Ultrastructural Features of Canine Distemper Virus Infection of the Chorioallantoic Membrane of the Hen’s Egg

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SUMMARY

The chick embryo chorio-allantoic membrane was examined 6 days after inoculation of the ONDESTEPOORT strain of canine distemper virus. Multinucleated cells containing cytoplasmic inclusions were observed in the chorionic epithelium. These inclusions consisted of randomly arranged nucleocapsid filaments. The filaments had an outer lightly staining layer of radial fibrils in addition to their dense core. Budding and mature virus particles were observed less frequently. The morphological stages of distemper virus replication resembled those reported for measles and rinderpest viruses in other systems.

INTRODUCTION

Myxoviruses are at present divided into two main groups (Waterson, 1962), the small myxoviruses (influenza group) and the large myxoviruses (parainfluenza group). Members of this second group have larger particles, with an internal component (nucleocapsid) of greater diameter than that of the influenza group particles. Canine distemper virus, measles virus and rinderpest virus, which are all members of the second group, are serologically related (cf. Warren, 1960). In this account the morphological features of infection of the chick embryo chorioallantoic membrane (CAM) by canine distemper virus are described and compared with those of the replication of closely related parainfluenza viruses.

METHODS

The infected chorio-allantoic membranes were supplied by Dr D. M. Berry (Glaxo Laboratories Ltd). Chick embryos were inoculated with the ONDESTEPOORT strain of canine distemper virus on the 8th day of incubation. The membranes were harvested after a further 6 days at 37°C and fixed in veronal acetate-buffered 1% osmium tetroxide solution containing sucrose. The fixed membranes were dehydrated and embedded in Araldite by conventional methods. Thin sections were stained with lead citrate (Reynolds, 1963), a mixture of uranyl acetate and potassium permanganate (Selman & Jurand, 1964) or by successive application of these stains.

RESULTS

Cytological change in regions containing replicating canine distemper virus varied from the presence of inclusion bodies with no other sign of cellular damage to a gross thickening of the CAM with infiltration of leukocytes, destruction of the normal bilaminar architecture of the chorionic epithelium and desquamation of dense flattened surface cells. The
Fig. 1. Multinucleated cell in the chorionic epithelium infected with canine distemper virus. The cytoplasm contains a large nucleocapsid inclusion (arrow). Note the abundance of desmosomes and intracellular fibrils.
Fig. 2. An intracytoplasmic nucleocapsid inclusion body (arrow). Note the abundant intracellular fibrils (f) and desmosomes (d).
altered surface cells had numerous desmosomes and contained many intracellular fibrils (Fig. 1). Multinucleated cells were found (Fig. 1), in which the presence of discrete intracellular desmosomes (Fig. 3a) suggested that they were formed by fusion.

Fig. 3. (a) An isolated desmosome from infected chorion. These formations suggest that multinucleate cells form by fusion. (b) Part of a cytoplasmic inclusion body. The nucleocapsid filaments are occasionally cut longitudinally (double arrows) or transversely (single arrows). Note the fibrous material between the dense cores of the nucleocapsid filaments. The transversely sectioned filaments are surrounded by radially arranged fibrous strands.
Inclusion bodies and budding particles were most easily found in regions where the CAM was minimally altered. After embedding in Araldite, such regions could hardly be distinguished from normal membrane, although they might have been distinguishable in the

Fig. 4(a). Part of a cellular protrusion with canine distemper virus nucleocapsid filaments. Some of the filaments closely aligned to the plasma membrane are sectioned transversely (radial lines) and others longitudinally (tangential lines). Unaligned nucleocapsid is present in the centre of the protrusion which has cytoplasmic fibrils in its neck. Most of its surface bears surface projections. (b) A portion of the border of two adjacent cells in the chorionic epithelium. One cell has dense nucleocapsid filaments aligned at intervals below the plasma membrane (arrows). At these sites the plasma membrane bears external projections. (c) A mature virus particle of canine distemper virus lying between two chorionic epithelial cells. There is an external layer of radial projections, beneath which is a single dense membraneous layer. Below this layer lie nucleocapsid filaments, two of which are sectioned transversely. (d) Canine distemper virus lying between epithelial cells; where the membrane of the particles is sectioned obliquely (arrows) the nucleocapsid filaments lying beneath it are seen to be arranged regularly.
fresh membranes. The most obviously altered regions often contained no evidence of virus replication and were presumably a response of the CAM to trauma (Cameain, Bres & Plagnol, 1960; Rich, Rogers & Leaders, 1965; Wyler & van Tongeren, 1957). The allantoic epithelium was not affected.

**Virus components**

Myxovirus assembly can be divided into two stages; the assembly of the nucleocapsid (internal component) and the association of this with altered cell plasma membrane to form mature particles. In the CAM, mature canine distemper virus was found only with difficulty, although nucleocapsid-containing inclusions were frequent. At the stage of development examined, nucleocapsid was found in the cytoplasm but not in the nucleus. Most of it was in relatively compact masses of irregularly arranged filaments (Fig. 1, 2). These cytoplasmic inclusions showed no special affinity for other cell components, except that they were often close to the nucleus.

Individual nucleocapsid filaments consisted of a dense core about 12 nm. in diameter surrounded by a diffuse region of lesser density which in cross-section of filaments well separated from neighbours (Fig. 3 b) extended to a diameter of about 90 nm. Neighbouring filaments seldom approached each other closer than 50 nm. between centres. The diffuse coat was often resolved into a fibrous material (with strands about 2·5 nm. in diameter) arranged radially with respect to the central core. This core was usually without any obvious central canal (Fig. 3 b). Nucleocapsid was also found at the cell surface. Sometimes small masses of filaments lay beneath the surface, but more usually there was a single layer of filaments here, bearing a constant relationship to the plasma membrane from which they were separated by a region of low density about 10 nm. in width (Fig. 4a). Where nucleocapsid filaments were arranged in this regular relationship to the plasma membrane, its external surface bore surface projections. In some cases these were sharply localized to regions adjacent to individual filaments (Fig. 4b). In many infected cells a large proportion of the surface was modified in this way, although no protrusions or free virus particles were present. Where the latter were present (Fig. 4c) they were almost uniformly coated with uniform projections. Tangential sections (Fig. 4d) showed that the nucleocapsid lying beneath their surface was fairly regularly arranged. The fibrous outer layer of the filaments appeared to be present, although it was generally obscured by non-specific cytoplasmic fibrils, similar to those beneath the normal plasma membrane which were included in the budding particle. This layer was not present between the core and the plasma membrane but only on the surface facing the interior of the protrusion or particle. Occasionally a protrusion remained attached to the cell surface by a desmosome. This stage in the assembly of particles was only rarely observed, more commonly between neighbouring cells than at the surface facing the shell membrane, where microvilli were normally found.

**DISCUSSION**

Morphologically, the assembly of canine distemper virus bears an extremely close resemblance to that of measles virus (Kallman et al. 1959; Tawara et al. 1961; Matsumoto, 1966; Raine et al. 1969). Previous electron microscopy of canine distemper virus infected cells (Tawara et al. 1961) and of cells infected with rinderpest virus (Tajima et al. 1967) demonstrated similar cytoplasmic inclusion bodies, but not the fibrillar outer layer of the filaments in these inclusions. Raine et al. (1969) illustrate an outer less dense layer associated with measles nucleocapsid after fixation with glutaraldehyde and osmium
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...tetroxide, but do not describe radial fibrils which may be a peculiarity of fixation with osmium tetroxide alone. This outer layer may be common to measles, rinderpest and canine distemper viruses and would explain why filaments in the cytoplasmic inclusions of these viruses approach each other less closely (nearest approach 40 to 50 nm. between centres as measured from these micrographs and published micrographs of other authors) than do those of some other myxoviruses (Kuhn & Harford, 1963; Prose et al. 1965; Compans et al. 1966; Howe et al. 1967). In sections of fixed and embedded CAM, the diameter of the dense core of the nucleocapsid (about 12 nm.) is apparently smaller, the diameter of the outer diffuse layer (up to 90 nm.) larger than the diameter of negatively stained nucleocapsid (15 to 17 nm. Cruickshank et al. 1962). An outer layer has sometimes been observed in the nucleocapsid in negatively stained preparations of measles virus (Almeida & Howatson, 1963; Norrby & Magnusson, 1965). However, the divergences between the appearance of nucleocapsid in sections and its appearance when negatively stained are not easily explained.

Parainfluenza virus SV5 can produce different types of effect in vitro, depending on the host cell (Compans et al. 1966; Holmes & Choppin, 1966). The effect of canine distemper virus on the CAM compares with the virulent infection of BHK21-F cells by SV5 where there is much cytoplasmic nucleocapsid but not with the moderate interaction between this virus and MK cells where much budding occurs and the amount of cytoplasmic nucleocapsid is reduced. When measles virus is injected into the brain of mice (Wear et al. 1968), budding and mature virus cannot be found, only intracellular nucleocapsid. Such imbalances in the virus replication cycle could result from inefficient assembly at the surface of cells other than those naturally infected by the virus.

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REFERENCES


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