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VISUALIZATION OF THE EXTRACAPSID COAT IN CERTAIN BLUETONGUE-TYPE VIRUSES

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ABSTRACT

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Electron microscopic studies employing the negative staining technique have demonstrated an extracapsid coat in Corriparta, Eubenangee, D'Aguilar, Warrego, Mitchell R, Wallal and M9/71 viruses. These observations are substantiated by the fact that coated and uncoated virus particles exhibit different diameters.

INTRODUCTION

Verwoerd, Els, De Villiers & Huismans (1972) have demonstrated the presence of an extracapsid protein layer covering the bluetongue virus (BTV) nucleocapsid. According to these workers the layer consists of two polypeptides. Negatively stained BTV from sucrose gradients invariably showed poorly defined surface detail whereas on certain caesium chloride gradients the particles were clearly defined as a result of the removal of the extracapsid protein layers.

We report here the presence of the extracapsid protein coat in viruses isolated in Australia (Doherty, 1972) which have been shown by Schnagl & Holmes (1971) and Gorman, Goss, Sayers & Symons (1971) to have properties in common with viruses included in the group of bluetongue-type viruses (Verwoerd, 1970). In addition, similar results are recorded on an undescribed member of the group isolated locally from a horse and designated M9/71.

MATERIAL AND METHODS

Mouse brain-derived Corriparta (MRMI), Eubenangee (In1074), D'Aguilar (B8112), Warrego (Ch9935), Mitchell R (MRM10434) and Wallal (Ch12048) viruses were inoculated on to cultures of BHK 21 cells grown in Roux flasks in Eagle's medium, supplemented with 5% normal bovine serum. For virus growth serum was omitted. The local virus strain M9/71, was adapted to and subsequently grown in BHK 21 cells for electron microscopic examination. Cells were pelleted at low speed, the medium poured off and the tube drained of medium by means of filter paper. A small portion of the pellet was then mixed with a small quantity of distilled water and the suspension so formed used for negative staining according to the following procedure. A drop of the suspension was mixed with a drop of 3% phosphotungstic acid (PTA) at pH 6 and then mixed using a Pasteur pipette. A drop of this mixture was then put on to a formvarcarbon coated grid, the excess fluid removed with filter paper and the grid allowed to dry. The grids were examined in a Siemens Elmiskop 1A electron microscope at an accelerating voltage of 80 kV and an instrumental magnification of 40 000. Particle diameters were measured on negatives using a Nikon model 6C comparator.

RESULTS

All the viruses examined showed the presence of the extracapsid layer which masks the capsomeres and gives the virion a larger diameter than the nucleocapsid form. The diameters of the complete particles including the extracapsid layer were within a narrow range of 65 nm to 77 nm. Nucleocapsid diameters (Table 1), however, varied from 61 nm (M9/71) to 67 nm (Warrego). The two forms of each virus are demonstrated in Fig. 1 to 7.

Although Corriparta, Mitchell R and D'Aguilar viruses gave similar results, relatively few virus particles were found which precluded standard error estimates for these viruses. It is interesting that Corriparta, Mitchell R and Eubenangee showed unclear particles which were bounded by a membrane, probably derived from the cell membrane. If so, this suggests that the virion, after budding, possesses the extracapsid layer. Such particles are indicated in Fig. 3b, 5b and 6b. In support of this observation is the fact that enveloped particles without the extracapsid layer could not be found in any of the virus preparations.

DISCUSSION

The extracapsid layer appears to consist of a mesh of fine threads. These have not been observed to date in sections of cells infected with various bluetongue-type viruses studied in our laboratories. The thickness of this layer appears to be approximately 100Å, and is probably responsible for the different diameters reported for individual members of the bluetongue-type group of viruses. No information is available on the nature of attachment of the extracapsid layer to the capsid although Verwoerd *et al.* (1972) have indicated

TABLE 1 Virus particle size (nm) by negative staining

	M9/71	Wallal	Warrego	Eubenangee	Corriparta	D'Aguilar	Mitchell R
Nucleocapsid	61 ± 2,2	23 ± 2,4	67 ± 1,3	65 ± 1,6	61*	63 ± 2	59 ± 1,7
Nucleocapsid + Extracapsid layer	75 ± 5,5	76 ± 1,5	76 ± 2,2	77 ± 1,2	66 ± 2,5	68*	65*

*Approximate values, since limited number of particles precluded statistical evaluation

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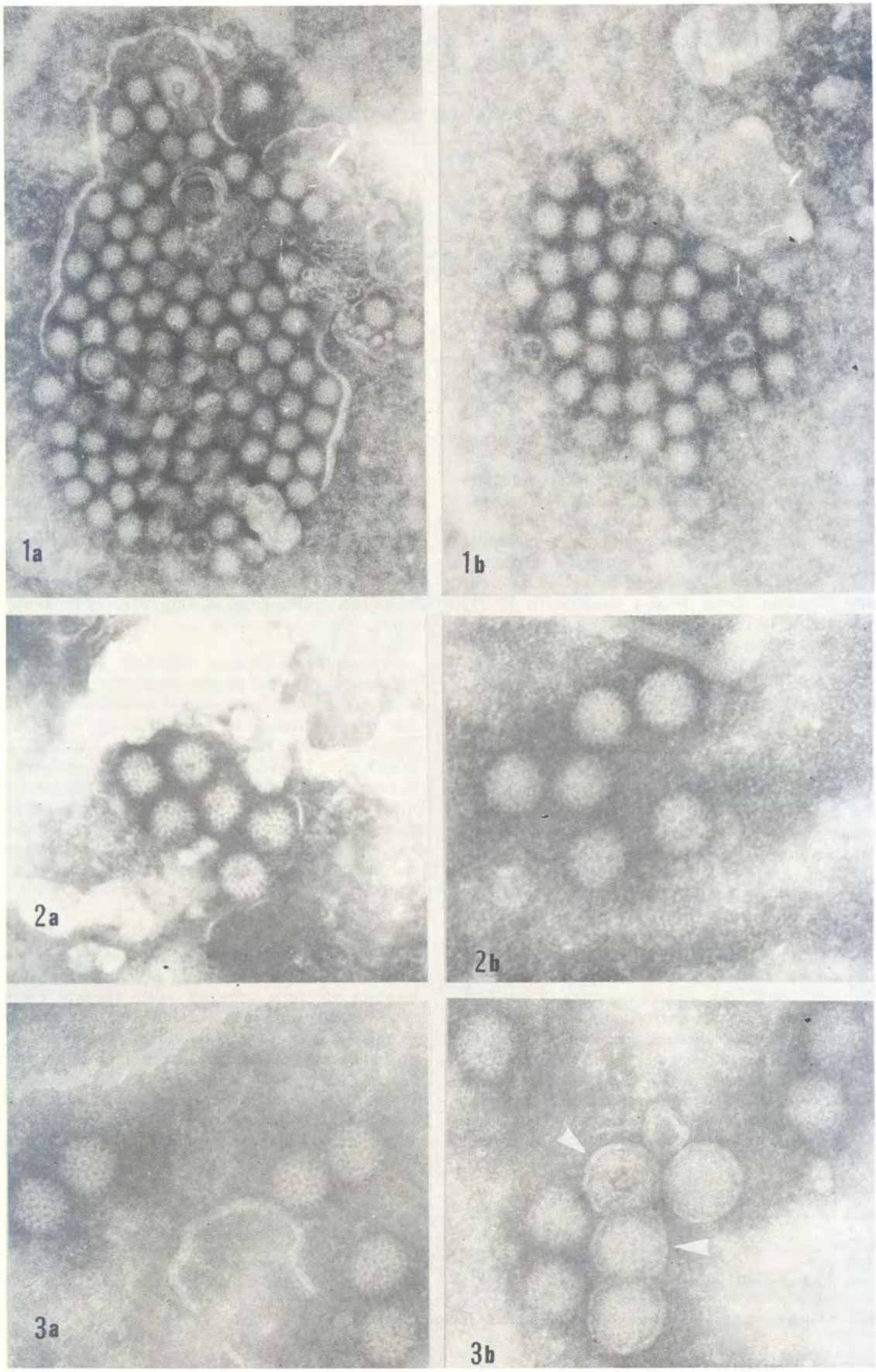

FIG. 1 M9/71 virus (a) clear nucleocapsids, (b) particles with the extracapsid layer. Magnification × 80 000
FIG. 2 Warrego virus (a) clear nucleocapsids, (b) particles with the extracapsid layer. Magnification × 160 000
Fig. 3 Eubenangee virus (a) clear nucleocapsids, (b) particles with the extracapsid layer, some enveloped (arrows) and the others not. Magnification × 160 000

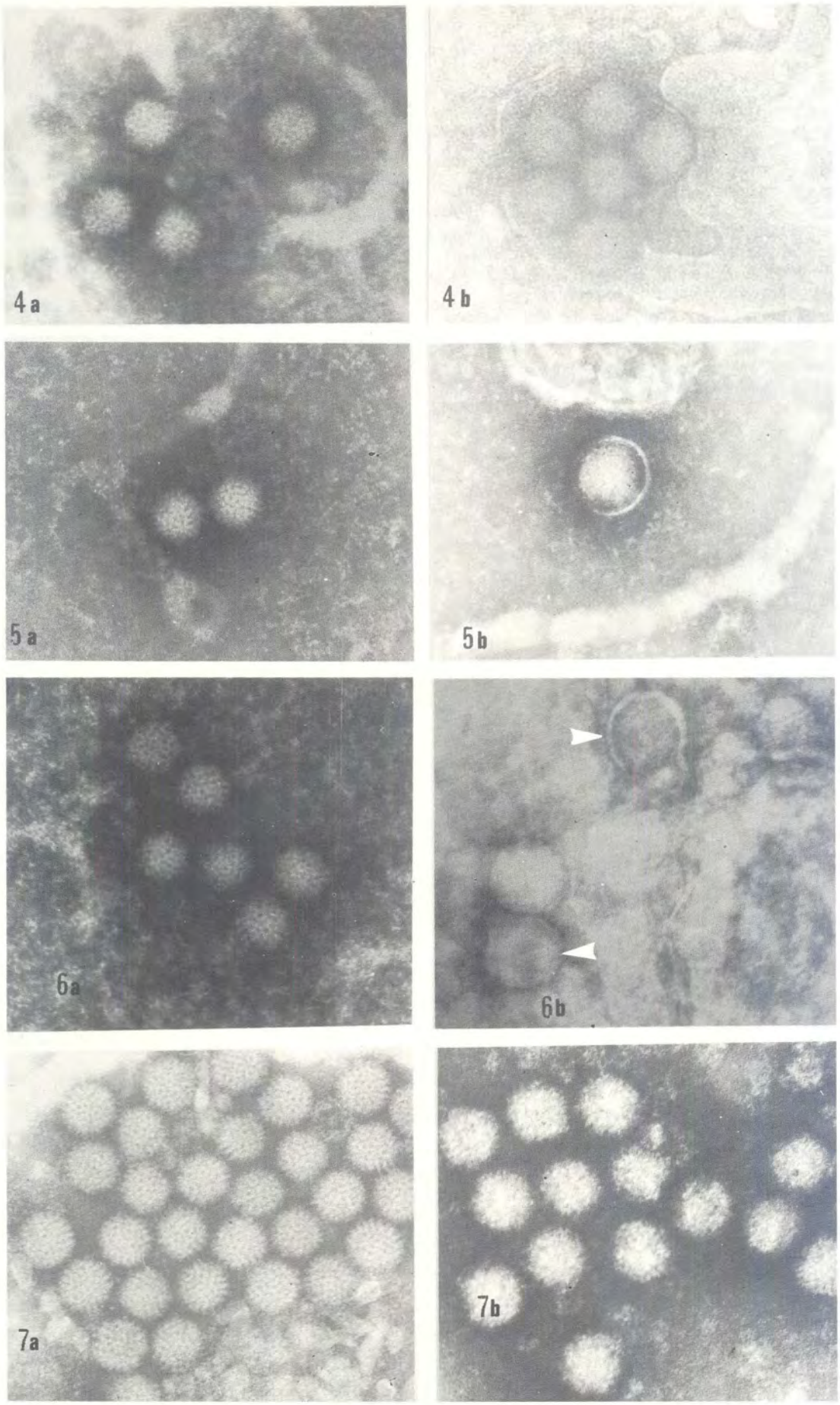

FIG. 4 Corriparta virus (a) nucleocapsids (b) particles with the extracapsid layer. Magnification × 160 000
FIG. 5 D'Aquilar virus (a) nucleocapsids, (b) enveloped particle with the extracapsid layer. Magnification × 160 000
FIG. 6 Mitchell R virus (a) nucleocapsids (b) enveloped particles (arrows) with the extracapsid layer. Magnification × 160 000
Fig. 7 Wallal virus (a) nucleocapsids, (b) particles with the extracapsid layer. Magnification × 160 000

that removal of this layer results in a loss of infectivity of the virus particles. The existence of outer layer proteins appears to be necessary for infectivity in other bluetongue type viruses. Its presence in enveloped Eubenangee, Corriparta, D'Aguilar and Mitchell R viruses would strongly substantiate this contention.

Preliminary investigations of the polypeptide composition of Wallal virus suggest the existence of extracapsid proteins. The virus composition resembles that of bluetongue virus but detailed comparison of polypeptide components has not been made.

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