Entry of Infectious Bovine Rhinotracheitis Virus into Cells

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Holmes & Watson (1963), Dales & Silverberg (1969) and Hummeler, Tomassini & Zajac (1969) showed that herpes simplex virus enters the host cell by being engulfed via the phagocytic process. Equine abortion virus enters the cell similarly (Abodeely, Lawson & Randall, 1970). An alternative mechanism of virus entry has been proposed by Morgan, Rose & Mednis (1968) who suggest that the stages of herpes virus entry into the host cell consist of attachment, digestion of the virus envelope, digestion of the cell membrane, passage of the capsid into the cytoplasm, breakdown of the capsid with the subsequent release of the DNA core into the cytoplasm. We describe here the early stages of infection of kidney cells by infectious bovine rhinotracheitis virus, another member of the herpes virus group.

Infectious bovine rhinotracheitis virus was kindly supplied by the Cell Culture Division of the Naval Biomedical Laboratory, Oakland, California. The virus was plaque-purified three times in our laboratory and propagated on Madin Darby bovine kidney cells grown in Eagle’s medium supplemented with 10% tryptose phosphate broth and 5% foetal calf serum (Madin & Darby, 1958). Infectious virus was assayed by the plaque method under Tragacanth Gum overlay (Mirchamsy & Rapp, 1968). Confluent monolayers of cells in 3 oz. prescription bottles were infected with the virus at an input multiplicity of 100 p.f.u./cell. The cells were previously chilled and the virus inoculum was allowed to adsorb at 4° for 1 hr with occasional gentle agitation. The unadsorbed

![Fig. 1, 2. Attachment of IBR virus to the cell surface. CM, cell membrane; CY, cytoplasm.](image-url)
virus was removed by washing twice with cold Eagle's medium. The infected cells were rapidly brought to 37°C by the addition of pre-warmed Eagle's medium and incubated at 37°C. At intervals samples were removed for electron microscopy. Cells were fixed as a monolayer by the direct addition of 2% glutaraldehyde followed by 2% buffered-osmium tetroxide and were then scraped off the glass, dehydrated and embedded in Maraglas (Zee, Hackett & Talens, 1970). Thin sections were stained with lead citrate and uranyl nitrate and examined with an AEI EM 6B electron microscope.

Fig. 3 to 6. Stages of fusion between the virus envelope and cell membrane releasing the virus capsid into the cytoplasm of the infected cell. ER, endoplasmic reticulum; VE, virus envelope; CM, cell membrane; CY, cytoplasm.

After adsorption for 1 hr at 4°C and before the addition of pre-warmed medium (time zero) the envelope, capsid and internal DNA core of the virus were clearly defined (Fig. 1). The virus particle was in close contact with the cell membrane and there was sometimes a slight depression of the cell membrane at the region of attachment (Fig. 2). After 10 min. incubation at 37°C the cell membrane and virus envelope at the site of virus attachment disintegrated and fusion began between the two structures (Fig. 3, 4). Within 30 min. after the addition of medium at 37°C fusion appeared to be complete and the capsid and the cytoplasm of the infected cell were in direct contact (Fig. 5, 6).

The unenveloped capsid was usually found free in the cytoplasm of the infected cell (Fig. 7). Occasional virus particles were observed within intact cytoplasmic vacuoles, even
Fig. 7. Section of a cell 15 min. after adsorption showing a naked capsid in the cytoplasm and an enveloped virus particle at the cell surface.

Fig. 8. A disintegrating virus particle in the cytoplasmic vacuole 45 min. after adsorption. V, vacuole; M, mitochondrion; ER, endoplasmic reticulum.
**Fig. 9.** A capsid close to the nuclear pores of the nuclear membrane. NP, nuclear pore; N, nucleus; ER, endoplasmic reticulum; CY, cytoplasm.

**Fig. 10.** Disintegration of a capsid near the nucleus of an infected cell 45 min. after adsorption. N, nucleus; CY, cytoplasm.
though the virus within the vacuole seemed to be in the process of disintegration (Fig. 8). We saw very few virus particles within vacuoles.

The movement of unenveloped capsids from the cytoplasm into the nucleus of the infected cell was not entirely clear. These capsids were frequently observed close to the nuclear membrane but not associated with membranous structure (Fig. 9). However, different degrees of capsid dissolution have often been seen with release of the nucleic acid core into the cytoplasm (Fig. 10).

Our studies incline us to the view that entry of herpes viruses into susceptible cells is by a process of fusion of virus and cell as described by Morgan et al. (1968). Our observations suggest that viropexis (Dales & Siminovitch, 1961; Dales, 1962) or phagocytosis is a rare phenomenon.

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REFERENCES


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