The Morphology of Bovine Syncytial Virus

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Bovine syncytial virus (BSV) was isolated by Malmquist, Van Der Maaten & Boothe (1969). Their description of the agent included thin section studies which indicated that the virus has some features in common with leukaemia viruses and the Bittner agent. However, the structure described corresponds closely to that described for foamy agents (Clarke, Attridge & Gay, 1969; Clarke, Gay & Attridge, 1969) and, furthermore, a recent fluorescent antibody study of foamy virus (Fleming & Clarke, 1970) showed a distribution of antigen similar to that found for BSV (Malmquist et al. 1969). In an attempt to clarify the relationship of BSV to foamy virus we obtained a strain of BSV from Dr M. J. Van Der Maaten and examined its structure by negative staining.

The virus was propagated in bovine embryonic spleen cultures, as described by Malmquist et al. (1969). When over 50% of the monolayer (in 180 ml. bottles) showed a cytopathic effect, the cells were scraped from the glass, centrifuged and resuspended in a few drops of water or 4% sodium phosphotungstate, pH 7.0. The preparations were applied to carbon-coated grids and examined directly or, for cells lysed with water, applied to grids and dried before staining with sodium phosphotungstate.

The virus particles were usually spherical and, when unpenetrated by stain (Fig. 1), had an electron transparent centre surrounded by a ring of projections. Such particles measured 100 to 110 nm. in diameter with projections about 15 nm. long. Particles were frequently ruptured and exuded material (Fig. 1) which often assumed a shape (Fig. 2, arrow) similar to that of the virus particle. The stained exudate was normally less electron-transparent than the virus, and occasionally carried a few projections (Fig. 8). Unpenetrated structures, variable in shape but larger than standard particles, were occasionally seen (Fig. 3).

Standard virus particles penetrated by stain showed a single internal component surrounded by an envelope (Fig. 4). Although not consistently resolved, the projections were occasionally seen on the outer surface of broken envelopes (Fig. 5).

Groups of free internal components were frequently seen (Fig. 6) as spheres about 70 nm. in diameter, with a discontinuous unstained line in the shell (Fig. 4, 7). An occasional internal component appeared to be hexagonal (Fig. 7, arrow) but this may be fortuitous. However, damaged internal components (Fig. 7) showed no tendency to release a linear helical structure. More than one internal component is occasionally seen in an envelope (Fig. 3), and the larger unpenetrated particles (Fig. 3) probably contain several internal components.

Although we had no difficulty in demonstrating the internal component of the virus, especially in preparations lysed by phosphotungstate, it was more difficult to identify complete particles. This was partly due to the difficulty in resolving projections: thus a group of possibly complete particles and internal components is shown in Fig. 8. Several of the unpenetrated particles are associated with exuded material (arrows), and the projections are poorly resolved. Quantitative study is difficult since such structures in isolation cannot be identified unequivocally as virus particles.

The morphology of BSV described here differs in no basic feature from that established for foamy virus by negative staining (Clarke & Attridge, 1968). However, the micrographs
Fig. 1 to 8. For legend see page opposite.
of unpenetrated particles of BSV (Fig. 1, 2, 3, 8) show less detail than those seen initially with foamy virus (strain MK 5) grown in monkey kidney cultures (Clarke et al. 1967). Since unpenetrated complete particles of strain MK 5, but not the internal components, were less well defined when grown in HEp2 cells (Clarke & Attridge, 1968) it is possible that the host cell membrane plays a part in the staining characteristics of enveloped agents like foamy virus and BSV.

BSV can be distinguished from the Bittner agent since the latter has an internal component 75 to 80 nm. diameter with projections 7 nm. long (Almeida, Waterson & Drewe, 1967). In the case of leukaemia viruses, no projections similar to those of BSV have been seen, and the internal component of the virus, while difficult to demonstrate by negative staining, probably exhibits helical symmetry (De-Thé & O'Connor, 1966). We therefore conclude that BSV has more structural characteristics in common with foamy agents than with any other group of viruses, and that it should be considered for classification within the foamy virus group.

The type of symmetry, if any, possessed by the internal component of this group of agents merits further investigation. Experimental variations in the concentration of pH of phosphotungstate or in the use of uranyl acetate have not been advantageous (J. K. Clarke, unpublished).

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REFERENCES


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Legend for Fig. 1 to 8.

Fig. 1 to 8. Phosphotungstate stained bovine syncytial virus.

Fig. 1, 2. Unpenetrated standard virus particles. Note that the lower particles are ruptured.

Fig. 3. Unpenetrated particles larger than standard particles are seen at the left. A broken envelope with two or three internal components is seen at the right.

Fig. 4, 5. Penetrated standard virus particles each containing a single internal component. Envelope projections are seen on some particles (Fig. 5).

Fig. 6, 7. Free internal components. One of these looks hexagonal (Fig. 7, arrow).

Fig. 8. Various forms of the virus. Note the material (arrows) which was exuded from unpenetrated standard particles. In one case (centre left) this material carries envelope projections.