Virus-like Particles in Bovine Sera for Tissue Culture

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SUMMARY

Virus-like particles were found in nine different bovine sera for tissue culture from commercial suppliers. These particles were spherical with an overall diam. between 70 and 95 nm. After negative staining, surface projections of about 11 to 12 nm were clearly seen. One of the nine sera was positive in an Ouchterlony test with antisera against bovine viral diarrhoea virus.

In many studies on virus production in tissue culture it is important to search for virus particles in the medium (Todaro, Zeve & Aaronson, 1970). In cases where the virus production of the cells is very low or the morphology of the virus particles is not well established it is necessary that the tissue culture medium itself, especially the serum component, does not contain virus-like particles.

In a previous report (Calafat, Hageman & Ressang, 1974) we described the morphology of bovine leukaemia virus (BLV) in short-term cultures of leukocytes from cows with persistent lymphocytosis. These particles were found in thin sections of the cells and after negative staining of material concentrated from cell culture medium. The yield of virus-like particles from the cells culture fluid was very low but after negative staining, particles could nevertheless be recognized on account of their clear surface projections. Thin sections from the same pellet showed also BLV particles. The diam. of the BLV particles was about 100 nm. However, in several pellets made from medium of monolayer producing BLV many particles with a diam. of about 80 nm and with surface projections were also found. These smaller particles were not observed in thin sections from the cell cultures and we therefore investigated whether the calf serum used for the preparation of the culture medium contained these particles.

In this report we describe virus-like particles found in nine different bovine sera from commercial suppliers used for tissue culture.

The samples of serum were obtained from commercial suppliers: calf serum (membrane filtered) batch no. 000160 and batch no. 000174 from Bio-cult Laboratories Ltd, Glasgow, Scotland; newborn calf serum no. 8353o from Microbiological Associates Inc., Bethesda, Md; foetal calf serum and newborn calf serum control no. C 536103 from Grand Island Biological Co., Grand Island N.Y.; foetal bovine serum control L 40284, foetal bovine serum no. 42211, new born calf serum control L 40282 and new born calf serum no. 40611 from Flow Laboratories, Irvine, Scotland.

Thirty five ml of each sample were centrifuged at 59164 g for 2 h and 2 pellets were obtained. One pellet was fixed with glutaraldehyde, postfixed with osmium tetroxide, dehydrated and embedded in a mixture of Epon and Araldite. Thin sections were stained with uranyl acetate and lead hydroxide.

Part of the other pellet was resuspended in a small quantity of 0.01 M-EDTA in order to dissolve protein aggregates from the medium and to get a cleaner specimen. A drop of the preparation was placed on a carbon-coated grid and allowed to dry. The grid was placed
Fig. 1. Virus-like particles after negative staining from different bovine sera. (a) Newborn calf serum from the Microbiological Associates; (b) foetal calf serum from Grand Island Biological Co.; (c) and (d) foetal bovine serum from Flow Laboratories. The particles show clearly surface projections (arrows). The surface projections in (c) seem a little curled. The virus-like particles are numerous as can be seen in (a).

on a drop of 3.5% glutaraldehyde in 0.1 M-phosphate buffer, pH 7.1, for 10 min and washed with distilled water and then the material on the grid was negatively stained by applying a drop of 2% sodium phosphotungstate, pH 7.2.

The preparations were examined with a Philips EM-300 electron microscope operating at 80 kV; to prevent specimen contamination, a cooling device was used.

All samples of bovine serum examined contained virus-like particles of a similar morphology even after inactivation of the sera for 30 min at 56 °C. These particles were numerous in all preparations. After negative staining the particles were easily recognized by the surface projections of about 11 to 12 nm. The particles were spherical with an overall diam. of 70 to 95 nm (Fig. 1). In thin sections of the pellets, the same particles were found surrounded by granular material (Fig. 2). The surface projections of about 11 to 12 nm were also seen in the majority of these preparations.
Benz & Moses (1974) were the first to describe virus-like particles in thin sections of 22 samples of bovine sera. These particles had no surface projections and the central core was of varying density. It is possible that these particles are the same as the ones we describe here because surface projections are not always as easily shown in thin sections as after negative staining. We had the same experience with the mammary tumour virus.

Bovine sera are frequently contaminated with bovine virus diarrhoea virus (BVDV), infectious bovine rhinotracheitis virus (IBRV), para-influenza virus 3 (PI-3; Kniazeff, 1968; Molander et al. 1972) and bovine syncytial virus (BSV; Van der Maaten et al. 1973). IBRV has a diam. of about 100 to 150 nm (Melnick, 1973); the diam. of PI-3 virus is between 150 to 300 nm (Melnick, 1973); the diam. of BVDV is about 60 nm according to Horzinek, Maess & Laufs, (1971) or 80 to >100 nm according to Ritchie & Fernelius (1969); and the diam. of BSV is about 90 to 115 nm (Malmquist, Van der Maaten & Boothe, 1974). The particles described here have a diam. of 70 to 95 nm (40 to 65 nm without surface projections). On the basis of these measurements, we can exclude that the particles found in our material are IBR of PI-3 virus.

After negative staining BSV has a morphology similar to the presently described particles and it also has surface projections of about the same length (Malmquist et al. 1974). The diam. of BVDV is similar to our virus particles, but it is not known yet whether BVDV has surface projections (Dutta, Shope & Pomeroy, 1965) or not (Ritchie & Fernelius, 1969).

Because it was difficult to determine by morphological studies alone the type of virus these particles represent—if these particles are indeed a virus—a double diffusion test in 0.7% agarose gel was carried out with samples of each bovine serum against undiluted anti-BVDV, PI-3, IBRV, BSV and BLV sera. Only the sample from Bio-cult Laboratories produced a precipitation line against anti-BVDV serum, but it is possible that the negative results are due to the destruction of the virus antigens during the preparation of the sera for the tissue culture.

When morphological studies are made on virus particles isolated from tissue culture medium, it is important to know whether virus-like particles are present in the bovine serum used for the preparation of the tissue culture medium. This is especially important when...
the morphological studies are based on negative staining, the morphology of the virus is not well known and the production of the virus is low. We have described as BLV in another paper (Calafat et al. 1974) particles isolated from tissue culture media of short-term cultures of leukocytes from cows with persistent lymphocytosis. These particles probably were contaminating particles from the foetal bovine serum.

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