PROCEEDINGS OF THE SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE 166, 282–286 (1981)

Enhancement of Infectivity of Cell-Free Varicella–Zoster Virus with Diethylaminoethyl-Dextran (41059)

KEIKO SASAKI,[1] TORU FURUKAWA, AND STANLEY A. PLOTKIN

The Wistar Institute of Anatomy and Biology, 36th Street at Spruce, Philadelphia, Pennsylvania 19104

Abstract. Diethylaminoethyl-dextran (DEAE-D) increased the cytopathic effect of varicella–zoster (V–Z) virus, and the plaquing efficiency observed 4 days after infection of MRC-5 human diploid fibroblasts with cell-free V–Z. When DEAE-D was added either before or after virus inoculation there was an increase of two- to fivefold in the number of plaques. The highest value of enhancement was obtained when DEAE-D was added to the maintenance medium at a final concentration of 5.0 μg/ml immediately after a period of virus adsorption. DEAE-D also had an augmenting effect on the adsorption and penetration kinetics of cell-free V–Z virus.

DEAE-D has been used extensively to facilitate the uptake of infectious viral nucleic acid by cells in tissue culture (1, 2). However, it does not appear always to have a corresponding enhancing effect on the infectivity of intact virus. Polio (1), rubella (3), rabies (4), the avian sarcoma virus transforming system (5), and mouse hepatitis virus (6) are enhanced, but DEAE-D has no effect on foot-and-mouth disease (7) and inhibits simian virus 40 (8).

In this paper we report experiments with DEAE-D that show enhanced uptake of cell-free V–Z virus in MRC-5 cells.

Materials and Methods. *Virus strain.* The studies described were performed with the VZ 1073 strain of V–Z virus, isolated by this laboratory and used at the 12th to 30th passage levels. The virus was identified by its cytopathic effect (CPE) and by specific indirect immunofluorescent using human convalescent serum in which the CF titer was 1:128 against V–Z and absent for cytomegalovirus.

Cell cultures. Monolayers of MRC-5 diploid human lung fibroblasts at the 25th to 40th subculture level were grown in Eagle's minimum essential medium (MEM) supplemented with penicillin (100 μg/ml), streptomycin (100 μg/ml), gentamicin (50 μg/ml), and 7.5% fetal calf serum. Cultures were used for virus production after 4 to 6 days growth and for plaque assay after 3 to 4 days of incubation at 37°.

DEAE-D. DEAE-D with a molecular weight of 2×10^6 was purchased from Pharmacia, Uppsala, Sweden (9). A stock solution containing 2.5 mg/ml was made in phosphate-buffered saline (PBS) and sterilized by autoclaving (3) or filtration.

Cytotoxic test. Two-tenths percent trypan blue in filtered PBS was used to determine cytotoxicity by addition to cell suspension held at room temperature for 3 to 5 min. The number of cells with blue stained nuclei in a total of 200 cells was recorded.

Preparation of cell-free virus (10, 11). When the infected cultures showed a 2–3+ viral CPE, the cells from each 75-cm² flask were removed with a rubber scraper and suspended in 2 ml of medium consisting of MEM supplemented with 2% fetal calf serum. The cell suspensions were sonically treated for 15 sec at intensity 6 with a Branson disintegrator and then clarified by centrifugation at 1500 rpm for 15 min. The supernatant fluid was removed and served immediately as the source of cell-free V–Z virus. Titers of cell-free V–Z virus were from 7.5×10^2 to 1.2×10^3 PFU/ml.

Plaque assay for cell-free virus and infected cells. The plaque assay for V–Z virus was carried out as described by Caunt and Shaw (12). MRC-5 cells were seeded in 35-mm Falcon tissue culture dishes and incubated at 37° for 3 to 4 days prior to use. Virus was inoculated at 0.2 ml per dish. After a 2-hr adsorption period at 37°, the

[1] Permanent address: The Kitasato Institute, 5-9-1, Shirokane, Minato-ku Tokyo, 108 Japan.

0037-9727/81/020282-05$01.00/0

inoculum was removed and washed three times with PBS. Plaques were counted at 4 days postinfection without an overlay using a light microscope since plaque formation due to a second cycle of infection is not yet present at that interval postinfection.

Addition of DEAE-D to cells. All tests were done in 35-mm Falcon tissue culture dishes. Three methods of adding DEAE-D were tested. When added before inoculation of virus (pretreatment) 0.2 ml of DEAE-D at a concentration of 5.0 or 50 μg/ml was used. For "simultaneous" treatment, 0.1 ml of DEAE-D (10 μg/ml) was added to 0.1 ml of virus inoculum. When cells were treated after virus adsorption (post-treatment) 3 ml of DEAE-D at 5.0 μg/ml was added.

Results. *Cytotoxic effect of DEAE-D on MRC-5 cells.* Since DEAE-D is moderately toxic for cultured cells (13), experiments were done to determine the toxic concentrations. For the pretreatment tests, six cultures were exposed to 0.2 ml of DEAE-D at 0, 5, 25, 50, 100, and 300 μg/ml for 2 hr at 37° and cultures were then washed three times with PBS. For the post-treatment tests, the cultures were incubated for 4 days at 37° with maintenance medium containing various concentrations of DEAE-D (0, 0.5, 2.5, 5.0, 10.0, 15.0, and 30.0 μg/ml). At the end of the incubation period, cultures were examined for evidence of cytotoxicity. The presence of DEAE-D in concentrations up to 50 μg/ml during pretreatment at 37° for 2 hr was not detectably injurious to MRC-5 cells, although some granulation was occasionally observed. In the case of post-treatment, the maximum concentration that was not injurious to MRC-5 cells over a 48-hr period was 5.0 μg/ml.

Effect of DEAE-D treatment on V–Z infection of MRC-5 cells. Whether added pretreatment, post-treatment, or at the same time as virus (simultaneous treatment), DEAE-D increased the number of plaques that appeared 4 days after infection from 1.2- to 5-fold (Table I). The highest values of enhancement were obtained when

TABLE I. EXPOSURE OF MRC-5 CULTURES TO DEAE-D FOR VARIOUS LENGTHS OF TIME DURING PRETREATMENT AND POST-TREATMENT

DEAE-D treatment (5.0 μg/ml)	DEAE-D exposure time relative to infection[a]		PFU/0.2 ml[b]	Enhanced ratio (fold)
	From	To		
Pretreatment	−15 min	0	18.5	1.6
	−1 hr	0	24.0	2.1
	−2 hr	0	28.0	2.4
	−3 hr	0	25.5	2.2
	−6 hr	0	27.5	2.4
	−12 hr	0	20.5	1.8
Simultaneous treatment	0	2 hr[c]	19.0	1.7
Post-treatment[d]	2 hr	4 days	59.5	5.2
	2 hr and 15 min	4 days	47.0	4.1
	3 hr	4 days	26.5	2.3
	4 hr	4 days	21.0	1.8
	5 hr	4 days	17.5	1.5
	8 hr	4 days	14.0	1.2
	14 hr	4 days	15.0	1.3
	26 hr	4 days	14.0	1.2
Control	None		11.4	1.0

[a] Cultures were infected at 0 time with the VZ 1073 strain, passage level 26.
[b] Each value represents the average of two determinations.
[c] DEAE-D added to the virus inoculum and kept during the time of virus adsorption for 2 hr.
[d] DEAE-D was added at various times after completion of the 2-hr adsorption and allowed to remain on the cultures until 4 days postinoculation.

DEAE-D was added to maintenance medium within 15 min after 2 hr of virus adsorption. Enhancement of about twofold was seen with pre- and simultaneous treatment. The same degree of enhancement was seen with post-treatment when the DEAE-D was added within 1–2 hr after the 2-hr virus adsorption. Removal of DEAE-D at any time after it had been present for 15 min still resulted in enhancement. Nevertheless, for maximal effect the DEAE-D not only had to be added to the medium immediately after the virus inoculum was removed but also had to remain in the medium until the plaques were counted (Table I).

The degree of enhancement resulting solely from pretreatment addition of DEAE-D was dependent upon the concentration of DEAE-D employed, as shown in Table II. A 50 μg/ml solution of DEAE-D gave the maximum number of plaques without toxicity, whereas a 100 μg/ml solution showed more enhancement but had a cytotoxic effect on the monolayers. DEAE-D enhancement of CPE consisted of more rapid and clearer plaque development as well as an increase in counts.

When whole infected cells were used as an inoculum, there was no enhancement.

Effect of DEAE-D on V–Z virus penetration. In order to test the effect of DEAE-D on the penetration of V–Z virus, an amount of antiserum sufficient to inactivate extracellular virus was added to infected cultures. MRC-5 cell monolayers were infected with cell-free V–Z virus strain VZ 1073 and allowed to adsorb the virus for 2 hr. The cultures were washed once after adsorption then incubated for 2 hr at 37° with convalescent serum from a herpes zoster patient. At the end of this antiserum treatment, DEAE-D was added to the cultures at a final concentration of 5.0 μg/ml in maintenance medium. The effect of the antiserum treatment on total plaque-forming units (PFU) is shown in Table III. When infected cultures were treated with antiserum for 2 hr, DEAE-D did not enhance PFU. Control cultures treated with normal fetal calf serum in place of the antiserum showed a twofold enhancement of total PFU by DEAE-D.

The results suggest that the action of DEAE-D is eliminated by neutralization of surface virus and that it acts at the penetration stage of the interaction between cell-free V–Z virus and MRC-5 cells.

The rate of adsorption of cell-free V–Z virus to MRC-5 monolayers pretreated with DEAE-D. In an effort to determine what effect DEAE-D has on the interaction between the cell and cell-free V–Z virus, the adsorption kinetics of cell-free virus were analyzed. MRC-5 cell monolayers were pretreated with 0.2 ml of a 50 μg/ml solution of DEAE-D, and control cultures were pretreated with 0.2 ml of PBS. After a 2-hr pretreatment period, both sets of cultures were washed three times with PBS then inoculated with cell-free V–Z virus. The rate of virus adsorption to cells was studied by

TABLE II. EFFECT OF PRETREATMENT OF MONOLAYERS WITH VARIOUS CONCENTRATIONS OF DEAE-D

DEAE-D (μg/ml)	CPE days after infection[a]			PFU/0.2 ml[b]	Enhanced ratio (fold)
	1	2	3		
100	–[c]	+	+++	129.5	4.7
50	–	+	++	80.5	2.9
25	–	+	++	53.0	1.9
5	–	+	++	45.0	1.6
0	–	?	+	27.5	1.0

Note. Monolayers of MRC-5 cells were treated with 0.2 ml of various concentrations of DEAE-D for 2 hr at 37°, then washed three times with PBS and inoculated with cell-free V–Z virus.

[a] CPE, cytopathic effect. Infection with the VZ 1073 strain, passage level 15, CPE in cell cultures: (–) none, (?) doubtful, (+) 1–5 PFU/dish, (++) 6–50, (+++) 51–100.

[b] Each value represents the average of two determinations.

[c] Visual evidence of cell toxicity.

TABLE III. EFFECT OF DEAE-D ON V–Z VIRUS PENETRATION

Treated with antiserum[a]	DEAE-D (5.0 μg/ml)	Average[b] (PFU/0.2 ml)	Enhancement of control virus (fold)
+	+	6.5	0.3
−	+	34.0	1.8
+	−	7.0	0.4
−	−	19.0	1.0

[a] Zoster patient convalescent serum (CF = 1:25).
[b] Infection with the VZ 1073 strain, passage level 12; average of two determinations.

varying the adsorption period at 37° from 0 to 9 hr. After incubation for each adsorption time, the supernatant fluid was removed from the dish, the cells were washed three times with PBS, and 3 ml of maintenance medium was added to the dish. These dishes were routinely incubated at 37°.

The results (Fig. 1) show that the rate of adsorption both in the presence and absence of DEAE-D was remarkably slow, particularly during the first hour. After pretreatment with 0.2 ml of 50 μg/ml solution of DEAE-D for 2 hr, virus adsorption was markedly increased during the first hour, yielding a 6- to 20-fold increase in the number of PFU as compared to untreated controls. After the first hour, enhancement gradually decreased until 9 hr after adsorption the enhanced ratio was reduced to 2-fold.

Effect of DEAE-D on the yield of V–Z virus in MRC-5 cell monolayers infected with cell-free virus. To determine whether there is any difference between viral growth kinetics in DEAE-D-treated and untreated MRC-5 cells, a growth curve experiment was performed. Monolayer cultures containing 9.5×10^5 cells/25 cm² flask were infected with 7.5×10^2 PFU per flask of cell-free virus. After 2 hr incubation at 37°, the cultures were washed three times with 5 ml of PBS, then 5 ml of maintenance medium containing DEAE-D at a final concentration of 5.0 μg/ml was added to half of the cultures, and all cultures were again incubated at 37°. Yields of infected cells and cell-free virus were titrated as follows. At 24-hr intervals up to 144 hr postinfection, cells from each culture were trypsin dispersed and part of the suspension was plated on MRC-5 cell monolayers for assay of infected cells, while the remaining cells were sonically treated as described under Materials and Methods for production of cell-free virus. Cell-free virus was also assayed in the fluid phase of the cultures.

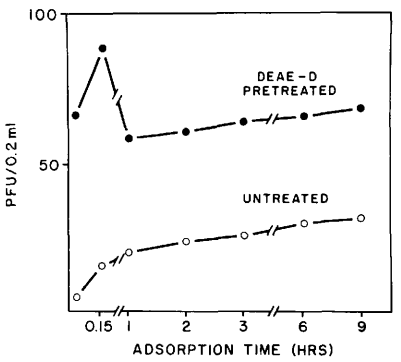

FIG. 1. The rate of adsorption of cell-free V–Z to MRC-5 monolayers pretreated with DEAE-D. MRC-5 cell monolayers were pretreated for 2 hr with either 0.2 ml of a 50 μg/ml solution of DEAE-D (●) or with 0.2 ml of PBS (○). After pretreatment, all cultures were washed three times with PBS and inoculated with the VZ 1073 strain of V–Z virus, passage level 21.

Results of a representative experiment are shown in Fig. 2. The only discernible differences were the greater number of infected cells in the DEAE-D treated cultures during the first 24 hr and the larger amount of cell-free virus released by sonication during the first 72 hr. At 96 hr the number of PFU of infected cells and cell-free virus in both cultures reached maximum. At this time, little or no spontaneously released cell-free virus was detected in any of the culture fluids either in the presence or absence of DEAE-D.

Discussion. Addition of DEAE-D to monolayers of MRC-5 cells before, at the same time, or after infection with V–Z

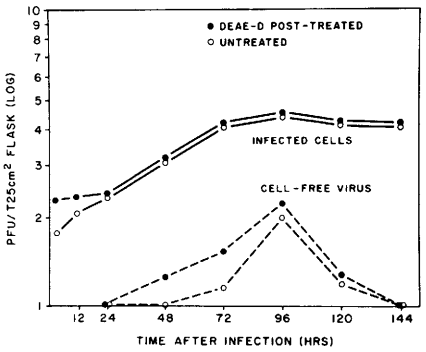

FIG. 2. Effect of DEAE-D on the yield of V–Z virus in MRC-5 cell monolayers infected with cell-free virus. MRC-5 cells (9.5×10^5/flask) were infected with 7.5×10^2 PFU/flask of cell-free V–Z virus, strain VZ 1073, passage level 18. Two hours after incubation at 37°, cultures were washed three times with PBS and treated with 5 ml of maintenance medium containing DEAE-D at a final concentration of 5.0 μg/ml (●) or left untreated (○). All cultures were again incubated at 37° and yields of infected cells and cell-free virus were titrated at 24-hr intervals as described under Materials and Methods.

virus increased the plaquing efficiency of cell-free V–Z virus both in terms of numbers and clarity of the plaque. The enhancement was greatest when DEAE-D was added to cells just after viral adsorption, but was also seen when the cells were treated before infection or when DEAE-D was added together with the virus. DEAE-D treated cultures showed an earlier CPE and plaque formation than control cultures. When DEAE-D was added to the maintenance medium of infected cells, virus yield was only slightly enhanced and there was no increase in spontaneously released virus.

We believe that the effect of DEAE-D is on viral penetration. Support for this belief is based on the following evidence. First, viral adsorption, as measured by plaque formation, is accelerated by DEAE-D pretreatment. Next, addition of DEAE-D just after viral attachment exerts the most profound enhancing effect. Third, when adsorbed, but not penetrated, virus was neutralized by the addition of antiserum to infected cultures, the effect of DEAE-D was eliminated. Finally, when DEAE-D was added to infected cultures 4 hr postinfection, the compound no longer influenced viral plaque formation.

The mechanism of action of DEAE-D is not known. The compound might bind to the cell surface thus creating a favorable ionic charge for virus attachment, or it might form complexes with the viral particles, allowing them to penetrate the MRC-5 cell surface more efficiently.

We anticipate that this DEAE-D assay method will be applicable to increasing the efficiency of plaque formation of cell-free V–Z virus.

The authors wish to thank Ms. Betsy Hornberger and Ms. Stephanie A. Kut for their assistance, and Ms. Barbara Cohen for her help in the preparation of the manuscript.

1. Pagano, J. S., and Vaheri, A., Arch. Ges. Virusforsch. **17**, 456–464 (1965).
2. Smull, C. E., and Ludwig, E. H., J. Bacteriology **84**, 1035–1040 (1962).
3. Vaheri, A., Sedwick, W. D., and Plotkin, S. A., Proc. Soc. Exp. Biol. Med. **125**, 1092–1098 (1967).
4. Kaplan, M. K., Wiktor, T. J., Maes, R. F., Campbell, J. B., and Koprowski, H., J. Virol. **1**, 145–151 (1967).
5. Vogt, P. K., Virology **33**, 175–177 (1967).
6. Takayama, N., and Kirn, A., Arch. Virol. **52**, 347–349 (1976).
7. Bachrach, H. L., Proc. Soc. Exp. Biol. Med. **123**, 939–945 (1966).
8. McCutchan, J. H., and Pagano, J. S., J. Nat. Cancer Inst. **41**, 351–357 (1968).
9. Pagano, J. S., McCutchan, J. H., and Vaheri, A., J. Virol. **1**, 891–897 (1967).
10. Caunt, A. E., and Taylor-Robinson, D., J. Hyg. (Camb.) **62**, 413–424 (1964).
11. Schmidt, N. J., and Lennette, E. H., Infect. Immun. **14**, 709–717 (1976).
12. Caunt, A. E., and Shaw, D. G., J. Hyg. (Camb.) **67**, 343–352 (1969).
13. Tilles, J. G., Proc. Soc. Exp. Biol. Med. **133**, 1334–1341 (1970).

Received October 17, 1978. P.S.E.B.M. 1981, Vol. 166.