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CANINE DISTEMPER VIRUS REPLICATION IN CELLS ON MICROCARRIERS

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Summary. – Chick embryo and Vero cells were grown on Gelaspher M gelatin microcarriers in suspension culture. The microcarriers had no adverse effects on cell morphology and growth. Microcarrier cell cultures were used for large-scale production of canine distemper virus. Virus yields ($TCID_{50}$ per ml) were more than 10-times higher as compared to stationary cell culture.

Key words: canine distemper virus; chick embryo cells; Vero cells; gelatin microcarriers; large-scale production

Cell cultures on microcarriers is a technique with enormous importance for the large-scale production of animal cells, viruses or cell products. This technique can produce high cell yields in relatively small culture volumes. The idea of cultivation of cells on microcarriers has been first introduced by van Wezel (1967). The microcarrier culture technology was used for large-scale production of foot-and-mouth disease virus vaccine (Spier and Whiteside, 1976; Meignier, 1978) and for small-scale growth of several other viruses (Giard *et al.*, 1977). The use of gelatin microcarriers for large-scale production of infectious bovine rhinotracheitis virus was reported earlier (Leško *et al.*, 1993). The advantages of using gelatin microcarriers for cultivation of fibroblasts and endothelial cells were described by Wisseman and Jacobson (1985).

In this study we report on the use of gelatin microcarriers Gelaspher M for the preparation of large amounts of canine distemper virus (CDV).

Virus and cells. F-BN 10/83 strain of CDV was used. Primary chick embryo (CE) and Vero cells were cultivated in Eagle's minimal essential medium (MEM, USOL, Prague) supplemented with 10 % inactivated calf serum. CE cells were prepared from 10–11 day-old embryonated eggs by a standard procedure.

Microcarriers Gelaspher M (Lachema, Brno), consisting from chemically modified gelatin with particle size 150–200 μm were used. They were obtained in autoclaved form as a suspension in phosphate buffered saline pH 7.4 (PBS). For cultivation of both types of cells a concentration of 30 ml of Gelaspher M sediment per one liter of cultivation medium was employed. One ml of the microcarrier suspension corresponded to approximately 260 000 particles with surface area 250 cm^2. Microcarriers were seeded with 4×10^5 viable primary CE cells or 3×10^4 Vero cells in suspension per cm^2 surface area.

Culture vessel. Both types of microcarrier cell culture were kept in a special 5 liter bottle (Leško *et al.*, 1993). The bottle was siliconized to prevent the adherence of cells and beads, and it contained

a magnetic spinbar driven at 50 rpm.

Cultivation of cells on microcarriers. Approximately 1/3 of the final volume (1 liter) of cultivation medium and the appropriate amount of microcarriers were preincubated in the bottle at 37 °C. Then cells were added at the optimal concentration and allowed to attach for 12–24 hrs at 37 °C. During the attachment period the cultures were stirred discontinuously, every 30 mins for 1 min. After that about 80–90 % of cells attached to the bead surface. The attachment period was controlled in samples of microcarrier suspension for cell adherence, pH and microbiological sterility. Then the rest of the final volume (2 liters) of warm cultivation medium was added and the suspension was kept continuously stirred for additional 48–72 hrs. During this period about 90 % of microcarrier beads were covered by cell monolayer.

Virus infection of cells. The stirring was stopped, microcarriers settled down and the medium was exhausted. Then microcarriers were washed with 3 liters of PBS and infected with CDV in 600 ml of warm cultivation medium without serum at a multiplicity of 0.01 $TCID_{50}$ per cell. The suspension was stirred for 1 hr, the volume of the culture was made to 3 liters by addition of the complete cultivation medium and the incubation was continued. The cells were controlled for cytopathic effect (CPE) and the cultivation medium was assayed for infectious virus at 24 hr intervals. When a complete CPE was observed in all cells (after 2–3 and 4–5 days in CE and Vero cells, respectively), the stirring was stopped, the microcarriers were left to settle down, and the cultivation medium was saved.

Virus titration. The infectious titer of CDV ($TCID_{50}$/ml) was assayed in tube cultures of the same type of cells as those used for propagation of virus in microcarrier culture by inoculating them with serial tenfold dilutions of virus material and reading CPE after 2–7 days.

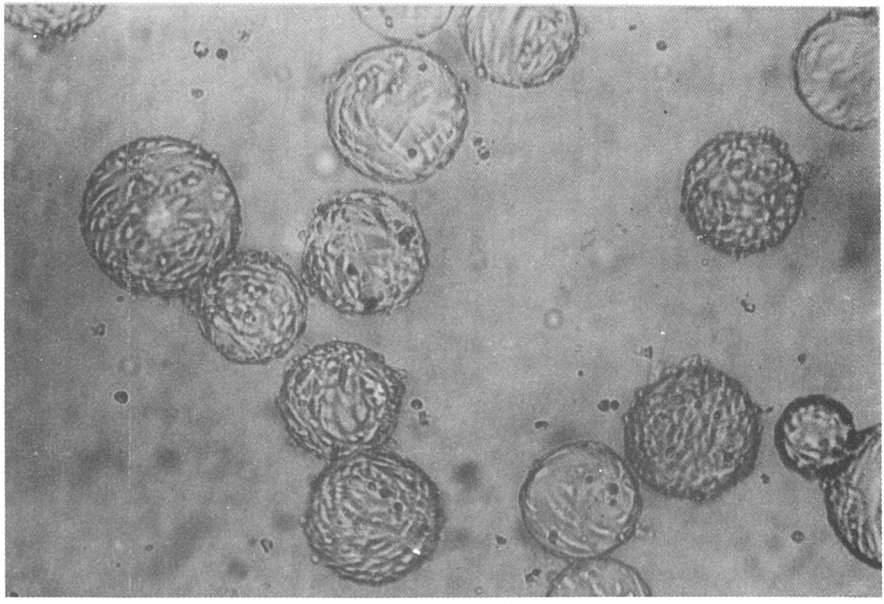

Fig. 1
CE cells after 48 hrs of cultivation on microcarriers

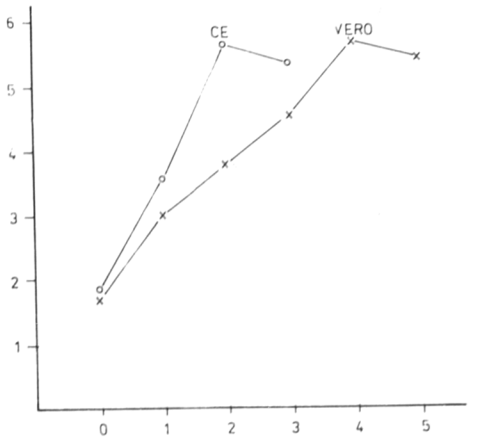

Fig. 2
Growth curves of CDV in CE and Vero cells on microcarriers
Abscissa: days p. i.; ordinate: log $TCID_{50}$/ml

For propagation of CDV two types of cells were used in our experiments: primary CE cells and stabile line of Vero cells. The attachment period of these cells to Gelaspher M microcarriers was 12–24 hrs. During this period 80–90 % cells attached to the surface of the microcarrier beads. Thereafter the cells began to spread out and proliferate. Our experiments showed that both types of cells were able to grow on Gelaspher M microcarriers exhibiting the same morphology as that normally observed in stationary Roux flask cultures.

The proliferation of CE cells on microcarrier beads after 48 hrs of cultivation is presented in Fig. 1, where 90 % of beads display a confluent monolayer on their surface. The growth curves of CDV in CE and Vero cells on microcarriers are demonstrated in Fig. 2. The highest infectious virus titers were reached on the 2nd day p. i. in CE cells and on the 4th day in Vero cells. First signs of CPE were visible after 24 hrs in both types of cells (Fig. 3) and CPE became complete after 2–3 days in CE cells (Fig. 4) and after 4–5 days in Vero cells (results not shown). The stationary cultures infected with CDV displayed a complete CPE and the highest virus titer after 4–5 days with CE cells, and after 5–7 days with Vero cells.

Table 1. Infectious titers of CDV propagated in stationary and microcarrier cell cultures

Cells	$TCID_{50}$/ml (days p. i.)	
	Stationary culture	Microcarrier culture
CE	$10^{3.9}$ (4–5)	$10^{5.1}$ (2–3)
Vero	$10^{3.7}$ (5–7)	$10^{4.9}$ (4–5)

$TCID_{50}$/ml values are averages from 10 experiments.

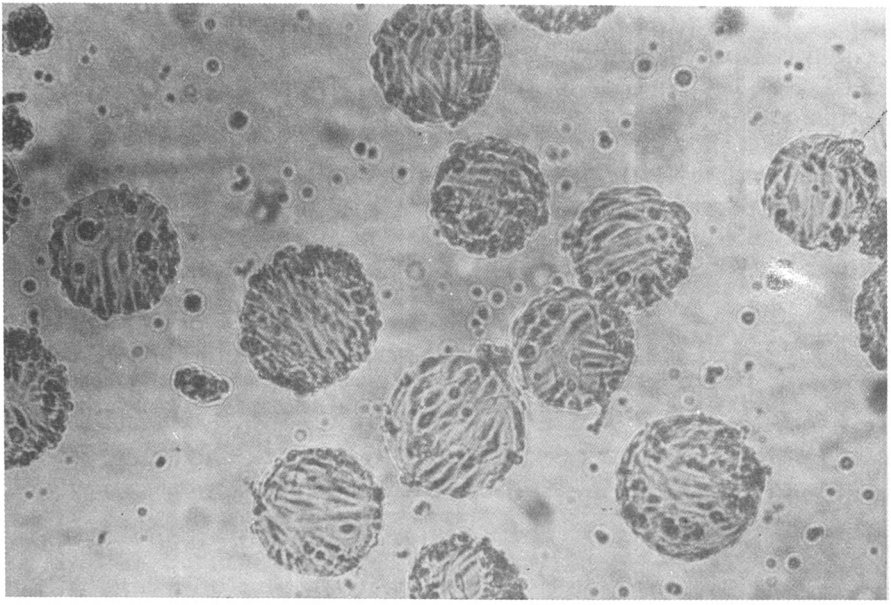

Fig. 3

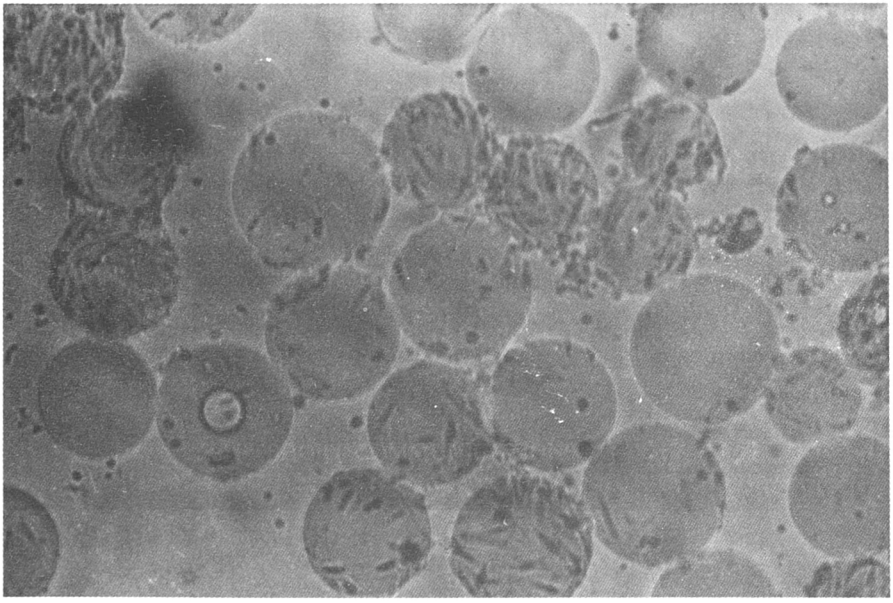

Fig. 4

For legends see page 416

A comparison of the highest virus titers obtained in microcarrier and stationary cell cultures (Table 1) demonstrates that they are by more than 1 log higher in the microcarrier cultures.

Our results show that gelatin microcarriers Gelaspher M can be successfuly employed for large-scale cultivation of high titer infectious CDV in CE or Vero cells in suspension culture. The yields of both cells and virus per ml of cultivation medium are substantially higher than in the classical stationary culture. The Gelaspher M beads are mechanically stabile and non-toxic for cells. Their transparency enables an easy microscopic control of cell morphology. The advantages of this microcarrier system can be taken in consideration in producing large amounts of CDV for preparation of a vaccine.

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Fig. 3
CPE of CDV on CE cells on microcarriers 24 hrs p. i.

Fig. 4
CPE of CDV on CE cells on microcarriers 48 hrs p. i.