An Electron Microscopic Study of Haemagglutinating Encephalomyelitis Virus of Pigs

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The haemagglutinating encephalomyelitis virus (HEV) of pigs was isolated in Canada by Greig et al. (1962). Three serologically identical strains of the virus (HEV 1, 2 and 21) were detected by these workers (Greig & Girard, 1969). Cartwright (1969) suggested that HEV was a myxovirus, but Phillip, Cartwright & Scott (1971) have recently examined the virus using negative contrast methods and concluded that it belonged to the coronavirus group. This present study examines the structure of HEV as seen in thin sections, but some results obtained by negative staining are also recorded.

Cell cultures in 30 ml. bottles were prepared from the kidneys of 3- to 5-week-old pigs. When a monolayer had just formed the cells were washed with Earle's lactalbumin medium and inoculated with 0.5 ml. of virus (strain HEV2) which was initially obtained from Dr A. S. Greig. The inoculum was absorbed for 16 hr at 37° and then 4.5 ml. of Earle's lactalbumin medium was added. For negative staining the medium was removed 2 days after inoculation and the cells were lysed by the addition of 0.5 ml. of water. The resulting fluid was applied to carbon-coated grids and stained with sodium phosphotungstate pH 6.5. For the preparation of thin sections, inoculated cells were fixed with glutaraldehyde followed by osmium and processed by standard techniques. Cultures were taken 16 hr after the initial inoculation and at intervals thereafter.

Most particles of the virus are roughly spherical (Fig. 1) and measure about 120 to 160 nm. in diameter including the projections. The projections are club-shaped, 15 nm. long and were normally seen only at the periphery of the virus. Occasional virus particles lost projections at some points and this process may well go to completion because structures were frequently seen which could have been virus particles without projections. Some virus structures, recognized by the presence of projections, appeared as short filaments which appeared to be fragmenting (Fig. 2) and this probably accounts for the presence of occasional small particles (Fig. 2).

In section structures which (since they were not found in uninoculated cells) we believe to be virus particles were seen outside the cell (Fig. 4) and in cytoplasmic vacuoles. The vacuoles varied markedly in size (Fig. 3, 6) and appeared to be derived from both the Golgi complex (Fig. 3) and the endoplasmic reticulum. Most vacuoles were electron lucent and almost empty, apart from containing variable numbers of complete particles which had apparently entered by budding (Fig. 5). Some vacuoles, however, were filled with electron dense material. Dense vacuoles were also present in uninoculated cells but in inoculated cultures especially at a late stage of infection, i.e. 48 hr after inoculation a few of these dense vacuoles contained variable numbers of complete particles (Fig. 8, 9 and 11). The electron dense material in virus-containing vacuoles were more granular than that of the other dense vacuoles (Fig. 8) and their origin is uncertain. Virus was not seen to enter electron dense vacuoles by budding and some of these vacuoles contained internal membranes (Fig. 8, 13). Structures which resemble virus particles were occasionally seen free in the cytoplasm but some at least of these may represent pinocytic vesicles or Golgi vesicles because similar structures were also found in uninoculated cells.
Fig. 1. A particle of HEV stained by sodium phosphotungstate.

Fig. 2. A filamentous particle of HEV (right) is apparently fragmenting to give small particles (left).

Fig. 3. Virus particles in Golgi vesicles (48 hr after inoculation).

Fig. 4. Extracellular virus particles (24 hr after inoculation).

Fig. 5. Virus particles budding into a cytoplasmic vacuole (48 hr after inoculation).
Fig. 6. Cytoplasmic vacuoles containing virus particles (24 hr after inoculation).

Fig. 7. Part of Fig. 6 enlarged to show the envelope projections. These projections tend to stain lightly.

Fig. 8. Electron dense vacuoles containing granular material and varying numbers of virus particles. Internal membranes (arrow) are seen in one vacuole. Some vacuoles (V) contain amorphous material but similar structures were frequently present in uninoculated cells.
Fig. 9. An electron dense vacuole containing virus particles limited by a 'unit membrane'. Some virus particles (arrows) appear to be incomplete (48 hr after inoculation).

Fig. 10. Intracytoplasmic vacuoles containing virus. One vacuole (arrow) has fused with the plasma membrane (16 hr after infection).

Fig. 11. A vacuole containing virus particles. The virus cores vary in staining properties. Some cores (arrows) contain granules which may represent a linear structure seen in transverse section (48 hr after inoculation).

Fig. 12. An inclusion seen in virus infected cells (48 hr after inoculation).

Fig. 13. An electron-dense vacuole containing membranous structures and virus-like particles (48 hr after inoculation).
In section virus particles varied in their staining characteristics and in size but there was a preponderance of particles 60 to 80 nm in diameter excluding projections. The complete structure of individual particles was not resolved in detail but the morphology of the virus can be inferred from an examination of a number of particles. The envelope of the virus consisted of a membrane bearing projections. The projections were 15 nm long but were resolved on only a small proportion of particles (Fig. 7). The membrane appeared as a normal 'unit membrane' which was best seen in particles with electron lucent cores (Figs 9, 11). The cores varied in morphology: many appeared to be homogenous and electron dense (Fig. 6 to 8); a few were electron lucent apart from the presence of a few 'granules' (Fig. 11) and many were intermediate between these two extremes and tended to be cleared to varying degrees at the centre (Fig. 3, 4). Vacuoles containing virus appeared to fuse with the plasma membrane (Fig. 10) and thus virus was released without cell lysis. Cytoplasmic inclusions of unknown origin and significance were frequently seen at later stages of infection. They consisted of electron dense vacuoles containing tubular material (Fig. 12).

Our negative staining results agree with the conclusion of Phillip et al. (1971) that HEV is a coronavirus. However, not all virus structures were identical in morphology (compare Fig. 1, 2). This variation is of some practical significance if negatively stained unpurified preparations are used for virus identification. The morphology of HEV as seen in section also varies both in size and in staining characteristics. The virus core when cleared at the centre appeared as a 'shell', as described for infectious bronchitis virus (Becket et al. 1967), but nevertheless it probably consists of a linear structure. This is best seen in relatively empty particles where the 'granules' (Fig. 11, arrow) probably represent a linear structure seen in transverse section.

The morphogenesis of coronaviruses is not as yet completely established: thus, although Becker et al. (1967) showed that virus formed by budding into cytoplasmic vacuoles, other workers (Hamre, Kindig & Mann, 1967; Uppal & Chu, 1970) found so few budding particles that they doubted if this process played a significant role in virus formation. Our results show that HEV could form by budding into electron-lucent vacuoles but budding structures were not frequently seen probably because the budding process is rapid. Nevertheless, although many HEV particles formed by budding into vacuoles it is not yet certain that all do; thus many particles were seen in electron-dense vacuoles but none were seen to enter these vacuoles by budding. The dense vacuoles resemble lysosomes and the structures shown in Fig. 8, 9 and 11 may represent lysosomal uptake preceding virus breakdown. However, the incomplete particles occasionally seen in the dense vacuoles (Fig. 9) could also be interpreted as particles in the process of formation using pre-existing intravacuolar membranes (Fig. 8, 13). Further work is required to clarify this problem.

Coronaviruses were originally thought of as 'respiratory' viruses but this may be misleading because they are also known to affect the reproductive tract (infectious bronchitis virus), the alimentary tract (transmissible gastro-enteritis virus) and the liver (mouse hepatitis virus). HEV is the first member of the group found to invade the central nervous system. It would be surprising if it is the only one to do so.

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