An A Particle, Probably of Bovine Origin, Detected in BHK 21 Cells Infected with Bovine Syncytial Virus

(Accepted 18 April 1971)

To initiate an investigation of bovine syncytial virus, two cultures of bovine embryonic spleen cells were obtained from Dr M. J. van der Maaten. One of these cultures was already inoculated with bovine syncytial virus. To propagate the virus, inoculated and unoinoculated cultures of bovine embryonic spleen cells were mixed. The cells were initially grown in Eagle’s medium with 20 % foetal calf serum, but this was changed after a few passages to Eagle’s medium plus 10 % tryptose phosphate broth and 10 % foetal calf serum (ETC).

After several passages the spleen cells ceased to grow well, so an attempt was made to propagate the virus in BHK 21 cells. Infected spleen-cell cultures were mixed in ETC medium with BHK 21 cells and syncytia developed in the mixed cultures. The cells were subcultured at 2- or 3-day intervals, and after 3 weeks the cytopathic effect was widespread. Fresh BHK 21 cells were added at intervals to some cultures. Most of the remaining cultures degenerated completely. BHK 21 cells and bovine embryonic spleen cells infected with bovine syncytial virus were fixed in 3 % glutaraldehyde in 0.1 M-Sørensen’s buffer pH 7.2 with 3 % sucrose, post-fixed in 1 % osmium tetroxide in buffer, dehydrated in ethanol and embedded in Maraglas. Sections were examined in an AEI EM6B electron microscope.

Three different virus-like structures were observed in the inoculated BHK 21 cells: bovine syncytial virus particles which are described elsewhere (Dermott, Clarke & Samuels, 1971); intracisternal particles known to be present in BHK 21 cells (Thomas, Delain & Hollande, 1967) and a third type of particle which is the subject of this communication. These particles
Fig. 2. Complete and incomplete (*) A particles scattered among cytoplasmic vacuoles and numerous small vesicles (V).
were found in the cytoplasm. They were seen as two concentric rings about 45 and 65 nm. in diameter (Fig. 1). Following the standard classification for this type of structure (Anon. 1966) we refer to them as 'A particles'. A particles were typically found in association with vacuoles (Fig. 2) and all cells which contained A particles were also seen to be infected with bovine syncytial virus. Some A particles were associated with vacuoles in a way which suggests that they may have been budding (Fig. 4) to form intravacuolar particles. The intravacuolar particles were frequently seen but were difficult to resolve (Fig. 5). They were about 80 nm. in diameter and had a dense central region surrounded by a non-spiked envelope.

![Image](https://via.placeholder.com/150)

Fig. 3. Cytoplasm containing bovine syncytial virus internal components (arrow) and A particles (A).
Fig. 4. A particles which may be budding at vacuolar membranes.
Fig. 5. An intravacuolar structure resembling C particles (arrow).
Fig. 6. An extracellular structure similar to a mature B particle.

Later passages of bovine syncytial virus in BHK 21 cells revealed no cytoplasmic A particles, but some extracellular particles which had an eccentric dense nucleoid within a spiked envelope (mature B particles, Anon. 1966) were found (Fig. 6). The A particles were detected twice with a three-month interval in BHK 21 cells inoculated with bovine embryonic spleen cells infected with bovine syncytial virus. On both occasions the particles disappeared.
following serial subculture of bovine syncytial virus in BHK 21 cells and the propagation of the virus in spleen cells was terminated because the cells ceased to grow after several passages. No A particles were seen in bovine embryonic spleen cells infected with bovine syncytial virus.

Since the A particles described were seen as two concentric rings, with an outer diameter of approximately 65 nm., they were readily distinguishable from the internal component of bovine syncytial virus which in section appeared as a single dense ring measuring 40 to 45 nm. in diameter (Fig. 3). These A particles also differ from complete bovine syncytial virus particles (Dermott et al. 1971) even those which are enveloped within the cell by smooth cytoplasmic membranes (Boothe, van der Maaten & Malmquist, 1970; Estes, Coote & Noronha, 1970; Dermott et al. 1971).

The particles seen indicate the presence of an agent or agents with some features in common with both the mouse mammary tumour (Bittner) virus and leukaemia viruses, but the agents cannot at present be unequivocally classified with either of these.

If we disregard the possibility that the A particle is a laboratory contaminant, a role for which it seems poorly qualified, there are two possible sources of origin: it could be latent in BHK 21 cells or it could have been introduced into the BHK 21 cells when these were inoculated with bovine syncytial virus. It seems unlikely that the A particle is a virus latent in BHK 21 cells because it has not been previously reported, despite the widespread use of these cells by virologists. Furthermore, we have not seen A particles in BHK 21 cells infected with simian foamy virus. Hence if the ‘A particle virus’ is latent in BHK 21 cells it would have to be assumed that its replication is temporarily stimulated by bovine syncytial virus but not by another member of the same virus group. We therefore think it likely that the virus was introduced into the BHK 21 cells by mixing the latter with a suspension in ETC medium of bovine embryonic spleen cells infected with bovine syncytial virus. If so, the A particle is of bovine origin regardless of whether it came from the calf serum, the embryonic spleen cells or the original inoculum used to isolate bovine syncytial virus.

Attempts to isolate an agent from lymphosarcomatous cattle led to the detection of bovine syncytial virus (Malmquist et al. 1969). However, there is little evidence to suggest that bovine syncytial virus is the causative agent of the disease. In view of the present results it may be worth while to attempt to isolate the ‘A particle virus’ from lymphosarcomatous cattle by fusing cultured bovine cells with BHK 21 cells using inactivated virus rather than replicating bovine syncytial virus.

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(Received 8 February 1971)