Virus-Like Particles in Bovine Turbinate Cells Infected with Bovine Virus Diarrhoea/Mucosal Disease Virus

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Summary
Thin sections of bovine turbinate cells grown in culture and infected with the NADL strain of bovine virus diarrhoea/mucosal disease virus have been examined by electron microscopy. Infected cells exhibited ultrastructural modification of the rough endoplasmic reticulum and associated with this small numbers of virus-like particles were observed. Advanced stages of cytopathic effect were illustrated by cells showing a marked increase in electron-density. These features were not observed in uninfected control cultures.

Introduction
Bovine virus diarrhoea/mucosal disease (BVD) virus is important not only as a naturally occurring infection of cattle but also because of its possible involvement in teratogenic disease syndromes of other species (e.g. Border disease of sheep), its interference with the serological diagnosis of hog cholera and its occurrence as a common contaminant of laboratory cell culture systems. The causative agent is an RNA virus, the type species of the pestivirus genus of the Togaviridae (2, 6, 7, 13), and is serologically related to both hog cholera virus and Border disease virus of sheep (1, 12). However, morphological information is unclear.

Examination by electron microscopy of negatively stained BVD virus preparations has revealed pleomorphic, approximately spherical, forms consistent with enveloped particles (4, 16). Although such particles have been identified with infectious fractions from infected cell culture material (16) there is a considerable spread in reported size range (14). More importantly, examination of thin sections of infected cells has failed to reveal morphological forms that can be identified as BVD virus (15).

In the study reported here, thin sections of bovine turbinate (BT) cells, infected in culture with BVD virus, have been examined by electron microscopy.
Materials and Methods

Cell Culture

Serially propagated bovine turbinate cells (10) were obtained from the USDA Veterinary Services Laboratories at Ames, Iowa, and grown in a modified Eagle's medium with Earle's salts enriched with sodium pyruvate, non-essential amino acids, lactalbumin hydrolysate, antibiotics and 10 per cent foetal calf serum. Both cells and serum were screened for BVD virus and the serum was checked to ensure absence of antibodies to BVD virus. Cells at the 23rd and 24th passage level were grown in 25 cm² plastic flasks and were inoculated with virus after 24 hours when the cell sheets were almost confluent.

Virus

The cytopathogenic NADL strain of BVD virus (3) supplied by the American Type Culture Collection as the fourth passage in bovine embryonic kidney cells was passaged twice in BT cells and the clarified supernatant cell culture fluid obtained after one cycle of freezing and thawing was stored at −70°C. Cultures were harvested when approximately 70 per cent of cells showed cytopathic effects.

Procedure

One day old subconfluent monolayer cell sheets of BT cells grown in 25 cm² plastic flasks were infected with $10^{4.1}$ to $10^{4.3}$ cell culture infectious doses of BVD virus contained in 0.5 ml to 1.0 ml of inoculum (representing multiplicities of infection between 0.02 and 0.03) after decanting the growth medium. One hour was allowed for adsorption at 37°C; the cells were then overlaid with medium and incubated at 37°C. Control cultures were treated similarly except for the inoculation of virus.

The cell sheets were harvested at 48 hours after infection and processed for electron microscopy.

Fig. 1. a Uninfected monolayer of bovine turbinate (BT) cells. Phase contrast × 96. b Monolayer of BT cells 48 hours after infection with the NADL strain of BVD. Large refractile cells are a major feature of the extensive cytopathic effect. Phase contrast × 96