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Transmissible Gastroenteritis (TGE) of Swine: In Vitro Virus Attachment and Effects of Polyanions and Polycations

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ABSTRACT

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Four transmissible gastroenteritis virus (TGEV) strains (Purdue-115, D-52, 188-SG and Gep-II) and two cell lines (swine testis-ST and pig kidney-RPD) were used to study virus attachment and cell susceptibility. Virus attachment was partially thermodependent and the rate varied, depending on the strain. Identical TGEV inocula produced a higher plaque number by plaque assay in the swine testis cell line (ST) than in the pig kidney cell line (RPD) but [^{3}H]uridine-labelled virus was found associated equally well with both cell lines. A field TGEV strain (Gep-II), which was unable to multiply in cell cultures, appeared able to inhibit the attachment of radiolabelled cell-passaged virus. Therefore, the susceptibility to TGEV infection was apparently not determined at the virus-to-cell attachment stage.

The attachment sites on the cell surface were specific, however, differences in TGEV attachment determinant between strains were not observed. Attachment of all the virus strains tested was enhanced by DEAE-dextran and inhibited by dextran sulfate, poly-L-lysine (PLL), poly-L-α-ornithine (PLO) and protamine sulfate.

INTRODUCTION

Attachment of virus to the host cell plasma membrane is the first step of the virus replication cycle and has been suggested to be one of the major determinants of cell susceptibility to virus infection. In most cases, this process occurs when viral protein(s) bind specifically to cell surface receptors (Tardieu et

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al., 1982). It was also demonstrated that the degree of virus virulence can be determined at this level (Mak et al., 1970; Mims and White, 1984).

Transmissible gastroenteritis virus (TGEV) is a member of the Coronaviridae. In the natural host, it infects intestinal epithelial cells, causing severe diarrhea in newborn piglets, but only mild diarrhea in adult swine (Bohl, 1981). The virus replicates in vitro in several porcine cell culture types (Bohl, 1981). However, viral isolation in cell cultures from field outbreaks is not always successful (Dulac et al., 1975). In contrast to virulent infection, peroral vaccination of sows, using attenuated virus, resulted in poor immunity (Saif and Bohl, 1979; Bohl, 1981). In the latter cases, it was not clear whether or not virus attached to target cells. In this paper, we report the results of experiments on TGEV attachment in vitro using four virus strains and two cell culture types. The effect of polycations and polyanions on virus attachment was also examined.

MATERIALS AND METHODS

Virus and cells

The four TGEV strains: Purdue-115, D-52, 188-SG and Gep-II and two cell lines: swine testis (ST) and pig kidney (RPD) have been described elsewhere (Laude et al., 1981; Aynaud et al., 1985). All but the Gep-II strain can multiply in these two cell lines. Strain 188-SG is a TGEV derived from D-52 by 188 passages in cell cultures and stomach juice of adult swine alternatively (Aynaud et al., 1985). Minimal essential medium (MEM) containing 100 IU ml^{-1} of penicillin and 100 μg ml^{-1} of streptomycin sulfate was used. For cell growth, the medium was supplemented with 10% fetal calf serum. Virus stock was produced by infecting confluent monolayers of RPD cells in 150-cm^2 plastic tissue-culture flasks (Falcon) with the cell-passaged strains and by inoculation of non-immune newborn piglets for the Gep-II strain.

Kinetics of attachment of infectious virus particles to the cells

A plaque assay (Aynaud et al., 1985) was used with the following modifications. A volume of 0.2 ml of virus suspension containing 50–100 plaque forming units (P.F.U.), was inoculated onto the cell monolayers ($\sim 3 \times 10^6$ cells per well) in six-well plastic plates (Costar). Virus attachment was allowed to occur at 37 °C. At the times indicated in Fig. 1, the inoculum was removed from the cell sheets by washing twice with MEM and replaced with MEM containing 1% agarose (Indubiose).

Attachment of [^3H]uridine-labelled virus

The [^{3}H]uridine-labelled virus was prepared by growing virus in monolayer cultures of RPD cells (in 150-cm^2 plastic flasks, Falcon) in the presence of 0.2

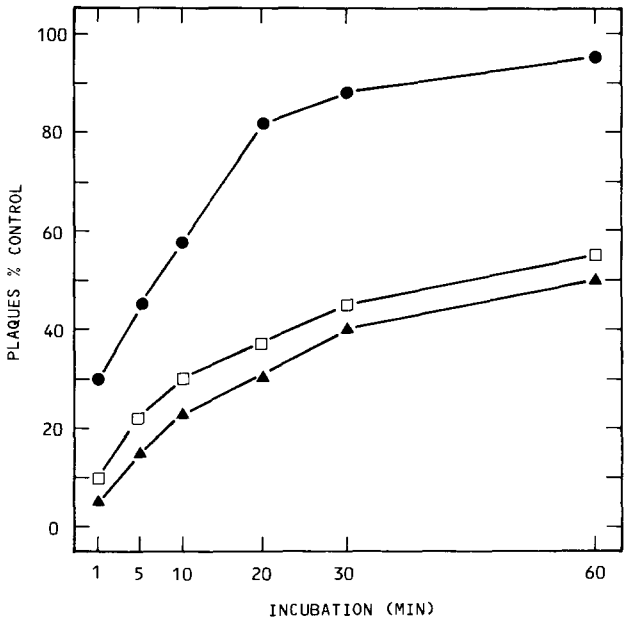

Fig. 1. Kinetics of attachment of infectious TGEV particles of strains Purdue-115 (●), D-52 (▲) and 188-SG (□) at 37 °C indicated by plaque formation. Virus inoculum (50-100 P.F.U.) was incubated with the ST cells for the indicated times, removed by washing and replaced by MEM containing 1% agarose. Plaque number observed in the cells where agar-overlay medium was added without removing the inoculum represents 100% infectious virus particles. Similar results (not shown) were obtained using RPD cells. Each point represents the mean of 12 replicates.

μCi ml^{-1} of [5-^{3}H]uridine (Amersham, sp. act. 26 Ci mol^{-1}). The virus harvested was then partially purified by pelleting in an R-30 rotor centrifuge (Beckman) at 85 000$\times g$ for 4 h at 4 °C. The pellet was resuspended in MEM by vortexing and diluted to give a titer of $\sim 10^{8}$ P.F.U. ml^{-1}, corresponding to 10^{4} c.p.m. ml^{-1}. The attachment of labelled virus was carried out at 4 °C. Confluent monolayer cells ($\sim 3\times 10^{6}$ cells per well) grown in 24-well plastic plates (Costar) were incubated with the radiolabelled virus suspension (0.1 ml per well) for 60 min with gentle rocking. Following three washings with MEM to remove the inoculum, the cells were solubilized with 1 N NaOH then neutralized with 0.1 N HCl. The whole content of a well was transferred to a scintillation vial. The radioactivity was measured using ACS scintillation liquid (Amersham) in an LKB liquid scintillation counter.

Competition for attachment sites between virus strains

The unlabelled TGE virus (strains Purdue-115, D-52, 188-SG and Gep-II) was first inoculated onto the cell monolayers, grown in 24-well plastic plates,

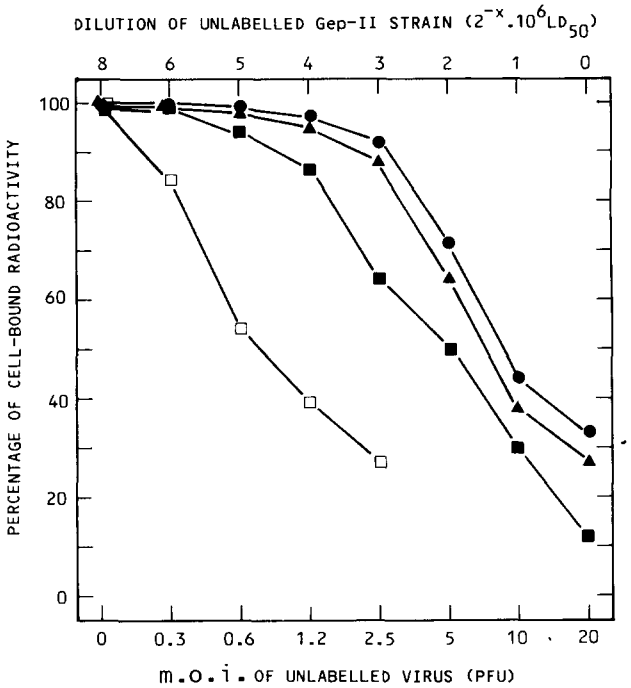

Fig. 2. Competition for attachment sites on ST cell surface between [^{3}H]uridine-labelled virus strain Purdue-115 and unlabelled TGEV strains Purdue-115 (●), D-52 (▲), 188-SG (□) and Gep-II (■). See the text for method. Each point represents the mean of three replicates.

at varying multiplicities of infection (m.o.i.) and dilutions as indicated in the legend to Fig. 2. Following incubation for 15 h at 4 °C, the virus was removed by extensively washing the cells with cold MEM. The radiolabelled virus was then inoculated and further steps in the experiment were performed as described above. Results are expressed as $A/A_0 \times 100$, A being the radioactivity associated with the cells which were first inoculated with different amounts of unlabelled virus and A_0 the radioactivity bound to the control cells.

Effects of polyanions and polycations on virus attachment

The following products were used. Polycations: DEAE–dextran (Pharmacia, mol. wt. = 500 000), protamine sulfate (Sigma, salmonine, grade X), poly-L-lysine (Sigma, mol. wt. = 240 000), poly-L-α-ornithine (Sigma, mol. wt. = 110 000). Polyanions: heparin (Choay-France), 5000 IU ml^{-1} and dextran sulfate (Sigma, mol. wt. = 500 000) and an amorphous product: dextran T 500 (Pharmacia, mol. wt. = 500 000). These were prepared immediately before use by dilution in MEM. The minimum toxic concentration for the cells had been determined previously. The effect of these chemicals on virus attach-

TABLE I

Attachment of [^{3}H]uridine-labelled TGEV to ST and RP.D cells

Virus strains	Cell-associated [^{3}H]uridine TGEV	
	ST cells	RP.D cells
Purdue-115	313[a]	382
D-52	588	678
188-SG	262	258

[a]c.p.m. of radiolabelled virus attached to the cells following incubation for 30 min at 4°C.

ment was tested by using the plaque assay as described above, except that the inoculum containing 50–100 P.F.U. was prepared in the presence of the chemical at appropriate concentrations and incubated with confluent monolayer cells for 30 min at 37°C. The inoculum was then removed by extensive washing and replaced with MEM containing 1% agarose. The chemicals were omitted from control cultures.

To test whether the chemicals had an effect on viral replication step(s) following attachment, after incubation with virus alone and removal of the inoculum, the cells were incubated with the chemicals for 30 min at 37°C. The cells were then washed free of the chemicals and MEM containing 1% agarose was added.

RESULTS

Virus attachment

Plaques were formed by infectious virus particles which were attached to the cells and not removed by washing. The percentage of infectious virus particles in the inoculum which attached to the cells, as a function of incubation time, is indicated in Fig. 1. It can be seen that the attachment rate of TGEV strain Purdue-115 to the cells was much greater than that of other strains (D-52 and 188-SG). After 1 h of incubation with the cells, most of the infectious virus particles ($>90\%$) of the Purdue-115 strain were found attached to the cells, whereas attachment was only 50% in the case of the D-52 and 188-SG strains. Results of attachment of [^{3}H]uridine-labelled virus to the ST and RPD cells showed that for any strain tested, the radioactivity was found equally associated with both cell types (Table I).

Differences in susceptibility to TGEV infection, indicated by plaque formation, between the ST and RPD cells and effects of temperature on virus attachment were studied by incubating virus inoculum with the cells for 30 min at either 4 or 37°C. It was found (Table II) that at 37°C, the infectious

TABLE II

Effect of temperature on TGEV attachment: identical inocula, following incubation with the cells for 30 min at 37 or 4 °C, were removed by washing and replacing with MEM containing 1% agarose

Virus strain	ST cells		RPD cells	
	4 °C	37 °C	4 °C	37 °C
Purdue-115	90[a] ± 8.2[b]	103 ± 9.4	28 ± 3.6	34 ± 6.3
D-52	24 ± 4.6	43 ± 6.6	8 ± 2.0	16 ± 3.7
188-SG	26 ± 5.3	40 ± 5.5	5 ± 2.1	9 ± 2.4

[a]Plaque number (average from 30 replicates).
[b]Standard deviation.

virus particles attached to the cells better than at 4 °C ($P < 0.05$). The plaque numbers observed also showed that the ST cells were significantly ($P < 0.05$) more susceptible than the RPD cells. This difference was also observed in infected cells to which agar-overlay medium was added without removal of the inoculum (Table III).

Virus attachment at specific sites on the cell surface

The attachment of the labelled virus was reduced by pre-incubation of the cells with unlabelled virus. The reduction was inversely proportional to the m.o.i. (for Purdue-115, D-52 and 188-SG strains) and dilutions (for the Gep-II strain) of the unlablled virus previously inoculated. Figure 2 shows a typical experiment using the labelled virus strain Purdue-115 and the ST cells. Experiments using the RPD cells and other labelled virus strains (D-52 and 188-SG) gave similar results (not shown). These results suggested a specificity of TGEV attachment sites on the cell surface. Moreover, the strain Gep-II, which

TABLE III

Susceptibility of ST and RPD cells to the TGEV infection measured by plaque numbers induced by a similar inoculum in monolayer cells

Virus strain	Plaque number	
	ST cells	RPD cells
Purdue-115	96[a] ± 9.1[b]	30 ± 3.5
D-52	34 ± 9.4	11 ± 3.8
188-SG	38 ± 6.9	7 ± 3.2

[a]Plaque number (average).
[b]Standard deviation.

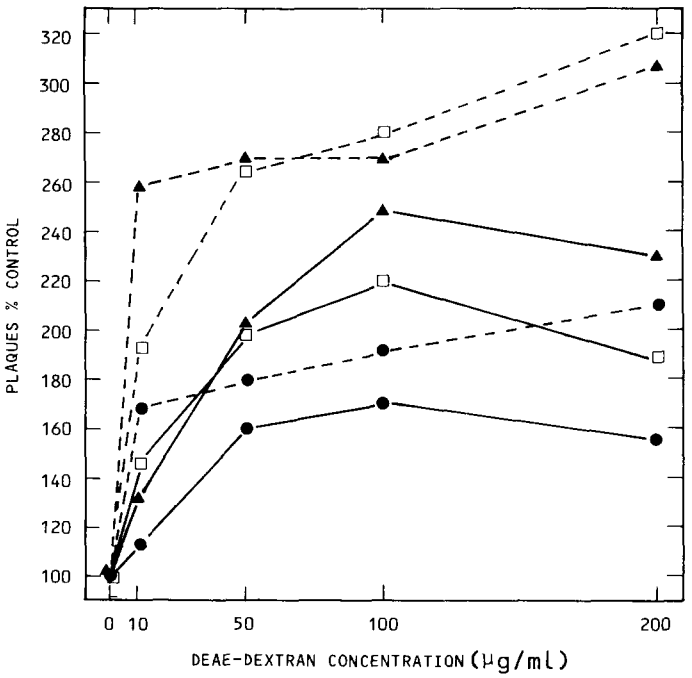

Fig. 3. Enhancement of TGEV attachment by DEAE-dextran: virus strains Purdue-115 (●), D-52 (▲) and 188-SG (□) in appropriate concentrations of DEAE-dextran were incubated with the ST (—) and RPD confluent monolayer cells (- - -) for 30 min at 37°C. Virus inoculum was removed and replaced by agar-overlay medium. Plaque number observed in the cells when DEAE-dextran was not used represents 100% of plaque number. Each point represents the mean of 16 replicates.

does not multiply in either ST or RPD cells, was found to be able to attach to the cells and to inhibit the subsequent attachment of labelled virus.

Effects of polyanions and polycations on TGE virus attachment

DEAE-dextran enhanced virus attachment (Fig. 3), when TGEV partially purified by pelleting was used. The curves reach a plateau beginning at a concentration of 50 μg ml^{-1} of DEAE-dextran.

An inhibitory effect on virus attachment was obtained when poly-L-lysine, protamine sulfate and dextran sulfate were used (Figs. 3, 4 and 5). The effect of poly-L-α-ornithine was similar to that of poly-L-lysine (data not shown).

Similar plaque numbers were observed in the control and in the pre-infected cells which were incubated with polyanions or polycations (for 30 min at 37°C) after the removal of inoculum.

No effect of heparin and dextran was observed. The effect of all chemicals was consistent for all TGEV strains tested.

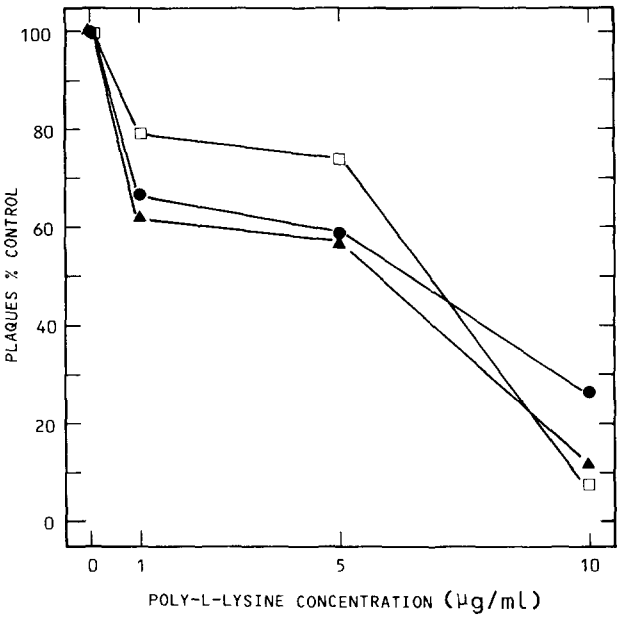

Fig. 4. Inhibitory effect on TGEV attachment to the ST cells of poly-L-lysine. See Fig. 3 for legends; similar results (not shown) were observed in RPD cells, as well as when poly-L-α-ornithine was used.

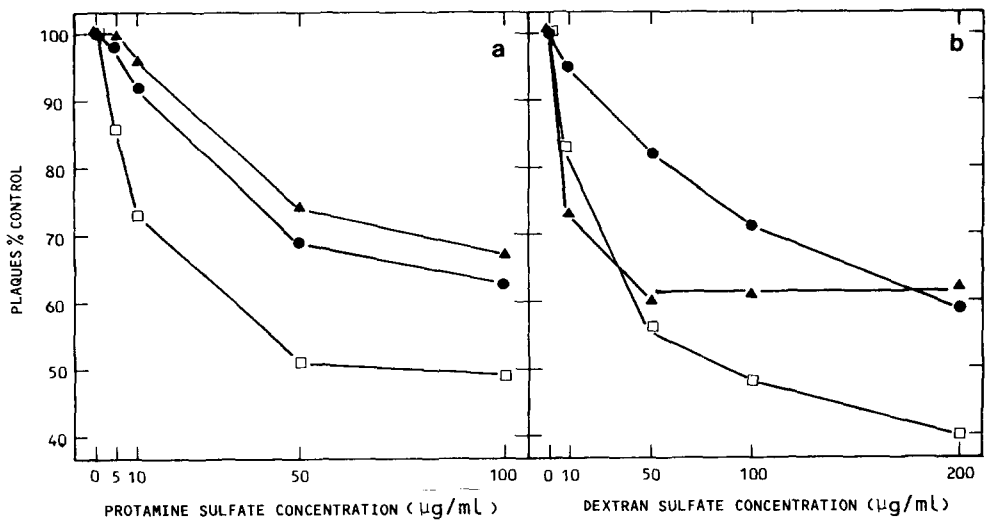

Fig. 5. Inhibition of TGEV attachment to ST cells by protamine sulfate (a) and dextran sulfate (b). Similar results (not shown) were observed in RPD cells. For legends, see Fig. 3.

DISCUSSION

Our determination of an incubation period (30 min) that preceded maximal virus attachment allowed the effects of temperature, polyanions and polycations on virus attachment to be studied. The results shown in Table I suggest that the attachment of TGEV is partially thermodependent. Differences in attachment rate between strains were also observed. The TGEV strain Purdue-115 attached to the cells much faster than the others. Although both the TGEV strains Purdue-115 and 188-SG were established as high passage strains (115 and 188 passages, respectively), the attachment rates of these strains were different (Fig. 1). The attachment rates of D-52 and 188-SG were found to be similar, however. Differences betweeen these two strains have been described (Aynaud et al., 1985), 188-SG having higher resistance to acidity and digestive enzymes and smaller plaques in ST cells. It is evident that these two characteristics are not related to attachment rate. No explanation is available for the difference in virus attachment rate between strains. Interference with infectious virus attachment by varying numbers of defective particles which are present in the different virus inocula is unlikely. Although the infectious virus titre of the 188-SG strain is lower (by 1 log, Aynaud et al., 1985), the intensity of virus–antibody reaction in ELISA is similar for the three virus strains (unpublished data), suggesting a 10-fold greater number of defective particles for the 188-SG strain compared to the others. The incubation of ~ 50–100 P.F.U. with 3×10^6 cells in a well (for plaque formation) would have rendered such an interference insignificant.

All three virus strains produced a higher number of plaques in ST cells than in RPD cells (Table II), but the radioactively labelled virus was found to be similarly associated to both cell types. This strongly suggests that the difference in plaque number observed between the two cell lines is not determined at the level of virus attachment. Also, the field TGEV strain Gep-II, which was unable to multiply in cell cultures even in the presence of DEAE–dextran, was able to attach to the cells, thereby inhibiting the subsequent attachment of labelled virus. A higher synthesis of viral RNA was observed for the three virus strains in ST cells than in RPD cells (Nguyen, 1986).

A defective multiplication of TGEV may be an explanation for the low susceptibility of pig kidney cell lines which has been described by Garwes et al. (1984). According to these authors, the multiplication of TGEV in LLC-PKl cells is stopped at the maturation step. Their observations and our present findings tend to reinforce the statement that genetic susceptibility or resistance to coronavirus is not determined at the level of virus receptors (Shif and Bang, 1970).

The results shown in Fig. 2, on the other hand, demonstrate a specificity of the attachment sites of TGEV on the cell surface. It is interesting to note that the difference in attachment site between virus strains which has been dem-

onstrated for other viruses, e.g., reovirus (Weiner et al., 1980), was not observed in the case of TGEV. Moreover, until now only one antigenic determinant of TGEV has been reported (Bohl, 1981) and the present study suggests that this also applies to the attachment determinant. Studies of foot and mouth disease virus showed that attachment and antigenicity determinants are different (Meloen et al., 1983). It is likely that TGEV possesses the same characteristic as neutralizing polyclonal antibodies from infected swine were unable to inhibit virus attachment (Nguyen et al., 1986).

Since the absolute ratio of TGEV particles to P.F.U. has not been established, we could not determine the number of virus attachment sites per cell. At an m.o.i. of ~4–5 P.F.U. per cell, the unlabelled virus strain Purdue-115 inhibited attachment of nearly 50% of the same radiolabelled virus strain at a m.o.i. of ~10 P.F.U. per cell. In agreement with Sturman and Holmes (1983), who demonstrated that the coronavirus receptors/cell ratio was low (~700), our results suggest that the ratio of TGEV attachment sites per cell could not be high, in contrast to many other viruses, for which the host cell possesses as many as 5×10^5 attachment sites (Armstrong et al., 1984).

Effects of polyanions and polycations on the virus cycle as well as on cellular uptake have been studied. These substances are believed to change the electric charges of ligands or cell receptors by combining with them and in that way enhancing or inhibiting the macromolecule (virus)–cell interactions (Ryser, 1967; Toyoshima and Vogt, 1969).

The infectivity of other coronaviruses has been reported to be enhanced by DEAE–dextran (Bradburne and Tyrrell, 1969; Takayama and Kirm, 1976; Hirano et al., 1978; Sato et al., 1983). Our results on TGEV have identified another coronavirus member with this characteristic. The effects of DEAE–dextran (polycation, enhancer), dextran sulfate (polyanion, inhibitory) and dextran (amorphous, no effect) are compatible with the explanation for the mechanism of action by electric charges of these molecules, but if the activity of polycations and polyanions relies only on their electric charges, contradictions can be seen in our results. Among polycations used, DEAE–dextran had an enhancing effect whereas others (PLL, PLO, protamine sulfate) were inhibitory. Such contradictions might be explained by the size of molecules used, since the effect of these molecules on cell uptake depends upon size as well as charge (Ryser, 1967).

Differences between strains in the level of enhancement or inhibition of virus infectivity by polyanions and polycations (Figs. 3, 4 and 5) might be explained by differences in their rate of attachment to the cells. The most important observation was the one-way effect of these molecules on virus attachment with all the strains studied. This again leads to the conclusion that there is no difference in the nature of attachment sites between TGEV strains.

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