Type 2 PPA of Cal-1 strain of MDV. Cytoplasmic inclusion bodies were found in almost all cell cultures, but more frequently in the cells inoculated with the FC 126 strain of HVT.

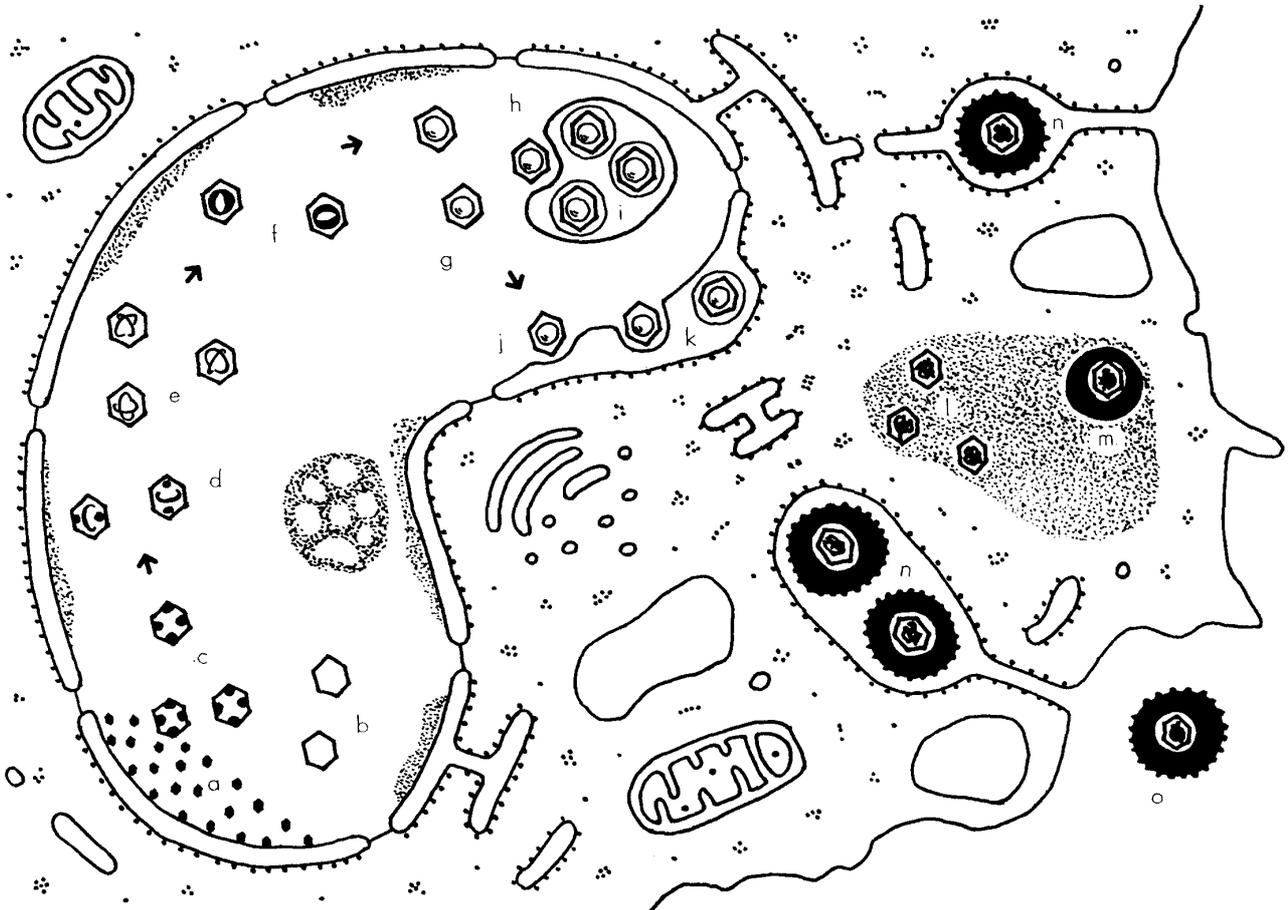
TABLE 1 *Incidence of various types of virus particles*

TYPES OF VIRUS PARTICLES		STRAIN OF VIRUS AND CELL CULTURE				
		Type 1 (Cal-1) CKC	Type 2 (Cal-1) CKC	JM CKC	JM QF	FC126 HVT QF
Small nuclear particle		+	+	+	+	##
Empty nucleocapsid		29 (9.0%)	38 (10.5%)	7 (16.7%)	46 (9.3%)	100 (20.7%)
Cross-shaped capsid		5 (1.6%)	114 (31.5%)	0	9 (1.8%)	213 (44.1%)
Capsid with horseshoe-shaped core		2 (0.6%)	23 (6.3%)	3 (7.1%)	11 (2.2%)	18 (3.7%)
Capsid with peculiar-shaped core		46 (14.3%)	32 (8.8%)	3 (7.1%)	45 (9.1%)	28 (5.8%)
Double shell nucleocapsid		83 (25.8%)	39 (10.8%)	14 (33.4%)	251 (50.5%)	17 (3.5%)
Nucleocapsid with dense core		106 (32.9%)	102 (28.2%)	15 (35.7%)	86 (17.3%)	28 (5.8%)
Incomplete enveloped particle		8 (2.5%)	5 (1.4%)	0	9 (1.8%)	1 (0.2%)
Enveloped particle in nuclear vesicle		42 (13.0%)	3 (0.8%)	0	40 (8.0%)	42 (8.7%)
Enveloped particle in cytoplasm		1 (0.3%)	6 (1.7%)	0	0	36 (7.5%)
Cytoplasmic inclusion body		+	+	-	+	##
Photographs examined		98	77	21	94	91

DISCUSSION

Small nuclear particles were observed in cells infected with various strains of MDV^{1,7,9,10,24,33)}, HVT^{15,25)} and other herpesviruses^{21,32,34,35,38)}. Crystalline structures of these small nuclear particles were also found in cells infected with MDV or HVT in the present study. These small nuclear particles were also found *in vivo*, such as nuclei of Schwann cells by CALNEK et al.⁴⁾, in virus-bearing lymphoid cells and epithelial cells of the feather follicle by NAZERIAN²³⁾ and epithelial cell nuclei in the bursa of Fabricius of chickens inoculated with the JM strain of MDV by the authors¹³⁾. Since we did not examine chronologically the culture cells inoculated with MDV or HVT, whether various types of immature particles in the nuclei were present in the stage of maturation process of these viruses or unusual structures of aberrant viral replication, is not known. However, shape and size of these small nuclear particles were closely similar to those of the particles which were found in the inner capsid wall of the cross-shaped capsids. Thus it might be assumed that capsomers may surround the six small particles and consequently form a capsid. NAZERIAN et al.²⁵⁾ considered that the small particles in the nucleus might be related to viral replication and perhaps part of the viral core, because they observed the presence of crystals of the small particles prior to the appearance of viral nucleocapsids, the close proximity of incomplete nucleocapsids to the edges of such crystals, and the dispersion and partial disappearance of these particles in cells with more nucleocapsids. SETTNES also considered that these particles were believed to be aggregates of core materials. NAZERIAN et al.²⁵⁾ reported that cross-shaped capsids was found in about 30% of all nucleocapsids of HVT, whereas no such structure was seen in preparations of the JM strain of MDV. In our present study, however, cross-shaped capsid could be seen not only in cells infected with HVT (44.1%), but also in cells infected with Type 1 (1.6%) and Type 2 PPA (31.5%) of Cal-1 strain of MDV and JM strain (1.8%) of MDV. Similar nucleocapsids are seen in cells infected with canine herpesvirus¹⁴⁾, laryngotracheitis virus⁴⁰⁾ and equine abortion virus²⁹⁾. Therefore, these structures may represent one of general properties of the herpesvirus group. However, the significance of these structures is not known. So far as we know, there is no discussion on nucleocapsid with horseshoe-shaped or peculiar-shaped cores as those we have reported in this paper. These nucleocapsids were found in all cell cultures. Under the present three-dimensional observations, horseshoe-shaped cores on thin sections appeared to represent an image of tangential section of one doughnut-like ring which was placed between the two particles. From this structural appearance, it might be considered that 4 out of 6 particles

FIG. 1 Hypothetical scheme of the maturation of MDV and HVT



Intranuclear forms of the virus particles —

a: small nuclear particle, b: empty capsid, c: cross-shaped capsid, d: nucleocapsid with horseshoe-shaped core, e: nucleocapsid with peculiar-shaped core, f: nucleocapsid with core which looks a hollow ball with vertical stripes, g: nucleocapsid with a hollow ball-shaped core, i.e. double shell nucleocapsid or nucleocapsid with dense core, h: budding process at the inner nuclear membrane of the nuclear vesicle, i: enveloped particle in the nuclear vesicle, j: budding process at the inner nuclear membrane, k: enveloped particle in the perinuclear space

Intracytoplasmic forms of the virus particles —

l: immature particle within cytoplasmic inclusion body, m: enveloped particle within cytoplasmic inclusion body, n: enveloped particle with peripheral spikes in the endoplasmic reticulum, o: enveloped particle with peripheral spikes in the extracellular space

of cross-shaped capsid developed into one doughnut-like ring and the other 2 particles remained (figs. 14 & 15). In the next stage of the development, the 2 remaining particles may also have developed into the other doughnut-like ring (fig. 24). These two combined rings must be no more than the nucleocapsid with the peculiar-shaped core which we described above. The width of the margin of round plates or doughnut-like rings may grow wide, so that they may look like a ball with vertical stripes (fig. 25). Consequently, these cores may grow into a hollow ball, which may be seen as a double shell capsid in thin section. The double shell capsid appeared to grow nucleocapsids with a dense core as NII et al. described. The nucleocapsids with the dense core appeared to acquire an envelope by budding through the inner nuclear membrane, as DARLINGTON & MOSS described. It is interesting that recently CAMPBELL & WOODE reported MDV particles which were closely associated with intranuclear filaments, and they were thought to represent the product of aberrant viral replication.

Enveloped particles in the nuclear vesicle were seen more in cells infected with Type 1 PPA of Cal-1 strain or JM strain of MDV, than in those infected with Type 2 PPA of Cal-1 strain of MDV. According to MIKAMI & BANKOWSKI¹⁷⁻¹⁹), Type 2 PPA was less virulent for chickens than did Type 1 PPA. As the envelope plays an essential role in the infectivity of the agent^{11,20,36}), these findings are very interesting.

We observed the similar size and shape of enveloped particles in the cytoplasm and cytoplasmic inclusion bodies as described by NAZERIAN²³). Furthermore, enveloped particles with a granular or fibrillar electron dense outer coat (peripheral spikes), as observed in Epstein-Barr virus infected cells by STEEL & EDMOND, were observed (fig. 11). It is interesting to note that the particles observed above were approximately same size as cell free MDV seen in negatively stained feather follicle preparation^{3,27}). Relation among enveloped particles in the nuclear vesicles or perinuclear spaces, the enveloped particles in the cytoplasmic inclusion bodies and the enveloped particles with peripheral spikes in the endoplasmic reticula or extra-cellular spaces are still unknown. The significance of these particles is a topic for further discussion.

In the figure 1, a hypothetical scheme of the maturation of MDV and HVT is shown. The proof of this hypothetical scheme has still to be established.

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EXPLANATION OF PLATES

PLATE I

Fig. 2 CKC inoculated with Type 1 PPA of Cal-1 strain of MDV
The nuclear contour is irregular and margination of chromatin at the nuclear envelope is seen. Large nucleolus (Nl) is also evident. An especially large number of virus particles are found in the maginal areas of the nucleus. Single arrows (↓): immature particles, Double arrows (↓↓): enveloped particles in the nuclear vesicle. The cytoplasm has many cytoplasmic processes and many variable-sized vacuoles (V). × 14,250

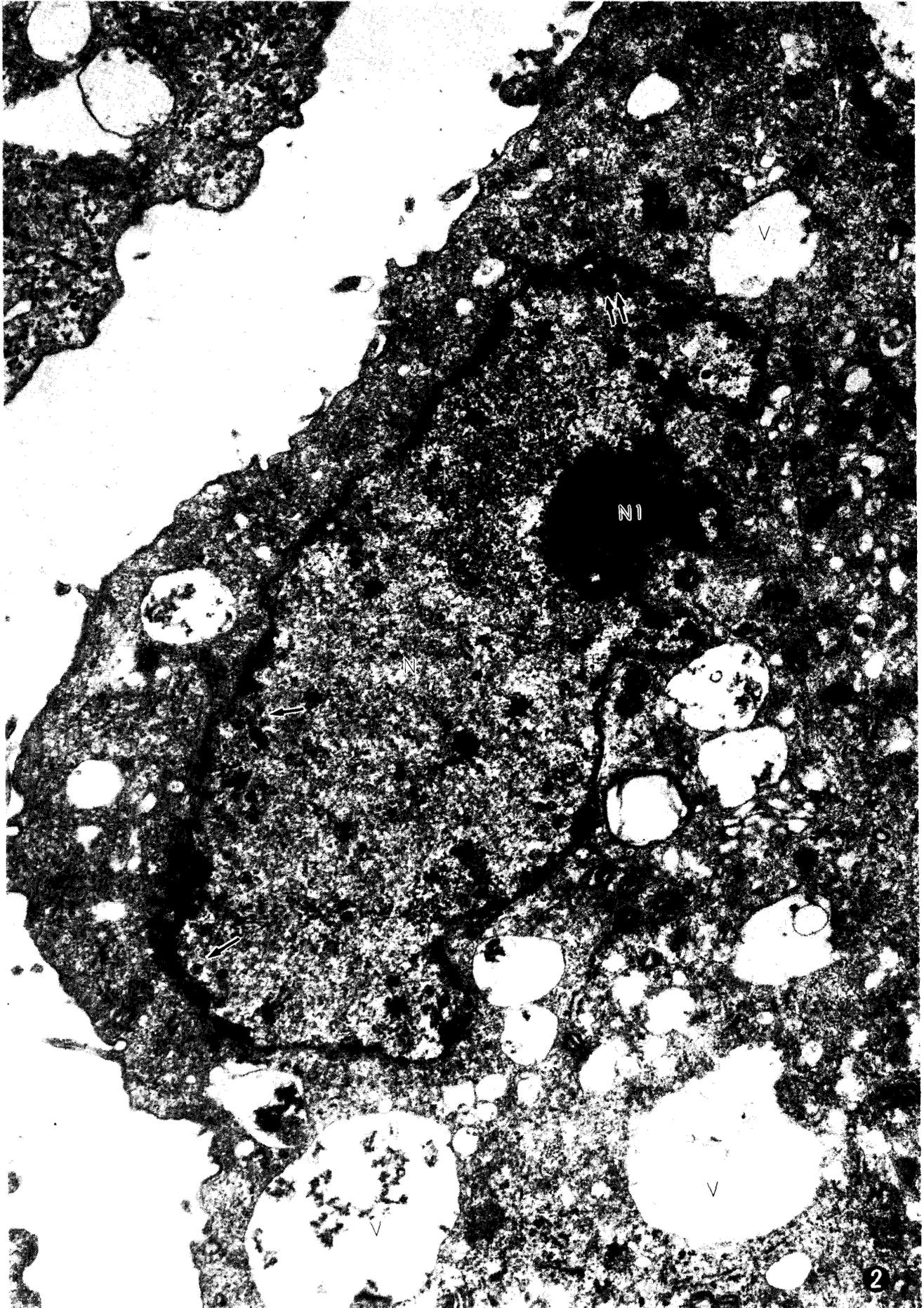


PLATE II

- Fig. 3 QF inoculated with FC 126 strain of HVT
Numerous small nuclear particles are found in the nucleoplasm. Empty capsids (a), a capsid contained one particle (b) and cross-shaped capsids (c) are seen. A particle in the capsid (b) shows almost the same size and shape as the small nuclear particles. $\times 66,000$
- Fig. 4 CKC inoculated with Type 2 PPA of Cal-1 strain of MDV
Cross-shaped capsid in the nucleoplasm $\times 165,000$
- Fig. 5 QF inoculated with FC 126 strain of HVT
Budding of cross-shaped capsid through the inner nuclear membrane of the nuclear vesicle $\times 66,000$

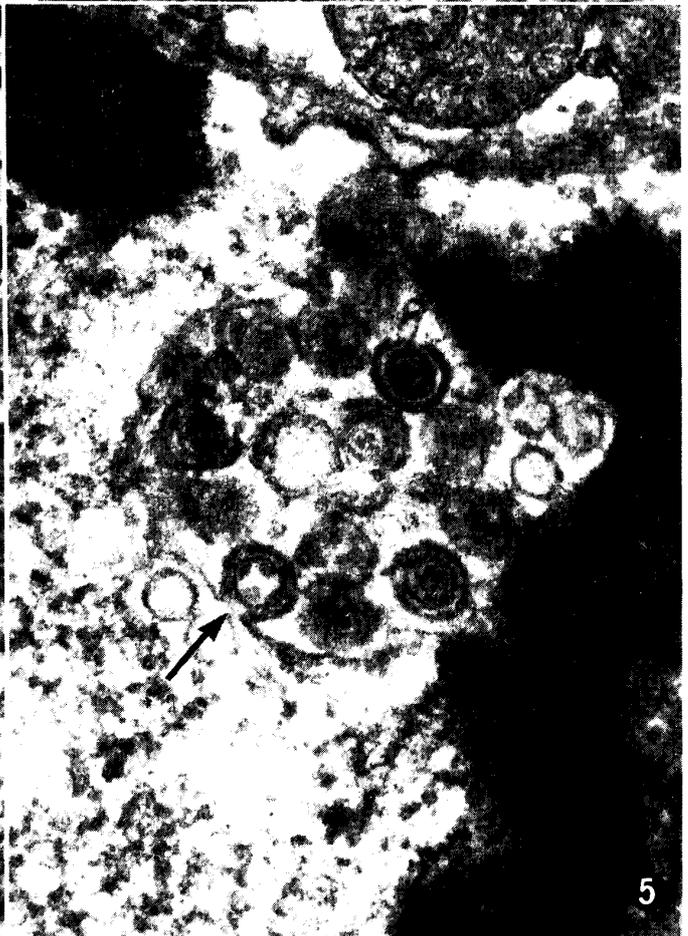
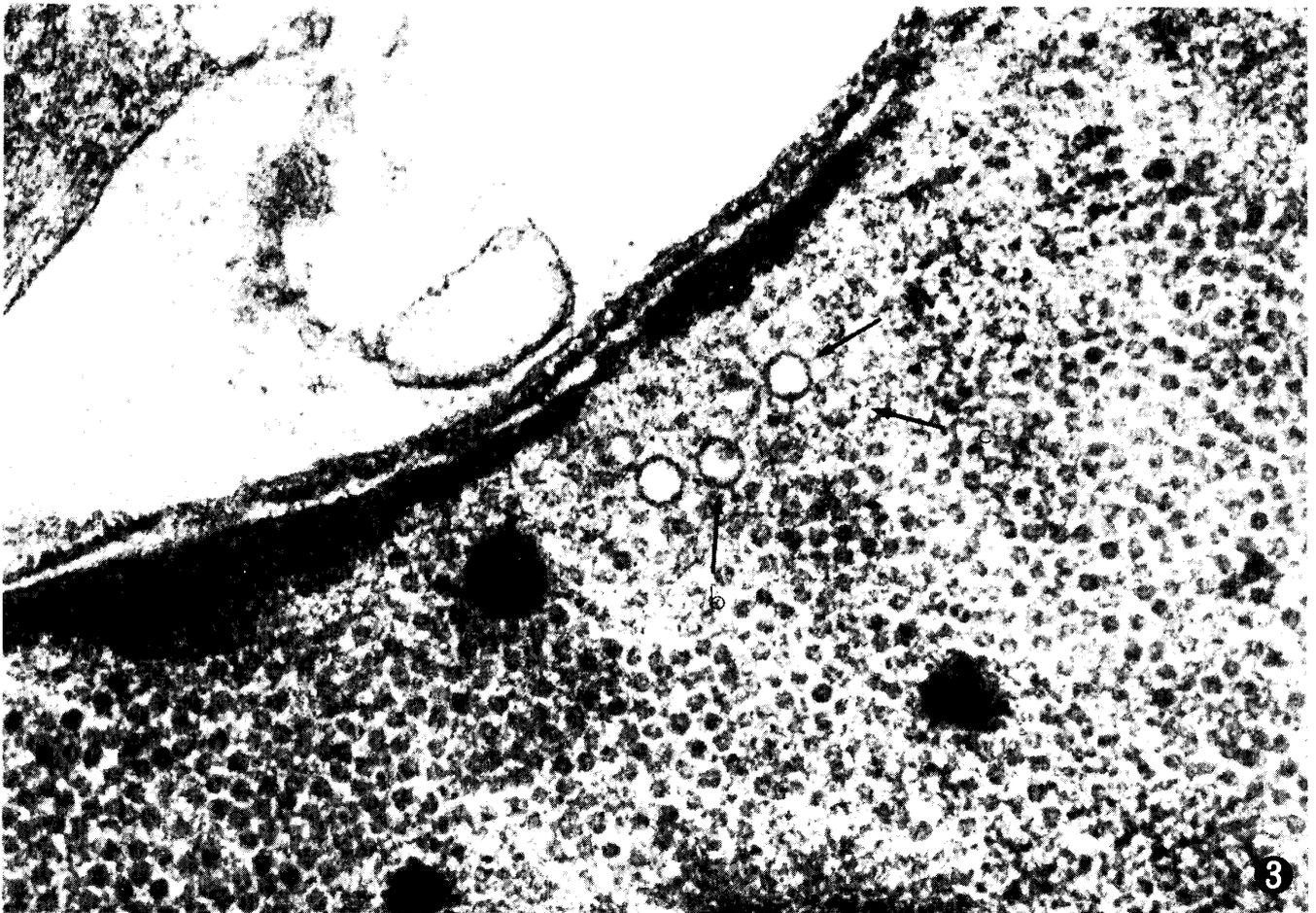


PLATE III

- Fig. 6 QF inoculated with JM strain of MDV
Nucleocapsid with horseshoe-shaped core
This capsid contains half of ring and one particle (↓). $\times 165,000$
- Fig. 7 CKC inoculated with Type 2 PPA of Cal-1 strain of MDV
Nucleocapsid with peculiar-shaped core.
This capsid contains one rod with some crossed structures with
low electron density. $\times 83,000$
- Fig. 8 CKC inoculated with Type 1 PPA of Cal-1 strain of MDV
Nucleocapsid with peculiar-shaped core
This capsid contains two parallel rods. $\times 83,000$
- Fig. 9 CKC inoculated with Type 1 PPA of Cal-1 strain of MDV
Numerous types of virus particles are seen in the nucleus and
the cytoplasm — a: empty capsid, b: double shell nucleocapsid,
c: nucleocapsid with dense core, d: enveloped particle in the
nuclear vesicle, e: incomplete enveloped particle in the
endoplasmic reticulum. $\times 66,000$

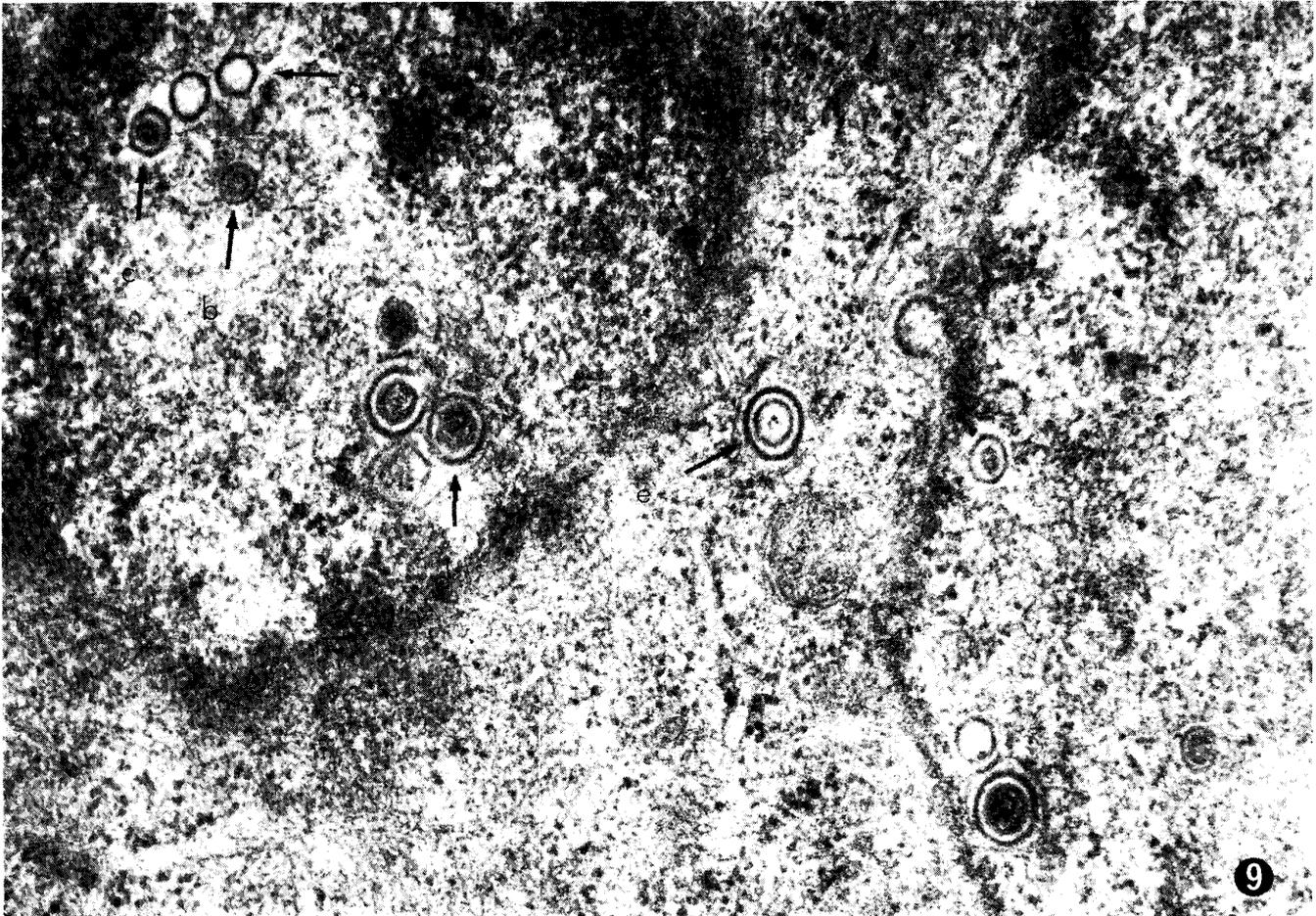
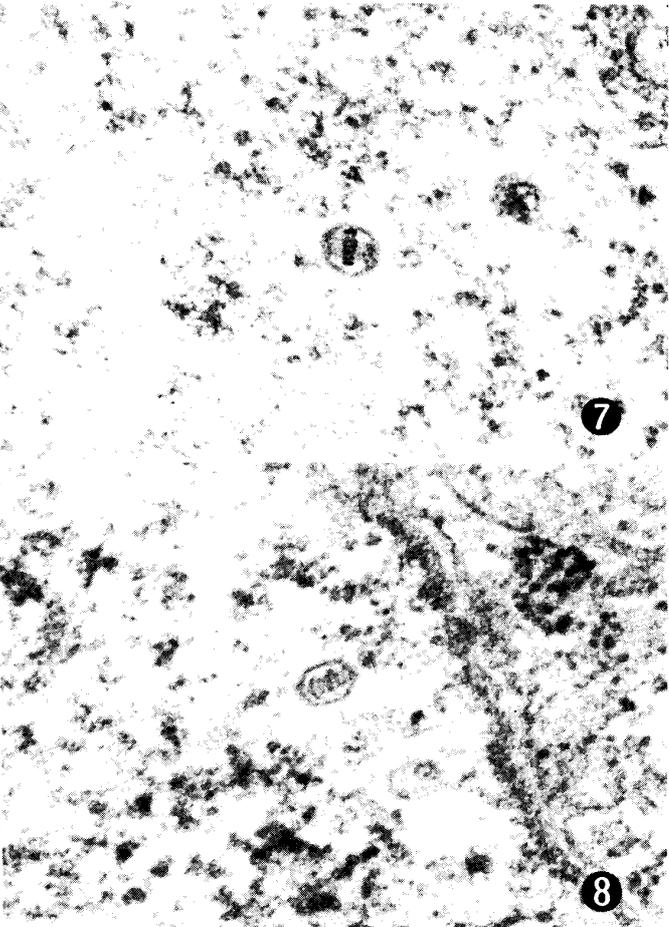
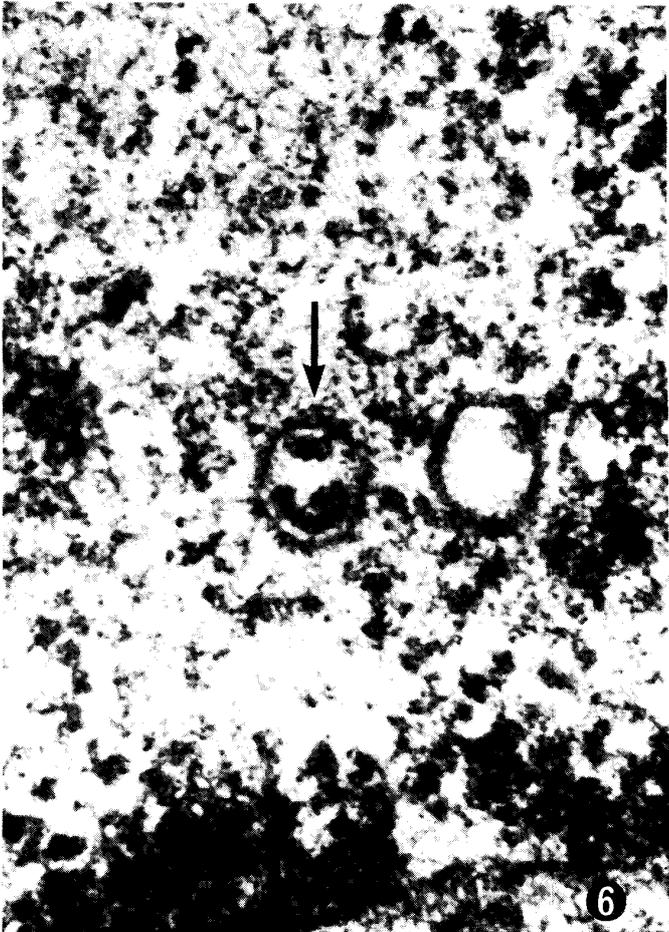


PLATE IV

Fig. 10 QF inoculated with FC 126 strain of HVT

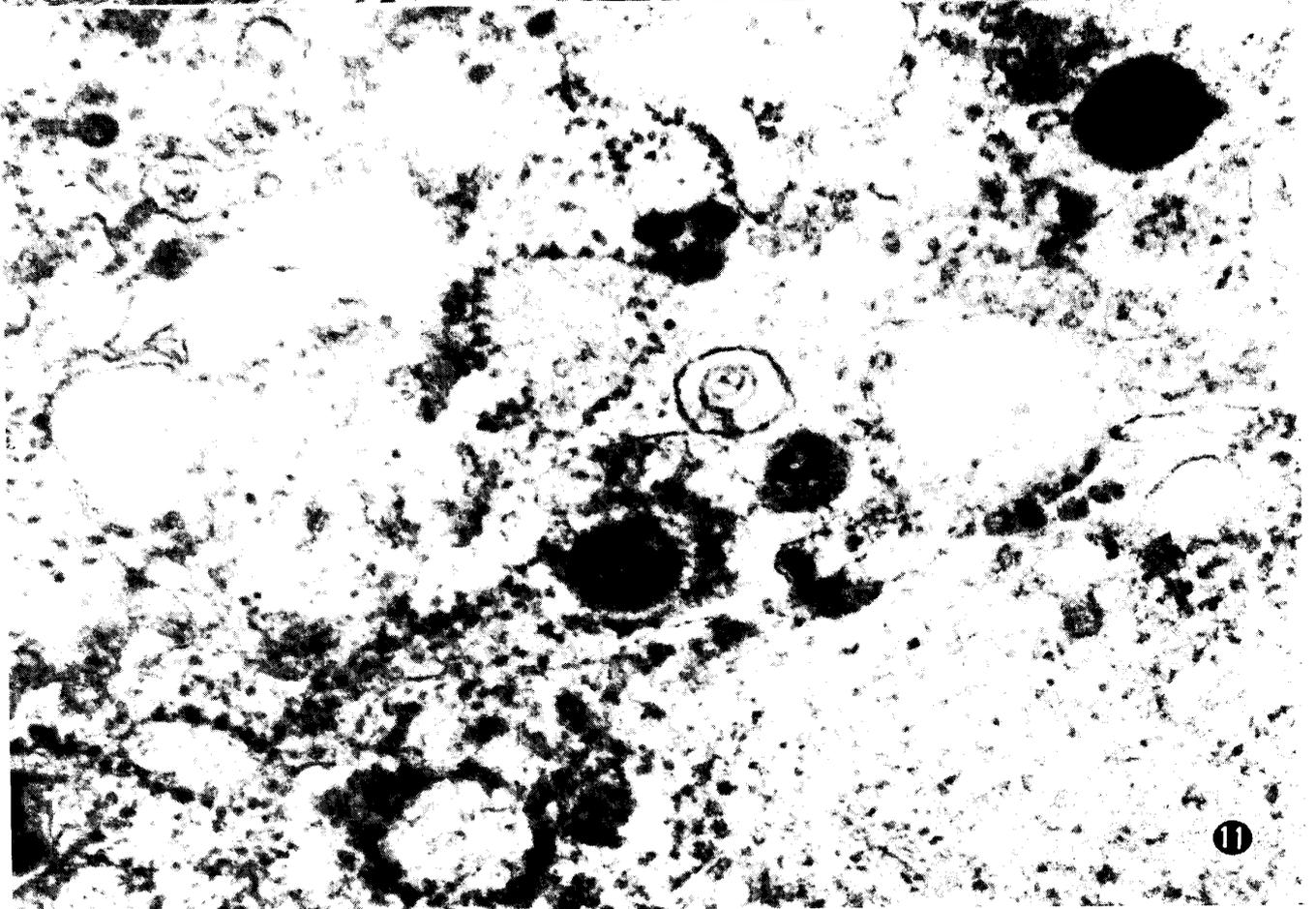
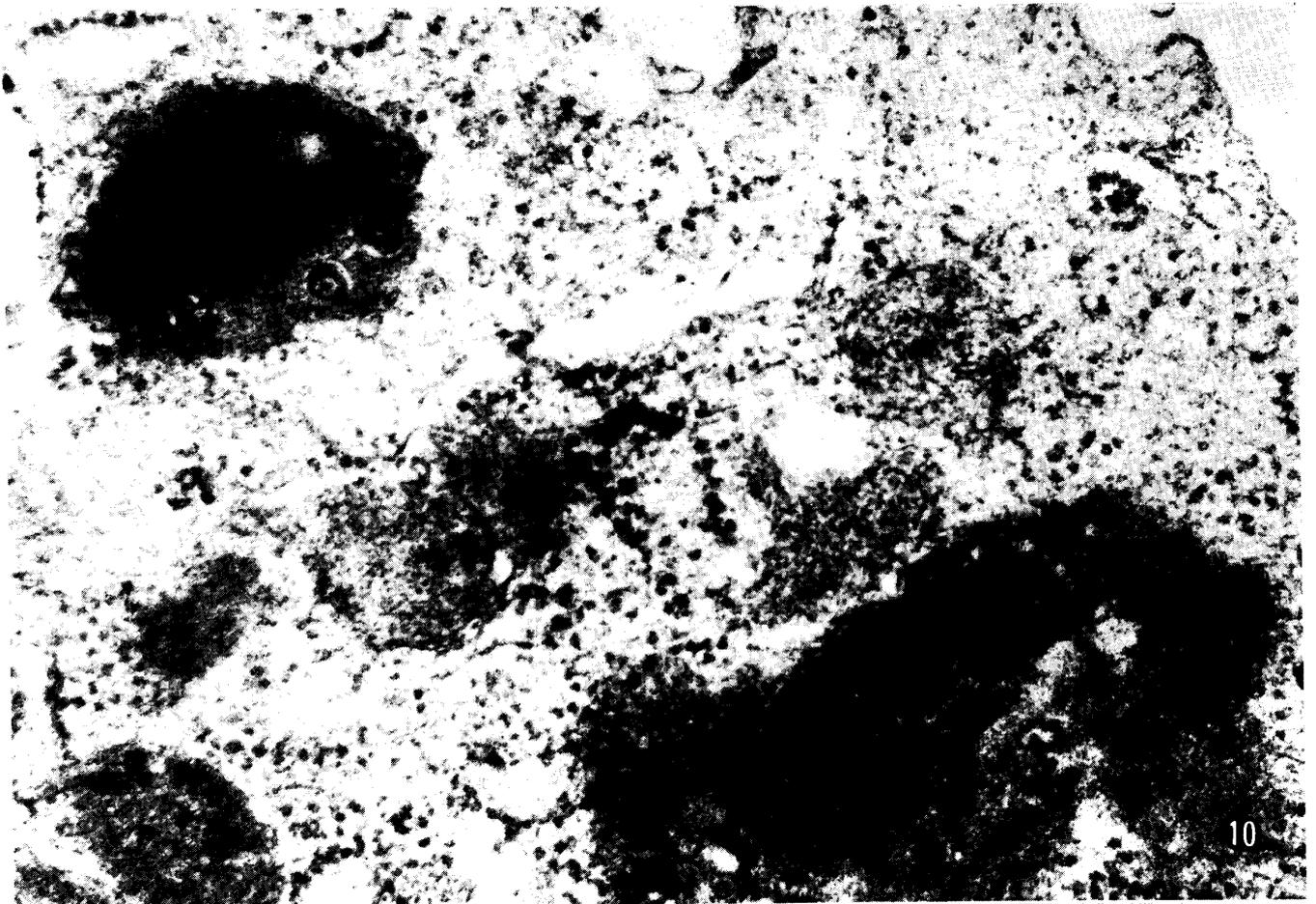
Cytoplasmic inclusion bodies

Five immature particles are seen in the inclusion body of the left upper part of the figure. Two enveloped particles are seen in the inclusion body of the right lower part of the figure. $\times 66,000$

Fig. 11 QF inoculated with FC 126 strain of HVT

Enveloped particles in the cytoplasm

One particle is present in the endoplasmic reticulum. $\times 66,000$



* PLATE V

Figs. 12 & 13 QF inoculated with FC 126 strain of HVT

Photographs of one cross-shaped capsid taken from different angles ($-35^{\circ} \sim 0^{\circ}$) $\times 231,000$

Fig. 14 Scheme of one cross-shaped capsid

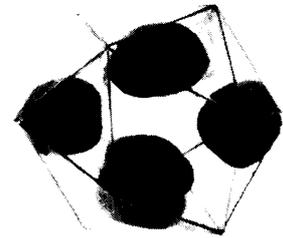
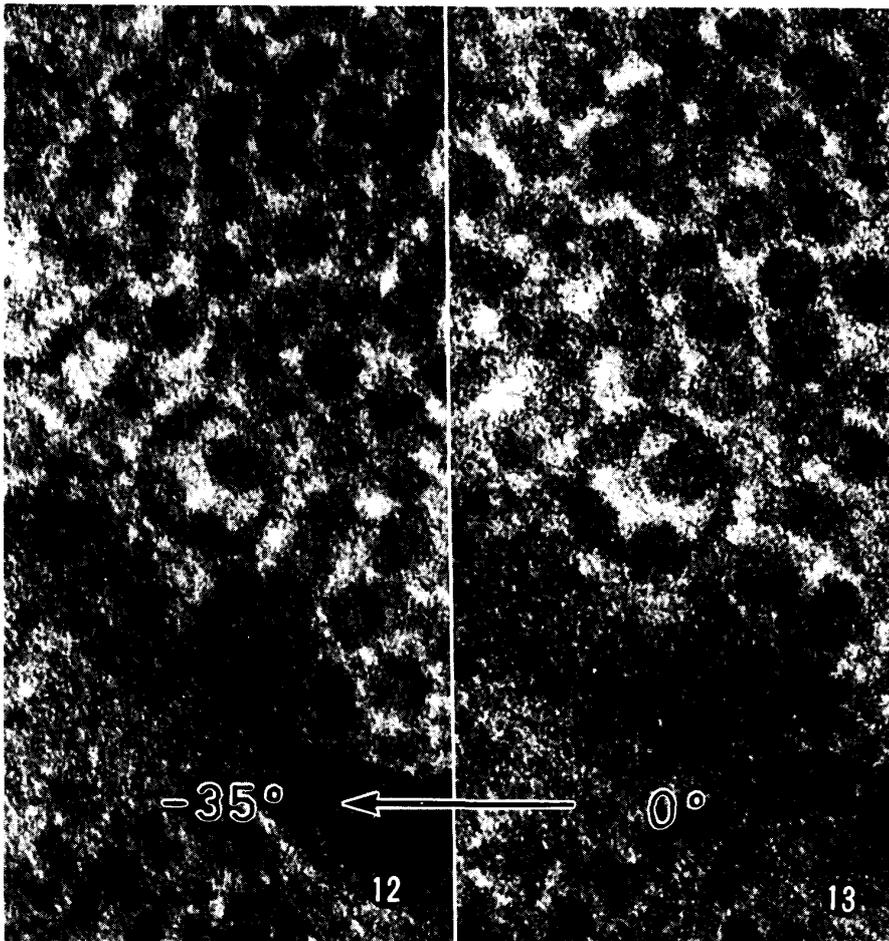
This capsid contains six spherical (icosahedral) particles.

Fig. 15 Scheme of nucleocapsid with horseshoe-shaped core

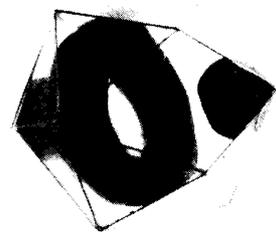
This capsid contains one doughnut-like ring and two particles. Fig. 6 represents an image of tangential section of this structure.

Figs. 16~18 QF inoculated with FC 126 strain of HVT

Photographs of one nucleocapsid with horseshoe-shaped core taken from different angles ($-20^{\circ} \sim 0^{\circ} \sim +35^{\circ}$) $\times 231,000$



14



15

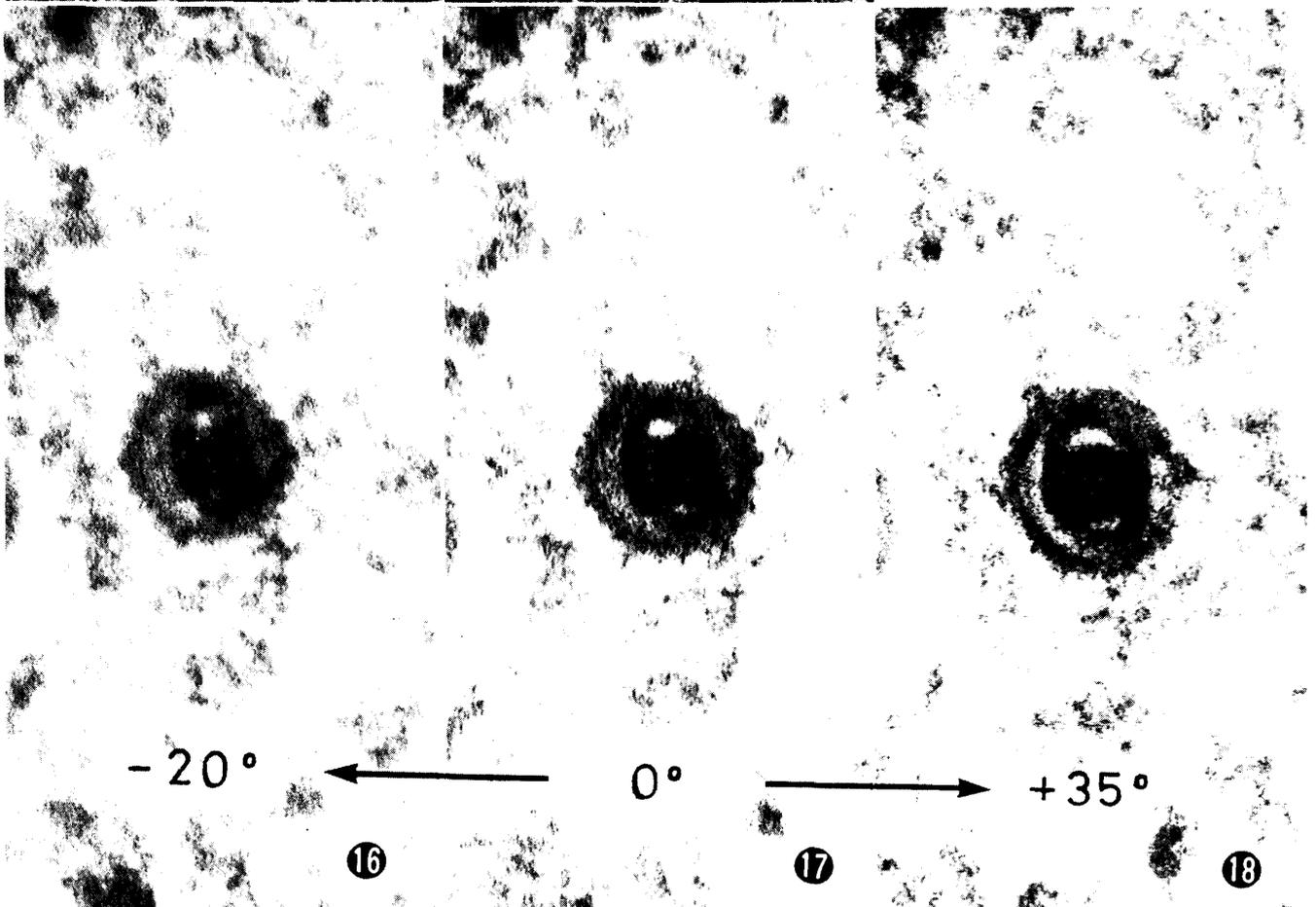


PLATE VI

- Figs. 19~21 QF inoculated with FC 126 strain of HVT
Photographs of one nucleocapsid with peculiar-shaped core taken
from different angles ($-20^{\circ} \sim 0^{\circ} \sim +35^{\circ}$) $\times 231,000$
- Fig. 22 QF inoculated with JM strain of MDV
Nucleocapsid with peculiar-shaped core $\times 200,000$
- Fig. 23 CKC inoculated with Type 1 PPA of Cal-1 strain of MDV
Nucleocapsid with core which looks like a hollow ball with
vertical stripes $\times 165,000$
- Fig. 24 Scheme of one nucleocapsid with peculiar-shaped core in fig. 22
- Fig. 25 Scheme of one nucleocapsid with core in fig. 23

