Observations on Complete and Empty Capsids of Foot-and-Mouth Disease Virus

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Recent reports have described the similarities and differences in the composition and occurrence of infectious virions and empty capsids (or top components) of several small RNA viruses (Maizel, Philips & Summers, 1967; Longley & Leberman, 1966; Fabiyi, Engler & Martin, 1964; Halperen, Eggers & Tamm, 1964). A previous report (Breese & Graves, 1966) on observations of crystalline arrays of foot-and-mouth disease virus type A, strain 119, has been followed by extensive experimentation with three field strains from Argentina (A1, O2 and C3, CANEFA), which have had fewer tissue culture passages. An effort was made to find a combination of multiplicity of infection, time of harvest, and cell culture which would regularly result in the observation of crystalline arrays of virus particles. During the course of these studies, the A1 strain was found to form arrays of both complete and recently described empty particles. Graves, Cowan & Trautman (1968) have reported on the physical properties and some of the serological activities of the RNA-free 75S foot-and-mouth disease virus particle. The electron microscopic observations on the development and appearance of the two forms of virus particle are reported here.

Primary swine kidney cell cultures were prepared from kidneys taken from swine of approximately 12 to 16 weeks of age. Virus stocks were made from bovine virus which had undergone 7 passages in primary bovine kidney cells grown in cultures and 1 passage in tissue cultures of baby hamster kidney (BHK-21) cells immediately before use. Four oz prescription bottles were inoculated with virus purified by the method of Graves et al. (1968), which combines precipitation by ammonium sulphate and fractionation on a DEAE column followed by CsCl isodensity centrifugation, zone recovery and dialysis. Cells, which had been drained of fluid, were inoculated with 0.1 ml of virus to give a multiplicity of 10 to 100 p.f.u./cell. After 30 min. incubation at 37°, 10 ml of Hanks’s balanced salt solution with 0.5% lactalbumin hydrolysate was added and the bottles returned to the incubator. At 4½ to 6 hr after inoculation the cells were rinsed once with Sorensen’s buffer, pH 7.3, and scraped off the glass into 2 ml of 1% glutaraldehyde in phosphate buffer (Millonig, 1961) and held overnight at 4°C. Post-fixation was with osmium tetroxide (Palade, 1952) and the samples were embedded in Epon (Luft, 1961). Sections were made with a diamond knife in a Porter–Blum MT-2 ultramicrotome (Ivan Sorvall, Norwalk, Conn.) and examined with an RCA-EMU-3G microscope.

Early appearance of virus was difficult to determine in the presence of ribosomes; however, 4 hr after inoculation, virus particles were visible in significant concentrations (Pl. 1, fig. 1). Samples taken at 4½ to 6 hr after inoculations yielded regular arrays of virus particles (Pl. 1, fig. 2, 3, 4). In Pl. 1, fig. 2, the particles are evenly stained throughout and appear to be complete. These particles are similar to those previously reported for purified foot-and-mouth disease virus (Bachrach & Breese, 1958; Breese & Bachrach, 1960). In Pl. 1, fig. 3 and 4, the appearance of the particles is quite different
in that the centres are less dense than the periphery. These particles are presumably empty and correspond to the 75 S antigen reported by Graves et al. (1968). They found the A1 strain of foot-and-mouth disease virus produces a greater proportion of this particle than it does infectious virions, while the other two strains, O2 and C3, produce very small amounts. While both complete and empty particle arrays are found in the same section in the electron microscope, they have not been found in the same cell. In those specimens where crystalline formations are seen, approximately one cell in 40 to 50 shows a crystal.

Samples of purified A1 virus were subjected to differential centrifugation (Graves et al. 1968) to separate the complete 140 S virus particle from the 75 S particle. These purified samples were stained as loop droplets with 1% phosphotungstic acid and examined in the electron microscope. Pl. 2, fig. 5, shows the 75 S particle at high magnification in which the particle centres are penetrated by stain. In Pl. 2, fig. 6, the 140 S particle which contains RNA is shown with particles of uniform density and not penetrated by stain.

Further studies are under way to determine the relationship of the complete and incomplete virus particles. There is no explanation for the apparent difference in formation of viral crystalline arrays by the type A1 foot-and-mouth disease virus strains and the lack of such formation by the O and C type strains under the same biological conditions. The formation of regular arrays may occur with any foot-and-mouth disease virus strain when the proper combination of inoculum, cell type, and time of incubation has been found.

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REFERENCES


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Short communications

Explanation of Plates
(See overleaf)
EXPLANATION OF PLATES

PLATE 1

Fig. 1. An accumulation (arrows) of strain A1 foot-and-mouth disease virus particles in the cytoplasm of a primary swine kidney tissue culture cell 4 hr after inoculation.

Fig. 2. A definite crystalline array of complete strain A1 foot-and-mouth disease virus particles 5 hr after inoculation into primary swine kidney tissue culture cells. There is one particularly orderly close-packed array (arrow).

Fig. 3. An example of a crystalline array (arrows) of 'empty' virus particles, strain A1 foot-and-mouth disease virus, near the periphery of the cytoplasm of a primary swine kidney tissue culture cell 5 hr after inoculation.

Fig. 4. Another example of 'empty' virus particles in an orderly array apparently almost surrounding a mitochondrion (arrow). Strain A1 foot-and-mouth disease virus 5 hr after inoculation in primary swine kidney tissue culture cells.

PLATE 2

Fig. 5. A dispersion of 75 S 'empty' strain A1 foot-and-mouth disease virus particles in phosphotungstic acid. Note that the electron-dense stain appears in the interior of the particles (arrows).

Fig. 6. A dispersion of 140 S strain A1 foot-and-mouth disease virus particles in phosphotungstate. In this case the virus is not penetrated by stain and has a more uniform density.