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A Comparative Study of Ultrastructural Changes due to Two Bovine Herpes Viruses

(Infectious Bovine Rhinotracheitis Virus and Malignant Catarrhal Fever Virus)

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Introduction

Members of the herpes virus group are relatively large, ether-sensitive, DNA viruses. Intranuclear inclusions of Cowdry's type A are charactristically present in infected cells[3]. The herpes simplex virus and some related to it are released from infected cells. Others, e. g. varicella and malignant catarrh, are so closely associated with cells that infectivity of cell-free preparations is hard to demonstrate[1]. The complete virus when negatively stained is seen to have an icosahedral core with 162 capsomeres on its surface[26]. These are hollow prisms 12.5×9.5nm in diameter. Outside the capsid is a membrane appearing up to 180nm across. Particles may be found with and without nucleoids and/or membranes[23]. There is dispute as to whether naked particles are infectious. They are certainly much less so than those with envelopes. The non-enveloped particles are said to have 3 shells round the core: with envelopes they acquire further 3 shells[18].

The earliest electron microscopic studies on the morphology of herpes viruses using shadowcast preparations of herpes zoster virus and of herpes simplex virus served only to confirm the size (100—150nm) deduced by ultrafiltration, and still later the negative staining method of Brenner and Horne (1959) revealed the morphological detail which has become so characteristic of the group.[8]

Morphology: Wildy *et al*,[26] used a negative staining method to study herpes simplex virus. They described two basic types of particles, distinguished by the presence or absence of an outer coat or envelope. The naked particle or capsid has an overall diameter of 105nm and corresponds to single ringed particles described in thin sections.[12]

In negative stain, the capsids show varying degrees of angularity, some being hexagonal and others almost spherical. The capsid is composed of capsomeres which are icosahedrally arrayed with five capsomeres along each edge of the triangular faces of the icosahedron, and thus 162 $[10(n-1)^2+2$ where $n=5]$ in the whole structure.

The general model of virus architecture of Caspar and Klug[2] would describe the 162 capsomeres of herpes virus as resulting from hexamer and pentamer clustering of structure units. Polygonal capsomeres can be seen on high-magnification micrographs, but it is usually not possible to equate the pentagonal ones with positions on the fivefold axes. The capsomeres are almost 10nm in diameter and are noteworthy for the apparent axial hole which is about 4nm in diameter and thus much larger than the "holes" described in the capsomeres of other viruses. This feature of the capsomeres gives the capsid a highly characteristic appearance. In negative stained peparations, the envelope is frequently distorted and hence has a very variable shape and size. Diameters vary from almost 150nm to over 200nm. Most envelopes contain only one capsid, but occasionally two or more capsids are seen inside one envelop. Some preparations of herpes virus contain particles which have the appearance of enveloped particles which have not been penetrated by negative stain and, consequently, the internal capside can not be clearly seen in these particles. In thin sections, the enveloped particles correspond to the double ringed particles described.

Cultivation: i) Infectious bovine rhinotracheitis virus (IBR virus) does not grow in fertile eggs. The virus has, however, been cultivated in bovine embryo tissue kidney, testis, lung, and skin, producing a cytopathic effect (CPE) within 1~2 days.[10] The disease was reproduced with culture material after 21 but not 40 passages.[20] Growth also occurs in pig, sheep, goat and horse kidney tissue cultures, in rabbit spleen, in human amnion and, after adaptation, in Hela cells. Plaques may be found in bovine kidney monolayers.

ii) Malignant catarrh fever virus (MCF virus). No continued cultivation in eggs is convincingly reported. The virus may be grown in cultures of thyroids or adrenals from infected cattle.[15] Syncytia and Cowdry A-type inclusions form in cultures. Once growth has been thus initiated cultivation has proved possible in sheep thyroid, calf testis or adrenal, rabbit and wildebeest kidney. Cultures up to the nineteenth passage in calf kidney were still infectious. In thyroid cultures transmission by means of cell-free fluids was possible.

Pattern of Replication: The early stages of the growth cycle are defined as those before newly formed particles are seen in the nucleus.[24] A number of different structures have been observed in the nuclei of infected cells, and most of these structures are described in the published literature by a number of workers in this field. It is, of course, difficult to distinguish structures merely representing break-down products of cellular organelles. Fine-grained dense aggregates were observed early in the nuclei of BHK 21 cells infected with herpes simplex virus.[24] These aggregates differed in staining characteristic and granularity from aggregates of interchromatin granules or nucleolar fragments, and they seem to be characteristic of the virus-infected cells. Naked particles are often found near these granules later in infection. These aggregates may correspond to the A-granules described in cytochemical studies of herpes-infected Hela cells.[9] and shown by them to contain both DNA and non-histone protein.

The role of these aggregates is, of course, uncertain, but because they seem to contain virus antigen and DNA and because virus particles are often found near them, they seem likely to be related to the formation of new particles.

All the structures so far described in this section are intranuclear, although Fujiwara and Kaplan[7] showed that structural proteins of the virus were first formed in the cytoplasm. Such a transfer would agree with the evidence that herpes virus proteins are made in the cytoplasm and transferred to the nucleus.[21] The enveloping of the capsid may occur at the nuclear membrane or within the cytoplasm.

Polykaryocyte formation may be of two types; early (usually within three hours after infection), or late, which occurs a number of hours or days after infection and usually coincides with the period of maximum virus multiplication. Productive infection is not always required for polykayocyte formation by the herpes virus. Similarly, IBR virus which grows at 37C° but not at 42C° in bovine kidney cells, produces polykaryocytes at both temperatures although at the higher temperature no evidence of viral DNA synthesis was detected.[22] Viral protein synthesis was detected at both temperatures. These experiments indicate that synthesis of complete virus particles is not required for polykaryocyte formation by the herpes viruses. As can be seen from the growth curves, after the end of the latent period, virus replication occurs exponentially for a variable period of time. Variables which influence virus growth are the virus strain, the type of cell, and the type of culture (monolayer, suspension, or single cell). The increasing rates of an essential precursor or increasing levels of activity of an essential enzyme are probably responsible for the observed kinetics. The ultimate yield of virus produced by infected cells also varies depending on the type of cell, the type of culture, the thermostability of the virus, and the temperature of incubation.[8]

Electron Microscopy in Diagnosis of Herpes Virus Infection: The characteristic morphology of herpes virus has led to an increasing use of electron microscopy in clinical diagnosis. Of course, speed is obviously of great importance, and electron microscopic examination of vesicular material by negative staining can be done very rapidly.[8] Electron microscopy proved more sensitive than the other rapid test (gel diffusion). Similarly, electron microscopy has proved to be very successful in identifying herpes virus particles in specimens taken from cases of Varicella.[4]

But electron microscopy can not differentiate between different herpes viruses or different pox virus (with the exception of orf virus) but this is usually less important than the differentiation of herpes and pox viruses.

In correlating information on virus particles based on thin sections with that obtained by negative staining electon microscopy, some allowance for technical distortion is necessary.

Negative-staining: These preparations contained a substantial number of particles with undoubted viral characteristics. They were of two distinct morphological types, enveloped and naked: the two types, occu rred in clusters with about equal frequency but were rarely intermingled. Enveloped particles possessed a loose external membrane of irregular outline, enclosing a capsid having a well-defined and regular profile. Overallsize of enveloped particles range from 140 to 220nm, while capsids measured about 100nm. Naked particles appeared circular or hexagonal in outline. They measured 100nm across and closely resembled the central bodies of enveloped particles. Each subunit had a diameter of about 9.5nm, and the length of those viewed in profile round the particle periphery was about 12.5nm. Shrinkage tends to occur during dehydration and embedding before cutting sections, while collaspse and spreading of unsupported membranes and other non-rigid components can be expected in specimens allowed to dry in phosphotungstate. There is a reasonable correlation of detail between the single and double-ringed particles seen in thin sections, and the naked and enveloped forms revealed by negative staining.

Materials and Methods

Virus Cultivation

a) Infectious Bovine Rhinotracheitis Virus(CT3 Strain): The isolate under investigation was recovered from a nasal swab taken from an experimental calf. The first cytopathic effect (CPE) was seen in bovine kidney cells (MDBK cell line) ten days after absorption of the transport fluid expressed from the swab. After three passages in MDBK cells the typical rounding of cells appeared within 3~4 days of inoculation, and a 1 litre flat was harvested as described below at day 6 when 20 to 80% of the cell sheet showed CPE.

b) Malignant Catarrhal Fever Virus (C500 Strain): The MCF (C500) virus was grown in bovine testis (BT) cells seeded into a 1 litre medical flat and inoculated when the cells formed a confluent sheet. The inoculum consisted of 5.0ml of tissue culture supernatant containg cell free virus and having a $TCID_{50}$ of 4.7/ml. Syncytia formation and rounding of cells was observed at day 10 and the preparations were harvested at day 12.

c) Rabbit Lung Macrophages: Preparations of lung macrophages were made after the technique of Watt *et al*,[25] for cultivating pig lung macrophages. The lungs of a rabbit showing the characteristic clinical features of MCFV infection were washed out three times with Hank's solution. The cells were pelleted by centrifuging for minutes at 1,000 r.p.m., and after two washes with Hank's the material was fixed with glutaraldehyde and Palade's solution as described below.

In addition, a portion of lung was taken from another terminal rabbit so that lung macrophages might be examined *in situ*.

Preparation of Thin Sections: Viruses in monolayer of bovine kidney cells and bovine testic cells were prepared for electron microscopy as described below after methods used by Davis and Sharmd[6], Pease[14] and Zee *et al.*[27]

a) Fixing and Harvesting Cultures: The cells were rinsed three times with phosphate buffered saline (PBS) and fixed in pre-cooled 3% glutaraldehyde at 4°C for 30 minutes. This was followed by 0.1M sodium cacodylate buffer pH 7.25 at 4°C for one hour.[19] Cells were then harvested with a rubber policemen and pelleted in PBS at 1,000 r.p.m. for 10 minutes. This pellet was post-fixed in Palade's solution (1.0% buffered osmium tetroxide) at 4°C for 30~60 minutes.

b) Processng: The fixed cell suspension was drawn up into capillary tubes and one of the tube sealed with plasticine. The capillary tubes were centrifuged in a microhaematocrit centrifuge for 5 minutes to make pellets. The pellets were extruded into molten 2% agar on a glass slide and then the pellets solidified at 4°C overnight in an incubator. The pellet could then

be cut out coated with agar.

c) Embedding in Araldite: Transferring the pellet to propylene oxide was the first step, and then a series of steps towards fresh araldite followed, in which incubation was at 60°C for 30 minutes and the percentage of araldite was progressively increased. In the third step pellets were embedded in araldite in BEEM capsules. These were left at 60°C for 4~5 days to harden.

d) Section Cutting: The embeded pellets were sectioned with an ultramicrotome using 45° glass knives (Reichert Om UZ) and cutting at 70nm thickness. Sections were floated onto water, picked up on formvar coated grids and allowed to dry and attach firmly before staining. Phases 1~1.5μm thick were always examined first to select appropriate fields, using 1% toluidine blue as the stain.

e) Staining of Thin Sections: The section which was attached to the grid was stained for 15 minutes in a 1% solution of uranyl acetate, rinsed three times in distilled water, and counter-stained with lead citrate solution.

Microscopy: The sections were examined in an AEI electron microscope (Type EM 6B).

Photography: It is obvious that only a small fraction of what the observer sees on the fluorescent screen of the microscope can be recorded on photographic film. Indeed, the time it takes to focus and otherwise adjust the microscope for picture taking, and to replace exposed plates is a major production bottleneck. Prints were made using standard photographic techniques.

Results

Morphology of Virions

1. IBRV/CT3: Intranuclear capsids were generally hexagonal or oval in outline, measuring up to 65nm along their long axis. Some were hollow, while others contained a core (25~35nm long) which, in its turn, was either solid or ring-like (fig. 1). Capsomere arrangement could be seen along some of the free borders of the intranuclear capsids, but no attempt was made to enumerate these subunits, or to calculate their size. The capsid measurements ranged from 65~75 nm, the variation occurring randomly in intranuclear, intracytoplasmic or cell-free sites.

Within the cytoplasm the virion had a double envelope measuring up to 175nm in diameter (fig. 3). The outermost membrane was not observed in cell free virus (fig. 4), where the overall diameter varied from 110~130nm (fig. 5). The structure of the envelope was most easily observed in the extracellular virions with the castellated projections radiating from the darkly staining basal layer.

2. MCFV/C500: Capsids in bovine testis(BT) cells varied from 65~75nm. Many were devoid of a central nucleoid and the nucleoid itself was seen predominantly in a ring form rather than a dense aggregate(fig. 7). Doubly enveloped virions up to 130nm in size were seen within the cell nucleus (fig. 7), but more often the appearance was of a single envelope measuring from 90~110nm, while in extracellular virus the range increased up to 125nm (fig. 12). The preparations indicated that the envelope had two layers with rounded projections emanating from the outerlayer.

Enveloped virions conforming to the pattern of mature virus were also seen in rabbit lung macrophages, but most often the particles were enclosed in single or multiple cell membrances (fig. 16). Within these intracytoplasmic vacuoles some naked particles showed a loss of structural definition, becoming rounded and uniformly opaque.

Morphogenesis of IBRV/CT3 and MCFV/C500

1. Development of IBRV/CT3 in MDBK Cells: Nucleocapsids first appeared in the nucleus of the kidney epithelial cells, with 50~75% of them containing a central nucleoid, separated from the capsid membrane by a clear space. Initially the units were diffusely distributed throughout the nucleus (fig. 3), but it was not unusual for aggregates to form(fig. 1). Enveloped virions were never observed inside the cell nucleus.

Naked capsids were next observed in the perinuclear cytoplasm where they were associated with the formation of a disc shaped vacuole lined by cell cytoplasmic membrane (figs. 2 & 3). Progressive stages of encapsulation could be seen in which either the virion could be said to invaginate the vacuole, or conversely the vacuole to surround the virion (figs. 2 & 3); the end result being an enveloped unit lying within a cy-

toplasmic vacuole. At the outer cell membrane the external layer of the vacuole was lost from the virion, giving rise to cell free virus surrounded by a single envelope. This maturation is represented in figures 1~5 inclusively.

2. Development of MCFV/C500 in BT Cells: Capsids remained disseminated throughout the cell nucleus, showing a tendency to be concentrated in the peripheral areas (fig. 8). Less than 50% contained a nucleoid (fig. 7). A striking feature of MCFV maturation was the invagination of cytoplasmic tubles into the cell nucleus, pushing the nuclear membrane before them (fig. 8). Intranuclear capsids either move into or are enveloped by these processes, obtaining an enveloping membrane as they gain access to the cytoplasm. It was unusual for them to appear to have a double envelope (fig. 10): It was also unusual to see intracytoplasmic enveloped virus outside these tubules although disruption of mitochondria occurred at an early stage of intranuclear viral replication(fig. 11). Naked capsids were seen in the cytoplasm (fig. 10), but did not appear to evoke membrane formation. Enveloped cell free virus was observed lying amongst disrupted cell fragments (fig. 12).

3. MCFV/C500 in Rabbit Lung Macrophages: The normal lung macrophage has a large nucleus with an open chromatin pattern; the cytoplasm projects into the numerous active pseudopods and contains an abundant rough endoplasmic reticulum, with many lysosomes and scattered mitochondria (fig. 13). In cells from rabbits dying due to MCFV infection, there was retraction of cytoplasmic projections and an increase in the size and density ef the lysosomes, several of which could be seen to contain virions(fig. 14). This was equally true of macrophages obtained from washing out the lung (fig. 15) or from cells fixed *in situ* (fig. 15). Intranuclear virus was not observed in any of these cells in any form. In the cytoplasm a wide range of forms were seen as has been mentioned under the description of virus morphology. A most striking feature was the multiple layering of cell membranes round units, giving rise to myelin-like figurations (fig. 16). Lysosomes often contained several virions which could be at the same or various stages of degeneration (figs. 14, 15 & 16).

Discussion

The CT3 virus was recovered from a nasal swab taken 7 days after the calf had been inoculated parenterally with IBRV/CT3. While the virus produced a typical herpetic cytopathic effect in BK cells, with syncytia formation and intranuclear inclusion bodies, it was thought unlikely that it was MCFV as all previous attempts to recover virus from nasal excretion have failed[16] An indirect fluorescent test using a known antiserum against Infectious Bovine Rhinitis Virus (IBRV) and an antibovine *r*-globulin preparation indicated that the agent was an isolate of IBRV. The calf subsequently developed antibodies to this virus.

As a further confirmation of CT3's identity, ultrastructural studies were made to describe both the morphogenesis. The objective of this study was to compare CT3 with MCFV/C500 in tissue culture and with MCFV/C500 *in vivo*. This latter aspect presented problems for while the arteritis and lymphoid proliferation of MCFV infection are quite specific[6] and virus can be isolated from lymphoid tissues, there is, so far, no record of virus being detected morphologically in these tissues. However, virus has been detected in the lung macrophages of rabbits dying from MCFV infection, and these were therefore used as the source of MCFV in the host animal.

Both CT3 and MCFV conform to the pattern of herpes viruses[1] with a central nucleoid enclosed in a capsid of icosahedral symmetry which in maturation became enclosed in an envelope derived from cell membranes. The capsids of both viruses (65~75nm) were within the lower range recorded for other herpes viruses. as were the enveloped virions (90~130nm). However, there were certain morphological distinguishing features inasmuch as most of the MCFV/C500 capsids lacked cores and when a nucleoid was present it was present it was most frequnetly ring like whereas CT3 nucleoids were present in most capsids and were most often uniformly dense. A further distinguishing feature was that MCF virions were rarely observed to have more than one envelope whereas a double envelope was characteristic of intracytoplasmic CT3 virions.

The differences in morphogenesis were even more striking. While in each case the capsids first appeared in the nucleus CT3 virus units showed a distinct tendency to aggregate in the periphery of the nucleus and then to migrate through the nuclear membrane into the cell cytoplasm without obtaining an envelope. MCFV/C500 on the other hand remained as disseminated particles in the cell nucleus, and it was an invagination of the nuclear membrane that engulfed it; thus the envelope of MCFV/C500 was nuclear in its origin and single in its formation. CT3 units became enveloped in the cytoplasm, seeming in evoke the formation of saucer shaped vacuoles which eventually surround the icosahedron with the inner membrane forming virion envelope and the outer membrane being shed when the virus is released from the cell. We were unable to ascertain whether the outer membrane is discarded or whether it fuses with the cell plasma membrane.

These ultrastructural studies thus confirmed that the two viruses were both herpes viruses, but did have different patterns of replication with CT3 comparable to those described for IBRV[11] and the MCFV conforming to that described by Plowright *et al*[17]. It is interesting to note that two avian herpetic diseases, infectious laryngotracheitis (ILT) and Marek's disease (MD), show an analogous pattern of viral maturation, with ILT virus deriving viral envelope from cell plasma membrane while MD virus is encased in cell nuclear membrane.[7] That the two plasma membrane virions (ILTV and IBRV) are both associated with upper respiratory tract lesions, while the two viruses enveloped in nuclear membrane (MDV and MCFV) are associated with additional lymphoproliferative disorders is another fascinating aspect of the analogy.

The examination of MCFV/C500 virus in rabbit lung macrophages revealed bizarre forms of virus. As no intranuclear virions were detected it is most likely that this virus was phagocytosed virus. If this is so, then it raises an interesting question as to the origin of the virus since it is notoriously difficult to find in other cells from rabbits dying of the disease. It is possible that there is cell free or cell associated virus which is being produced in the lung epithelium. Alternatively, virus may be associated with the extensive lymphoblast invasion that occurs in the febrile phase of the illness. The layering of membranes have been described in association with the morphogenesis of herpes simplex, but as a rare rather than a characteristic feature.[13]

Conclusion

The morphology and development of two bovine herpes viruses (infectious bovine rhinotracheitis virus and malignant catarrhal fever virus) were examined in tissue culture preparation. The both viruses had the characteristic structure of herpes virions but differences in morphogenesis were recorded.

The IBRV/CT3 isolate obtained its envelope from cell plasma membrane.

Enveloped virions were never observed inside the cell nucleus.

The MCFV/C500 particles derived an envelope from the nuclear membrane of the cell.

In addition, MCFV/C500 was examined in rabbits dying from infection. Virions were only found in the cytoplasm of lung macrophages, and it is suggested that this was phagocytosed material.

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Legends for Figures

Fig. 1. Aggregates of intranuclear capsids: some with dence cores (↑), some with ring cores (▲), and some empty. CT3 Virus in MDBK cells. ×40,000

Fig. 2. Two intracytoplasmic capsids (↑) are associated with the formation of membranous vesicles. The vesicle on the left curves round the capsid while the right capsid is virtually engulfed. CT3 Virus in MDBK cells. ×40,000

Fig. 3. Both intranuclear and intracytoplasmic capsids are present. Two intracytoplasmic virions show partial (↑) and complete (▲) envelopment by the cytoplasmic membranes. CT3 Virus in MDBK cells. ×40,000

Fig. 4. The extracellular virion (↑) lacks the outermost cytoplasmic membrane which can be seen on particle B. CT3 Virus in MDBK cells. ×40,000

Fig. 5. Mature CT3 virions lying between the microvilli of the epithelial cells. The castellation of the outer layer of the membrane can be distinguished (▲). CT3 Virus in MDBK. ×40,000

Fig. 6. In this infected cell virions can be seen at all stages of replication; intranuclear capsids are clumped (↑); intracytoplasmic particles aggregated (A) or attached to vacuoles (V), while enveloped CT3 virus lies outside the cell between microvilli (▲). CT3 Virus in MDBK cells. ×5,000

Fig. 7. Intranuclear capsids of MCFV in bovine testis (BT) cells. The majority of the particles are empty. When present the nucleoid has a translucent centre, appearing as a ring structure. MCFV in BT cells. ×40,000

Fig. 8. A peripheral and diffuse scattering of the capsids is the prevailing pattern in this infected BT cell. The intracytoplasmic organelles have lost their structural detail. MCFV/C500 in BT cells. ×7,500

Fig. 9. Finger-like invaginations protrude into the nucleus (A). Virions lying in their lumen are enveloped in a single membrane (▲). MCFV/C500 in BT cells. ×40,000

Fig. 10. Transverse sections of virions lying within the invaginations gives the appearance of a double envelope (↑). Naked capsids can also be seen in the cytoplasm of this infected cell (▲). MCFV/C500 in BT cells. ×40,000

Fig. 11. At an early stage of virion formation the organelles are disrupted, although there is very little virus in the cytoplasm (↑) in comparison with the abundant intranuclear particles. MCFV/C500. ×10,000

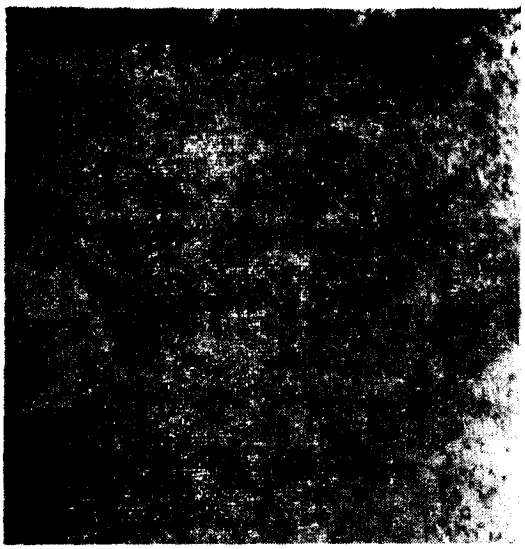
Fig. 12. MCFV/500 virions lying amongst cell debris. The nucleoid is more condensed (↑) and the two layers of the envelope can defined (▲). MCFV/C500 in BT cells. ×40,000

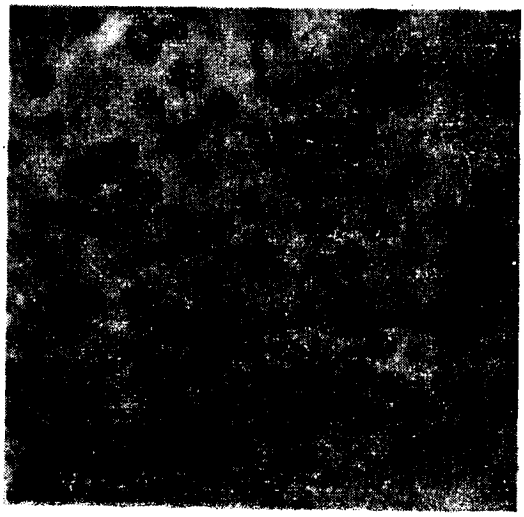
Fig. 13. An active rabbit lung macrophage (RLM) which has been maintained in tissue culture for 72 hours. There are many cytoplasmic projections and "resting" vacuoles within the cytoplasm. RLM *in vitro*. ×5,000

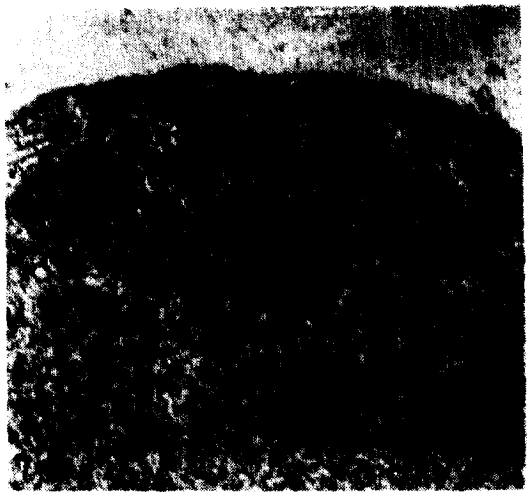
Fig. 14. An *in vitro* macrophage containing numerous electron dense vacuoles. There are phagosomes or lysosomes, and in several of them capsids can be distinguished (↑). Viral units cannot be seen in the nucleus. RLM *in vitro* ×5,000

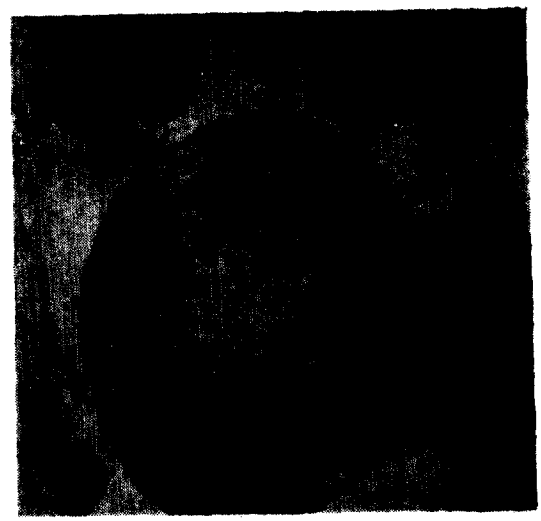
Fig. 15. Macrophage (M) lying in the alveolus of a rabbit which had a fatal MCFV/C500 infection. The cytoplasm contains many vacuoles containing virions. An enlargement of this figure is made in the next figure. RLM *in vitro*. ×5,000

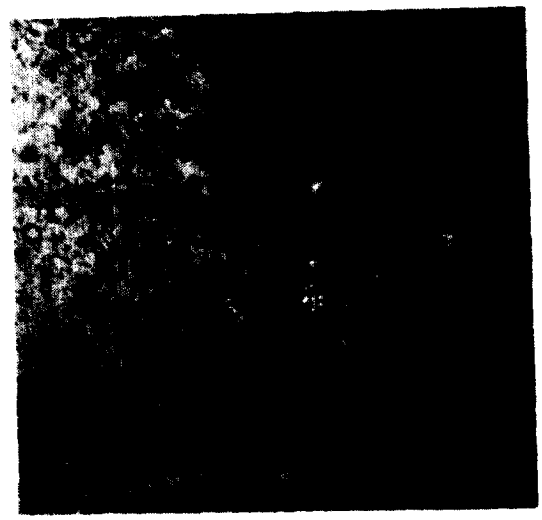
Fig. 16. This is an enlargement of fig. 15. Enveloped and uneveloped virions, singly and grouped, occur both within vacuoles and lying free in the cytoplasm. Some of the vacuoles have several membranes (▲) while in some cases myelin like figures develop (A). It is suggested that these are all phagocytosed virions with which the macrophage is coping with varying degrees of success. *in vitro*. ×40,000

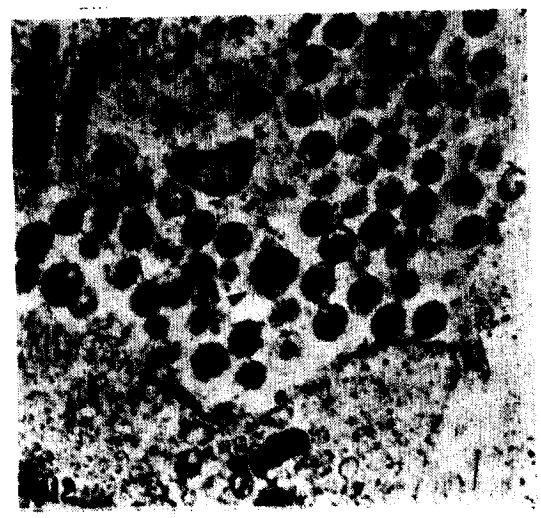


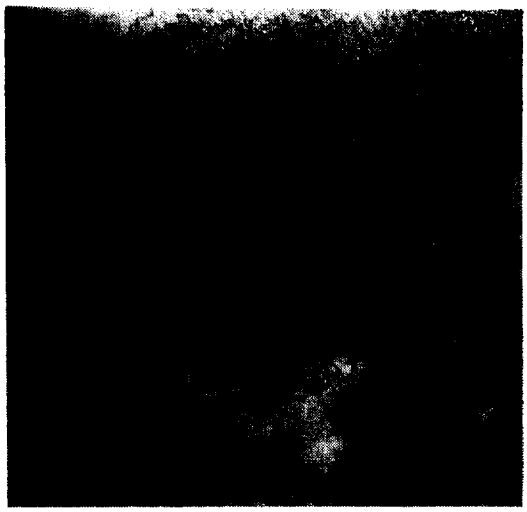












References

1. Andrewes, C. and Pereira, H.G.: Viruses vertebrates. 3 ed. Bailliere Tindall, London (1972) p. 342.
2. Caspar, D.L.D. and Klug, A.: Cold Spring Harbor Symp. Quant. Biol., (1962) 27:1.
3. Cowdry, E.V.: Nuclear inclusions in the kidneys of *Macaeus rhesus* monkeys. Am. J. Path.,(1935) 11:659.
4. Cruickshank, J. G., Bedson, H.S. and Watson, D.H.: Electron microscopy in the rapid diagnosis of smallpox. Lancet (1966) 2:527.
5. Daubney, E. and Hudson, J.R.: Transmission experiments with bovine malignant catarrh, J. Comp. Path. Ther. (1936) 49:63.
6. Davis, W.C. and Sharma, J.M.: Ultrastructural comparison of morphogenesis and fate of Marek's disease and infectious laryngotracheitis viruses in chicken kidney cells. Am. J. Vet. Res., (1973) 34:873.
7. Fujiwara, S. and Kaplan, A.S.: Site of protein synthesis in cells infected with pseudorabies virus. Virology, (1967) 32:60.
8. Kaplan, A.S.: The Herpes viruses. Academic Press, New Yerk and London. (1973) p. 430.
9. Love, R. and Wildy, P.: Cytochemical studies of the nucleoproteins of Hela cells infected with herpes virus. J. Cell Biol., (1963) 17:237.
10. Madin, S.H., York, C.J. and Mckercher, D.G.: Isolation of the infectious bovine rhinotracheitis

virus. Science, (1956) 124:721.
11. Mckercher, D.G.: Infectious bovine rhinotracheitis. Advan. Vet. Sci., (1959) 5:299.
12. Morgan, C., Ellison, S.A., Rose, H.M. and Moore, D.H.: Structure and development of viruses as observed in the electron microscope. I. Herpes simplex virus. J. Exp. Med., (1954) 100:195.
13. Nii, S., Morgan, C. and Rose, H.M.: Electron microscopy of Herpes simplex virus. II. Sequence of development. J. Virol. (1968) 2:517.
14. Pease, D.C.: Histological techniques for electron microscopy, 2ed. Academic Press, New York and London. (1964).
15. Plowright, W., Ferris, R.D. and Scott, G.R.: Bule wildebeest and the aetiological agent of bovine malignant catarrhal fever. Nature. (1960) 188: 1167.
16. Plowright, W.: Studies on malignant catarrhal fever of cattle. East Africa Veterinary Organization, Muguga, Kenya (1964).
17. Plowright, W., Macadam, R.F. and Armstrong, J.A.: Growth and characterization of the virus of bovine malignant catarrhal fever in East Africa. J. Gen. Microbiol. (1968) 39:253.
18. Roizman, B., Spring, S.B. and Schwartz, J.: The Herpes virion and its precursors made in productively and in abortively infected cells. Fed. Proc., Fed. Am. Soc. Exp. Biol., (1969) 28:1890.
19. Sabatini, D.D., Bensch, K. and Barrnett, R.J.: Cytochemistry and electron microscopy. The preservation of cellular ulterastructure and enzymetic activity by aldehyde fixation. J. Cell Biol.,(1963) 17:19.
20. Sehwartz, A.J.F., York, C.J. Zirbel, L.W. and Estela, L.A.: Modification of infectious bovine rhinotracheitis (IBR) virus in tissue culture and development of a vaccine. Proc. Soc. Exp. Biol. Med., (1957) 96:453.
21. Spear, P.G. and Roizman, B.: The proteins specified by herpes simplex virus. I. Time of synthesis, transfer into nuclei, and properties of proteins made in productively infected cells. Virology, (1968) 36:545.
22. Stevens, J.G.: Selective inhibition of herpes virus DNA synthesis at elevated temperature. Virology, (1966) 29:570.
23. Watson, D.H. and Wildy, P.: Some serological properties of herpes virus particles studied with the electron microscope. Virology, (1963) 21:100.
24. Watson, D.H., Wildy, P. and Russell, W.C.: Quantitative electron microscope studies on the growth of herpes virus using the techniques of negative staining and ultramicrotomy. Virology,(1964) 24:523.
25. Watt, R.G., Plowright, W., Sabo, A. and Edington, N.: A sensitive cell culture system for the virus of porcine inclusion body rhinitis (cyto megalic inclusion disease). Res. Vet. Sci., (1973) 14:119.
26. Wildy, P., Russel, W.C. and Horne, K.W.: The morphology of herpes virus. Virology (1960) 12: 204.
27. Zee, Y.C., Hackett, A.J. and Talens, L.: Vesicular stomatitis virus maturation sites in six different host cells. J. Gen. Virol. (1970) 17:95.

두 種類의 소의 Herpes Virus에 대한 微細構造變化의 比較研究

(소의 전염성비기관염 바이러스와 악성카탈열 바이러스)

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Herpes virus인 소의 전염성 비기관염 바이러스와 악성 카탈열 바이러스의 形態와 그 形成過程(形態發生)을 組織培養에서 각기 試驗하였다. 이 두 種類의 바이러스는 herpes virus의 特徵的인 構造를 가지나 그 形態發生이 相異함을 밝혔다.

1. 소의 傳染性鼻氣管炎 바이러스 (IBRV/CT3)배양에서 그 바이라스의 envelope는 細胞質膜으로부터 由來됨을 밝혔다.

2. IBRV/CT3 배양에서 envelope를 가진 바이러스는 細胞核內에는 전혀 나타나지 않았다.

3. 소의 惡性카탈熱 바이러스(MCFV/C500)의 배양에서 그 바이러스의 envelope는 細胞核膜으로부터 由來됨을 밝혔다.

4. 소의 惡性카탈熱 바이라스(MCFV/C500)에 感染된 토끼의 肺臟에서 얻은 macrophage에 대해 바이러스의 所在를 追究한바, 이 바이라스는 macrophage의 細胞質內에만 存在함을 밝혔다. 그리고 이 바이라스는 貪食된 바이라스임을 알았다.