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ELECTRON MICROSCOPIC STUDIES ON REOVIRUS TYPE 1 IN BHK 21 CELLS

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INTRODUCTION

Studies on the morphological development of all three types of reovirus have been carried out by a number of workers (Tournier & Plissier, 1960; Gomatos, Tamm, Dales & Franklin, 1962; Harford, Hamlin, Middelkamp & Briggs, 1962, Rhim, Jordan & Mayor, 1962; Dales, 1963; Dales, Gomatos & Hsu, 1965; Mayor & Jordan, 1965; Anderson & Doane, 1966).

The crystalline appearance of reovirus aggregates in culture cells seems to be well established as is the association of the virus with spindle tubules. Pleomorphism of the nucleoid has been reported by Harford *et al.* (1962). These workers also reported coreless particles with indications of a double layered capsid. Coreless particles in culture cells have also been reported by Anderson & Doane (1966) while Mayor & Jordan (1965) have suggested a double layered capsid, an observation supported by Mayor, Jamison, Jordan & Van Mitchell (1965) and Müller, Schneider & Peters (1966).

MATERIALS AND METHODS

Virus and cells

BHK 21/13 cells grown in 500 ml roller bottles in Eagles medium were used in the study. Cells were inoculated with reovirus Type 1, "Lang " strain, at a multiplicity of 5 to 10 pfu per cell.

Electron microscopy

After infection with the virus, cells were harvested after 5 minutes, 2, 7, 16, 20 and 24 hours. Before harvesting, cells were washed with fresh Eagles medium to remove cell debris left from the virus suspension. Approximately $1x10^8$ attached cells were freed from the surface of roller bottles by vigorous, manual shaking. The suspensions were centrifuged at 300 g for 5 minutes and pellets of cells obtained in this way were fixed for one hour in 1 per cent buffered solution of osmium tetroxide (Millonig, 1961) and sectioned with glass knives on a Porter Blum MT—2 ultramicrotome. Sections were double stained with 5 per cent uranyl acetate for 45 minutes followed by lead citrate for 15 minutes (Reynolds, 1963). A Siemens Elmiskop 1A electron microscope was used in the investigation.

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Results

The uninfected BHK cell maintained in our laboratory possesses large, lobed nuclei, a well developed rough endoplasmic reticulum (ER), numerous polysomes and clusters of fine filaments in the cytoplasmic matrix, a few mitochondria and a fairly poorly developed Golgi complex.

At two hours the infected cell is characterized by incipient swelling of the rough ER to form cisternae lined by ribosomes. The mitochondria often contain dense inclusion bodies similar to those reported in studies on African horsesickness virus and bluetongue virus. Myelin figures are commonly found in the cytoplasm and fine filaments do not increase significantly at this stage.

Mature progeny particles appear at 16 hours for the first time. They occur in crystalline array and are often found in association with spindle tubules as reported by previous workers [Plate 1 (1 and 2)].

Fine filaments and " kinky " filaments (Dales *et al.*, 1965) are found in profusion at this stage while the Golgi complex is also prominent.

Pleomorphism of the nucleoids of progeny particles is common, many particles showing two nucleoids and a few showing more than two [Plate 1(1)].

Fine filaments are often observed to be attached to the cores of the progeny particles, especially those with small cores [Plate 2 (3 and 4)].

Groups of particles lacking a core are also commonly encountered [Plate 3 (5 and 6)]. Within these groups are found some particles with cores of varying sizes.

Discussion

The swelling of the rough ER to form cisternae has been observed in BHK 21 cells infected with bluetongue virus or African horsesickness virus (Lecatsas, 1967; Lecatsas & Erasmus, 1967). This suggests that it may represent a general cell reaction to infection by different viruses. While mitochondria with inclusion bodies have also been noted in bluetongue virus and African horsesickness virus studies using BHK 21 cells, myelin figures, commonly found in the present work, have not been noted in cells infected with bluetongue virus.

Mature progeny particles appear in crystalline form in 16 hour cells and lie in the cytoplasmic matrix. The groups are often large, usually a number of groups appearing in a single section of a cell.

The increase in amount of fine and " kinky " filaments at this stage is unexplained but the increase in the number of Golgi zones may suggest some correlation with the condensation of capsid protein.

The presence of more than one nucleoid per virus particle seems to be conclusively shown and supports the observations of Harford *et al.* (1962). Those particles showing only one nucleoid presumably do so due to superimposition of one nucleoid on the other, Similar observations have been made in attenuated strains of bluetongue virus in BHK 21 cells (unpublished work).

The attachment of fine filaments to the cores of mature progeny particles appears to be well established from the photographs and is further supported by the fact that similar observations have been made in bluetongue-infected BHK 21 cells (Lecatsas, 1967). Three possible explanations can be advanced to account for this phenomenon. First, the RNA moiety may remain in a partly uncoiled form at the time of condensation of capsid protein around the nucleic acid. Secondly, the RNA moiety may be incompletely inserted into a preformed capsid. In this respect Smith & Hills (1962) and Xeros (1964) have made similar observations in the *Tipula* iridescent virus. Thirdly, the nucleic acid moiety may leak out of the formed virus particle as a result of abnormal assembly of the particle. It appears that a central scaffold is not necessary for the assembly of the protein capsid (Kellenberger, 1966). This contention supports the first and second possibilities cited here. The presence of a double layered capsid appears to make the second and third possibilities less likely.

Electron microscopic autoradiographic studies employing tritiated uridine are in progress in an effort to identify the chemical nature of the attached filaments. At present a minimum grain size of 200 Å can be expected in autoradiographic methods employing the presently available photographic emulsions. Since the diameter of the attached filaments is in the region of 30 Å, they are unlikely to be identified by this method. Digestion of the filaments with ribonuclease is also being attempted but this method is of little use if the filaments represent double stranded RNA. In addition, OsO_4 fixation of cells and tissues would not appear to favour the action of ribonuclease owing to its firm attachment to lipoprotein and nucleoprotein structures in the cell. Fixation with aldehyde fixatives only, on the other hand, would reduce contrast so markedly that 30 Å filaments are not likely to be visible, even with adequate double staining after fixation.

However, since the core of icosahedral viruses is generally accepted to contain the nucleic acid moiety of the virus particle, it seems very likely that filaments attached to apparently incomplete cores of virus particles represent a form of the nucleic acid of the particle.

Since the phenomenon of attached filaments has also been noted in bluetongue virus-infected BHK 21 cells, it may occur in a number of spherical RNA viruses. Alternatively, BHK 21 cells may be instrumental in producing incomplete progeny particles of various viruses with respect to the nucleic acid content.

Summary

BHK 21 cells infected with reovirus Type 1 were sampled at various stages during a single cycle of virus replication, sectioned and studied with the electron microscope. Attention is drawn to cytopathic characteristics shown by the infected cell which are absent from uninfected cells.

On the basis of similarities with bluetongue virus it is suggested that the nucleic acid moiety of the virus particles may be incompletely inserted in the capsid. This may be due to incomplete coiling up of the nucleic acid before condensation of the capsid around the nucleic acid or incomplete packing in of the nucleic acid into a preformed capsid or possible leaking out of the nucleic acid from the formed virus particle.

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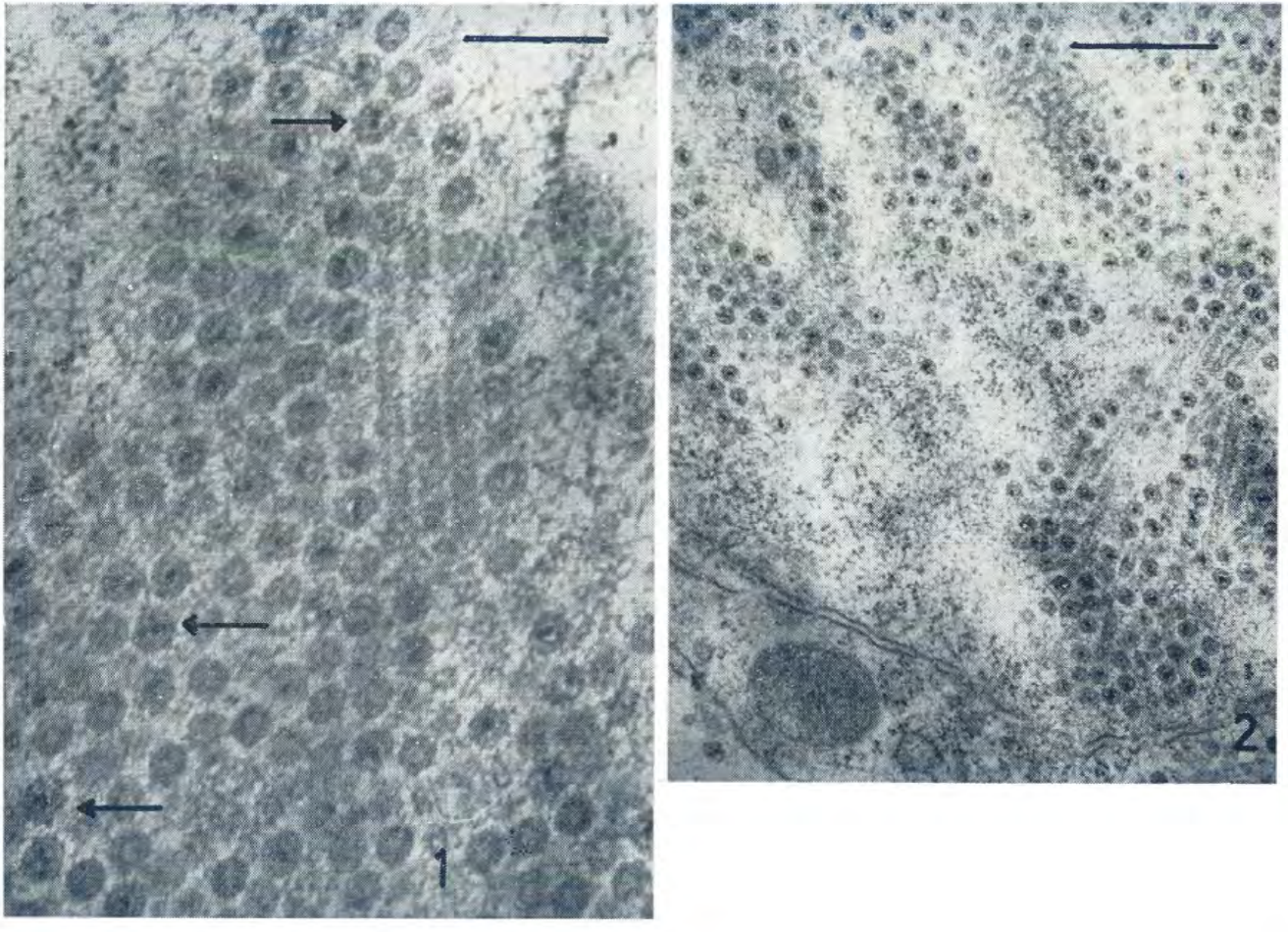


PLATE 1

1. Reovirus particles in crystalline array associated with spindle tubules. Arrows indicate particles with more than one nucleoid. Empty particles are also evident. Final magnification x80,000. Bar equals 250 mμ.
2. Crystalline array of reovirus particles and association with spindle tubules. Final magnification x40,000. Bar equals 500 mμ.

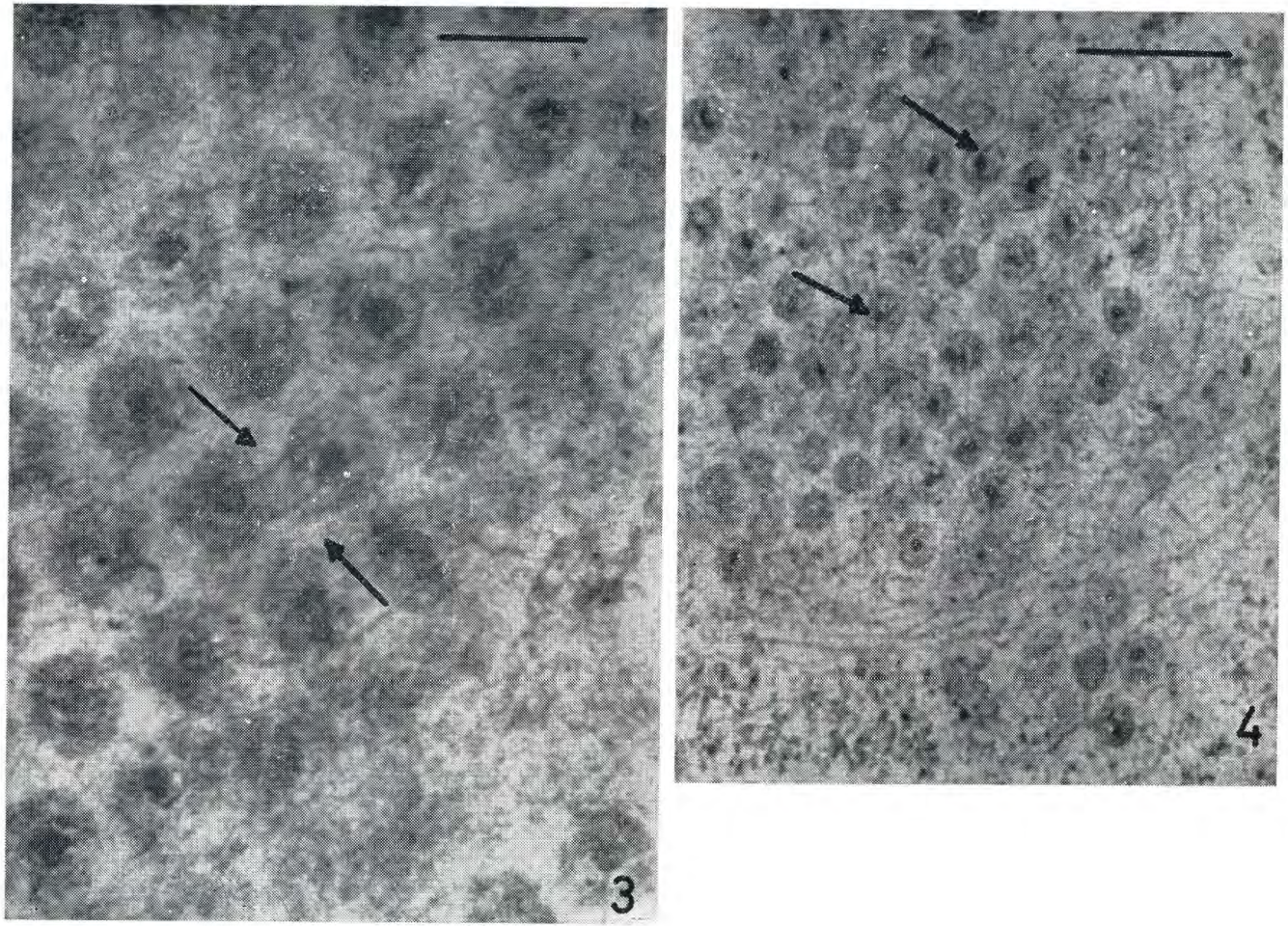
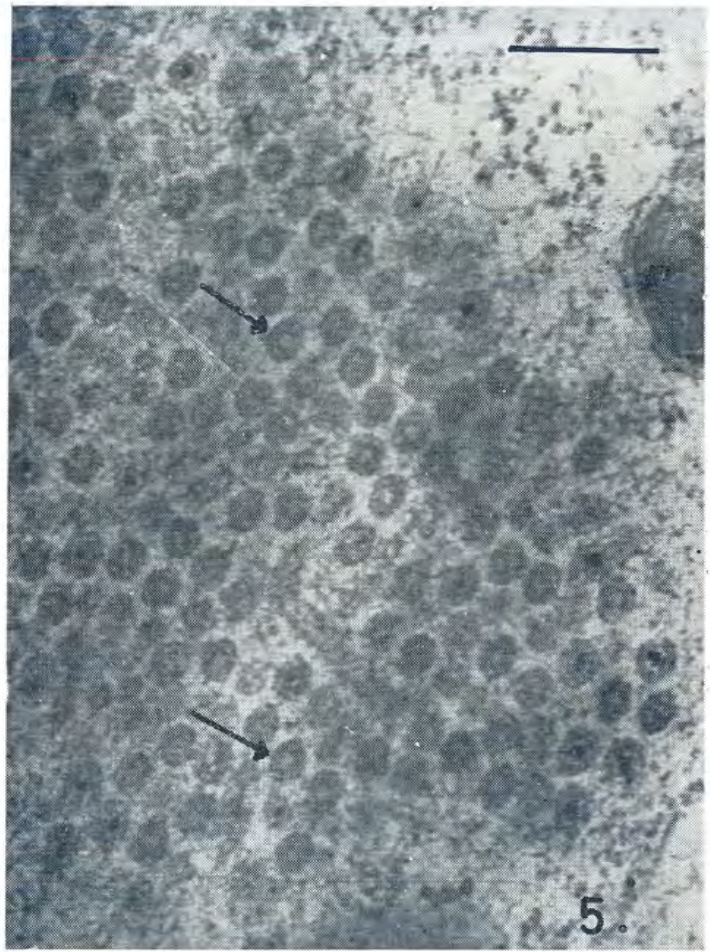


PLATE 2

3. Fine filaments attached to the nucleoids of two particles (arrows). Other particles in figure show suggestions of such attached filaments. Final magnification x160,000. Bar equals 100 mμ.

4. Reovirus particles showing fine filaments attached to nucleoids (arrows). Final magnification x80,000. Bar equals 250 mμ.



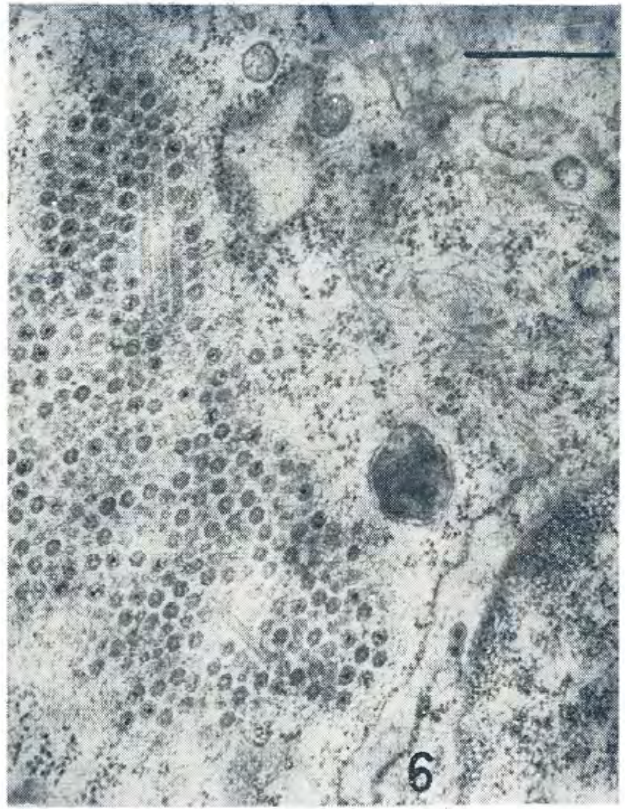


PLATE 3

5. Group of reovirus particles devoid of nucleoids (arrows). A few particles in the group contain nucleoids. Final magnification x80,000. Bar equals 250 mμ.

6. Group of reovirus particles mostly devoid of nucleoids but some showing dense centres. Final magnification x40,000. Bar equals 500 mμ.