Direct Cell to Cell Transfer of Bittner Virus

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Bittner virus consists of an internal component which arises in the cytoplasm and becomes enveloped by budding through a cell membrane (Moore, 1962). During a study of this process we obtained evidence that the virus could be transmitted directly from cell to cell and that in some instances an unusual form of particle was involved. In this communication we describe this unusual form and the engulfment of virus particles.

Pieces of spontaneous mammary tumours from C3H mice, originally obtained from the Christie Institute, Manchester, were fixed in 3% glutaraldehyde in Sorensen's phosphate buffer of pH 7.2 for 2 hr at 4°C, post-fixed in 1% osmium tetroxide for 2 hr at 4°C, and embedded in Maraglas by standard techniques. Sections were stained by alcoholic uranyl acetate followed by lead citrate.

Budding particles were found at cytoplasmic vacuoles and at the plasma membrane. Particles which budded at the plasma membrane were not randomly distributed at the periphery but tended to concentrate at free cell surfaces (Fig. 1). Some particles, however, budded where cell surfaces were in apposition, and these were frequently undergoing pinocytosis by the adjacent cell (Fig. 2A, B, C). Only 'immature B' particles have been observed within pinocytic vesicles. Initiation of pinocytosis did not require the presence of a free virus particle since vesicles began to form around particles before completion of the budding process (Fig. 2D, E). Many empty pinocytic vesicles were seen (Fig. 2F) while some appeared to be engulfing part of the cytoplasm of an adjacent cell (Fig. 2G).

About 1% of all the particles budding at the plasma membrane had an elongated internal component which extended from the tip to the base of the bud (Fig. 3A). These unusual forms had a diameter of 60 to 65 nm, with an internal component of diameter 50 to 55 nm. Some were expanded at their distal end and may release normal 'immature B' particles. In contrast to the more usual form of budding particle these forms (stems) were found only where the plasma membranes of adjacent cells were apposite. The tip of the stem was frequently situated in a pinocytic vesicle forming in an adjacent cell (Fig. 3B, C).

All the pinocytic vesicles involved in the processes described had membranes which were thicker and denser than the original, normal plasma membrane (Fig. 2, 3). In those best resolved the thickened membrane appeared as a central electron dense line with projections which, though present on both sides, were more prominent on the convex surface (Fig. 2D).

Enveloped mammalian viruses may enter a cell in a pinocytic vesicle or by fusion of the virus envelope with the plasma membrane. Herpes virus (Morgan, Rose & Mednis, 1968) and influenza virus (Morgan & Rose, 1968) utilize both methods. There are, however, two morphologically different types of pinocytic vesicle: those surrounded by a normal 'unit membrane' structure and those (fuzzy vesicles) in which the membrane is thickened (Fawcett, 1966). Influenza (Morgan & Rose, 1968) and Sendai viruses (Morgan & Howe, 1968) are occasionally found within fuzzy vesicles whereas herpesviruses (Morgan et al. 1968) are found in vesicles showing no thickening of the membranes. It is not known which method of uptake is more efficient in initiating virus replication but, if the cell to cell transmission of virus which we describe is a biologically significant process, Bittner virus must be released from fuzzy vesicles in a viable state. The process of direct cell to cell transfer could allow...
spread of virus with little interference from immune processes. If so, the observation that stems are produced mainly, if not exclusively, where cell membranes are in close apposition suggests that they are significant in dissemination of virus within an animal.

Fig. 1. Bittner virus in a mouse mammary tumour cell. Virus particles budding (B) at the free cell surface or into small cytoplasmic vacuoles (V). Where adjacent cells are in apposition no budding virus is seen. Both immature B (IB) and mature B (MB) forms are present.
Fig. 2. A, B, C. Various stages of the engulfment of free virus in fuzzy vesicles. (D), (E) Virus partly engulfed by vesicles before budding is complete. (F) Two apparently empty pinocytic vesicles. (G) A pinocytic vesicle apparently engulfling part of the cytoplasm of an adjacent cell.
Fig. 3. (A) An unusual form of Bittner virus showing elongation of the internal component. The normal double shell structure is represented by two parallel lines at each side of the stem. (B) A Bittner virus stem. The enlarged tip is partly engulfed in a fuzzy vesicle. A stem cut obliquely is also present (arrow). (C) A Bittner virus stem partly engulfed in a fuzzy vesicle.
Short communications

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