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Received Jan. 2, 1968. P.S.E.B.M., 1968, Vol. 128.

Interaction of Respiratory Syncytial Virus with Polyions: Enhancement of Infectivity with Diethylaminoethyl Dextran* (32969)

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For working on the plaque isolation of respiratory syncytial (RS) virus, a high rate of infectivity of virus is very desirable. Polyions were examined as substances which would stimulate the interaction between RS virus and cells. Polyions, such as diethylaminoethyl (DEAE) dextran and protamine sulfate, have been used extensively to facilitate the uptake of intact virus (1,2) and infectious viral ribonucleic acid by cells in tissue culture (2–6), and thus increase the rate of infectivity. The experiments in this paper show that after exposure to DEAE dextran, the infectivity of RS virus markedly increased, whereas the opposite effect was obtained after treatment of the virus with dextran sulfate, heparin, and protamine sulfate. The experiments used HEp-2 and African green monkey kidney (AGMK) cell cultures.

Materials and Methods. Virus. The AGMK-adapted, plaque-purified stock of RS virus (21113-38) was used throughout this study.

Tissue cultures. The HEp-2 cells were grown as monolayers in tissue culture dishes (35 × 10 mm, Falcon Plastic) in Eagle's minimum essential medium containing Earle's balanced salt solution (BSS) with 10% fetal bovine serum, 2 m*M*/ml of glutamine and antibiotics. The AGMK cells were grown in tissue culture dishes in Earle's BSS containing 0.5% lactalbumin hydrolyzate with 2% fetal bovine serum, 2 m*M*/ml glutamine and antibiotics.

Polyions. Stock solutions of DEAE dextran (mol. wt. 2×10^6, Pharmacia, Uppsala, Sweden), protamine sulfate (Upjohn Co.), dextran sulfate (Pharmacia) and heparin (Calbiochem) (kindly supplied by Dr. K. K. Takemoto, NIH) were prepared in distilled water and kept under refrigeration.

Plaque assay. Plaque assays were performed on HEp-2 and AGMK cell monolayers as described by Coates, *et al.* (7) with slight modification. Plates (HEp-2,3–4 days old; AGMK, 7–9 days old) were washed and exposed to 1.0 ml of diluted virus. After incubation at 36°C in the CO_2 incubator for a minimum of 2 hours, the inoculum was removed and the cell sheet was overlaid with 4 ml of a fluid medium consisting of Cooper's (8) or L-15 medium (9), 5% heat-inactivated fetal bovine serum, 1% methylcellulose (Fischer Scientific Co., 4000 Centipoise), 2 m*M*/ml of glutamine and antibiotics. Cultures were incubated in the regular incubator without CO_2 for 3 days (HEp-2) or 8 days (AGMK) at 36°C. At that time, the overlay was replaced with 10% formaldehyde solution to fix the cell sheet, and cells were stained with Giemsa stain. The plaque count was made with the aid of a Bausch and Lomb stereozoom dissecting microscope.

Results. Effect of polyions on RS virus infectivity. The HEp-2 cell monolayers were exposed to virus containing various concentrations of DEAE dextran, protamine sulfate, dextran sulfate, or heparin. After incubation at 36°C for 2 hours, the inoculum containing polyion was removed, and the cultures were

* This work was supported by contract PH43-64-943 from the Vaccine Development Branch, National Institute of Allergy and Infectious Diseases, NIH, Bethesda, Maryland.

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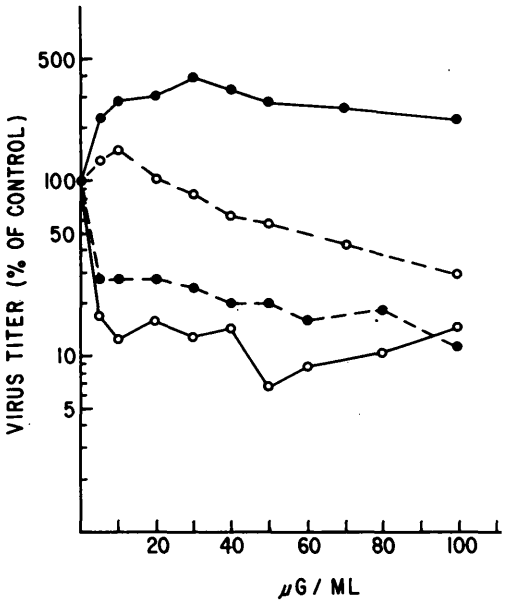

FIG. 1. Effect of polyions in the inoculum on RS virus infectivity. The HEp-2 cell monolayers were inoculated with virus (approx. $10^{5.7}$ pfu/ml) and polyions were added separately at the same time. (●——●) DEAE dextran; (O----O) protamine sulfate; (●----●) dextran sulfate; (O——O) heparin.

washed and overlaid with the fluid medium. Graphs showing the effect of the four polyions on RS virus infectivity are shown on Fig. 1. The number of plaque forming unit (pfu) that appeared 3 days after infection in HEp-2 cells with DEAE dextran was increased more than twofold. Although DEAE dextran showed an enhancing effect on RS virus infectivity at a concentration of 5 μg/ml, maximal effect was obtained with 30 μg/ml in HEp-2 and 10 μg/ml in AGMK cells. Further increase in the concentration of this polyion was not toxic to cells (up to 400 μg/ml), but failed to improve its performance. The increase in the number of plaques was paralleled by an increase in titer of infectious virus in the culture medium. Protamine sulfate inhibited the infectivity by 50% at a concentration of 60 μg/ml after a slight increase in the infectivity with 5 to 10 μg/ml. At a concentration of 70 μg/ml, it showed toxicity for HEp-2 cells. Dextran sulfate and heparin decreased the pfu markedly (28.3%, 17.5%) with a concentration as low as 5 μg/ml followed by further decrease in the infectivity at higher concentration. No alteration of plaque characteristics was observed with any of the polyions.

To study further the differences between the polycations, RS virus was mixed with various polycation concentrations and incubated at 36°C for 2 hours before inoculation. At the end of the incubation, the mixture was diluted 100 and 1000 times with Hanks' BSS and inoculated on HEp-2 plates. As shown in Fig. 2, 30 μg/ml of DEAE dextran enhanced the virus infectivity about twofold. Protamine sulfate also enhanced the infectivity as much as DEAE dextran, i.e., about twofold increase in the pfu for a concentration of 20 μg/ml. Dextran sulfate and heparin have an inhibitory effect on the infectivity in the mixture.

Effect of DEAE dextran on absorption and penetration of the virus. The enhancing effect of DEAE dextran was further investigated in relation to time of infection. The estimated 100 pfu of virus dilution in Hanks' BSS or Hanks' BSS containing 30 μg/ml of DEAE

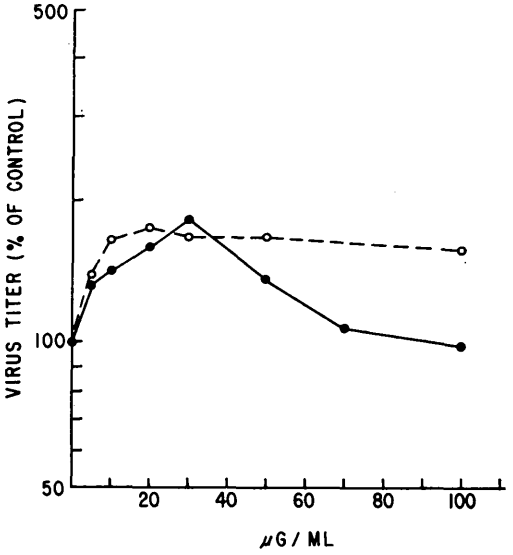

FIG. 2. Effect of polycations on RS virus infectivity. Virus (approx. $10^{5.5}$ pfu/ml) was mixed with various concentrations of polycations and incubated at 36°C for 2 hours before inoculation. The mixture then was diluted and inoculated on HEp-2 cell monolayers. (O) Protamine sulfate; (●) DEAE dextran.

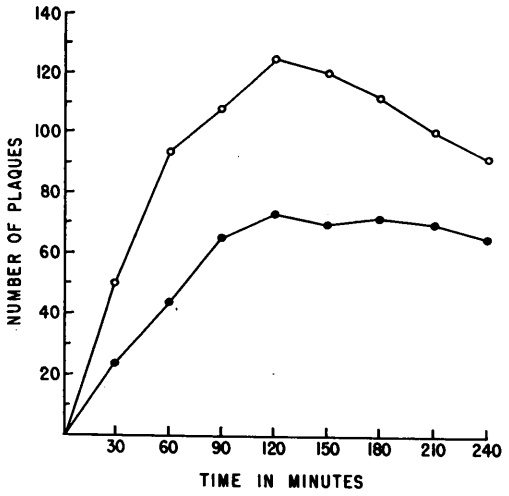

FIG. 3. Effect of DEAE dextran on adsorption of RS virus. Estimated 100 pfu of virus dilution in Hanks' BSS (●) or Hanks' BSS with 30 μg/ml DEAE dextran (○) were added to HEp-2 cell monolayers.

dextran, were added to HEp-2 monolayers. Infected monolayers were incubated at 36°C and at 30-min intervals were washed with Hanks' BSS. The cells were then overlaid and incubated as in a plaque assay. The number of pfu adsorbed from inoculum in Hanks' BSS with DEAE dextran in 2 hours, expressed as percentage of pfu adsorbed from inoculum in Hanks' BSS, was between 170 and 200%. About 50% of infective virus adsorbed in 45 min and maximal adsorption was observed 120 min after infection. The results of one such experiment are shown in Fig. 3.

The HEp-2 cells were exposed to 30 μg/ml DEAE dextran at the times indicated in Table I, and the number of plaques formed 3 days after infection was determined. The data indicate that pretreatment of cells with DEAE dextran (at −2 and −1 hour) has the same enhancing effect on RS infectivity as had treatment at the time of adsorption of the virus (0 hour), but if the cell monolayers were washed twice with Hanks' BSS, there was no significant increase in the infectivity. The DEAE dextran had no enhancing effect when it was added after initial exposure to virus (+1 and +2 hrs).

Effect of DEAE dextran with $MgSO_4$. Plaque formation was slightly enhanced by increasing Mg^{2+} to 1—20 mM. There was, however, no additive effect with DEAE dextran.

Discussion. Kaplan *et al.* (1) showed that the effect of the polyions on the virus infectivity appears to be related to their ionic charge: the infectivity of rabies virus is enhanced by polycations (DEAE dextran and protamine sulfate) and is inhibited by polyanions (dextran sulfate and heparin). In the experiments reported here, however, protamine sulfate enhanced RS virus infectivity if the virus was treated with this polyion in the mixture prior to inoculation, but it inhibited the infectivity if it was applied to the virus inoculum as Kaplan *et al.* did, although the effects of the other polyions were consistent with their results.

In the case of Mengo encephalomyelitis virus (10), protamine sulfate was observed to enhance virus infectivity when it was added to the agar overlay: this is usually attributed to reversal of the inhibition which is known to be in agar. When protamine sulfate was added to a suspended L cell–Mengo virus mixture, infectivity was again inhibited.

Considering only those cases where the complicating inhibitory effect of agar was not present in the system, we see that protamine sulfate may act as an enhancing agent, as in

TABLE I. Exposure of HEp-2 Cultures to DEAE Dextran before and after Infection with RS Virus.

Exposure of culture to DEAE dextran (30 μg/ml) in relation of time of infection at 36°C (hours)		pfu/ml (× 10^4)
−2[a]	Without washing	5.2
	With washing	3.3
−1	Without washing	5.2
	With washing	3.6
0		5.3
+1[b]		1.5
+2		2.5

[a] Cells were exposured to DEAE dextran at the time indicated and infected with the virus with or without washing of cell sheets.

[b] Cells were treated with DEAE dextran after removal of virus inoculum.

the case of rabies virus, or an inhibitor as in the case of Mengo and RS virus. Therefore, it seems that the ionic charge itself is not the sole factor in the effect of polyions on virus infectivity. Further investigation is necessary to show what the significant characteristics of the polyions are that influence infectivity. Possible suggestions may be found in Campbell and Colter (11) who showed that molecular weight is a factor in determining the ability of polyions to affect the infectivity, and in Ryser (12) who showed that the influence of polyions on the permeability of animal cell membranes varied with molecular weight of the polyions.

Although the exact site of the enhancing action on RS virus infectivity by polycations has not been determined, DEAE dextran could possibly act by complexing with the virus particles and by binding to the cellular surface to make virus attachment more efficient. Protamine sulfate works by binding to the cell in such a way that the virus particles are denied access to cellular receptor sites. This process, and not direct binding to the virus particles, is most likely the mechanism of inhibition by protamine sulfate. The direct effects of DEAE dextran and protamine sulfate in the mixture on RS virus may suggest the stabilization of virus particles by these polycations.

The enhancement of the infectivity with DEAE dextran should be advantageous not only in plaque isolation, but also in any other studies in which plaque procedures are used.

Summary. The infectivity of RS virus was enhanced by the addition of DEAE dextran to the virus inoculum or in the mixture. The maximal effect (2- to 5-fold) was obtained with 30 μg/ml in HEp-2 cells and 10 μg/ml in AGMK cells in the virus inoculum. The enhancing action of this polyion with RS virus was apparently exerted at the early stages of virus–cell interaction and involved direct complexing with virus particles. Although protamine sulfate enhanced the infectivity in the mixture as much as DEAE dextran, it inhibited the infectivity to 50% at a concentration of 60 μg/ml after a slight increase with 5–10 μg/ml in the virus inoculum. Dextran sulfate and heparin decreased markedly the infectivity of the virus (28 and 18%) with a concentration as low as 5 μg/ml in the virus inoculum in HEp-2 cells, and also showed an inhibitory effect in the mixture.

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Received Jan. 2, 1968. P.S.E.B.M., 1968, Vol. 128.