VISUALIZATION OF THE EXTRACAPSID COAT IN CERTAIN BLUETONGUE-TYPE VIRUSES

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ABSTRACT


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 poly peptides. 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 Schagel & 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 (88112), Warrego (Ch9935), Mitchell R (MRM10434) and Wallal (Ch12048) viruses were inoculated on to cultures of BHK 21 cells grown in Eagle’s medium, supplemented with 5% normal bovine serum. For virus growth serum was removed with filter paper and the grid allowed to dry.

Negatively stained according to the following procedure. A drop of tannic acid staining 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 formvar–carbon coated grid, the excess fluid removed with filter paper and the grid allowed to dry.

Table 1 Virus particle size (nm) by negative staining

<table>
<thead>
<tr>
<th></th>
<th>M9/71</th>
<th>Wallal</th>
<th>Warrego</th>
<th>Eubenangee</th>
<th>Corriparta</th>
<th>D’Aguilar</th>
<th>Mitchell R</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nucleocapsid</td>
<td>61 ± 2.2</td>
<td>23 ± 2.4</td>
<td>67 ± 1.3</td>
<td>65 ± 1.6</td>
<td>64*</td>
<td>63 ± 2</td>
<td>59 ± 1.7</td>
</tr>
<tr>
<td>Nucleocapsid + Extracapsid layer</td>
<td>75 ± 5.3</td>
<td>76 ± 1.5</td>
<td>76 ± 2.2</td>
<td>77 ± 1.2</td>
<td>66 ± 2.5</td>
<td>64*</td>
<td>65*</td>
</tr>
</tbody>
</table>

*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’Aguiar and Mitchell R viruses would strongly substantiate this contention.

Preliminary investigations of the polypeptide composition of Wallall 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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References