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ELECTRON MICROSCOPIC STUDIES ON EQUINE ENCEPHALOSIS VIRUS

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ABSTRACT

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Electron microscopic studies carried out on thin sections of BHK 21 cells infected with equine encephalosis virus (EEV) have revealed a fine cytopathology closely resembling that shown by bluetongue and African horsesickness viruses. The characteristics include the presence of granular inclusion bodies from which mature virus particles appear to arise, swelling of the rough endoplasmic reticulum and the presence of inclusion bodies in the mitochondrial matrix. The diameter of the sectioned virus particle is approximately 73 nm, and 60 nm when negatively stained. The experimental observations suggest that the capsid is composed of 32 morphological subunits.

INTRODUCTION

The presence of granular inclusion bodies containing maturing virus particles has been reported in cells infected with bluetongue virus (Bowne & Jochim, 1967; Lecatsas, 1968a), African horsesickness virus (Lecatsas & Erasmus, 1967) and lately in a new virus, XBM/67 (Lecatsas & Erasmus, unpublished observations). Other cytopathic characteristics common to these viruses include swelling of the rough endoplasmic reticulum (RER) and changes in the matrix of the mitochondria. BHK cells infected with bluetongue virus show stacks of tubular elements in the cytoplasm as do BHK cells infected with XBM/67 virus, while BHK cells infected with African horsesickness virus show the presence of thick filaments in the cytoplasm. The fine structure studies described in the present work were carried out in order to characterize equine encephalosis virus (EEV) (Erasmus, Adelaar, Smit, Lecatsas & Toms, in press) structurally. In addition, the fine cytopathology of BHK cells infected with the virus is compared with that produced by other viruses of the bluetongue type.

MATERIALS AND METHODS

Virus and cells

BHK-adapted EEV was seeded onto roller tubes of BHK 21/C13 cells in modified Eagle's medium supplemented with 5% foetal calf serum. Details of the isolation of the virus are supplied in our initial publication (Erasmus *et al.*, in press).

Electron microscopy

Ultramicrotomy: After infection with EEV, cell monolayers were harvested at 15 minutes and at 2, 12, 20, 28, 36, 44 and 48 hours. The suspensions were centrifuged at 300 *g* for 5 minutes, the supernatant drained off and the pellet of cells resuspended in 1% OsO_4 (Millonig, 1961). Fixation was allowed to proceed for 1 hour. Pieces of the pellet were embedded in Epon 812 resin (Luft, 1961) and the final blocks sectioned with glass knives. Sections were double-stained with 5% uranyl acetate for 45 minutes and lead citrate for 3 minutes (Reynolds, 1963). A Nikon model 6C comparator was used to determine the approximate diameter of the virus particle from negatives.

Negative staining: BHK cells were suspended in the growth medium by scraping them from the roller tube and centrifuging them at low speed. The supernatant was discarded and the cells resuspended in a few drops of distilled water. A drop of this suspension was added to a drop of 2% phosphotungstic acid (pH 6,5) and a formvar-carbon-coated grid placed on the mixture. The excess fluid was drained by means of filter paper. Grids were examined in a Siemens Elmiskop 1A electron microscope employing a double condenser illumination and operated at 80 KV. Micrographs were taken at an instrumental magnification of 40 000.

RESULTS

Attachment and penetration of the cell by virus occur soon after inoculation and at 2 hours the Golgi complex is abundantly dispersed in the perinuclear area. The cytoplasm shows masses of fine filaments but relatively few polysomes. At 12 hours the polysomes increase markedly in number, nucleoli become prominent and myelin figures are occasionally found. At 20 hours some mitochondria show dense inclusion bodies. At 28 hours the progeny particles (Fig. 1) become apparent in limited numbers, the RER appears swollen while the polysome content of the cells remains high. These characteristics are retained in 36, 44 and 48 hour cells except that the presence of granular inclusion bodies (Fig. 2) becomes evident, usually in the perinuclear area, where the Golgi apparatus is commonly situated. These granular inclusion bodies are irregular in shape, are not bounded by a membrane and contain apparently maturing virus particles embedded in their matrix. Mature particles sometimes possess fine strands attached to their cores (Fig. 3) but such strands are not seen in negative contrast (Fig. 4). The presence of electron-dense mitochondrial inclusion bodies is indicated in Fig. 5. The occasional association of the inclusions with fine tubules is also found. The tubules are demonstrated in both positive and negative contrast (Fig. 6 and 7).

Aggregations of virus particles occur in the cytoplasmic matrix and resemble large crystals. The liberation of virus particles can occur singly, or in groups if the cell membrane ruptures. It is noteworthy that particles with incomplete cores are likely to show attached fine threads, while those with full cores show no attached threads. Sectioned particles suggest a diameter of about 73 nm, while negatively stained particles have a diameter of 60 nm and suggest a low capsomere number. Enveloped particles were rarely found.

DISCUSSION

The gross fine cytopathology of BHK cells infected with EEV is very similar to that shown by infection with bluetongue virus, African horsesickness virus and XBM/67 virus. The swelling of the RER appears to be a basic prerequisite or consequence of the process of virus multiplication and may well be involved in the passage of virus protein from the ribosome to a specific

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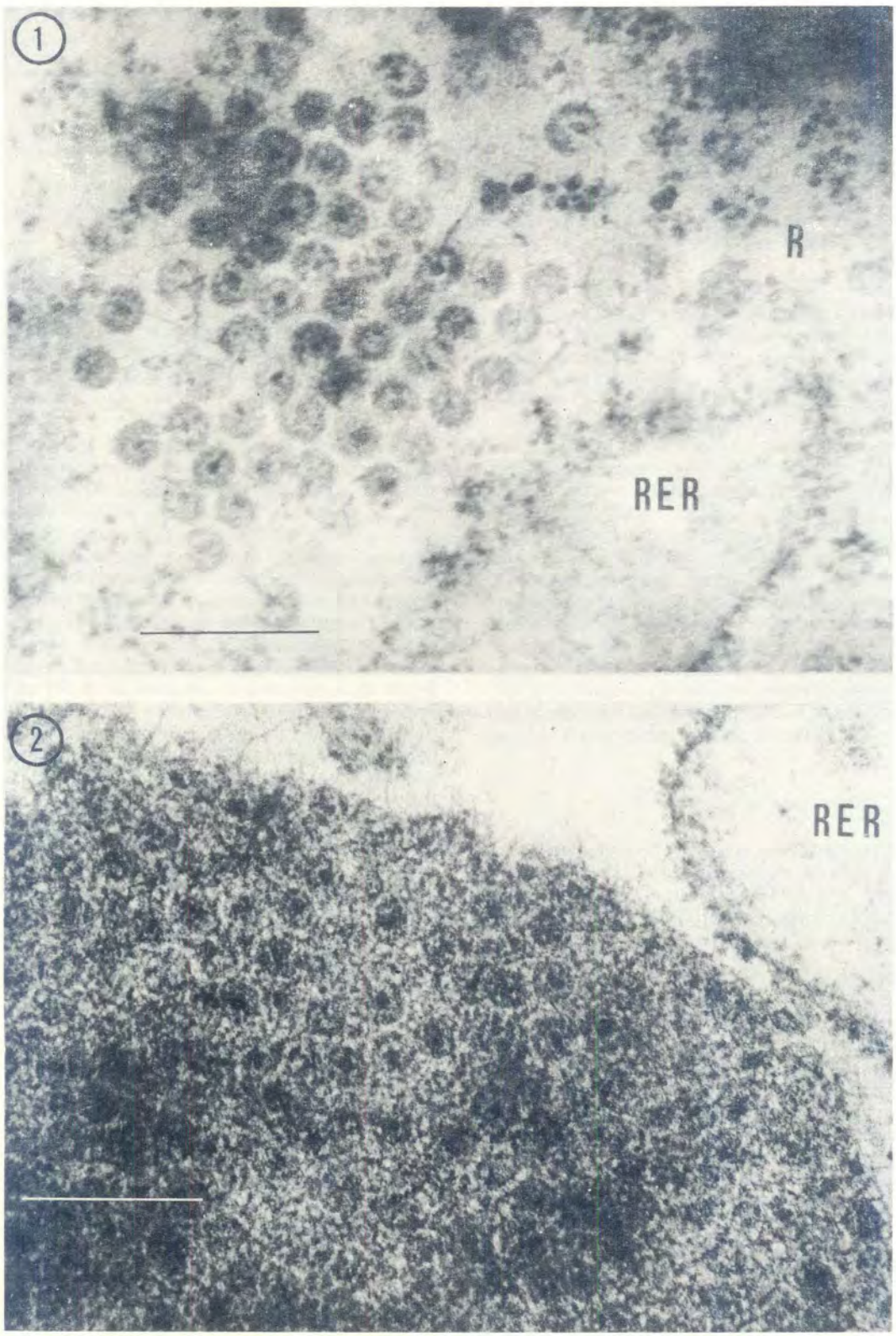
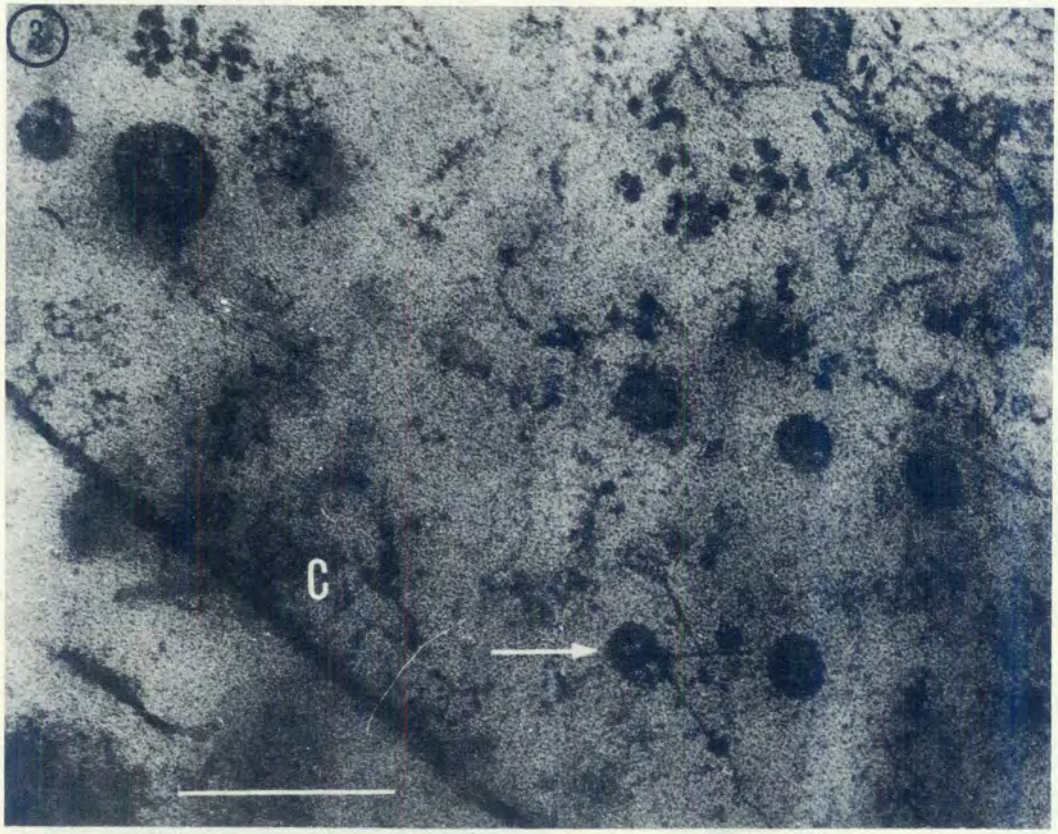


FIG. 1 Group of virus particles in cytoplasmic matrix of BHK cell. Particles appear to lie in crystalline array and some have dense cores while others have apparently incomplete cores with attached fine strands. Swollen, rough endoplasmic reticulum (RER), ribosomes (R). Bar equals 250 nm, 48 hour cell.

FIG. 2 Large, crystalline-like aggregation of virus particles in cytoplasmic matrix. Rough endoplasmic reticulum (RER). Bar equals 250 nm, 48 hour cell.



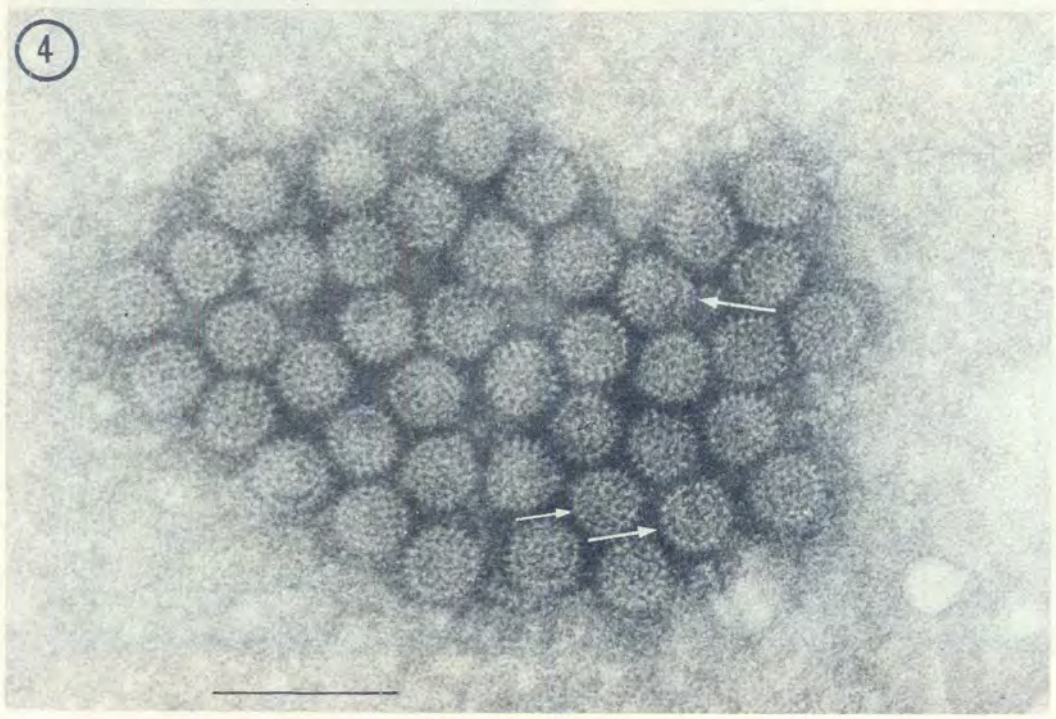


FIG. 3 Virus particles in cytoplasm near cell membrane (C). Arrowed particle has attached fine thread and relatively small core. Note group of filaments in upper right hand area of photograph. Bar equals 250 nm, 48 hour cell.

FIG. 4 Virus particles negatively stained with phosphotungstic acid. Arrowed particles suggest a low capsomere number. Bar equals 125 nm.

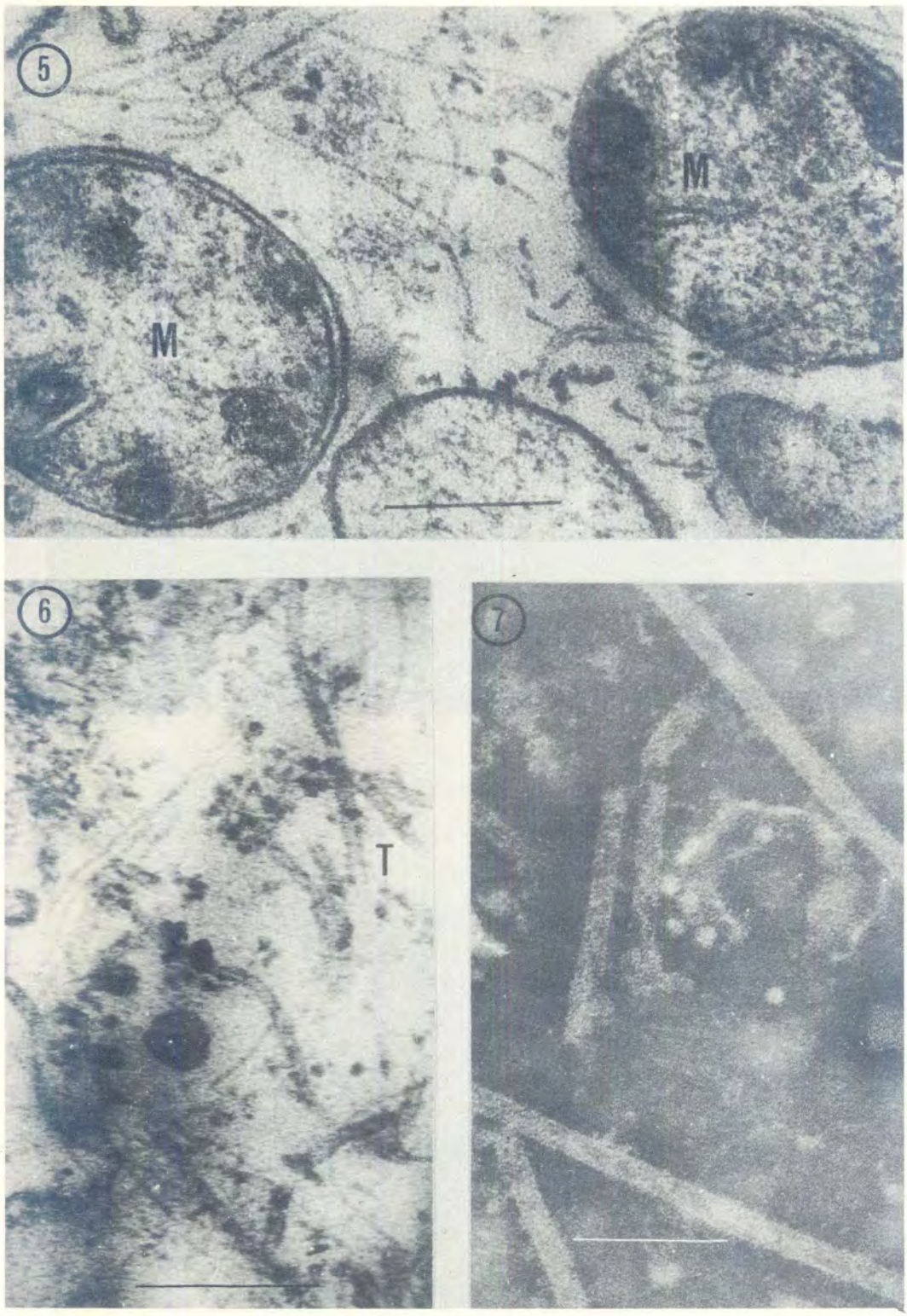


FIG. 5 Two mitochondria (M) lying in cytoplasm and showing dense inclusions in their matrix. Bar equals 250 nm, 48 hour cell.

FIG. 6 Tubular structures (T) in the cytoplasmic matrix of an EEV-infected cell. Bar equals 250 nm.

FIG. 7 Tubular structures negatively stained with phosphotungstic acid. The tubules do not appear to be composed of capsomeres but may represent aggregations of structural units. Bar equals 125 nm.

area for incorporation into the virion. In this respect, the Golgi complex appears to be actively involved in some way with the eventual appearance of complete virus particles. The common location of Golgi apparatus and viral inclusion bodies and viral crystals in the perinuclear area, coupled with the contention that the Golgi complex in some way mediates the incorporation of new protein into its eventual form, appears to enhance this possibility.

The significance of the increase in fine filaments is obscure as is the appearance of inclusion bodies in the mitochondrial matrix. The latter phenomenon would appear to be a side effect of virus infection and not a prerequisite for virus multiplication.

Prominent nucleoli in BHK cells infected with African horsesickness virus have been reported by us and their appearance in the present study suggests a common metabolic involvement.

The association of spindle tubules with the virus does not resemble reovirus-infected cells (Dales, 1963; Lecatsas, 1968b), where virus particles appear to align themselves along the length of the tubule. The tubules shown in the present study do not appear to be rigid structures.

The granular inclusion bodies are significant in that they suggest a common method of formation of the different viruses. Recent unpublished work in our laboratory shows that ^{3}H-uridine is incorporated into these bodies in actinomycin D-treated cells infected with bluetongue virus. It would appear, therefore, that the granular inclusion bodies contain viral cores around which the protein capsid is assembled, hence the apparently incomplete capsids of viruses embedded in these inclusion bodies.

The arrangement of EEV particles in crystalline array is striking and has occasionally been noted in our studies with bluetongue virus. The appearance of crystals of virus particles may be dependent on the concentration of virus in the cell, although they do appear to be characteristic of bluetongue, horsesickness and EEV viruses.

The incompleteness of the virus particles shown here is of interest since this phenomenon has been noted in bluetongue-infected BHK cells (Lecatsas, 1968a). This characteristic may be associated in some way with the cell type used in growing the virus.

Negatively stained EEV particles closely resemble those of African horsesickness virus (Oellermann, Els & Erasmus, 1970). Our preliminary investigations suggest strongly that the EEV capsid consists of 32 hexamer-pentamer morphological subunits with icosahedral symmetry, or a dimer or trimer arrangement of the capsomeres. In negative contrast EEV resembles Colorado tick fever virus with respect to the ring visible at the base of the capsid. (Murphy, Coleman, Harrison & Gray, 1968.)

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