The Structure of Visna Virus Studied by the Negative Staining Technique

An electron microscope study on sections of cells infected with visna virus has revealed the presence of spherical bodies emerging from the cell surface and ultimately becoming detached as virions averaging approximately 800 Å in diameter (1). No virus-type structures were seen inside the cell membrane. A more recent study (2) showed that, at any time during visna virus release, about half or less of the total progeny virus was associated with the host cells. It furthermore indicated that most of the cell-associated virus was attached to the external cell surface.

The above findings, and the fact that visna virus has been found to be ether sensitive (3), indicate that in its mode of formation visna virus is similar to those animal viruses which mature at the external mem-

brane of their host cells at the time of release. Since a number of these viruses, which include for example the myxoviruses and certain tumor viruses, have been found to possess capsids of helical symmetry (4–6), it seemed of interest to apply the negative staining technique in an attempt to find out whether the structure of visna virus would also fit this symmetry pattern. The purpose of the present communication is to present the results of a study of the visna virus particle by the negative-staining technique.

Visna virus, strain K485, which had been passed about 40 times in tissue culture, was propagated in sheep cell cultures as described previously (7). The infectious fluid was clarified at 2500 rpm for 15 minutes and was then concentrated and partially purified by two cycles of alternating high speed (80,000 g for 30 minutes) and low speed (10,000 g for 20 minutes) centrifugation in a Spinco model L ultracentrifuge. The pellets containing the virus were resuspended in a small volume of medium 199 with 1% sheep serum. The final suspension had an infectivity titer of about 10^9 TCID50/ml, corresponding to an approximately 100-fold concentration of the virus. Before preparation for the electron microscope the suspension was dialyzed against a 1% ammonium acetate solution for 1–2 hours. The dialyzed virus concentrate was then mixed with an equal volume of a 2% solution of potassium phosphotungstate buffered to pH 7.0 with 1% KOH and sprayed onto carbon-coated grids with a Vaponephrine nebulator. The grids were examined in a Siemens Elmiskop I at an instrumental magnification of 40,000 times, using double condenser illumination.

Moderate numbers of particles were seen in the phosphotungstate droplets lying singly or in small clumps (Fig. 1). They were all approximately spherical in outline but varied in size, most of them being about 900–1000 Å in diameter. The surface of the particles was stippled, and the stippling was seen in profile to be due to the presence of numerous projections measuring about 100 Å in length (Fig. 2). Most of the particles were intact, and no internal structure could be made out. In some however, a concentric arrangement of the interior was suggested (Figs. 1 and 3). On treatment with ether for 1 hour the particles underwent complete disintegration and no structure could be identified as derived from the virus. After 10 minutes of ether treatment a few disrupting particles could be identified. In one group of disintegrating particles only were found "rosette"-like structures similar to those found in ether-treated influenza (4). In the same group a small number of helical rods about 90 Å in thickness was seen.

Ultrathin sections of partially purified visna virus sedimented from infectious fluid by high speed centrifugation were examined previously in the electron microscope (8). This examination revealed a number of virus particles of the type observed in cell cultures infected with visna virus (7). When in the present study a similar preparation was studied by the negative staining technique, the surface of the particles was seen to be covered with numerous projections like those covering the outer envelope of myxoviruses. Morphologically, visna virus therefore seems to resemble this group of viruses, at least in its external appearance. A similarity to the influenza virus subgroup (5) is indicated by the size range of the particles and from the finding that they do not seem to undergo spontaneous rupture to a demonstrable extent. However, the symmetry of the internal structure of the virus could not be definitely established as being of the helical type.

In spite of the apparent morphological relationship to the influenza viruses, visna virus differs from these viruses in various respects. It does not hemagglutinate or cause the hemadsorption of a variety of red cells, and the growth of visna virus in tissue culture is inhibited by 5-bromodeoxyuridine (9). The extreme slowness of the visna disease in sheep and the peculiar course of the infection (10), combined with the finding that visna virus multiplies relatively slowly in tissue cultures (2, 11), might suggest some relationship to tumor viruses. It is noteworthy that those tumor viruses which like visna virus are formed by budding at the external membrane of their host cells, e.g., Gross leukemia virus, avian tumor viruses, and particularly the Bittner virus, also show an external appearance similar to that of
Fig. 1. A clump of visna particles. Concentric structure is indicated in the interior of some of the particles (arrow). Magnification: X 164,000.

Fig. 2. Visna particles showing an outer envelope covered with numerous spikes about 100 Å in length. Magnification: X 250,000.

Fig. 3. A partly disrupted particle showing concentric ring structure. Magnification: X 300,000.
influenza viruses (12–17). However, as in the present study of visna virus, the negative-staining technique has as yet failed to reveal the structure of the inner component of the tumor viruses.

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REFERENCES


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