Demonstration of Virus-like Particles in a Bovine Cell Line

(Accepted 5 November 1968)

Virus-like particles have been observed in permanent cell lines derived from malignant mouse fibroblasts (L cells) and from baby hamster kidneys (BHK 21 cells). Dales & Howatson (1961), Kindig & Kirsten (1967) and Cromack (1968) reported virus-like particles of type C and type A in lines of cultured strain L mouse cells. Bernhard & Tournier (1964), McGee-Russell, Vizoso & Sanders (1965) and Compans et al. (1966) described the presence of virus-like particles with a diameter of 85 to 120 nm. in baby hamster kidney cell lines (BHK 21 clone 13, and BHK 21-F).

In the present studies we observed virus-like particles in a cell line derived from calf kidney cell culture. The primary cultures of calf kidney were prepared 2½ years previously in a medium consisting of Earle's salt solution with 10% calf serum and 10% lactalbumin hydrolysate (but 5% in Hanks's salt solution) and with penicillin (100 u./ml.) and streptomycin (0.1 mg./ml.). The cultures were incubated at 37° for 7 days and then stored at room temperature. After 2 weeks the cell layers were suspended, treating them with a mixture of EDTA and trypsin, and bottle cultures were set up using the same medium. Two days later this medium was replaced by the medium described by Stoker & Macpherson (1961); kanamycin (0.4 mg./ml.) was added. This medium was used until the 9th subculture of the cells, and was then reinforced with 10% lactalbumin hydrolysate (5% in Hanks's salt solution) for subcultures during the next 2 years. In the primary cultures and the early subcultures epitheloid cells were predominant. Spindle-shaped cells appeared in the 8th subculture and soon replaced the original cells. Cultures containing this cell-type continued to grow on serial subculture without apparent variation in growth rate. Usually subcultures were prepared at intervals of 3 to 4 days, and the cell line was designated CK-66. Cytopathic changes developed in CK-66 cells when they were infected with the following viruses: vesicular stomatitis virus, type Indiana; foot-and-mouth disease virus, types O, A and C; enteroviruses of cattle; mouse Elberfeld (ME) virus; bovine rhinovirus (SD-1 strain); Sindbis virus; an influenza A virus strain; and pseudorabies virus.

CK-66 cells of the 190th to 210th subcultures were used for electron microscopic studies. Cell layers of bottle cultures were fixed using glutaraldehyde and 2% osmium tetroxide. Specimens were embedded either in an epoxy resin (Epon 812, Serva, Heidelberg) or in Vestopal (Mikropal, Ferak, Berlin). Sections were cut on a Servall MTI-Porter-Blum ultramicrotome and stained by 1 min. applications of a saturated solution of uranyl acetate diluted with an equal volume of ethanol, followed by a solution of lead citrate (Reynolds, 1963). The sections were examined in a Siemens Elmiskop I electron microscope. Morphologically different types of virus-like particles were observed. Spherical particles with a diameter of about 90 nm. containing an electron-dense central core about 45 nm. in diameter, from which lines radiated to the outer border of the particle (Pl. 1a,b), were frequently seen within the nuclear envelope (Pl. 1g) or in cisternae of the endoplasmic reticulum often arranged in a chain-like order (Pl. 1f), and resembled the virus-like particles described in BHK 21 cells.
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(Bernhard & Tournier, 1964; McGee-Russell et al. 1965; Compans et al. 1966). Round or slightly oval particles, with diameters ranging from approximately 100 to 150 nm., were seen with a double-layered membrane, about 15 nm. thick, observable with good resolution. Closely apposed to the inner layer of this membrane an electron-dense region was observed. In this region often some small electron-transmitting areas were present (Pl. 1 e, h, i). These particles resembled elementary bodies of mycoplasmas as demonstrated by Hummeler, Tomassini & Hayrick (1965) and were found in the extracellular space sometimes associated with elongated forms which were similar in diameter and structure to the particles (Pl. 1 i, right side). Particles such as those seen in Pl. 1 d averaged 100 to 110 nm. in diameter and contained a dense central 'nucleoid' and an outer region of intermediate density surrounded by a double membrane. These particles were localized in the extracellular space and were thought to be type C particles according to Bernhard's & Guérin's (1958) classification. Finally there were spherical particles measuring about 110 nm. in diameter characterized by a concentric double membrane and an eccentric 'nucleoid' (Pl. 1 e). These were assumed to be type B particles (Bernhard, 1958). In the sections viewed the first two types of particle were seen frequently and in about the same quantity. In contrast, the type C and B particles were detected very rarely.

Tissue culture fluids of CK-66 cells of the 202nd subculture were centrifuged in a Spinco model L ultracentrifuge, rotor 21, at 19,000 rev./min. for 1 hr. The sediment was suspended in isotonic NaCl 0·01M-phosphate buffer, pH 7.2, to give 100-fold volume concentration and was negatively stained. The negatively stained preparations were made with 2% phosphotungstic acid adjusted to pH 6·0 with 5N-KOH. Pl. 1 j, k, l illustrate the particles viewed in the prepared specimens. Two types of particles were encountered. The one type (Pl. 1 j) was round, approximately 120 nm. in diameter and consisted of a membrane and a core, about 50 nm. in diameter. The other type (Pl. 1 k, l) appeared as spherical bodies 115 to 120 nm. in diameter with inner structures (about 90 nm.), suggesting a coiled component. At present, it cannot be decided whether the objects seen by negative staining are viruses, and, if they are, which of them represents one or the other of the four types of particle detected in ultrathin sections of CK-66 cells (Pl. 1 a to i).

EXPLANATION OF PLATE

a. An extracellular virus-like particle with an electron-dense core from which lines radiate to the membrane of the particle.
b. A virus-like particle.
c. Two extracellular virus-like particles.
d. An extracellular type C virus-like particle.
e. An intracellular type B virus-like particle.
f. Virus-like particles in a vacuole of the cytoplasm.
g. Some of these particles are seen within the nuclear envelope.
h. Virus-like particles observed in the extracellular space. Some of the particles reveal a double membrane.
i. Is similar to h and shows elongated forms besides spherical particles.
j. A negatively stained virus-like particle with a central spherical core separated from a relatively broad envelope.
k and l. Negatively stained virus-like particles with an envelope which surrounds an inner structure suggested to consist of a coiled component.
The origin and significance of the virus-like particles is unknown. The particles shown in Pl. 1 d, e, k, l are similar in morphology and size to viruses found in association with mouse mammary tumours, murine leukaemias and lymphomas, Rous sarcoma and avian leukaosis (Bernhard, 1960).

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REFERENCES


(Received 23 September 1968)