Onderstepoort J. vet. Res. (1969), 36 (2), 321-324.
Printed in the Republic of South Africa by T. W. Hayne Ltd. (H. & G.), Johannesburg for the Government Printer, Pretoria

RESEARCH NOTE

ELECTRON MICROSCOPIC STUDIES ON CORRIPARTA VIRUS

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Corriparta virus, an unclassified arbovirus isolated in Australia from *Culex annulirostris* (Doherty, Carley, Mackerras & Marks, 1963), has been likened in its structure to reovirus (Carley, 1967). It is relatively ether-sensitive compared to reovirus, while its size has been given as 67 mμ.

The present investigation was carried out in order to compare the virus with bluetongue virus (BTV) and African horsesickness virus (AHSV) with respect to size, structure and fine cytopathology and to investigate further its relationship to reovirus.

BHK_{21}/C13 cells grown in Eagle's medium supplemented with 10 per cent normal bovine serum were used in the investigation. The fifth suckling mouse brain passage of the MRM 1 strain of Corriparta virus was adapted to BHK_{21} C13 cells. After three passages, the virus was seeded onto roller tubes and the cells harvested and processed for electron microscopy.

Our methods of preparation of thin sections for electron microscopy have been described previously (Lecatsas & Weiss, 1969). Cells used in negative staining preparations were ruptured in distilled water and one drop of this suspension mixed with one drop of phosphotungstic acid at a pH varying between 6.4 and 7.2. Formvar films coated with a carbon layer on 300-mesh copper grids were used as the specimen substrate. A Siemens Elmiskop 1A electron microscope, employing a double condensor system and operated at 80 kV, was used in the investigation.

Plate 1(1) shows a granular cytoplasmic inclusion containing embedded virus particles which appear to be incomplete in that they contain no nucleoid and suggesting that the granular material represents capsid protein. Liberation of virus particles from such an inclusion leads to masses of progeny virus particles in the cytoplasmic matrix [Plate 1(2)], some exhibiting dense nucleoids and others with empty or partially empty cores. Nucleoids with partially formed capsids contained in membrane bound bodies are depicted in Plate 1 (3 and 4), and may represent the breakdown of incoming virus particles in lysosome-like structures. Plate 2(5) shows a virus particle with a fine filament attached to the core, possibly representing genetic material which is incompletely incorporated in the progeny particle. Plate 2(6) shows negatively stained virus particles with no double capsid as found in reovirus and suggesting a low capsomere number.

Our investigations to date have indicated that the virus is pH sensitive, is not inhibited by bromodeoxyuridine and is partially sensitive to chloroform, suggesting, together with its size, that the virus resembles the "Diplornaviruses" (Verwoerd, 1969a,b). In addition, complement fixation tests showed no serological relationship between Corriparta virus and BTV or AHSV.

Size determinations in the present investigation suggest approximate values of 67mμ in section and 60 mμ in negative contrast preparations. The value of 67 mμ is in close agreement with that of Carley (1967) and also approximates that of AHSV (Lecatsas & Erasmus, 1967) and BTV (Els & Verwoerd, 1969). Our measurements for reovirus give a value of 80 mμ in section and 75 mμ in negatively stained preparations.

Morphologically, the virus appears to be similar to BTV, showing no double capsid as is commonly found in reovirus (Vasquez & Tournier, 1962) when negatively stained. In addition, while reovirus is thought to have 92 capsomeres, BTV has recently been shown to possess 32 (Els & Verwoerd, 1969). In section, the virus particle presents a dense nucleoid in "full" particles with a markedly less dense capsid. Empty particles having no visible nucleoid are commonly found as are particles having a fine filament attached to the core, a phenomenon characteristic of BTV and AHSV when grown in BHK_{21} cells. It is suggested that this fine strand may represent genetic material which has not been efficiently incorporated into the virus particle (Lecatsas, 1968a,b).

The presence of dense, granular, cytoplasmic inclusions containing apparently incomplete virus particles is clearly demonstrated. Similar bodies are found in BHK_{21} cells infected with BTV and AHSV, and are also reported by Holmes (1969) in mouse brain infected with Eubenangee virus. Myelin-type inclusions containing viral nucleoids are commonly found and may represent lysosomes in which particles are being degraded after initial entry into the cell.

We consider virus size and morphology, as well as the presence of virus-containing cytoplasmic granular inclusions as specific factors in distinguishing BTV, AHSV and Corriparta virus from reovirus. Other details of the fine cytopathology of virus-infected cells, such as the association of reovirus with spindle tubules (Dales, 1963), serve to characterize viruses more specifically. On this basis, for example, AHSV can easily be distinguished from BTV by the respective presence in BHK_{21} cells of coarse cytoplasmic filaments and bundles of cytoplasmic tubular elements (Lecatsas, 1968a,b). The lack of filaments or tubules associated with Corriparta distinguishes it from AHSV and BTV respectively.

ACKNOWLEDGEMENT

We are indebted to Dr. R. L. Doherty, Director, Queensland Institute of Medical Research for supplying us with a sample of Corriparta virus.

Received for publication on 29 August 1969. — Editor

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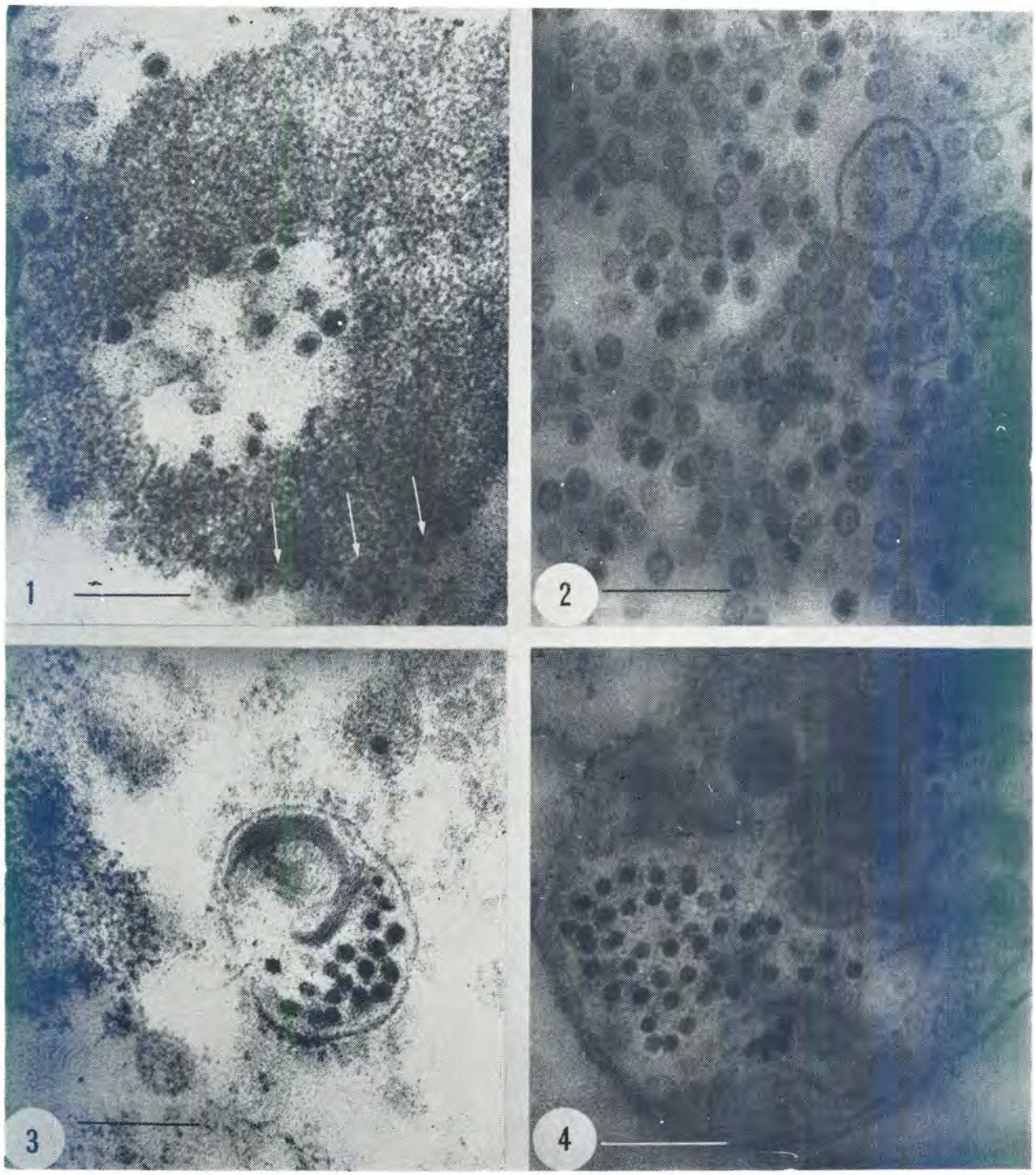

PLATE 1.—1. Granular, cytoplasmic inclusion containing developing virus particles embedded in the matrix (arrows). These particles show no nucleoid while particles at the periphery and centre of the inclusion show dense nucleoids. Bar equals 250 mμ. 2. Virus particles with and without dense nucleoids are evident in the cytoplasmic matrix. Bar equals 250 mμ. 3. Membrane-bound, lysosome-like inclusion body containing viral nucleoids. Bar equals 250 mμ. 4. Membrane-bound lysosome-like inclusion body containing viral nucleoids with indistinct capsids. Bar equals 250 mμ

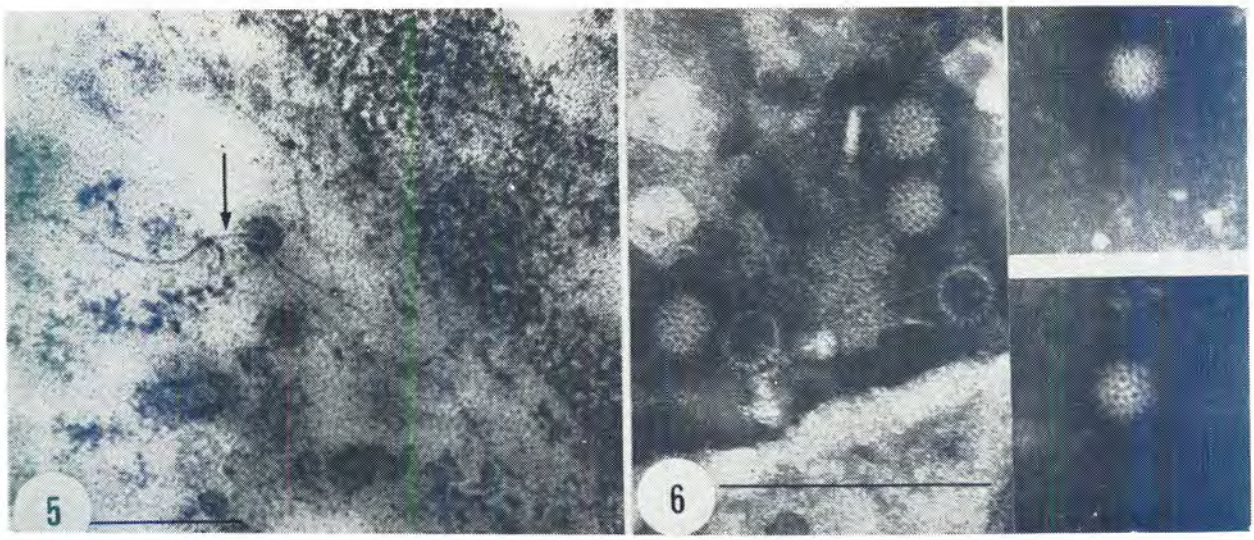

PLATE 2.—5. Virus particle with fine filament (arrow) attached to its core. The small core suggests that the filament represents viral nucleic acid which has not been efficiently incorporated into the virus particle. Bar equals 250 mμ. 6. Virus particles negatively stained with 2 per cent phosphotungstic acid. A low number of tubular capsomeres is suggested. "Empty particles" are also evident. Bar equals 250 mμ