Acta virol. **25** : 315—318, 1981

REPRODUCTION OF ATTENUATED MUMPS VIRUS IN DOG KIDNEY CELL CULTURES PRECULTIVATED AT 37 AND 34 °C

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Received February 11, 1981

Summary. — The titres of attenuated mumps virus reached in primary dog kidney cell cultures precultivated at 34 °C were on the average 8-fold higher than in cultures precultivated at 37 °C. This effect was probably due to adaptation of the cells to lower temperature, connected with changes in cell metabolism, which were also manifested by different growth and survival of the cell cultures on glasses of different composition.

Key words: attenuated mumps virus; primary dog kidney cells; incubation temperature

Introduction

Primary dog kidney cell cultures have been used in our Institute for many years in routine production of a live vaccine against measles (Mareš and Dřevo, 1965; Dřevo and Časný, 1971). In recent years the possibility has been investigated to use them for the preparation of a live mumps vaccine (Izbický and Fröhlichová, 1979; Izbický and Časný, 1981). We are reporting the results of experiments which showed that the reproduction of attenuated mumps virus and the quality of cultures are significantly affected by the temperature at which the cells had been grown.

Materials and Methods

Cell suspensions were routinely prepared by trypsinization of the renal cortex from beagle puppies up to 6 months old. Ten million cells in 150 ml of lactalbumin hydrolysate medium VEL (a commercial product of SEVAC, Prague), supplemented with 10% inactivated calf serum and 0.9 g $NaHCO_3$ and 0.03 g neomycin per liter were seeded into 1200-ml Roux bottles with a 220 cm^2 cultivation area. The stoppered cultures were then incubated stationarily in a horizontal position at 37 or 34 °C until a confluent monolayer was reached, usually for 7 days. Experiments on virus reproduction were done in Sial glass bottles. In experiments on cell growth and further maintenance of uninfected cultures, also Simax glass bottles were employed. The composition of both glasses is given in Table 1.

In experiments on virus reproduction we used the cell cultures immediately after the monolayers had formed. The attenuated AA strain of mumps virus (a variant of PVA strain; Izbický and Fröhlichová, 1979) at the 5th passage level in primary dog kidney cells was inoculated in the Roux bottle cultures at a multiplicity of 10^{-3} $TCID_{50}$ per cell, always in 150 ml of fresh medium 199 (Morgan *et al.*, 1950) supplemented with 5% inactivated calf serum and 1.65 g $NaHCO_3$ and 0.03 g neomycin per liter. Further incubation of all stationary cultures proceeded at 34 °C.

Table 1. Chemical composition (in %) of Sial and Simax glass*

Glass	SiO_2	B_2O_3	Al_2O_3 + Fe_2O_3	BaO	CaO	K_2O	Na_2O
Sial	74.40	8.30	5.30	4.40	0.70	2.00	4.80
Simax	79.45	12.50	3.15	—	0.35	0.95	3.48

*According to Volf *et al.* (1967).

Since the first harvest (6th day after inoculation), the cultures were maintained in a lowered volume (120 ml) of the same medium without the addition of calf serum and with $NaHCO_3$ concentration reduced to 1.2 g per liter. Virus suspensions were harvested and the media changed at intervals of 2 or 3 days.

Cell counts were determined in uninfected cultures grown and maintained under the same conditions as the infected cultures. The cultures were dispersed by a trypsin-EDTA solution and stained with crystal violet and the cells counted in a Bürker chamber.

Virus in the culture fluids was titrated in Vero cell tube cultures in modified Eagle's minimal essential medium with galactose and pyruvate based on haemagglutination and haemadsorption of chick erythrocytes and a cytopathic effect (Izbický and Fröhlichová, 1979). In addition to routine $TCID_{50}$ titres (log V) calculated according to Kärber, we compared the yields of infectious virus also based on log values of the cumulative titre (log V_c) of the total $TCID_{50}$ yield at the given day per ml culture fluid (Izbický and Fröhlichová, 1979). The significance of the results was evaluated by Student's t-tests.

Results

The routine (log V) and cumulative (log V_c) titres of the attenuated mumps virus in culture fluids harvested from the 6th to the 15th day after inoculation of primary dog kidney cell cultures that had been grown at 37 or 34 °C are presented in Table 2.

Routine titres were on the average by 0.9 log unit (difference significant at $p_{0.05}$), i. e. about 8-fold higher in medium from cultures grown at 34 °C (B) than in medium from cultures grown at 37 °C (A). The cell counts at the time of inoculation were in B cultures by 18% higher than in A cultures. The

Table 2. Comparison of titres (log V and log V_c) of attenuated mumps virus in the fluid phase of cultures of dog kidney cells that had been grown at 37 and 34 °C

Day p.i.	Cells grown at 37 °C		Cells grown at 34 °C		log V37 − − log V34*
	log V	log V_c	log V	log V_c	
6	5.0	5.00	5.4	5.40	−0.4
8	5.0	5.30	5.5	5.75	−0.5
10	5.0	5.48	6.2	6.33	−1.2
13	4.7	5.54	6.4	6.67	−1.7
15	5.2	5.71	5.9	6.74	−0.7

*$\bar{x} = -0.900$, $s = 0.486$.

At inoculation, the cell counts per Roux bottle were 28 (37 °C) and 33 (34 °C) millions.

final cumulative titre in B cultures was by 1.03 log unit, i. e. about 10-fold higher than in A cultures.

In a pilot experiment we tested also cultures grown at 32 °C and after inoculation maintained at 34 °C. In culture fluids harvested up to the 15th day after inoculation, the $TCID_{50}$ titres were significantly higher (on the average by 0.8 log unit at $p_{0.01}$) than in fluids from cultures grown at 37 °C.

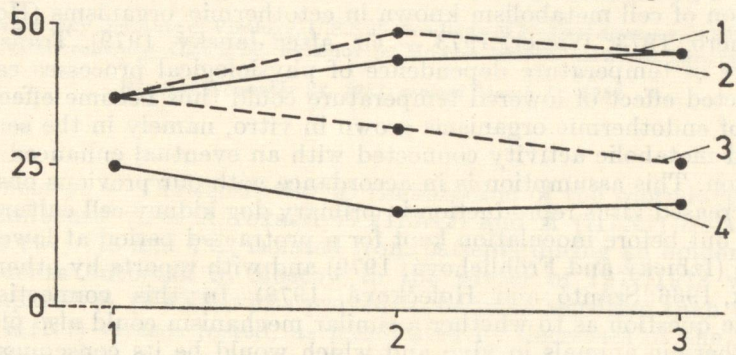

Fig. 1.

Cell counts in primary dog kidney cell cultures grown in Sial (— — —) and Simax (———) glass vessels at two temperatures

Abscissa: 1 — cell counts determined after 1 week of growth at 37 (curves 3, 4) or 34 (curves 1, 2) °C; 2 and 3 — cell counts after maintaining the cultures in protein-free medium at 34 °C for 1 and 2 weeks, respectively.

The effect of growth temperature on the cell counts after the monolayers had been formed and in the course of maintenance of uninfected primary cultures of dog kidney cells was studied in eight experiments. Fig. 1 illustrates the average cell counts after one week of growth at 37 (A) or 34 °C (B) and a further two weeks of maintenance of both types of culture at 34 °C. The cell counts in type A cultures at all three intervals were markedly higher in Sial glass vessels than in Simax glass vessels. The differences were significant in both the 1st and 2nd week of cultivation ($p_{0.05}$ and $p_{0.01}$, respectively). It is also evident that the cell counts in B type cultures were higher than in type A cultures, especially in the 2nd and 3rd week. The difference was especially marked in Simax glass vessels in the 2nd week (difference significant at $p_{0.01}$).

Discussion

Reproduction of attenuated mumps virus in primary cultures of dog kidney cells was clearly dependent on the temperature at which the cells had been grown before inoculation. In the fluids from cultures grown at 34 °C, the virus titres were on the average 8-fold and the cumulative infectious virus yields 10-fold higher than in fluids from cultures grown at 37 °C. The results obtained with cultures grown at 32 °C were similar.

The increased yields of virus from cultures grown at 34 °C could have been only partially due to higher cell counts at the time of inoculation. The increased infectious virus yields could evidently have been considerably affected by a changed quality of the cultures due to lowered incubation temperature during growth of the cultures. We assume that an important role was played here by a phenomenon similar to the slow temperature adaptation of cell metabolism known in ectothermic organisms (Hochachka and Somero, 1973; Wieser, 1973 — cit. after Janský, 1979). Translation of the curve of temperature dependence of physiological processes caused by a protracted effect of lowered temperature could thus become effective also in cells of endothermic organisms grown in vitro, namely in the sense of an increased metabolic activity connected with an eventual enhanced virus reproduction. This assumption is in accordance with our previous observation of an increased virus reproduction in primary dog kidney cell cultures grown at 37 °C but before inoculation kept for a protracted period at lowered temperature (Izbický and Fröhlichová, 1979) and with reports by other authors (Stanček, 1966; Szántó and Holečková, 1978). In this connection there arises the question as to whether a similar mechanism could also play a role in endothermic animals in vivo and which would be its consequences e. g. on prolonged local or total hypothermia of the organism.

The cell counts in cultures grown at 34 and 37 °C also indicate significant changes in cell metabolism due to lowered incubation temperatures. Our experiments showed that in addition to the chemical composition of the glass also the temperature of incubation significantly affects the growth and survival of dog kidney cell cultures.

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