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MORPHOLOGICAL DEVELOPMENT OF MUMPS VIRUS IN VERO CELLS

By

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SUMMARY

Sequential development of mumps virus vaccine strain in Vero cell was studied by electron microscope. The earliest alteration that could be observed inside the infected cell was the appearance of early granular inclusions within the cytoplasm. These inclusions were converted into the nucleocapsid strands which accumulated inside the cytoplasm. The process of virus maturation occured at the site of celluler membrane. These processes were deposition of viral antigens at the site of virus formation and accumulation of nucleocapsid beneath the altered membrane. At the late stage of infection some inclusion bodies appeared inside the nucleus and the virus was released by budding from the cytoplasmic membrane.

Introduction

Mumps virus as a member of paramyxoviruses is the causative agent of a disease characterized by fever and the involvement of parotids and other glands (Feldman, 1977). The virus is composed of a nucleo - protein core containing RNA which is surrounded by a lipid containing membrane (waterson and Almedia, 1966).

Morphologically mumps virus has three distinct components. The helical nucleocapsid, the envelope which surrounds the nucleocapsid in a double membraned shell and a layer of projections which cover the outer surface of the

membrane. The virus grows inside the sytoplasm of the host cell. During the multiplication it induces certain cellular lesions which have been studied in chick embryo fibroblast (Brandt 1958, Gresser & Enders, 1961) and in human conjuctiva (Walker and Hinze 1962) cells by light microscope.

Although morphogenesis of mumps virus in the host cell has been reported (Due-Ngvyen and Rosenblum 1967 , Mannweiler and Kand G. Rutter 1975), but these studies have been confined to the use of chick cell adapted virus strain in chick embryo fibroblast cells. In the present study we used a strain of mumps virus which has been adapted to grow in Vero cells. The multiplication process of this strain in Vero cells was determined by the electron microscope.

Material & Methods

Cell : Vero cells were cultivated in 8 0z. bottles. The medium used for cell growth was Eagles containing %5 CS and antibiotics maintenance media for virus cultivation was Eagles plus 0.2 % gelatine.

Strain of Biken vaccine virus (Lot No. M4-03B) obtained from Research Foundation for Microbial Disease of Osaka - University Japan was grown in monkey cells and after 3 passages in Vero cells was used for cell infection.

Infection of Cells

Vero cells in monolayer were infected with mumps virus at a multiplicity of infection of 0.1 TCID/50 per cell. The virus was allowed to adsorb at 37°C. for one hr. After the adsorption time excess virus was removed and fresh medium was added and incubated at 37°C.

Electron Microscopy

Following infection of different time intervals samples from the infected and non infected control cells were removed. The maintenance medium was decanted and the monolayer was washed with 5ml. of cold PBS (Phosphate Buffer Saline) and then fixed as monolayer in %3 glutaraldehyde in 0.1 M Phosphate buffer PH. 7. 2 as described previously (Shahrabadi and Morgante 1977).

Fixation was performed at 4°C. for two hr. then the fixed cells were scraped off the bottles by a rubber policeman and washed overnight in 0.1 M. Phosphate buffer. The cells were then fixed in %1 Osmium tetroxid and embeded in EPON 812. Sections were cut with a glass knife and stained with uranyl acetate and lead citrate and examined in EM 400 Philips electron microscope.

Preparation of Virus for Negative Staining

Monolayer of Vero cells were infected with mumps Virus. Five days after infection the medium of the infected cells was removed and centrifuged at low speed to sediment the loose cells and cell debries. The clarified supernatant was centrifuged at 25000 rpm. for 2 hr. in a Sorval model OTD-25 ultracentrifuge. The pellet was resuspended in 0.5 ml. of 0.5 M. ammonium acetate and negatively stained with phosphotungstic acid then examined in the electron microscope.

Results

Morphology of the Virus :

Mumps virus strain Biken Vaccine was partially purified and stained with phosphotungstic acid as described. Morphologically the virus appeared pleomrophic with particle size ranging from 120 to 250 nm. The virus had an envelope with distinct surface projections (Fig. 1). The envelope could be disrupted by suspending the virus particles in distilled water and the internal nucleocapsid could be released. The nucleocapsid was sedimented at 30,000 rpm. for 3 hr. Fig. 2 shows a partial purified preparation of the nucleocapsid which had herringbone characteristic with a diameter of 20 nm. similar to measles virus nucleocapsid.

Intracellular Multiplication

Samples from infected and non infected control cells were sectioned and examined by electron microscope. At O time the virus was being adsorbed to the host cells. In thin sections viruses could be seen in close association with the cellular membrane (Fig. 3). In some cells the virus appeared in the process of engolfment by the cytoplasmic membrane (Fig. 4, a, b).

The process of penetration of virus inside the host cell could not be determined. From this time on till 30 hr. after infection there was no detectable morphological alteration inside the infected cells. The first change at 30 hr. that could be observed inside the cytoplasm was the appearance of some dark staining material in the form of inclusions inside the cytoplasm (Fig. 5). At 38hr. after infection some coild tubule like structures were observed inside the cytoplasm (Fig. 6).

These structures had a diameter of about 150 A° and in cross sections appeared as electron dense circular or oval rings. The tubular structures which were viral nucleocapsid accumulated inside the cytoplasm and at 56 hr. occupied a major

portion of the cytoplasmic space (Fig. 7). By this time portions of cell membrane in some infected cells which had an electron dense appearance increased in thickness and some material deposited on the outer surface of the plasma membrane (Fig. 8). Some tubule like structures aligned parallel to the cell membrane and appeared beneath the electron dense area. (Fig. 9). The tubules in cross sections showed dense circular structures and some appeared as loosely interwoven filaments. From 56 to 74 hr. after infection the tubules aggregated and became loosely packed under the altered portion of plasma membrane. Virus particles started to release by budding and several buds in various stages of maturation could be seen at the surface of some infected cells, (Fig. 10).

It seemed that the process of virus maturation was not confined to budding, exceptionally some apparantly matured particles appeared inside the cytoplasm of some infected cells. (Fig. 11).

From 74 hr. to 115 hr. virus particles started to release from the infected cells and in thin sections several particles could be observed adjacent to the cell membrane. The nucleus remained apparently unchanged until 115 hr. after infection. At this time some inclusions composing of densely packed dark staining filements and some light staining inclusion were formed inside the nucleus of some infected cells. (Fig. 12). From this time on the cytoplasm appeared disrupted and many particles were in process of release.

Discussion

It has been reported that alteration induced in mumps virus infected cells and accumulation of nucleocapsides inside the cytoplasm varies with the strain of virus used and the type of cell infected (Due Nguyer and Rosebium 1967). In this study the strain of Biken vaccine virus was used to infect Vero cells. At the time of adsorption some virus particles were observed in close association with the cell membrane. The entery of virus into the cell was not clear. It seems that like other enveloped viruses the viral enveloped fuses with the cell membrane and subsequently part of viral envelope is removed and nucleocapside is librated inside the cytoplasm. The first alteration was observed in the cytoplasm at 30 hr. Post infection in the form of aggregation of granular material. These materials did not have tubular nucleocapsid structures but possibly they were some material which later on converted to nucleocapsid structures. In other virus cell system such as chick embry cells infected with Ricki strain of mumps virus (Dur-Nguyen and Rosenblum 1967). these inclusions were not observed prior to the appearance of nucleocapsid tubules. Formation of cytoplasmic nucleo-capsid and the alteration of cellular membrane occured within a certain period of time. The membrane change was limited to certain area of the cell surface and it seemed that the entire cell membrane was not altered as what was observed in influenza virus

infected cells. (Due-Nguyer et al 1966). It has been shown that (Rutter and Mannweiler 1973) the morphologically altered sites of the plasma membrane consist of viral surface spikes and viral antigens which form virus envelope.

These areas are the site of viral assembly where some nucleocapsids accumulate and the outfolding membrane surrounds the nucleocapsids and finally is released by budding process. Similar alternation have been found with parainfluenza type 1 virus grown in KB cells (Berkaloff 1963, Colbert & Berkaloff 1964).

Formation of intranuclear inclusions have been observed in chick embryo fibroblastic cells infected with the Ricki strain of mumps virus (Due- Nguyen et al 1966). The inclusions, present in Vero cells were morphologically different from that was observed in chick cells . These inclusions appeared at late stage of infection and their role in virus replication is not clear.

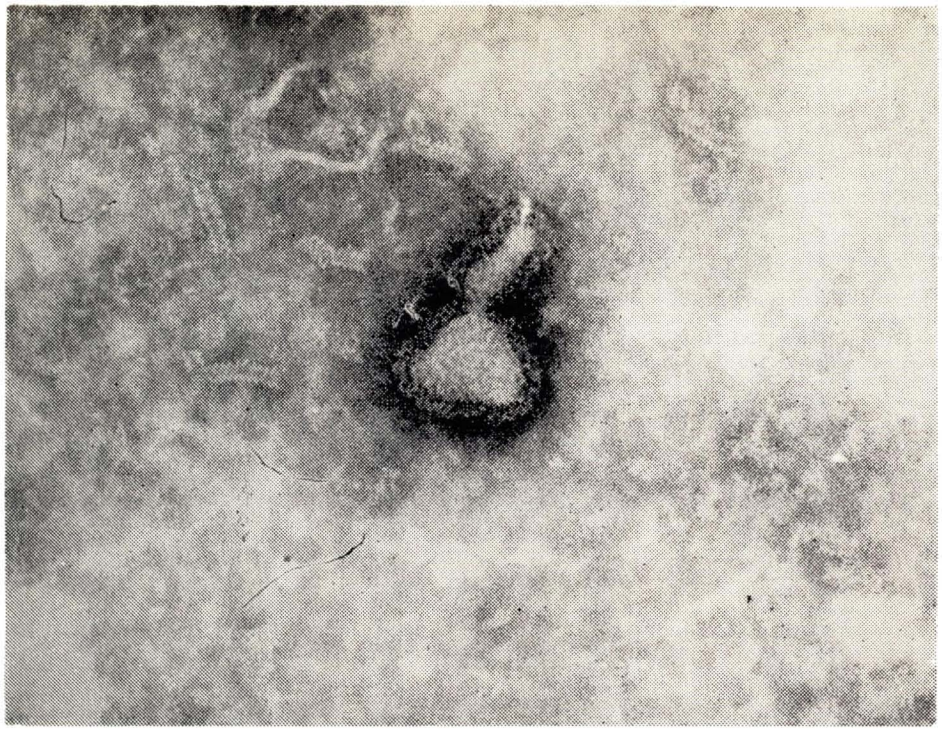

Fig. (1) Mumps virus partially purified and negatively stained with phosphotungstic acid. The virus is enveloped with surface projections. X 100000

Fig. (2) Preparation of mumps virus nucleocapsid extracted from the virions X 150000

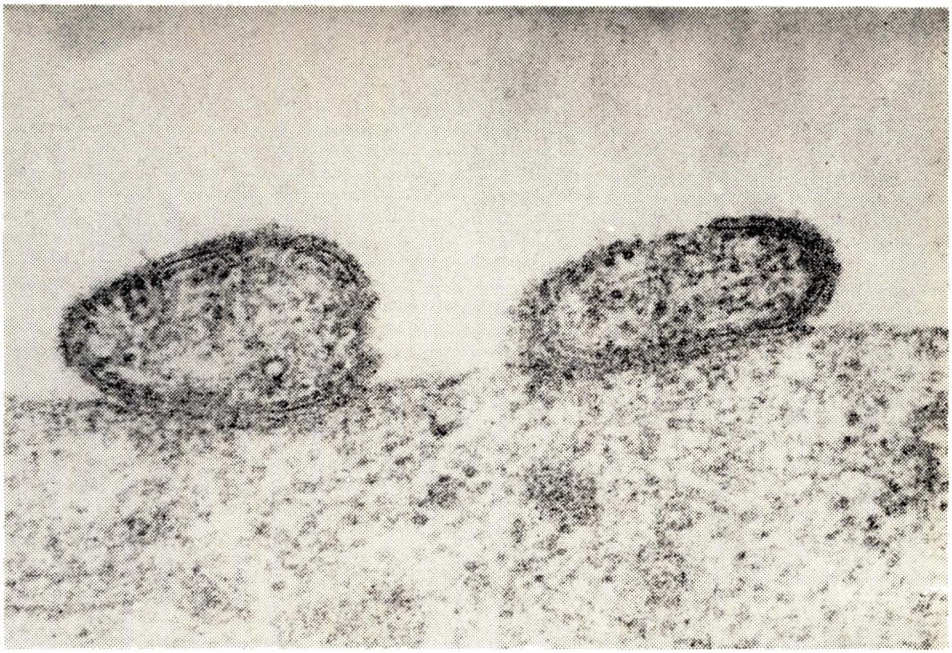

Fig. (3) Thin section of cell at 0 time infected with mumps virus. The virus is adsorbed to the surface of the cell X 130000

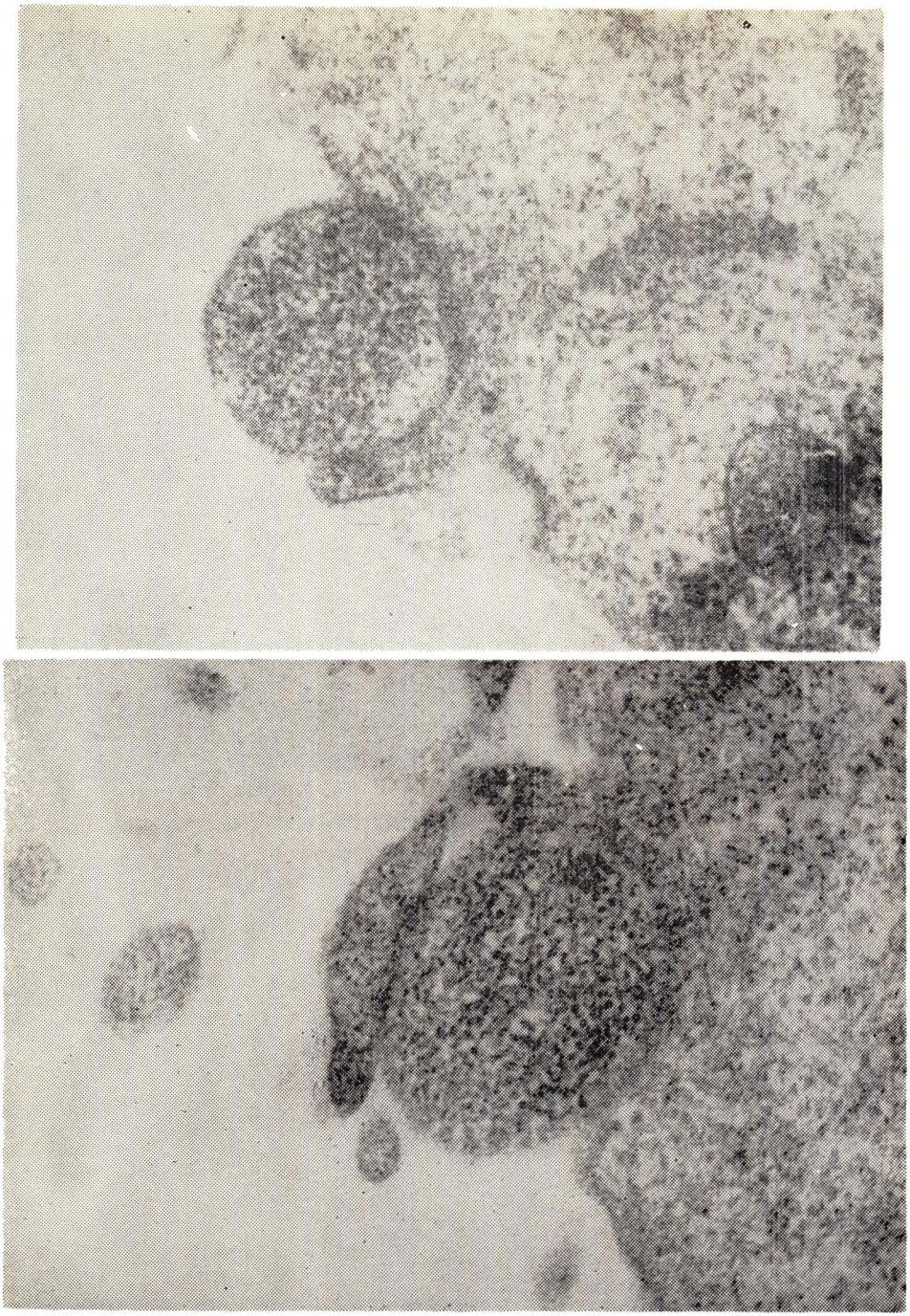

Fig. (4 a,b) Thin section of a cell during the first hr. of infection showing the engolfment of a virus particle X 130000

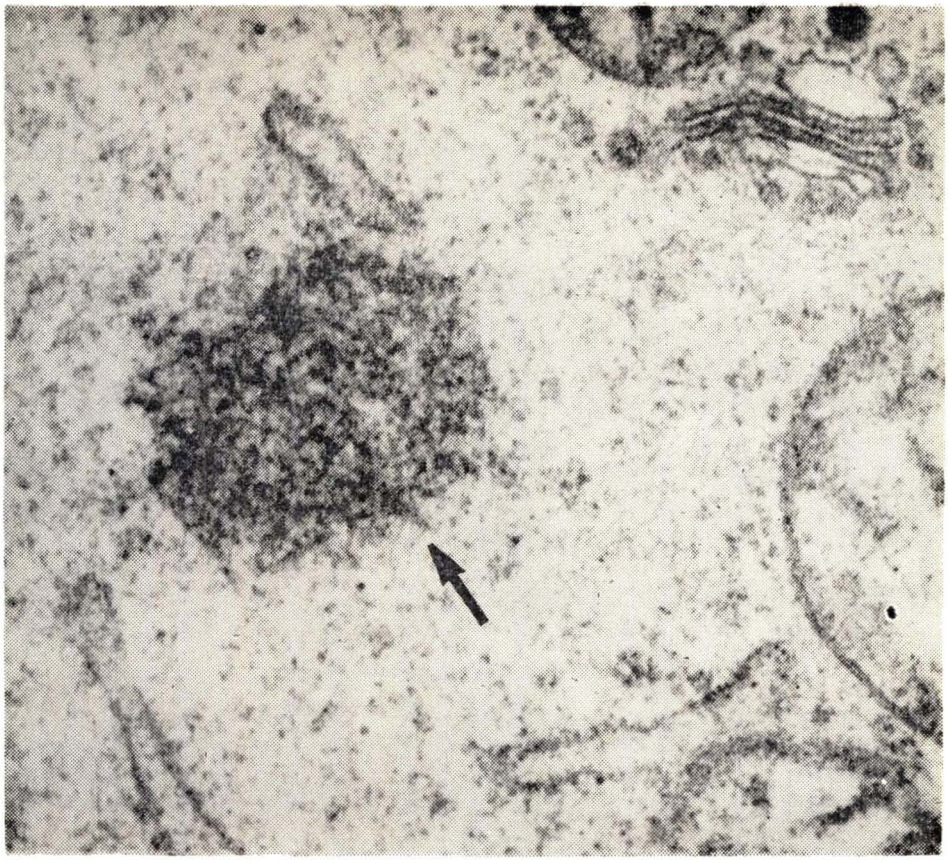

Fig. (5) Thin section of a cell 30 hr. after infection showing inclusion bodies at the early stage of infection (Arrow) X 60000

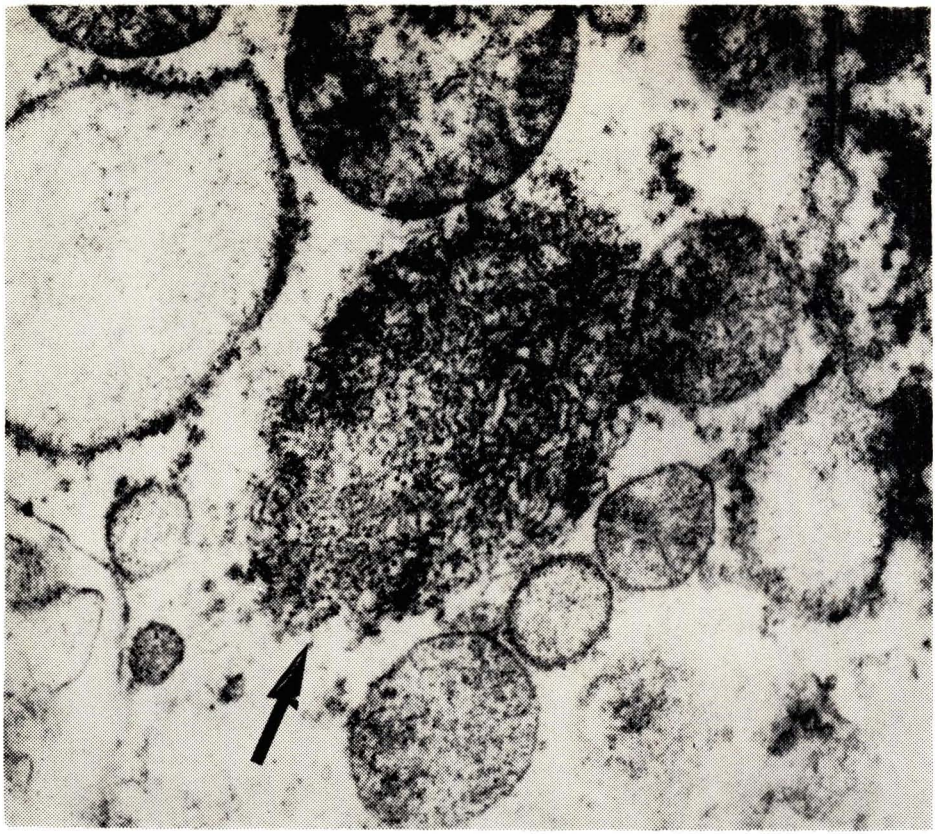

Fig. (6) Thin section of 38 hr. infected cell showing the appearance of nucleocapsid strands inside the cytoplasm (Arrow) X 120000

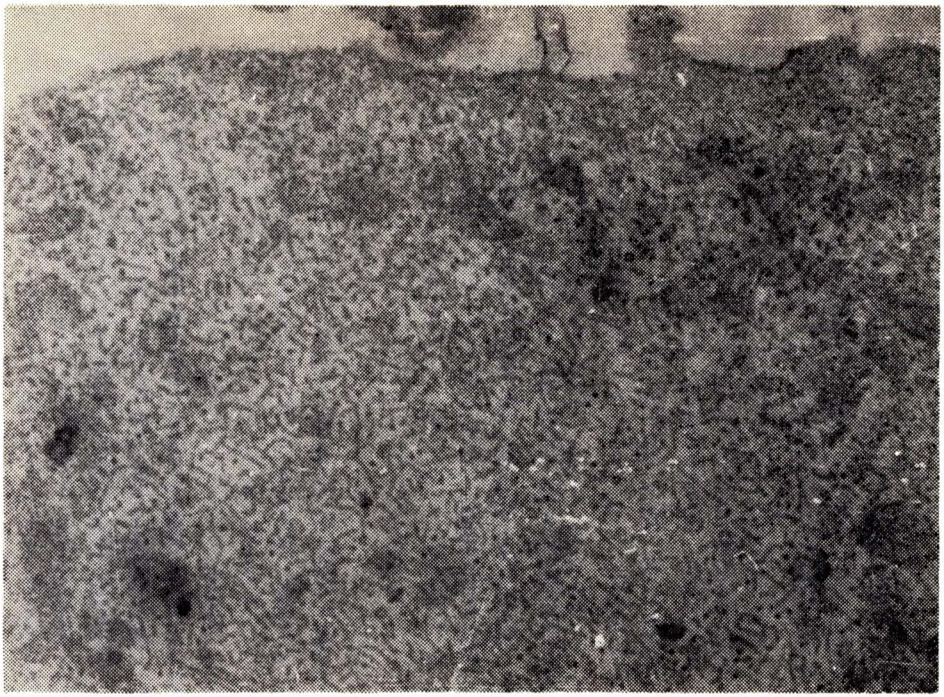

Fig. (7) Thin section of 56 hr. infected cell showing accumulation of nucleocapsid inside the cytoplasm X 130000

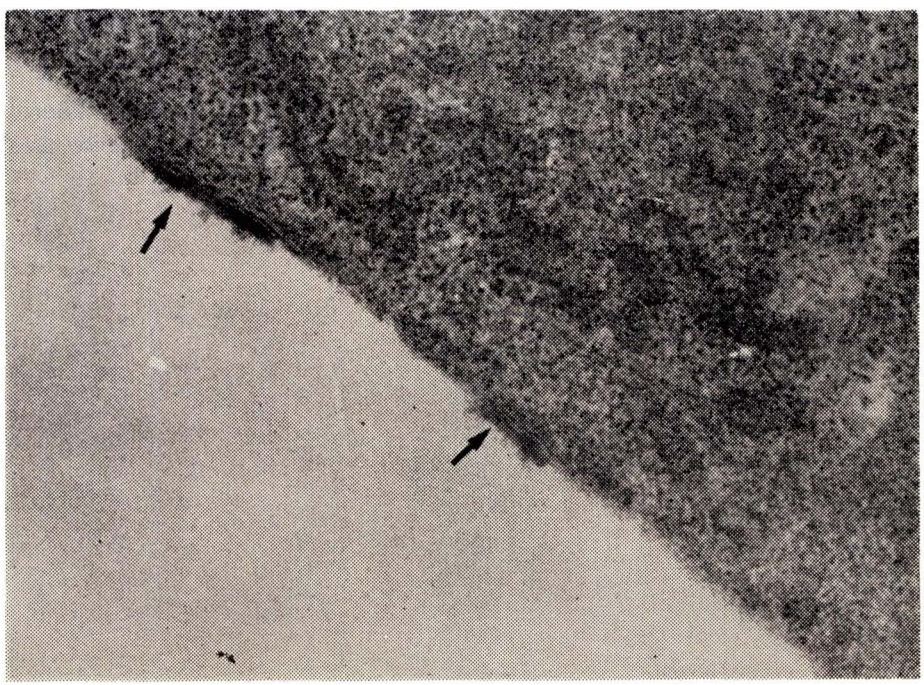

Fig. (8) Thin section of 46 hr. infected cell. Some areas of the cytoplasmic membrane has increased in thickness resulting from the deposition of viral antigens (Arrow) X 130000

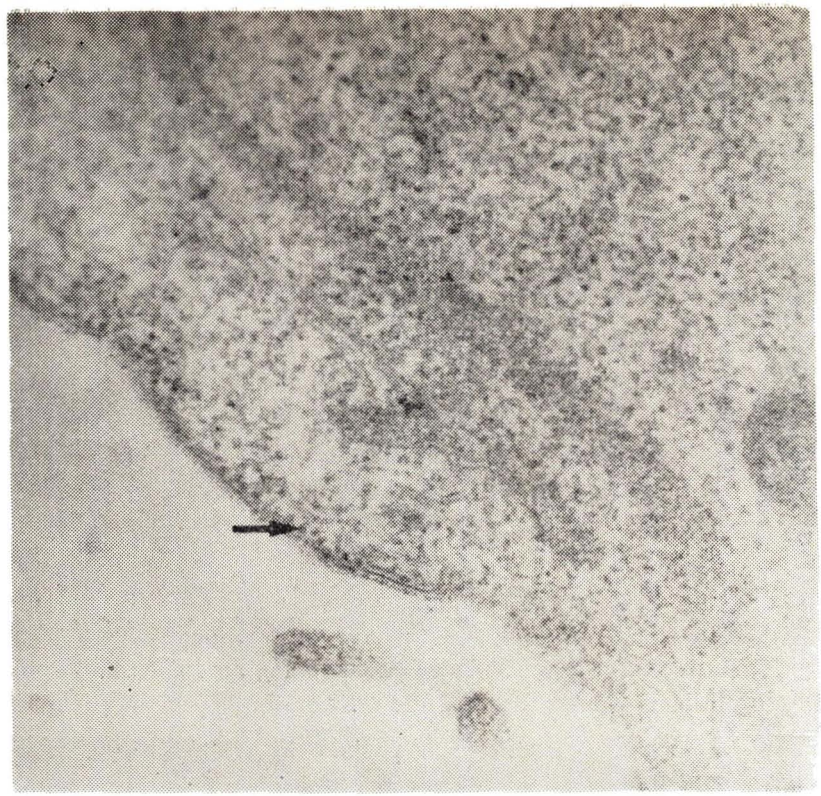

Fig. (9) Similar infected cell as in fig. 7 portion of the cell membrane is altered and viral nucleocapsids appeared beneath the electron dense area (Arrow) X 80000

Fig. (10) Thin section of 74 hr. infected cell showing several buds of virus particles in process of maturation X 80000

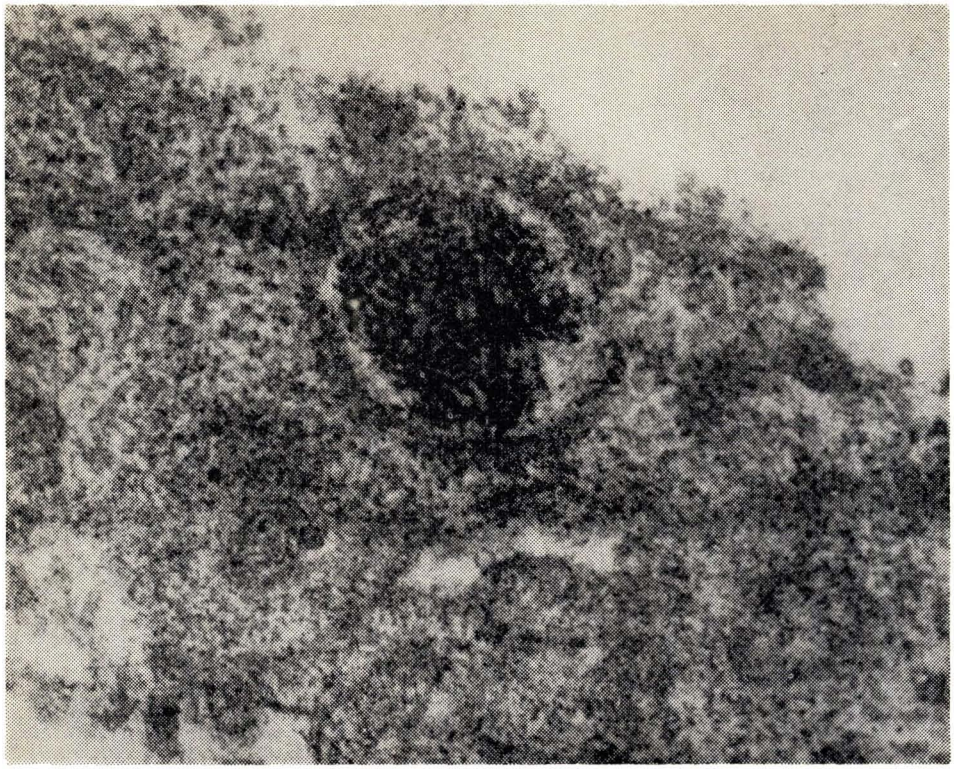

Fig. (11) Similar cell as in fig 10 showing an apparently matured virus particle inside the cytoplasm X 80000

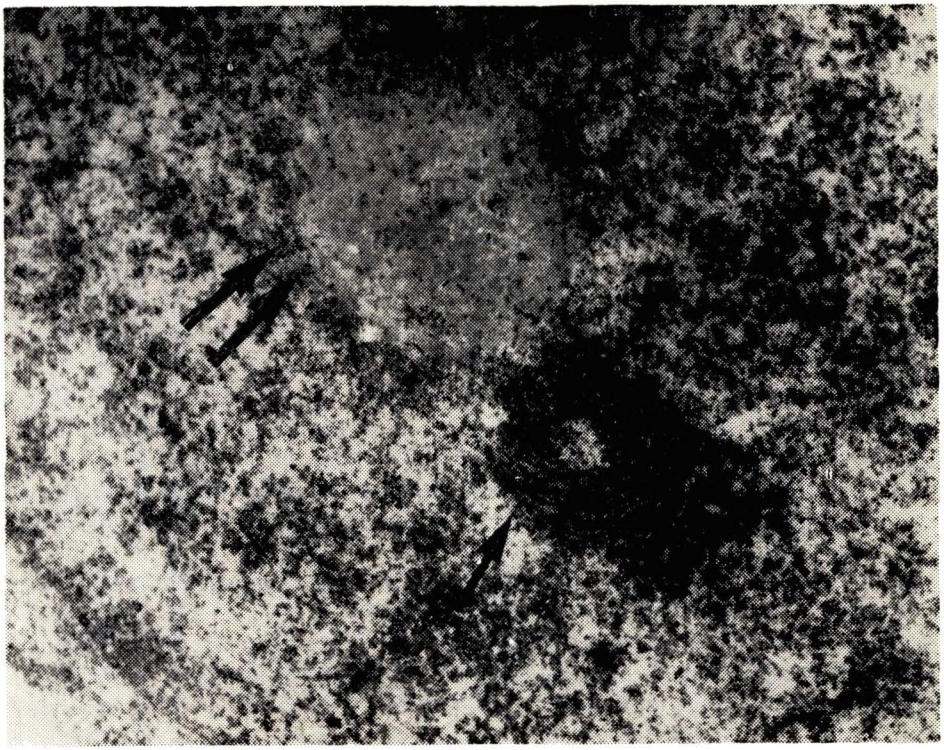

Fig. (12) Thin section of 115 hr. infected cells. A dark staining filementous (single arrow) and a light staining inclusion (Double arrows) are seen inside the nucleus X 100000

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