

## Effect of Sucrose on the Infectivity, Migration and Neutralization of Neurovirulent Poliovirus Type 1

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**Abstract:** Infectivity of neurovirulent poliovirus type 1, Brunhilde strain, was elevated more than 1 log on human rhabdomyosarcoma (RD) cells in the presence of 7.5 percent sucrose, although migration of the virus through 15 percent sucrose solution was not significant. Apparent inhibition of virus neutralization by rabbit antiserum was observed at all serum dilutions tested (1:100-1:1600) in the presence of 11.25 percent sucrose and at 1:800 serum dilution in the presence of 5.6 and 2.8 percent sucrose.

**Key words:** Neurovirulent poliovirus, Infectivity, Migration, Neutralization, Sucrose

### INTRODUCTION

It was shown that neutralized poliovirus was capable of adsorbing to HeLa cells (Joklik & Darnell 1961; Mandel 1967 a), and further analysis of the interaction between HeLa cells and neutralized poliovirus were reported (Mandel 1967 b, Lewenton-Kriss & Mandel 1972). Several hypotheses were postulated for the relationship between virus stabilization and its neutralization as well as for the one-hit nature of the neutralization reaction (Mandel 1976, Katagiri *et al.*, 1968). However, the mechanism of poliovirus adsorption to susceptible cells, its migration and neutralization in the presence of sucrose still remain unexplained. While, stabilization of attenuated poliovirus strains by sucrose have been reported (Mirchamay *et al.*, 1978; Srivastava *et al.*, 1988; Srivastava, 1989).

This paper reports the effect of sucrose on the infectivity of neurovirulent poliovirus on RD cells, its migration and neutralization.

## MATERIALS AND METHODS

*Virus and cells:* Neurovirulent poliovirus type 1, Brunhilde strain, was kept in the Enterovirus Research Unit, Institute of Hygiene and Epidemiology, Prague, Czechoslovakia, and was once passaged on human rhabdomyosarcoma (RD) cells. The cells were grown in Eagle's minimal essential medium (MEM, Eagle, 1959) with 10 percent heat-inactivated calf serum at 37 °C, and the serum concentration was reduced to 2 percent for the maintenance medium.

*Infectivity assay:* Two assay methods on RD cells were used. The first method of TCID<sub>50</sub> titration was used for virus infectivity in the presence of sucrose. The cell suspension ( $2 \times 10^5$  cells / ml in the maintenance medium) was distributed in several bottles and mixed with an equal volume of different concentration of sucrose solutions as well as physiological saline as a control. The pH of the physiological saline was adjusted to 7.0 and sterilized in all experiments in this study. Five ml of each mixture was distributed into replicate middle-sized tubes and added with 5 ml each of virus specimens serially diluted in 10-fold steps with physiological saline. Four replicate culture tubes were prepared from each middle-sized tube by distributing 2 ml of the cell-sucrose-virus mixture in each culture tube and tightly stoppered. After 6 hours of incubation at room temperature, the tubes were transferred to 37 °C and cytopathic effect of the cells was observed microscopically on the 7th day to calculate the virus titer by Reed and Muench's method. The second method of plaque titration was used for the migration and neutralization studies of the virus. Growth medium was removed from RD cell cultures in 6 cm diameter Petri dishes, and 0.1 ml of the virus specimen was inoculated to each dish. After 1 hour adsorption at 37 °C, the cells were covered by 5 ml of overlay medium containing 1 percent agarose in the maintenance medium with neutral red. When agarose was solidified, the dishes were incubated at 37 °C for 5 days to form plaques, and virus infectivity was shown by plaque forming unit (PFU).

*Sucrose solutions:* Weighed amount of sucrose (Lachema, Brno, Czechoslovakia) was spread on a sheet of aluminium foil and sterilized overnight by ultraviolet light from 4 lamps of 15 Watts at a distance of 30 cm. The sucrose was dissolved in physiological saline to prepare various concentration (w / v) of solutions.

*Neutralization test:* The procedure of Wallis *et al.* (1965) was modified using diluent of 5 percent calf serum in MEM. Immune rabbit serum was diluted in 2-fold steps from 1:50 to 1:800 and mixed with equal volumes of sucrose solutions or physiological saline as a control. Sucrose-serum or control mixtures were added with equal volumes of the virus diluted to 200 PFU / 0.1 ml and incubated at 37 °C for 1 hour, and virus infectivity was assayed as described above using 4 dishes for each mixture. Neutralization of the virus at each serum dilution was shown by percent of plaque reduction compared with the number of plaques in the control without antiserum.

*Antiserum:* Antipoliovirus serum was raised in rabbits by several intravenous injections of partially purified virus, and was heat-inactivated at 56 °C for 30 minutes.

## RESULTS

*Infectivity of poliovirus on RD cells in the presence of sucrose*

RD cell suspensions were mixed with equal volumes of sucrose solutions of 60, 30, 15, 7.5 and 3.75 percent, as well as physiological saline as a control. Each mixture was added to an equal volume of the diluted virus, so that final concentration of sucrose became 15, 7.5, 3.75, 1.87 and 0.93 percent, and the control did not contain sucrose. The virus infectivity in the final mixture was assayed by TCID<sub>50</sub> as described in the Materials and Methods. The result in Table 1 showed that in the presence of 15 percent sucrose the cells were damaged nonspecifically and the virus titer was not calculated. In the presence of 7.5 percent sucrose, the virus titer increased 1.3 logs compared with the control, while 3.75 percent or less concentration of sucrose showed little increase in the virus titer with less than 0.6 logs from the control.

**Table 1.** Infectivity of poliovirus assayed on RD cells in presence of sucrose

Percentage of sucrose	Virus Titer in log
15	Cells destroyed
7.5	8.5
3.75	7.8
1.87	7.5
0.93	7.0
Control	7.2

*Migration of poliovirus to RD cells in the presence of sucrose*

In replicate tubes,  $7 \times 10^5$  RD cell suspension in 0.1 ml of the maintenance medium was mix with 0.5 ml of 20 percent sucrose. The cell-sucrose mixture in each tube was covered by 1 ml of 15 percent sucrose with Bromphenol blue (BPB) and then by 1 ml of 9 percent sucrose containing 100 PFU of the virus. The tubes were tightly stoppered and incubated at 37 °C. After various times of incubation, one of the tubes was taken out and portion of each layer in the tube was collected by Pasteur pipett using BPB as a marker of the middle layer and kept frozen at -20 °C until infectivity was assayed by PFU.

The results in Table 2 showed that virus titer in the top layer increased slightly until 8 hours and then decreased at 16 hours of incubation. This slight increase in the virus titer, especially at 2 and 4 hours of incubation, may be due to the dispersion of virus clumps besides enhanced infectivity by sucrose. While decreased titer at 16 hours of incubation could be due to the thermal inactivation of the virus. Virus infectivity in the middle and the bottom layers was significantly lower than the titer in the top layer until 8 hours of incubation, indicating that migration of the virus through the middle layer

**Table. 2.** Migration of poliovirus in the presence of sucrose

Hours of incubation	Layers and virus infectivity (PFU/ml)		
	Top	Middle	Bottom
1	125	25	35
2	170	25	5
4	170	10	10
8	145	25	20
16	20	40	45

containing 15 percent sucrose was not significant. However, the virus titer in the middle and the bottom layers increased slightly and became higher than the titer in the top layer at 16 hours of incubation, indicating the virus growth in RD cells in the bottom layer caused by small number of the virus migrated through the middle layer.

*Neutralization of poliovirus by antiserum in different concentration of sucrose*

Antipoliovirus rabbit serum serially diluted in 2-fold steps from 1:50 to 1:800 was mixed with equal volumes of 45, 22.5, 11.25, and 5.6 percent sucrose solutions as well as physiological saline as a control, thus serum dilutions became 1:100 to 1:1600. Each mixture was added with an equal volume of diluted poliovirus thus final sucrose concentration

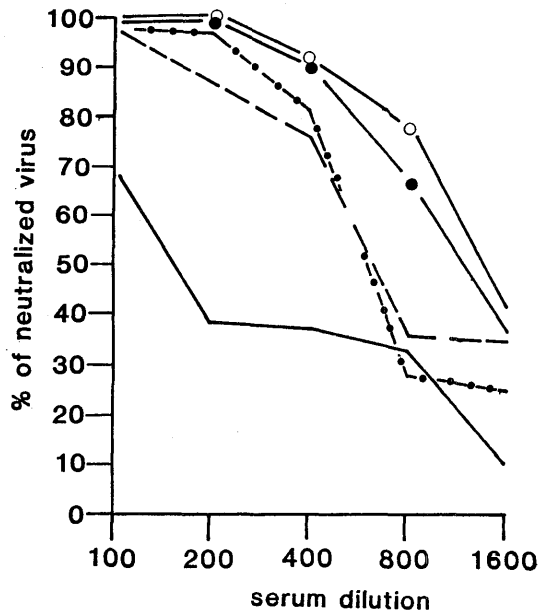


Fig. 1. Neutralization of poliovirus in the presence of sucrose. Sucrose concentration at final concentration of: 11.25 percent full line (—), 5.6 percent dashed line (---), 2.8 percent dotted line (●—●—●), 1.4 percent closed circles (●—●—●), 0 percent control open circle (○—○—○).

became 11.25, 5.6, 2.8 and 1.4 percent and the control was without sucrose. The final mixtures were incubated at 37 °C for 1 hour, and residual virus infectivity was assayed by PFU to calculate percent of neutralized virus compared with the control without antiserum.

The results in Fig. 1 showed that virus neutralization was apparently inhibited in the presence of 11.25 percent sucrose at all serum dilutions tested. On the other hand 5.6 and 2.8 percent sucrose inhibited virus neutralization at 1:800 serum dilution and 1.4 percent sucrose did not give significant effect on the virus neutralization.

#### DISCUSSION

Sucrose stabilized the infectivity of attenuated poliovirus strains at high temperatures for several weeks (Mirchamsy *et al.*, 1978; Srivastava *et al.*, 1988; Srivastava, 1989). The mechanism by which cell-adsorption and neutralization of neurovirulent polioviruses in the presence of sucrose is not fully understood yet. Nonspecific degradation of RD cells in the presence of 15 percent sucrose in this study could be due to the osmotic shock. Enhanced virus infectivity in the presence of 7.5 percent sucrose might be due to the enhanced adsorption and penetration of the virus to the cells, or it might also be due to the dispersion of the virus clumps. The latter possibility could be compatible with the finding in the virus migration study. Inhibition of the virus neutralization in the presence of 11.25 percent sucrose might be due to the reduced mobility of the virus particles to meet with antibody molecules, as supposed by the migration study. However, it is rather hard to explain the reduced neutralization in the presence of 5.6 and 2.8 percent sucrose at specific serum dilution of 1:800. Since 7.5 percent sucrose showed certain enhancing effect on poliovirus infectivity, apparently reduced level of the virus neutralization could be due to the presence of some sucrose in the inoculum. These possibilities should be analyzed by further studies.

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