THE EFFECT OF POLYSACCHARIDES ON THE REACTION BETWEEN ERYTHROCYTES AND VIRUSES, WITH PARTICULAR REFERENCE TO MUMPS VIRUS

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In the accompanying paper (1) it is shown that the capsular polysaccharides of type-specific Friedländer bacilli inhibit the multiplication of mumps virus in the allantoic sac of the chick embryo, yet have no demonstrable effect upon the multiplication of influenza A, influenza B, or Newcastle disease viruses. In an earlier report (2) it was demonstrated that polysaccharides of diverse origin, including the capsular polysaccharide of Friedländer bacillus type B, cause inhibition of the multiplication of pneumonia virus of mice (PVM) in the mouse lung. Because the active polysaccharides failed to cause inhibition of hemagglutination by PVM or to reduce the capacity of the normal mouse lung to combine with the virus (2), it was concluded that inhibition of the multiplication of PVM cannot be explained on the basis of blockade of virus "receptors." Recently, Green and Woolley (3) have reported that apple pectin inhibits the multiplication of influenza A virus in the allantoic sac of the chick embryo, and have found that the polysaccharide also causes inhibitions of hemagglutination by the virus. These workers suggested that inhibition of multiplication and hemagglutination of the virus by a polysaccharide might be closely related phenomena.

The results of preliminary experiments (4) with mumps, influenza A, influenza B, and Newcastle disease viruses indicated that the capsular polysaccharide of Friedländer bacillus type B causes inhibition of hemagglutination with each of the agents studied. Although polysaccharide-treated erythrocytes do not adsorb mumps virus, they do adsorb influenza A or B virus in a manner similar to untreated erythrocytes. There are evident discrepancies in the effect which polysaccharides exert on hemagglutination on the one hand, and on virus multiplication on the other. It appeared important, therefore, to carry out more detailed experiments, and to determine whether the inhibition of hemagglutination and multiplication of viruses by means of polysaccharides can be attributed to a single mechanism.

It will be shown that with mumps virus, as with PVM (2), there is no correlation between the capacity of polysaccharides to inhibit hemagglutination and

multiplication of the agent respectively. It will also be demonstrated that polysaccharides which fail to inhibit the multiplication of influenza A, influenza B, and Newcastle disease viruses markedly inhibit hemagglutination by these agents. Moreover, it will be shown that substances which block the effect of polysaccharides on the hemagglutination reaction with mumps virus do not have a similar action upon inhibition of virus multiplication. In addition, evidence will be presented which indicates that the mumps virus "receptors" present in the allantoic membrane of the living embryo are not blocked by active polysaccharide.

Materials and Methods

Viruses.—In these studies the following viruses were employed: mumps; influenza A, PR8 strain; influenza B, Lee strain; Newcastle disease; and PVM. The methods of cultivation and storage of each of these agents were identical to those already described (1, 5). Except for PVM each of the viruses was maintained by passage in the allantoic sac of the chick embryo; PVM was maintained by serial passage in the lungs of albino Swiss mice.

Hemagglutination Titrations.—Chicken, human group O, and mouse erythrocytes were in all instances freshly obtained, and washed 3 times with saline prior to their use. Hemagglutination titrations were performed as described in the accompanying paper (1).

Polysaccharide Preparations.—In most of the experiments the capsular polysaccharides of Friedländer bacilli type A (Fr.A), type B (Fr.B), and type C (Fr.C) (6, 7), respectively, were employed, although other polysaccharides previously studied (2) were also used. The products derived from the oxidation of Fr.B by periodic acid and by treatment with alkali as described in the accompanying paper (1) were also utilized.

Antiserum.—Sera from rabbits hyperimmunized with Friedländer bacillus type B (1) were used in specific precipitation experiments.

EXPERIMENTAL

It appears that with each of the agents employed in this study hemagglutination results from an interaction between the virus particles *per se* and erythrocytes. In order to determine the effect of polysaccharides on the hemagglutination reaction with these viruses, it was important to investigate the two components essential to the reaction as independently as possible. Therefore, certain experiments were designed to test the effect of treatment of virus with polysaccharide, while others were devised to test the effect of polysaccharide on erythrocytes. These experiments are described below.

I. The Effect of Polysaccharides on Mumps Virus.—The capacity of mumps virus to cause agglutination of chicken erythrocytes *in vitro* after the virus had been treated with various polysaccharides was determined. In the following experiment a large quantity of Fr.B was added to undiluted virus.

To 5 cc. of allantoic fluid infected with mumps virus was added 10 mg. of Fr.B dissolved in 1.0 cc. of saline. As a control, 1.0 cc. of saline alone was added to 5 cc. of infected allantoic fluid. This quantity of allantoic fluid was used because it is equal to the amount usually found in the allantoic sac of the embryos employed. The hemagglutination titer of each

mixture was determined immediately after preparation, following which the mixtures were held at 35°C. Aliquots were removed after 24 and 48 hours and their hemagglutination titers determined in duplicate in the usual manner.

As is shown in Table I it was found that there was no diminution in the capacity of mumps virus to cause hemagglutination even after holding the virus at 35°C. for 48 hours in the presence of 10 mg. of Fr.B. It should be pointed out that the quantity of polysaccharide employed in this experiment was 5 times greater than the largest amount used in experiments on the inhibition of multiplication of mumps virus *in vivo* and 2,000 times greater than the smallest quantity of Fr.B which is effective in causing inhibition of multiplication of the virus in the allantoic sac (1).

TABLE I

The Hemagglutination Capacity of Mumps Virus after Treatment of a Constant Quantity with the Capsular Polysaccharide of Friedländer Bacillus Type B

Treatment of mumps virus					Results of hemagglutination test								Hemagglutination titer*
					Dilution* of allantoic fluid								
Mixture			Time	Temperature	4	8	16	32	64	128	256	512	
5.0 cc.		*1.0 cc.*	*hrs.*	°C.									
MV allantoic fluid		Saline	0		4	3	3	3	3	2	±	0	128
"	"	"	"	24	35	3	3	3	3	3	2	1	0	"
"	"	"	"	48	"	3	3	3	3	2	2	±	0	"
MV allantoic fluid		Fr.B 10 mg.	0		4	3	3	3	3	2	±	0	128
"	"	"	" "	24	35	4	3	3	3	3	2	±	0	"
"	"	"	" "	48	"	4	3	3	3	3	2	±	0	"

* Expressed as the reciprocal.

In order to determine whether a great excess of polysaccharide would affect the hemagglutinating capacity of mumps virus, additional experiments were performed in which decreasing amounts of virus were treated with large quantities of various polysaccharides.

Serial twofold dilutions of allantoic fluid infected with mumps virus were prepared in saline solutions of Fr.B in a concentration of 2.0, 5.0, and 10 mg. per cc., respectively. In addition, similar dilutions of virus were prepared in solutions containing 5 mg. per cc. of Fr.B which had been treated with OH^- at pH 12.3 for 24 hours at 37°C. (1) and in solutions of the capsular polysaccharide of pneumococcus type III. The mixtures were held either at room temperature or at 37°C. Immediately after preparation, as well as at 1, 2, and 3 hours, aliquots of each dilution were tested for their capacity to cause agglutination of chicken erythrocytes. Similar experiments were also carried out with influenza A, influenza B, and Newcastle disease viruses diluted in a saline solution containing 5 mg. per cc. of Fr.B.

The results of these experiments are shown in Table II. In no instance was the virus hemagglutination titer lower than that of the saline control when the erythrocyte suspension was added immediately after dilution of virus in the polysaccharide solution. When each of the diluted virus-polysaccharide mixtures was held at room temperature, no reduction in hemagglutination titer occurred during 2 hours, and only in the case of mumps virus did two- to fourfold reductions in titer occur after 3 hours. When mixtures were held at 37°C.

TABLE II

The Hemagglutinating Capacity of Various Viruses after Treatment of Decreasing Quantities with Different Polysaccharides

Virus	Treatment			Hemagglutination titer*	Hemagglutinating units of virus inhibited	Degree of inhibition of virus multiplication obtained *in vivo*‡ Log
	Dilutions of virus prepared in	Time	Temperature			
	5 mg./cc.	*hrs.*	*°C.*			
MV	NaCl (control)	3	37	128	—	
"	Fr.B	0		"	0	−1.98
"	"	1	23–25	"	"	
"	"	3	"	32	2	
"	"	1	37	"	"	
"	"	3	"	16	4	
"	Fr.B treated with OH^- at pH 12.3	"	"	"	"	−0.13
"	Pneumococcus type III	"	"	8	8	−0.73
IAV (PR8)	NaCl	"	"	512	—	
" "	Fr.B	"	"	"	0	−0.52
IBV (Lee)	NaCl	"	"	1024	—	
" "	Fr.B	"	"	"	0	−0.15
NDV	NaCl	"	"	"	—	
"	Fr.B	"	"	512	1	−0.07

* Expressed as the reciprocal.

‡ *Cf.* accompanying paper (1).

no significant decrease in titer was observed with any virus except mumps. After 1 hour at 37°C. in the presence of Fr.B mumps virus showed a reduction in hemagglutination titer of two- to fourfold, while after 3 hours the titer was reduced four- to sixteenfold. It will be noted that, when tested against decreasing amounts of mumps virus, Fr.B treated with OH^- showed as striking an effect as untreated Fr.B in causing a reduction of the hemagglutination titer of the virus despite the fact that this degradation product is completely incapable of causing inhibition of mumps virus multiplication (1). Likewise, the capsular polysaccharide of pneumococcus type III caused marked inhibition of hemagglutination by mumps virus under the conditions of these experiments,

but failed to cause significant inhibition of the multiplication of the virus in the allantoic sac (1). The product of oxidation of Fr.B by HIO_4 and the untreated capsular polysaccharides of Friedländer bacilli types A and C were not used because each substance itself caused agglutination of chicken red blood cells under the conditions of these experiments.

II. The Effect of Treatment of Erythrocytes with Polysaccharide.—The capacity of mumps virus to cause agglutination of chicken erythrocytes *in vitro* after the red blood cells had been treated with polysaccharide was determined. In a similar manner, the effect of such treatment of erythrocytes derived from various species upon hemagglutination with influenza A, influenza B, and Newcastle disease viruses as well as PVM was also studied.

Fresh suspensions of chicken, human group O, or mouse erythrocytes were prepared in saline as well as in saline solutions of various polysaccharide preparations. The concentration of erythrocytes employed was either 3 or 10 per cent. The various polysaccharides employed were: (1) Fr.B; (2) the product of oxidation of Fr.B by HIO_4 for 160 minutes (1); (3) degradation product of Fr.B following treatment with OH^- at pH 11.6 for 24 hours at 37°C. (1); (4) capsular polysaccharides of Friedländer bacilli types A (Fr.A) and C (Fr.C), respectively. The concentrations used were either 2 or 5 mg. per cc. The various erythrocyte suspensions were held at room temperature for at least 3 hours. In certain instances they were held at 4°C. for an additional 12 to 18 hours. Following this treatment, the erythrocytes were washed 2 or 3 times with saline. It was found, however, that washing was not an important step because the results were the same whether the erythrocytes were washed or not. Following treatment, the erythrocytes were diluted with sufficient saline to give 0.5 per cent suspensions. Hemagglutination titrations were then carried out with serial twofold dilutions of the following viruses; mumps, influenza A, influenza B, Newcastle disease, and PVM.

The results of experiments with erythrocytes treated with 5 mg. per cc. of Fr.B are shown in Table III. It was found that treated chicken erythrocytes were, in most instances, entirely inagglutinable by either mumps or influenza B virus. Similarly, chicken erythrocytes treated with Fr.B were agglutinated by both influenza A and Newcastle disease viruses at significantly lower virus dilutions than were control cells. It will be noted that mouse red blood cells similarly treated were agglutinated by as high a dilution of PVM as control erythrocytes. However, the treated cells were not agglutinated by as high a dilution of influenza A virus as were control cells. Influenza B virus consistently failed to agglutinate untreated mouse red blood cells. Human group O erythrocytes treated with Fr.B showed no diminution in agglutinability with either influenza A or influenza B virus. That the polysaccharide had been adsorbed by both human and chicken erythrocytes was readily demonstrated by agglutination tests with Fr.B immune rabbit serum. The treated erythrocytes, even though washed repeatedly, were markedly agglutinated by anti-Fr.B serum.

The results of an experiment with mumps virus and chicken erythrocytes treated with Fr.B, the alkali degradation product of Fr.B, the product of oxida-

tion of Fr.B with HIO_4, or the capsular polysaccharides of Friedländer bacilli types A and C, respectively, are presented in Table IV. Each of the polysaccharide preparations was used in a concentration of 2 mg. per cc. in the treatment of the red blood cells.

It will be noted that both unaltered Fr.B and the product derived from it after alkaline hydrolysis altered red blood cells sufficiently so that the hemagglutination titers obtained were significantly lower than the control. The product of oxidation with HIO_4 caused only a twofold reduction in titer, whereas

TABLE III

The Effect of Treatment of Erythrocytes with the Capsular Polysaccharide of Friedländer Bacillus Type B on Hemagglutination Titration End Points with Various Viruses

Virus	Erythrocytes*	Hemagglutination titer determined with		Hemagglutinating units of virus inhibited	Degree of inhibition of virus multiplication *in vivo*§ Log
		Control RBC	Fr.B-treated RBC‡		
MV	Chicken	128	0	128	−2.11
IAV (PR8)	Chicken	1024	64	8	
	Human	256	256	0	−0.52
	Mouse	1024	128	4	
IBV (Lee)	Chicken	1024	0	1024	
	Human	128	128	0	−0.15
	Mouse	0			
NDV	Chicken	4096	64	32	−0.07
PVM (heat-released)	Mouse	128	256	0	−1.97

* 0.5 per cent suspension.

‡ Erythrocytes were treated with 5 mg. per cc. of Fr.B for 3 hours at room temperature.

§ *Cf.* references 1 and 2.

the polysaccharides Fr.A and Fr.C caused no demonstrable effect. As is shown in the accompanying paper (1), Fr.A, Fr.C, and the product of oxidation of Fr.B with HIO_4 inhibit the multiplication of mumps virus in the allantoic sac as effectively as does untreated Fr.B; however, the product of alkaline hydrolysis of Fr.B does not.

The results of the experiments described above indicate that the treatment of erythrocytes with polysaccharide preparations may lead to a variety of effects as regards the agglutination of such red blood cells by viruses. The species from which the erythrocytes were derived, the particular polysaccharide

employed, as well as the nature of the chemical treatment to which it had been subjected, and the identity of the virus itself are all factors which require independent consideration. A change in any one of these variables, with no change in either of the others, so altered the results as to make systematic prediction virtually impossible.

III. Adsorption of Viruses by Polysaccharide-Treated Erythrocytes.—In view of the fact that certain polysaccharides reduced or eliminated the agglutinability of chicken erythrocytes by various viruses, it was of interest to determine whether this alteration was accompanied by a corresponding reduction in the ability of treated red blood cells to adsorb viruses. The results of preliminary

TABLE IV

The Effect of Treatment of Chicken Erythrocytes with the Capsular Polysaccharides of Type-Specific Friedländer Bacilli and Certain Degradation Products on Hemagglutination Titration End Point with Mumps Virus

Erythrocytes treated* with	Hemagglutination titer	Hemagglutinating units of virus inhibited	Degree of inhibition of virus multiplication obtained *in vivo*‡ Log
2 mg./cc.			
Nacl (control)	128		
Fr.B	16	4	−2.11
" treated with OH^- at pH 11.6	32	2	−0.14
" " " HIO_4	64	1	−1.58
Fr.A	128	0	−1.36
Fr.C	"	"	−2.06

* 3 per cent suspensions were treated for 3 hours at room temperature and then washed 3 times with saline.

‡ *Cf.* accompanying paper (1).

experiments (4) indicated that with influenza A and B viruses the extent of the reduction in these two properties was not proportional. With mumps virus, on the other hand, the capacity of treated erythrocytes to adsorb and to be agglutinated by the agent was correspondingly diminished.

Ten per cent suspensions of chicken erythrocytes were prepared in saline solution containing 5 mg. per cc. of Fr.B; control suspensions were prepared in saline containing no polysaccharide. The suspensions were held at room temperature for 3 hours and then stored at 4°C. for 12 to 18 hours. Shortly before use the erythrocytes were washed 2 to 3 times with saline. The packed red blood cells were then made to 10 per cent suspensions in undiluted allantoic fluid infected with either influenza A, influenza B, or mumps virus. The cell-virus mixtures were again held at room temperature and frequently shaken. At intervals aliquots were removed, centrifuged at 4°C., and the supernates withdrawn. The hemagglutination titers of all supernates obtained from a single experiment were determined simultaneously.

The results of typical experiments are presented graphically in Fig. 1. It was found that both influenza A and B viruses were adsorbed by and eluted from treated erythrocytes in a manner closely similar to their adsorption by and elution from control red blood cells. This result was particularly surprising in view of the finding that erythrocytes similarly treated showed greatly diminished agglutinability with these two viruses. In the case of mumps virus

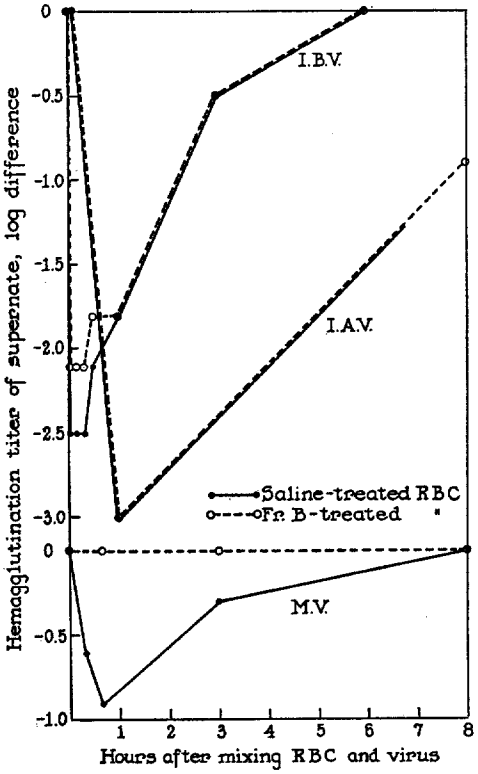

FIG. 1. The adsorption of influenza A (IAV), influenza B (IBV), and mumps (MV) viruses by chicken erythrocytes treated with the capsular polysaccharide of Friedländer bacillus type B.

it was found that Fr.B-treated erythrocytes failed entirely to adsorb demonstrable amounts of the agent, whereas control erythrocytes adsorbed most of the virus from the allantoic fluid.

Similar experiments were carried out with chicken red blood cells treated with the capsular polysaccharide of Friedländer bacillus type C (Fr.C). Such erythrocytes were capable of adsorbing mumps virus in a manner similar to control cells. It will be recalled that the agglutinability of red blood cells

treated with Fr.C also was undiminished when tested with mumps virus (*cf.* Table IV). It should be emphasized, however, that Fr.C was shown to be as effective an inhibitor of mumps virus multiplication as Fr.B (1).

The results of these experiments indicate clearly that polysaccharide-treated erythrocytes, even though completely inagglutinable by a particular virus, may or may not adsorb that virus. It appears of considerable interest that treated erythrocytes which failed to agglutinate visibly in the presence of more than 500 agglutinating units of influenza B virus were nonetheless capable of adsorbing the virus in apparently undiminished degree, whereas such red blood cells failed to adsorb mumps virus.

IV. Adsorption of Mumps Virus by the Allantoic Membrane.—The finding that chicken erythrocytes treated with Fr.B did not adsorb mumps virus *in vitro* raises the possibility that a similar effect might be demonstrable with the living chick embryo. Experiments were therefore carried out to determine whether mumps virus was adsorbed from the allantoic fluid by normal and polysaccharide-treated allantoic membranes.

Normal chick embryos 9 to 11 days of age were employed. About one-half the shell over the air sac was removed, and part of the shell membrane was torn away. All allantoic fluid possible was removed with a syringe and needle through an avascular area in the chorioallantoic membrane. Four cc. of saline solution containing 10 mg. per cc. of Fr.B was then injected into the allantoic sac. Control embryos likewise received 4 cc. of saline. After injection embryos were held at 35°C. for 3 hours. In some instances this period was prolonged to 12 to 14 hours. All fluid possible was again removed from the allantoic sac. Mumps virus was diluted either in buffered saline or in a saline solution of Fr.B, 10 mg. per cc., and 2.0 cc. was injected into the allantoic sac. The virus was diluted sufficiently to give the final fluid in the sac a hemagglutination titer ranging from 1:16 to 1:64. After thorough mixing of the fluid in the allantoic sac by drawing it back and forth into the syringe, 1.0 cc. was promptly removed. At intervals thereafter similar amounts were withdrawn. It was possible to obtain only a limited number of specimens, usually 3, from each embryo. The hemagglutination titer of each specimen was determined in the usual manner. In every instance it was possible to maintain the embryo in a living state throughout the entire experiment.

The results obtained in four separate experiments are presented graphically in Fig. 2. It was found that even when the allantoic sac had been treated for a number of hours with large quantities of Fr.B, mumps virus was nevertheless adsorbed from the fluid. Even when the allantoic membrane of the living embryo was treated with 40 mg. of Fr.B for 14 hours at 35°C. before injection of virus, and the latter diluted in a 1.0 per cent solution of the polysaccharide, no decrease in adsorption was demonstrable. It is obvious that these results are strikingly different from those obtained with chicken erythrocytes which had been treated with Fr.B *in vitro*. It appears that the polysaccharide, even in large amount, does not alter the living cells of the allantoic membrane as it does red blood cells, and that mumps virus "receptors" of allantoic membrane cells are not blocked by the presence of Fr.B.

V. The Effect of Ribonucleic Acid on the Activity of Polysaccharide (Fr.B).— In an attempt to find a substance which would neutralize or block the inhibitory action of polysaccharide (Fr.B) with respect to the hemagglutinating activity of mumps virus, the effect of ribonucleic acid was studied.

A solution of commercial yeast nucleic acid (Boehringer) having a concentration of 50 mg. per cc. in saline was prepared, and the pH adjusted to 7.1. The solution was sterilized by filtration. Equal volumes of nucleic acid solution and a solution of Fr.B, 10 mg. per cc. in saline, were mixed and held at room temperature for 15 minutes before use. Chicken erythrocytes were treated with the mixture as described above. The capacity of mumps virus to agglutinate treated red blood cells was then determined.

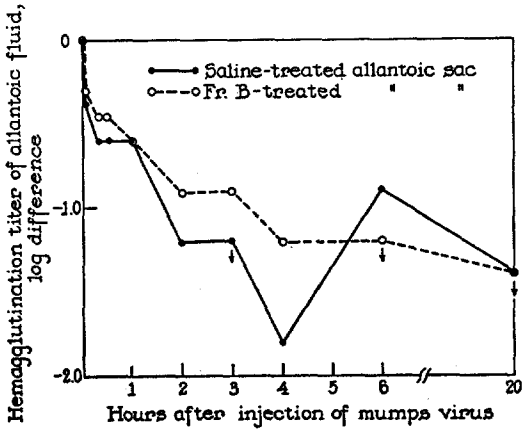

FIG. 2. The rate of adsorption of mumps virus by the living allantoic membrane following treatment with the capsular polysaccharide of Friedländer bacillus type B (Fr.B).

The results of a typical experiment are shown in Table V. It is seen that, although chicken erythrocytes treated with Fr.B alone were not agglutinated by mumps virus, cells treated with a mixture of the polysaccharide and ribonucleic acid were agglutinated by almost as small quantities of virus as control erythrocytes. Treatment with ribonucleic acid alone had no effect on the hemagglutination reaction.

Adsorption and elution of mumps virus with chicken erythrocytes previously treated with a mixture of Fr.B and ribonucleic acid were also studied. The results are presented graphically in Fig. 3. It was found that the virus was adsorbed and subsequently eluted from the treated red blood cells in a manner closely similar to that observed with control erythrocytes. Cells treated with Fr.B alone failed to adsorb mumps virus. In other experiments it was found that after red blood cells had been treated with Fr.B, the addition of ribonucleic acid in amounts as large as 50 mg. per cc. failed to cause dissociation of the

erythrocyte-polysaccharide combination. Cells treated in this manner remained inagglutinable by mumps virus.

Because ribonucleic acid blocked the *in vitro* activity of polysaccharide, it appeared of interest to determine whether this substance would also neutralize the *in vivo* activity of Fr.B with respect to mumps virus. The effect of a mixture of Fr.B and nucleic acid on the multiplication of virus in the allantoic sac was therefore studied.

TABLE V

Hemagglutination Titrations with Mumps Virus and Chicken Erythrocytes Treated with the Capsular Polysaccharide of Friedländer Bacillus Type B and Ribonucleic Acid.

Erythrocytes treated* with		Hemagglutination titer
Fr.B	Ribonucleic acid	
mg./cc.	*mg./cc.*	
0	0	128
5	0	0
"‡	25‡	64
0	"	128

* Treated for 3 hours at room temperature and then washed 2 times with saline.

‡ These substances were mixed and held at room temperature for 20 minutes before erythrocytes were added.

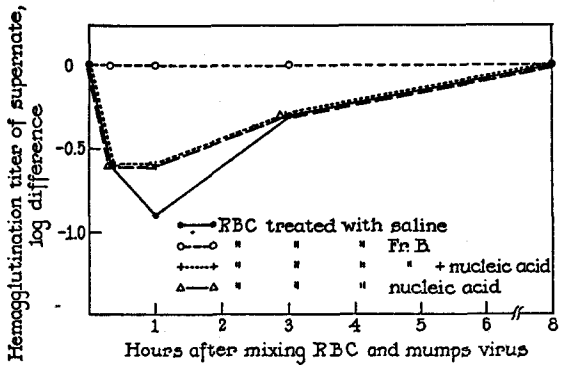

FIG. 3. The rate of adsorption of mumps virus by chicken erythrocytes following treatment with the capsular polysaccharide of Friedländer bacillus type B (Fr.B) and ribonucleic acid.

Saline solutions of Fr.B containing from 10 to 0.5 mg. per cc. were mixed with equal volumes of solutions of ribonucleic acid, 50 mg. per cc. After a period of 20 to 30 minutes at room temperature, 0.2 cc. of each mixture was injected into the allantoic sac of 7 to 9 day old embryos. Each embryo received from 0.05 to 1.0 mg. of Fr.B and 5 mg. of nucleic acid. In some experiments a mixture containing 2 mg. of Fr.B and 10 mg. of ribonucleic acid was injected. After an interval of 3 hours, either 10 or 100 E.I.D. of mumps virus was inoculated into the allantoic sac. The embryos were incubated at 35°C. for 6 days. They were then chilled overnight

at 4°C., the allantoic fluid removed, and the hemagglutination titer of each fluid determined (1). At least 4 embryos were used in each group, and at least 2 groups of control embryos were employed in each experiment.

The results are shown in Table VI. It will be noted that ribonucleic acid had no effect on the capacity of the polysaccharide to inhibit the multiplication of mumps virus even when 100 times more nucleic acid than Fr.B was employed.

The results of these experiments indicate clearly that ribonucleic acid is capable of blocking the inhibitory effect of polysaccharide (Fr.B) on the hemagglutination reaction with mumps virus, but has no blocking action on the inhibitory effect of Fr.B on multiplication of the virus.

TABLE VI

The Effect of the Capsular Polysaccharide of Friedländer Bacillus Type B and Ribonucleic Acid on the Multiplication of Mumps Virus in the Chick Embryo

1st injection Intra-allantoic	Interval	2nd injection Intra-allantoic	Incubation at 35° C.	Hemagglutination titer of allantoic fluids Log	Difference from controls Log
mg./embryo	*hrs.*	*E.I.D.*	*days*		
Fr.B, 2 mg.	3	MV 10	6	0	−1.52
Fr.B, 0.05 mg.	"	" 10^2	"	−0.99	−1.41
Fr.B, 2 mg. + ribonucleic acid, 10 mg.	"	" 10	"	0	−1.52
Fr.B, 0.05 mg. + ribonucleic acid, 5 mg.	"	" 10^2	"	−1.03	−1.37
Ribonucleic acid, 10 mg.	"	" 10	"	−1.81	+0.29
" " 5 "	"	" 10^2	"	−2.16	−0.24

DISCUSSION

The results of the experiments presented in this communication and those described in the accompanying paper (1) indicate clearly that there is no correlation between the effects of polysaccharides on the hemagglutination reaction with viruses and their inhibitory action on the multiplication of viruses. Had a definite correlation been demonstrated, it would have been possible to put forward a relatively simple hypothesis regarding the probable mechanism of the inhibitory action of polysaccharides with respect to virus multiplication.

Not only do some polysaccharides which markedly inhibit hemagglutination by one virus *in vitro* fail entirely to inhibit multiplication of the same virus *in vivo*, but others which are highly effective as inhibitors of the multiplication of of a virus are without discernible effect on the hemagglutination reaction with the same agent. Green and Woolley (3) also noted that some carbohydrates which inhibited hemagglutination with influenza A virus did not reduce multiplication of the virus.

The results obtained with the Friedländer type B polysaccharide after chemical alterations provide strong support for the concept that inhibition of hemagglutination and inhibition of multiplication of viruses are not closely related phenomena. Certain chemical changes induced in the carbohydrate, as for example treatment with OH^-, entirely abolish inhibitory activity with respect to virus multiplication without markedly diminishing the capacity of the carbohydrate to inhibit viral hemagglutination. Other chemical alterations, such as treatment with HIO_4, almost eliminate inhibitory activity with respect to hemagglutination but leave unaffected the capacity to inhibit multiplication of the agent. In the light of these observations it appears improbable that, with respect to inhibition by a polysaccharide, the erythrocyte component which reacts with a virus is closely related to the constituents of susceptible tissue cells essential for multiplication of that virus.

De Burgh and his coworkers (8) have recently shown that one component of human erythrocytes which reacts with influenza A virus is, in large part, polysaccharide. The nature of the component of susceptible tissue cells which appears to react with influenza A virus (9) in a similar manner is not yet known. However, evidence has been obtained indicating that the component present in normal mouse lung tissue which reacts with PVM is not polysaccharide but probably protein in nature (10). Nonetheless, the multiplication of PVM in the mouse lung is inhibited by certain polysaccharides (2).

Were the inhibition of hemagglutination to be satisfactory as a model for the study of the mechanism by which virus multiplication is inhibited *in vivo*, it appears necessary that inhibition of hemagglutination be associated with inhibition of adsorption of viruses by erythrocytes. It is evident from the results obtained that these two properties are not correlated, for some polysaccharides completely inhibit hemagglutination, yet have no demonstrable effect upon adsorption of the same virus by erythrocytes. Had these properties been associated, it would have been necessary to show that adsorption of virus by susceptible tissue cells is prevented by polysaccharide before the validity of the erythrocyte-virus model could be assumed. With mumps virus in the allantoic sac, as also with PVM in the mouse lung (2), it appears that such is not the case. In both instances, despite the presence of relatively large amounts of a polysaccharide highly active as an inhibitor of virus multiplication, apparently unaltered adsorption of virus by tissue cells occurs. Thus, the first step in the establishment of infection with a virus, *i.e.* combination or union between virus particles and susceptible cells, is not prevented by polysaccharides which are active as inhibitors of virus multiplication.

SUMMARY

Polysaccharides which cause inhibition of the multiplication of mumps virus in the allantoic sac may or may not cause inhibition of hemagglutination by

the virus. Moreover, such substances may or may not prevent adsorption of the virus by erythrocytes. The available evidence indicates that polysaccharides active as inhibitors do not block adsorption of mumps virus by cells of the living allantoic membrane. With influenza A, influenza B, and Newcastle disease viruses, as well as with PVM, there also appears to be a lack of correlation between the *in vitro* and *in vivo* inhibiting activity of polysaccharides.

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