INHIBITION OF HERPES VIRUS ADSORPTION
BY TWO FRACTIONS OBTAINED FROM
COMMERCIAL PROTAMINE SULFATE

SHIGERU YAMAMOTO, HIDEFUMI KABUTA
AND YOH NAKAGAWA

*Department of Microbiology, Kurume University School
of Medicine, Kurume, Japan*

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The commercial protamine sulfate could be separated into two fractions by Sephadex G-50 column which inhibited plaque formation by herpes simplex virus when cell monolayers were treated with them before adding the virus. One of them (Fr. I) contains high molecular weight proteins and the other (Fr. II) contains only protamine molecules. The former is more effective than the latter. The inhibitory effect disappears after incubation of the fractions with a large amount of cells. Both fractions do not directly reduce virus infectivity. Furthermore, they do not inhibit virus replication after virus adsorption. The virus particles remaining unadsorbed to cells pretreated with the fractions could be detected only when virus diluted in Earle's saline containing skim milk and lactalbumin hydrolysate was used for inoculation. These findings gave a clear evidence that protamine (Fr. II) and also high molecular weight proteins in Fr. I adsorb to cell surface or receptor and in consequence inhibit virus attachment to the cell surface resulting in the reduction of plaque formation.

The basic proteins and the synthetic basic polymers affect the interaction of virus or viral RNA with cells although they are different in effect among a variety of viruses. In some cases they interfere with the virus-cell interaction³⁾⁴⁾¹³⁾, but in others enhance it¹⁾⁹⁾¹¹⁾¹²⁾¹⁴⁾. Recently, the contrasting effects were found even between two variants of encephalomyocarditis virus⁵⁾.

It was revealed in the previous report that the plaque formation by herpes simplex virus was also reduced if the cell monolayers were exposed to

protamine sulfate before inoculation with the virus¹⁷⁾. It has been found that the commercial protamine sulfate can be separated into two protein fractions by gel filtration. This paper deals with the comparison of effects of the two fractions on virus-cell interaction, and the evidence obtained here demonstrates that both fractions bind to the cell surface and inhibit the adsorption of virus particles to cell resulting in the reduction of plaque formation.

MATERIALS AND METHODS

Cells. G-7 cells, a clone isolated from GMK cells (Established African grivet monkey kidney cells), were grown in 2-oz bottles with approximately one million cells using a mixture consisting of 7 parts of Hank's saline containing 0.5 % lactalbumin hydrolysate and 3 parts of Eagle's medium, supplemented with 10 % heated (56 °C, 30 minutes) bovine serum.

Virus. The M strain of herpes simplex virus had been transferred ten times in GMK cell cultures following its isolation from a case with Herpes labialis. Characteristics of this strain have been described previously^{8) 16)}.

Solution. Phosphate-buffered saline, pH 7.2 (PBS), and that deprived of MgCl₂ and CaCl₂ (PBS(-)) were prepared according to Dulbecco and Vogt⁶⁾. Earle's saline supplemented with 0.5 % skim milk and 0.125 % lactalbumin hydrolysate (MLE) was used in some experiments.

Protamine. Protamine sulfate (salmine) was obtained from Nutritional Biochemicals Corp.

Fractionation of protamine sulfate by Sephadex. Protamine sulfate was dissolved in PBS(-) at a concentration of 50 mg/ml and yielded a turbid solution which was centrifuged at 10,000 r.p.m for ten minutes at 25 to 28 °C, and filtered through a 0.22 μ Millipore filter. Two to four ml of the filtrate were applied on Sephadex G-50 column, 2 $\times$ 20 cm, and eluted with PBS(-).

Protein estimation. The protein content in each fraction was measured by the method of Lowry et al.¹⁰⁾ using bovine albumin as a standard.

Treatment of cell monolayers with the fractions. The two-day old monolayer cultures were washed twice with five ml PBS. The washed monolayers

were exposed to five ml of the fraction appropriately diluted with PBS for one hour at 37 °C. Control cultures were exposed to PBS under the same conditions. The cultures were then washed twice with PBS and followed by standing in five ml PBS for 30 minutes at room temperature to remove completely the substances in the fraction from culture bottles. After the fluid was drained off, the monolayers were inoculated with two tenth ml of virus suspension in PBS containing 50 to 100 PFU (plaque forming units). Four to five bottle cultures were used for one titration. The virus was allowed to adsorb for two hours at 37 °C and overlaid according to the method as described previously¹⁶⁾.

RESULTS

Fractionation of protamine sulfate by Sephadex G-50 and the effect on plaque formation. Two ml of prota-

Fig. 1 Fractionation of protamine sulfate by Sephadex G-50.

Two ml of protamine solution were applied on the column, 2 $\times$ 20 cm, and eluted with PBS(-). The first 15 ml were discarded. Every fraction (2 ml) was diluted 25 times with PBS and used for treatment of cell monolayers. Aliquots from diluted fractions were assayed for protein content.

mine sulfate solution were applied to Sephadex G-50 column. Fractions of two ml were collected and diluted with 48 ml PBS and the effect on plaque formation was tested. At the same time, aliquots from the diluted fractions were assayed for protein content. As illustrated in Fig. 1, two peaks of protein were obtained and the first one was more effective on the reduction in the number of plaques formed by M strain than the second peak, although it contained smaller amount of protein than the other. In addition, no alteration of plaque characteristics was observed with these peaks. The first peak appeared also in excluded fraction when it was rechromatographed through the column of Sephadex G-100.

The tubes 3 to 5 and 12 to 14 were pooled and referred to as Fr. I and Fr.

II, respectively, for the following experiments. As the eluants appearing after the tube 15 were contaminated with excess of SO_4^{--} ions derived from the original solution of protamine sulfate, they were not used for experiments.

Effect of the exposure time on plaque reduction. The washed monolayers were exposed to 45 and 130 $\mu\text{g/ml}$ of Fr. I and Fr. II, respectively, and PBS at 37°C. At the given time, the monolayers were washed and tested on plaque formation. Fig. 2 indicated that maximum reduction was achieved at 30 minutes in both cases. Incubation over 90 minutes reduced the number of plaques in the control monolayers which were exposed to PBS. Therefore, the incubation for 60 minutes seems to be adequate for estimating the reduction of plaques by Fr. I or Fr. II.

Fig. 2 Effect of the exposure time on plaque efficiency.

Cell monolayers were incubated with Fr. I or Fr. II for the indicated period before addition of virus.

Fig. 3 Effect of pretreatment of cell monolayers with different concentrations of Fr. I and Fr. II on plaque efficiency.

Reduction of plaques by various concentrations of Fr. I and Fr. II.

The relationship between the concentration of Fr. I or Fr. II with which the cell monolayers were treated and the number of plaques formed were illustrated in Fig. 3. Below the concentration of $6 \mu\text{g/ml}$, the reduction of the number of plaques was not so different between Fr. I and Fr. II, whereas, over this concentration, Fr. I markedly reduced the number of plaques formed in comparison with Fr. II. The effectiveness of Fr. I was not enhanced by increasing the concentration at higher than $50 \mu\text{g/ml}$. Fr. I and Fr. II were not toxic to cells at the concentration lower than $200 \mu\text{g/ml}$.

Effect of Fr. I and Fr. II on virus infectivity. The virus suspension which contained approximately 5×10^7 PFU/ml was mixed with an equal volume of PBS, 2 mg/ml of Fr. I and Fr. II. After incubation for one hour at 37°C with shaking, the mixture was diluted 100,000 times and inoculated on to the cell monolayers for plaque assay. The trace amount of Fr. I and Fr. II remaining in diluted mixtures should be too low in the concentration to affect the plaque formation. The PFUs of the diluted mixtures treated with Fr. I, Fr. II and PBS were 198.5, 176.5 and 191.5 per ml, respectively. The values are not significantly different indicating that the virus particle is not inactivated by either Fr. I or Fr. II.

Effect of Fr. I and Fr. II on virus replication. Monolayer cultures were washed twice with PBS and inoculated with 0.2 ml virus suspension. The inoculum contained 3 PFU per cell, which were allowed to adsorb for two hours at 37°C . The cell monolayers were then washed five times with PBS and incubated at 37°C in four ml of Eagle's medium or of that containing

Fig. 4 Virus replication in the presence of Fr. I and Fr. II.

Cells were inoculated with virus at a multiplicity of 3 PFU per cell. After adsorption for two hours, the cultures were washed five times and incubated at 37°C in four ml medium. At the time intervals indicated, the virus was harvested and assayed. The media were Eagle's medium (Δ), Eagle's medium containing $25 \mu\text{g/ml}$ of Fr. I ($\bullet$) and Eagle's medium containing $25 \mu\text{g/ml}$ of Fr. II ($\circ$).

$25 \mu\text{g/ml}$ of Fr. I or Fr. II. At each time interval indicated in Fig. 4, the cultures were frozen and thawed and followed by sonication for two minutes at 20 kc/sec. Fig. 4 shows that both fractions do not affect or slightly enhance virus replication after virus adsorption. Maximum yield of virus was observed 21 hours after adsorption in every case, and at this time more virus was found in cultures with Fr. I and Fr. II than in control (1200, 1300 and 850 PFU per cell, respectively).

Adsorption of Fr. I and Fr. II with cells. An experiment was designed to see if there was a possibility that the substances in Fr. I and Fr. II may bind to cell surface and result in the inhibition of virus attachment to the cell. The cells were dispersed by trypsin and washed twice with a large amount of PBS. The cells were suspended to give a suspension containing 5×10^7 cells in 25 ml of PBS, Fr. I and Fr. II ($15 \mu\text{g/ml}$), and incubated at 37°C for one hour with intermittent shaking. After incubation, the suspension was centrifuged to remove cells and the supernatant fluid was added to cell monolayers in order to see the effect on plaque formation. Further adsorption, when necessary,

was carried out by the same procedure as above. The results are shown in Table 1. It is clear that the plaque reducing effect of Fr. I was completely diminished by preincubation with cells, while five cycles of adsorption were required to reverse the inhibitory effect of Fr. II. The result may be attributed to the difference in molecular size of substances contained in Fr. II. As the behavior in Sephadex column showed that the substances in Fr. II was lower molecular size than those in Fr. I, the concentration in Fr. II may be higher on a molar basis. This may accordingly result in the requirement of larger amount of cells for adsorbing the substances in Fr. II.

TABLE 1
Effect of adsorption of Fr. I and Fr. II with cells on plaquing efficiency

	Before adsorption		After adsorption	
	av. no. of plaques	plaquing efficiency	av. no. of plaques	plaquing efficiency
Expt. 1			adsorbed $1 \times$ with cells	
Fr. I, $15 \mu\text{g/ml}$	39.3	33	118.4	107
Fr. II, $15 \mu\text{g/ml}$	51.4	44	55.0	50
PBS	118.2	100	110.0	100
Expt. 2			adsorbed $5 \times$ with cells	
Fr. II, $15 \mu\text{g/ml}$	51.3	55	77.0	86
PBS	93.0	100	89.3	100

Adsorption was carried out by incubating the suspension of 5×10^7 cells in 25 ml of Fr. I, Fr. II and PBS at 37°C for one hour.

Fate of unadsorbed virus. An attempt was made to study the fate of the virus particles. The inoculum which was adsorbed for two hours on cell monolayers pretreated with PBS, Fr. I or Fr. II ($25 \mu\text{g/ml}$) was removed and transferred to fresh cultures for estimation of unadsorbed virus. The virus for inoculation was diluted in PBS in one experiment, and in MLE in

the other one. As shown in Table 2, when the virus in PBS was used, the amount of virus remaining in the inoculum applied to monolayers pretreated with Fr. I was significantly less than the control and that in the case of Fr. II was roughly comparable to it.

When the virus diluted in MLE was inoculated on to the cells pretreated with Fr. I and Fr. II, the plaquing

TABLE 2
Inhibitory effect of pretreatment of cell monolayers on virus adsorption

Pretreatment of cultures with	Inoculation with virus in	Adsorbed virus		Unadsorbed virus		Recovery %
		total PFU	plaquing efficiency	total PFU	% of control	
Expt. 1						
Fr. I, 25 μ g/ml	PBS	59	14	61	80	24
Fr. II, 25 μ g/ml	"	209	50	84	111	60
PBS	"	415	100	76	100	100
Expt. 2						
Fr. I, 25 μ g/ml	MLE	189	26	201	216	48
Fr. II, 25 μ g/ml	"	615	86	168	181	97
PBS	"	714	100	93	100	100

After adsorption of virus to treated and untreated cell monolayers, the inoculum was removed and transferred to fresh cultures to estimate unadsorbed virus. For estimation of adsorbed virus, the cultures were overlaid immediately after removal of the inoculum.

efficiencies were 26 and 86%, respectively (Table 2, Expt. 2). These values are higher than those obtained in the experiment which carried out with the virus diluted in PBS (Table 2, Expt. 1). The reason is not clear but it seems that MLE can partially reduce the inhibitory effect probably by masking the substances contained in the fractions which previously adsorbed to the cell surface or by releasing them from the cell surface by competition with their binding site. In fact, MLE reacts with Fr. I and Fr. II and the precipitate is produced. Repeated experiments on Fr. II (25 μ g/ml) using the virus diluted in MLE showed the plaquing efficiencies to be 75 to 88%. Therefore, 86% plaquing efficiency (i.e., 14% reduction) seems to be significant although the difference from control is small. Furthermore, more numbers of PFU were detected in the inocula which were applied to monolayers pretreated with Fr. I and Fr. II in comparison with the control. These results clearly demonstrate that substances in Fr. I and Fr. II

inhibit adsorption of virus particles to cell surface resulting in the reduction of plaques. On the other hand, the discrepancy of the results with the unadsorbed virus obtained between experiments which were carried out with the virus in PBS and with that in MLE seems worthy of consideration. One possibility is that the virus particles remaining unadsorbed in the inoculum may be inactivated more rapidly in PBS than in MLE under the condition of the temperature of 37°C during adsorption period.

There exists another possibility that some component might be released from pretreated cells which would inactivate unadsorbed virus particles in PBS but not those in MLE. To test this possibility, the following experiments were undertaken. Two-tenth ml PBS were added to cell cultures pretreated with Fr. I, Fr. II and PBS. After incubation for two hours at 37°C, the 'conditioned' fluid was collected and diluted two-fold stepwise with PBS after centrifugation. In one experiment, 0.3 ml

dilutions of the conditioned fluid were mixed with 0.3 ml PBS and incubated at 37°C for 30 minutes and then 0.6 ml of virus suspension appropriately diluted in PBS were added. After further incubation for 30 minutes at 37 or 25°C,

infectivities were determined. The other experiment was carried out by the same procedures except that 0.3 ml dilutions of the conditioned fluid were mixed with 0.3 ml MLE instead of PBS. As shown in Table 3, the conditioned

TABLE 3
Effect of "conditioned" fluid from pretreated cell monolayers on virus infectivity

Final dilution of conditioned fluid		Conditioned fluid from cells pretreated with					
		Fr. I		Fr. II		PBS	
		Preincubation with					
		PBS	MLE	PBS	MLE	PBS	MLE
Expt. 1	1 : 4	70	71	69	90	86	94
	1 : 8	62	74	59	91	78	92
	1 : 16	45	71	48	99	66	94
	1 : 32	40	70	47	101	66	100
	1 : 64	17	81	26	101	56	95
Expt. 2	1 : 4	73	72			100	94
	1 : 8	102	97			102	100
	1 : 16	96	98			101	100
	1 : 32	84	100			82	99

Conditioned Fluid was obtained by incubating cell monolayers pretreated with Fr. I, Fr. II (25 µg/ml) and PBS in 0.2 ml PBS per culture bottle.

Three-tenth ml of diluted conditioned fluid was mixed with an equal volume of PBS or MLE, and incubated at 37°C for 30 minutes (Preincubation). Further incubation at 37°C (Expt. 1) or 25°C (Expt. 2) was done after adding 0.6 ml virus diluted in PBS.

The numbers represent per cent infectivities of control. Infectivity of control, which contained PBS instead of the conditioned fluid and was kept in ice water, was 75.2 PFU/0.2 ml.

fluid from PBS-treated cells is as effective as MLE in protecting virus and the protection decreases by dilution unless MLE is added in the fluid. However, the conditioned fluid from Fr. I- or Fr. II-treated cells inhibit weakly but significantly virus infectivity. It is evident that this inhibitory effect is not due to the interference with virus adsorption to cell surface at the time of plaque assay since the pretreatment of cell monolayers with the conditioned fluid from Fr. I- or

Fr. II-treated cells does not affect the plaque formation as shown in Table 4. On the other hand, addition of MLE reverse the inhibition by the conditioned fluid from Fr. II but cannot reverse the inhibition by the conditioned fluid from Fr. I. These results leads to a speculation that the cells treated with Fr. I or Fr. II release some factor into fluid which inhibit virus infectivity. Some component in MLE may probably combine with such an inhibitor released from Fr. II-treated cells making the

TABLE 4
Effect of pretreatment of cell monolayers with "conditioned" fluid on plaquing efficiency

Pretreatment of cell monolayers with conditioned fluid from cells treated with	Av. no. of Plaques	Plaquing efficiency
Fr. I	88.0	102
Fr. II	93.0	107
PBS	87.0	100
Control ^{a)}	86.5	100

Conditioned fluid was obtained as described in Table 3.
 Cell monolayers were treated with 1:2 dilution of the conditioned fluid before adding the virus.

a) Cell monolayers pretreated with PBS.

inhibitor inactive, whereas, it does not affect the inhibitor from Fr. I-treated cells. Such an event accounts for the finding in preceding experiment (Table 2, Expt. 2) that, when virus in MLE was used for inoculation, practically the same recovery (total amount of adsorbed and unadsorbed virus particles) was obtained in cell monolayers pretreated with Fr. II as in control monolayers, but only lower recovery in cell monolayers pretreated with Fr. I.

Effect of Fr. I and Fr. II on virus clones. An experiment was carried out

to confirm whether the reduction of plaques in pretreated monolayers was caused by some variants possibly contaminated in virus inoculum. Virus clones were isolated from the single plaques formed by adsorbed and unadsorbed virus to cell monolayers which were treated with PBS, Fr. I and Fr. II (Table 2, Expt. 2). Each clone was propagated passing once in cells, and tested on the plaque forming ability in treated or untreated cell monolayers. As shown in Table 5, the reduction of plaques formed in pretreated monolayers

TABLE 5
Effect of Fr. I and Fr. II on plaquing efficiency of virus clones derived from different origins

Clones derived from	no. of clones	Plaquing efficiency in cell monolayers pretreated with 20 µg/ml of	
		Fr. I	Fr. II
virus adsorbed to cells pretreated with Fr. I	4	19-28 (23)	57-73 (62)
" Fr. II	5	16-26 (20)	48-84 (66)
" PBS	5	20-27 (24)	47-75 (68)
virus unadsorbed to cells pretreated with Fr. I	8	12-28 (19)	40-73 (55)
" Fr. II	8	17-25 (20)	43-81 (61)
" PBS	7	16-28 (19)	44-81 (64)

Numbers in parenthesis represent the average values of plaquing efficiencies.

were roughly comparable in clones regardless of their origins. It is, therefore, unlikely that the virus stock consisted of the mixed populations which contained variants with and without ability to attach to the pre-treated cells.

Effect of heating on Fr. I and Fr. II. Fr. I and Fr. II (1 mg/ml) in PBS (-) were heated at different temperatures and exposed to cell monolayers after being diluted 40 times with PBS (25 μ g/ml in final concentration). The plaque reducing capacity of Fr. I and II was maintained after heating at 56°C for 30 minutes. As shown in Fig. 5, Fr. II was very stable for heating at 100°C. In contrast, Fr. I rapidly but partially lost the plaque reducing capacity at 100°C, and maintained it thereafter at a constant level.

Digestion of Fr. I and Fr. II by trypsin. Two ml of PBS (-), Fr. I and Fr. II in PBS (-) were mixed with equal volume of 0.3% trypsin (DIFCO, 1:250) in 0.02 M Tris (tris (hydroxymethyl) aminomethane) buffer, pH 8.0, and incubated at 37°C. After digestion for two hours, the mixture was heated in boiling water for five minutes to inactivate the enzyme. The mixtures were diluted 7 times with PBS and used for estimation of plaque reduction. Table 6 shows that the numbers of plaques formed in cell monolayers

Fig. 5 Effect of heating on plaque reducing capacity of Fr. I and Fr. II.

Fr. I and Fr. II containing 1 mg protein per ml in PBS (-) were heated in boiling water for indicated period. The cell monolayers were treated with heated sample after diluted 40 times with PBS.

treated with trypsin-digested Fr. I and Fr. II were as same as control. As Fr. I was partially heat labile (Fig. 5), another experiment in which the enzyme was inactivated by adding the bovine serum into reaction mixture after incubation was attempted and the same result was obtained. This clearly pointed out that the plaque reducing capacity was associated to the protein or protein moiety in substances contained in Fr. I and Fr. II.

TABLE 6
Effect of trypsin-digested Fr. I and Fr. II on plaquing efficiency

Pretreatment of cultures with	Av. no. of plaques	Plaquing efficiency
PBS (-)	103.6	100
" treated with trypsin	115.0	100
Fr. I, 25 μ g/ml	15.6	15
" treated with trypsin	124.6	108
Fr. II, 150 μ g/ml	25.6	25
" treated with trypsin	117.2	102

Amino acid composition of Fr. I and Fr. II. The qualitative amino acid analysis was carried out by two-dimensional paper chromatography on acid hydrolysates of Fr. I and Fr. II. Fr. I and Fr. II were desalted by eluting with distilled water through Sephadex G-10 column and hydrolyzed by 6 N HCl at 110 °C for 16 hours in sealed ampules filled with nitrogen gas. The hydrolysates were condensed under reduced pressure and served for chromatography. Phenol-water containing 1% ammonia (4 : 1) and n-butanol-acetic acid-water (4 : 1 : 2) were used as developing solvents. Fourteen spots corresponding to amino acids and two unidentified spots were clearly separated on the chromatogram of hydrolysate of Fr. I. On the other hand, only seven spots were recognized in the hydrolysate of Fr. II. Those were isoleucine-phenylalanine, valine-methionine, alanine, glycine, serine, proline and arginine, among which the spot of arginine was especially large and intense in color. The relatively small amount of arginine was detected in Fr. I by visual comparison of spots on the chromatogram. This was also confirmed by Sakaguchi reaction⁷⁾ carried out on the paper. The result obtained on Fr. II was in accord with the amino acid analysis of salmine reported by Ando et al²⁾. It is, thus, confirmed that Fr. II contains only protamine molecules. The behavior in Sephadex column and the amino acid analysis clearly demonstrates that Fr. I does not contain the aggregates of protamine molecules but is composed of different protein(s) with higher molecular weight.

DISCUSSION

The commercial protamine sulfate could be separated into two fractions

by Sephadex G-50 column which inhibited the virus-cell interaction, one of which (Fr. I) contains high molecular weight protein(s) and the other (Fr. II) contains only protamine molecules. Both fractions inhibit the plaque formation by herpes simplex virus in cell monolayers if the cells are treated with them before inoculation with the virus, although Fr. I inhibits much more efficiently than does Fr. II. As the plaque reducing capacity of Fr. I is partially diminished by heating, it may be reasonable to consider that it contains several kinds of proteins.

Wallis and Melnick¹⁵⁾ found that protamine sulfate increased the number and the size of plaques formed by herpes simplex virus when it was introduced in agar overlay medium. Our previous study showed that it slightly increased the size of plaques but did not affect the number of plaques when it was introduced in agar¹⁶⁾. In the present experiment, it was confirmed that Fr. I and Fr. II did not affect or slightly enhanced virus replication when either of the fractions was added in the medium after virus adsorption (Fig. 4) and that they did not directly affect virus infectivity. On the other hand, if the cells are treated with the fractions before adding the virus, the number of plaques is reduced (Fig. 1). These findings lead to the assumption that the fractions neither inactivate the intra- and extra-cellular virus nor inhibit virus replication but they bind to the cell surface and in consequence prevent virus attachment to or inactivate virus on the cell surface. Binding of the substances to cells could be clearly demonstrated by removal of plaque reducing capacity of Fr. I and II by preincubation with cells (Table 1). The fact that the plaque reduction is due

to the failure of virus attachment to pretreated cells became evident from experiments in which the number of PFU remaining in inoculum was determined after adsorption period (Table 2). This strongly supports the hypothesis proposed by Colter et al.³⁾ and by Craighead and Layne⁵⁾ that polycations block the receptor site by binding to the cell surface.

Craighead and Layne⁵⁾ failed to recover the unadsorbed virus from the inoculum which was applied to polycation-treated monolayers. In our experiment, such unadsorbed virus could be detected only when the virus diluted in MLE was used for inoculation but could not be detected when the virus diluted in PBS was used. The data obtained here indicate that some factor will be released from cells pretreated with Fr. II which inactivate virus but could not inactivate it in MLE (Table 3). Although such a factor has not yet been characterized, it seems probably that it is the complex of protamine molecule and cell receptor, which, if it is once released into fluid will be capable of binding to virus particle and will result in inactivation of virus. Such a complex will be also capable of binding to some substances, probably protein or peptide, which are contained in skim milk or lactalbumin hydrolysate, and in consequence will diminish its capacity of binding to virus particle. In the case of Fr. I, a similar complex will be released but not affected by MLE.

Protamine enhances adsorption of P³²-labelled fowl plague virus to cells¹⁾ and DEAE dextran enhances infectivity of poliovirus and its infectious RNA¹²⁾. The infectivity of rabies virus is remarkably enhanced by protamine or DEAE dextran⁹⁾ and that of respiratory syncytial virus is also enhanced by DEAE dextran and by low concentration of

protamine¹¹⁾. On the other hand, protamine reduces infectivity of Semliki Forest virus but increases RNA infectivity⁴⁾. In addition, infection of mengo encephalomyelitis virus is inhibited by protamine³⁾. Our preliminary experiments showed that protamine enhanced markedly the plaque formation by measles virus and to a lesser extent by poliovirus, but reduced that by Sindbis virus. Such the contrasting effects of polycations among various viruses are probably due to the differences of surface structure of virus particles. Many authors have attributed the effects of polycations on virus infectivity to ionic charge. The experiment on a variant of encephalomyocarditis virus by Craighead and Layne supports this hypothesis⁵⁾. They observed that plaque formation by r⁺ variant was inhibited by basic polyamino acid to a greater degree at low than high pH values. However, our repeated experiments by using Fr. I and II and herpes simplex virus carried out by strictly the same manner did not give the results comparable to that obtained by them. It seems, therefore, unlikely to consider that the ionic charge is the sole factor in the effect of polycations on virus adsorption or infectivity.

Although no evidence was obtained that Fr. I and II affect the virus-cell interaction by the different mechanism, the data obtained here demonstrate that both fraction inhibit virus adsorption by binding to the cell surface. However, the biochemical mechanism by which the virus-cell interaction is inhibited still remains to be elucidated.

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