Observations on the Morphology of two Rotaviruses

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SUMMARY

The negative staining technique was used to study the morphology of two rotaviruses, the epizootic diarrhoea of infant mice (EDIM) and the simian virus SA 11. It is proposed that the inner capsid of the virion has icosahedral symmetry and consists of 180 morphological units arranged in an open lattice formation with the 12 spaces at the apices being surrounded by 5 capsomeres and the other 80 spaces each surrounded by six capsomeres. The outer capsid of the virion consists of a honeycomb-like lattice which corresponds to the lattice arrangement of the inner capsid.

The term 'rotavirus' has become popular for a group of viruses of characteristic morphology which are frequently associated with acute enteritis of young animals. The term was first proposed by Flewett et al. (1974), who demonstrated a close serological and morphological similarity between viruses from acute gastroenteritis of children and calves. Subsequently, Lecatsas (1974) suggested that the simian virus SA 11 and the 'O' virus from sheep and calves should be included in the rotavirus group. Woode et al. (1976) have presented evidence to show that the virus causing epizootic diarrhoea of infant mice (EDIM) and the enteritis viruses from young pigs and foals are morphologically similar as well as antigenically related to human and bovine viruses.

The rotaviruses have a characteristic double capsid but there still remains some uncertainty about the exact morphology of both the inner and the outer capsid layers. We employed negative staining to study the morphology of two typical rotaviruses – the murine virus, EDIM and the simian virus SA 11. The EDIM virus was obtained from a homogenate of infected mouse gut and the SA 11 virus from infected primary monkey kidney cultures. Before electron microscopy, the virus suspensions were subjected to repeated differential centrifugation at 10000 g for 10 min and 50000 g for 1 h. Final pellets were suspended in a drop of distilled water, mixed with an equal vol. of 2% phosphotungstic acid and examined in a Siemens Elmiskop 1A.

Extensive examination of the EDIM and SA 11 virus particles by electron microscopy revealed no morphological differences between them, but, whereas particles with a double capsid layer were numerous in the SA 11 sample, they were found only very infrequently in the EDIM preparation. Fig. 1(a) shows a group of EDIM virus particles, each with a single capsid. One particle (Fig. 1a, single arrow) displays an organized surface structure with lattice-like arrangement. In an enlargement of this particle (Fig. 1b) it can be seen that the spaces in the lattice are surrounded by either 5 or 6 morphological units (capsomeres) and the lattice therefore appears to follow icosahedral symmetry with the space at each apex surrounded by 5 capsomeres, and the other spaces in the lattice surrounded by 6 capsomeres (Fig. 1c). Each capsomere thus borders on three equidistant spaces and is surrounded by 3 symmetrically placed capsomeres. Disintegrating particles (Fig. 1a, double arrow) clearly show the lattice arrangement and the tubular forms of the virus which are occasionally seen (Holmes et al. 1975; Woode et al. 1976) show a lattice structure which has the same spacing but is purely hexagonal.
Fig. 1. (a) A group of EDIM virus inner capsids. Lattice structure is apparent on the surface of one particle (single arrow) and in the disintegrating virus (double arrow). (b, c) Enlargement of particle arrowed in (a) showing central fivefold axis of symmetry. (d) Ring-shaped structures artificially produced by superimposing two negatives of the particle shown in (b). (e) Model of the proposed inner capsid viewed down the fivefold axis of symmetry. Arrows indicate 3 pairs of adjacent capsomeres where 2 faces of the icosahedron meet (cf. b). (f) Model of the rotavirus with the axis of symmetry slightly off-centre showing how the 'rota' effect may be produced on one side of the particle.
Interpretation of electron micrographs using transmission electron microscopy is frequently made difficult because the image represents a superimposition of top and bottom surfaces of the particles. A rotavirus positioned as in Fig. 1(b), so that electrons passed through the centre of the fivefold axis, would result in an enhanced image of the surface structure because top and bottom surfaces would then fortuitously coincide. When the electrons do not pass through an axis of symmetry, Moire pattern artefacts result.

These Moire pattern artefacts can be seen in a number of the particles in Fig. 1(a) which appear to have ring-like structures on their surface. These rings are unlike real structures in that they are irregularly spaced and sometimes overlap. Woode et al. (1976) reported that these rings seen in rotavirus particles could be made to appear or disappear by tilting the virus in an eucentric goniometer stage - an observation which supports their illusory nature. By superimposing two negatives of the particle in Fig. 1(b) we were able to produce artificially photographic images with exaggerated ring shapes (Fig. 1(d)). Martin, Palmer & Middleton (1975) described the morphology of infantile gastroenteritis virus and proposed that the surface of the particles were composed of 32 large ring-shaped morphological units. For reasons described above, we prefer to disregard the rings and consider the smaller units to be the true capsomeres. The inner capsid would therefore appear as in the model (Fig. 1(e)), an icosahedral structure with 9 capsomeres per face and thus a total of 180 capsomeres per particle. Where two faces of the icosahedron meet, one therefore finds 3 pairs of adjacent capsomeres, each pair containing one capsomere from each of the two faces. These paired capsomeres appear in the micrograph (Fig. 1(b)) as elongated structures near the top of the particle (arrows) and coincide with the capsomeres indicated by arrows in the model (Fig. 1(e)). Because of the overall lattice arrangement of the capsomeres, with spaces at the apices and along the face edges, the icosahedron is not outlined by a rigid array of capsomeres (as with most other icosahedral viruses). This must allow some elasticity in the shape of the particle so that at times it becomes almost spherical. The particles which have been penetrated by stain consequently have a 'rounded' hexagonal appearance.

The spoke-like projections characteristic of rotavirus morphology (and also responsible for the popular name) are not seen around the entire periphery of the inner capsid, but usually on one side of the particle only (Fig. 1(a)). Close examination shows that each 'projection' represents not one capsomere but two. The retouched photograph of the model (Fig. 1(f)) shows how this effect can be produced by stain penetrating the edge of the particle when it is positioned in such a way that the axis of symmetry is slightly off centre.

The outer capsid of the rotavirus can be clearly seen in the SA 11 preparation (Fig. 2(a)). It has a honeycomb-like appearance on the upper surface of the particles and viewed at the periphery it appears as a semi-translucent layer with a smooth outer edge which in parts seems to be discontinuous. There are short radial septa which extend inwards from the outer edge to meet the projections of the inner capsid. Fig. 2(b) shows an outer capsid which has separated from the virion and here the structure can be seen more clearly as there is no underlying inner capsid to add confusion. It appears to consist of a 3-dimensional honeycomb layer. The centre to centre dimensions of the spaces in the honeycomb of the outer capsid correspond with the dimensions of the spaces in the lattice of the inner capsid which suggests that the outer capsid is placed upon the virion in such a way that the septa of the honeycomb layer meet the capsomeres, and the spaces coincide. This is diagrammatically represented in Fig. 2(c). The diagram should be viewed as illustrating a structural concept rather than a model based on precise measurements. By measuring the difference in the diameters of the single- and double-coated particles, the height of the honeycomb can be deduced to be in the order of 3.5 to 4.0 nm.
Fig. 2. (a) A group of SA 11 virus particles with the outer layer intact. Particle A has been retouched to enhance the honeycomb-like surface structure. (b) SA 11 virus showing inner capsid (top); double capsid (bottom left) and free-lying outer capsid layer (arrow). (c) Diagram to illustrate the proposed honeycomb-like structure of the outer capsid and its positioning on the capsomeres of the inner capsid.
The structure proposed here for the inner capsid of rotaviruses resembles the interpretation given by Vasquez & Tournier (1964) for the outer surface of reovirus 3. Their observations were later supported by Amano et al. (1971). The honeycomb-like structure for the outer surface of rotaviruses has not previously been suggested.

**REFERENCES**


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