THE INHIBITORY EFFECT OF POLYSACCHARIDE ON MUMPS VIRUS MULTIPLICATION

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That certain polysaccharides, either of bacterial or non-bacterial origin, can modify the course of infection with pneumonia virus of mice (PVM) and inhibit multiplication of the virus in the mouse lung was demonstrated recently by Horsfall and McCarty (1). Very small amounts of polysaccharide, indeed but a few micrograms, if given by the intranasal route, cause striking inhibitory effects when injected either some days before or after inoculation of the virus. Green and Woolley (2) have shown that the multiplication of influenza A virus (PR8) in the allantoic sac of the chick embryo is inhibited as a result of the injection of apple pectin. Relatively large amounts of pectin, 25 to 50 mg., if given intra-allantoically, were found to be effective when injected either ½ hour before or 1 hour after inoculation of the virus. In a preliminary communication Ginsberg, Goebel, and Horsfall (3) recently reported that the capsular polysaccharide of Friedländer bacillus type B inhibits the multiplication of mumps virus in the allantoic sac of the chick embryo.

There are only a few prior reports which deal with the effect of polysaccharides on viruses or infections induced by these agents. Armstrong (4) demonstrated that after the intranasal injection of certain bacteria mice were less susceptible to intranasal inoculation with either St. Louis encephalitis virus or influenza A virus. In retrospect, it appears possible that this effect may have been due to a polysaccharide because the active component was present in sterile culture filtrates and withstood heating at 74°C. for 45 minutes. Levine and Frisch (5) found that certain bacterial extracts are capable of inhibiting the activity of bacteriophage strains which have the capacity to cause lysis of the parent bacterium. Subsequently, Burnet (6) confirmed and extended these observations, and Gough and Burnet (7) showed the phage-inactivating substance present in bacterial extracts to be a polysaccharide. Johnson (8) described the inactivation of tobacco mosaic and several other plant viruses by extracts of *Aerobacter aerogenes* as well as a variety of other microorganisms. Takahashi (9) obtained from yeast extract a polysaccharide which has the capacity to inactivate tobacco mosaic virus.

Our present interest in the inhibition of viral multiplication by means of polysaccharides derives from the idea that information concerning substances which block multiplication may provide clues to the nature of the unknown

substances in protoplasm which are essential for the multiplication of viruses. When it was found that a polysaccharide which is strikingly effective in blocking the multiplication of PVM in the mouse lung (1) is also capable of inhibiting the multiplication of mumps virus in the chick embryo (3), a detailed study of the phenomenon was undertaken. The results of this investigation form the subject of this report and that which accompanies it (10).

In the present communication it will be shown that capsular polysaccharides derived from type-specific strains of Friedländer bacilli cause a decrease in the susceptibility of the chick embryo to infection with mumps virus and, even in very small amounts, are effective as inhibitors of multiplication of the virus in this species. It will also be demonstrated that inhibition of mumps virus multiplication results when the appropriate polysaccharide is given before, or as late as 4 days after, inoculation of virus; that polysaccharides injected either into the allantoic or yolk sac inhibit multiplication of virus in the allantoic sac. In contrast, it will be shown that the multiplication of influenza A and influenza B, as well as Newcastle disease viruses is not inhibited by the polysaccharide which is active with respect to mumps virus and PVM. In addition, evidence will be presented which indicates that the specific serological properties of active polysaccharides are probably dependent upon certain chemical groupings distinct from those which mediate inhibition of viral multiplication. Evidence will be presented to show that the virus *per se* is not demonstrably inactivated by polysaccharide.

Materials and Methods

Viruses.—The following viruses were employed: mumps, influenza A, influenza B, and Newcastle disease. Mumps virus (MV) was obtained from Dr. Karl Habel, The National Institute of Health, Bethesda, Maryland, who had adapted it to multiplication in the allantoic sac of the chick embryo. As routine, the virus was cultivated in the allantoic sac of 7 to 9 day old embryos which after inoculation were incubated at 35°C. for 6 days. Thereafter, the infected embryos were chilled at 4°C. overnight, and the allantoic fluid removed.

The PR8 strain (11) of influenza A virus (IAV) and the Lee strain (12) of influenza B virus (IBV) were used. Both these strains have been passed many times through chick embryos and mice. Newcastle disease virus (NDV) was obtained from Dr. F. R. Beaudette, New Jersey Agricultural Experiment Station, New Brunswick, New Jersey. Each of these 3 viruses was cultivated in the allantoic sac of 9 to 11 day old embryos which after inoculation were incubated at 35°C. for 48 hours. Infected allantoic fluids were harvested after preliminary chilling, as with mumps virus. Between experiments each of the viruses employed was stored in a solid carbon dioxide cabinet at −70°C. (13). It was found that the addition of nine parts of sterile normal horse serum (previously heated at 56°C. for 30 minutes) to one part of infected allantoic fluid protected virus infectivity, and permitted storage of each of the agents employed at −70°C. in cellulose nitrate tubes for long periods without loss of titer.

Chick Embryos.—Fertile White Leghorn eggs were incubated at 39°C. for 7 to 11 days before inoculation depending upon the virus employed. All embryos used in a given experiment were of the same age, and the eggs containing them were received in the laboratory on the same date.

Virus Infectivity Titrations.—Serial tenfold dilutions of infected allantoic fluid were made in sterile broth containing 10 per cent normal horse serum. A volume of 0.1 cc. of the desired dilution of virus was inoculated through a small drill hole in the egg shell directly over the allantoic sac in a region where the blood vessels could be avoided. At least 4 embryos were inoculated with each virus dilution and a minimum of 16 embryos was employed in each titration. After inoculation embryos were incubated at 35°C. In the case of mumps virus the period of incubation was 6 days; with influenza A, influenza B, and Newcastle disease viruses this period was 2 days. In all instances allantoic fluid was withdrawn after chilling of the eggs. Each allantoic fluid was tested for its capacity to cause agglutination of chicken erythrocytes. Virus titration end points (E.I.50) were calculated by the 50 per cent end point method of Reed and Muench (14).

Virus Hemagglutination Titrations.—Serial twofold dilutions of infected allantoic fluids were made in 0.85 per cent NaCl solution buffered at pH 7.2 (0.025 M phosphate). To 0.4 cc. of each dilution was added 0.4 cc. of a 1.0 per cent suspension of washed chicken erythrocytes. Readings were made after the tubes had stood 1 hour at room temperature. The end point was taken as the highest dilution at which definite (2+) agglutination of the red blood cells occurred.

Polysaccharide Preparations.—The following bacterial polysaccharides were employed: (1) capsular polysaccharides of Friedländer bacillus type A (Fr.A), type B (Fr.B), and type C (Fr. C), respectively (15, 16); (2) capsular polysaccharide of pneumococcus type II; (3) capsular polysaccharide of pneumococcus type III; (4) capsular polysaccharide of streptococcus MG (17); (5) levan synthesized from sucrose *in vitro* by the action of cell-free enzyme derived from *Streptococcus salivarius* type II (18); (6) dextran synthesized from sucrose *in vitro* by the action of cell-free enzyme derived from *Leuconostoc mesenteroides* (19). The following polysaccharide preparations of non-bacterial origin also were used: (1) blood group A substance; (2) apple pectin; (3) commercial corn starch. Before injection in the chick embryo each polysaccharide was dissolved in saline and heated at 70°C. for 30 minutes. In addition, the capsular polysaccharide of Friedländer bacillus type B was autoclaved during the course of preparation at 15 pounds' pressure for 20 minutes.

Immune Serum.—Rabbits were hyperimmunized by repeated intravenous injection of formalinized Friedländer bacillus type B vaccine. Immune serum was obtained 8 days after the last injection.

Precipitin Tests.—The presence of the capsular polysaccharide of Friedländer bacillus type B (Fr.B) was determined by the capillary precipitin technique (20) with specific immune rabbit serum. This procedure is of sufficient sensitivity to detect the polysaccharide in a concentration of the order of 1 μg. per cc. In order to determine accurately the extent of the serological activity of various solutions containing the degradation products of Fr.B, a turbidimetric measurement of the quantity of precipitate developed upon reaction with immune rabbit serum was employed (21).

EXPERIMENTAL

I. Reproducibility of Hemagglutination Titration End Points with Mumps Virus.—Levens and Enders (22) demonstrated that mumps virus causes agglutination of chicken erythrocytes. As is shown below, the hemagglutination technique can be used to measure the concentration of virus in infected allantoic fluid. In order to assess accurately the significance of differences in hemagglutination titers in various experiments, it was necessary to determine the reproducibility of such titers under relatively constant conditions. Individual

pools of allantoic fluids infected with mumps virus (MV), stored at $-70°C$. as described above, gave relatively constant virus infectivity titration end points (E.I.50) in the chick embryo. With different preparations the E.I.50 ranged between 10^{-6} and 10^{-7}. The hemagglutination titration end points obtained with allantoic fluids from 33 groups of 4 chick embryos inoculated intra-allantoically with 10^2 embryo infectious doses (E.I.D.) of mumps virus were tabulated, and the reproducibility of the end point was determined in the usual manner (23). In Table I the essential data and the results of the appropriate computations are presented. It should be pointed out that dilutions of 3 different pools of infected allantoic fluid were employed as the inocula in these experiments. The observed variation in end points obtained with each pool used as inoculum was considerably smaller than that noted when all end

TABLE I

Reproducibility of Hemagglutination Titration End Points with Allantoic Fluids from Chick Embryos Infected with Mumps Virus

Preparations of inocula employed	No. of groups of embryos* inoculated with 100 E.I.D.‡ intra-allantoi-cally	Hemagglutination titer of allantoic fluids§ Log			Deviation			Standard deviation
					From geometric mean Log			
No.		Lowest	Highest	Geo-metric mean	Least	Great-est	Mean	σ
3	33	−1.20 (1:16)	−2.76 (1:576)	−2.12 (1:132)	+0.01	−0.92	±0.26 (1.8-fold)	0.342 (2.2-fold)

* 4 chick embryos per group.

‡ E.I.D = embryo infectious doses.

§ Allantoic fluids obtained 6 days after inoculation; infected embryos were incubated at 35°C.

points obtained with the 3 inocula were considered. It will be noted that the mean deviation of the hemagglutination titration end points for the entire series is log ±0.26, and that the standard deviation of the distribution of the end points is 0.342. It can be shown that with any two individual end points, determined in the manner described above, a difference of 0.97 log unit should occur by chance only once in 20 times. In a single experiment the probability that a difference of this magnitude between two end points is significant is much greater. Throughout this paper a difference of 1.0 log unit between end points obtained with 2 groups of 4 embryos each will be considered as significant.

II. Hemagglutination as a Measure of Mumps Virus Concentration.—Habel (24) showed that mumps virus is infectious for the chick embryo, and can be cultivated in the allantoic sac as well as in the amniotic or yolk sacs. Beveridge and Lind (25) found that the hemagglutination titer of allantoic and

amniotic fluids infected with mumps virus was directly related to the comple-
ment-fixing titer of such fluids, and concluded that the hemagglutination titer
was a function of virus concentration. However, Henle *et al.* (26) have shown
that embryos infected with mumps virus elaborate a soluble antigen distinct
from the virus *per se*, which, like the virus, is capable of fixing complement in
the presence of specific antibodies. In the light of these findings it seemed
necessary to determine by as direct means as possible the relationship between
hemagglutination and virus titers. This problem was investigated in two
different ways: (1) the rate of multiplication of the virus and the rate of in-
crease in hemagglutination titer were determined and correlated; and (2) the
effect of high gravitational fields upon both the virus titer and the hemag-
glutination titer was measured.

The rate of multiplication of mumps virus in the allantoic sac was studied in the following
manner: In each experiment a number of chick embryos, 8 or 9 days of age, were inoculated
intra-allantoically with 0.1 cc. of a 10^{-3} dilution of mumps virus (10^3 E.I.D.). Each day
thereafter allantoic fluid was removed from at least 4 embryos which then were discarded.
The hemagglutination titer of each fluid was determined. In certain experiments pools
were prepared containing equal volumes of allantoic fluid from each of a given group of
embryos. Both the infectivity and hemagglutination titers of such pools were determined
as described above.

The results of these experiments are presented graphically in Fig. 1 in which
both the hemagglutination and infectivity titers of the allantoic fluid are
plotted against time after inoculation with mumps virus. In Fig. 1 *B* the
hemagglutination titers obtained in four separate experiments are shown. It
is evident that during the first 3 days after inoculation the presence of virus
was not demonstrable by means of the hemagglutination technique. After the
3rd day there was a rapid increase in titer which reached maximal levels on
the 6th day. In Fig. 1 *A* both the infectivity and the hemagglutination titers
obtained simultaneously in one experiment are shown. It will be seen that
the rate of increase in the concentration of virus as determined by infectivity
titrations paralleled the rate of increase in hemagglutination titer. When the
experimental error of both titration techniques is taken into account, it can
be shown that there is no significant difference between the two curves shown
in Fig. 1 *A*. It appears from these results that virus and hemagglutination
titers increase with time after inoculation of mumps virus into the allantoic
sac in closely similar manners. Moreover, it appears that virus titers (E.I.50)
of the order of $10^{-4.30}$ or higher are necessary before hemagglutination is demon-
strable with mumps virus. Additional evidence on this point is presented
below (*cf.* Tables II and VI).

The effect of high gravitational fields upon both the virus and hemagglutination titers of
infected allantoic fluid was determined in the following manner: Allantoic fluids from infected

embryos were pooled, and the virus infectivity titer was immediately determined. The pooled fluid was then centrifuged at 15,000 R.P.M. for 30 minutes in a high speed vacuum apparatus (27). The centrifuge head was similar to that previously described (28) but was almost twice as large; the diameter = 30.1 cm. After centrifugation the supernate was decanted carefully and the sediment resuspended in a quantity of broth equal to the original volume. Virus infectivity titrations were then carried out with the supernate and the resuspended sediment
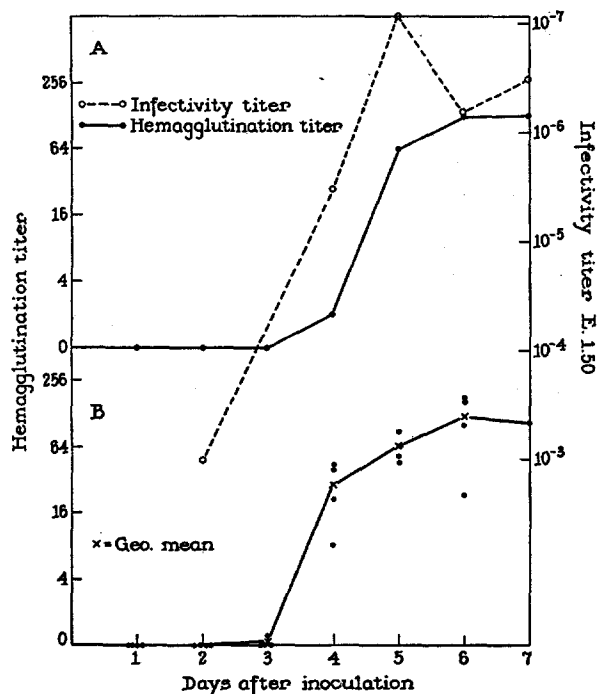

FIG. 1 A. Comparison of infectivity and hemagglutination titers of pools of allantoic fluids obtained from chick embryos in a single experiment at intervals after inoculation with mumps virus. Groups of 4 embryos were given 1,000 E.I.D. intra-allantoically.

FIG. 1 B. Hemagglutination titers of allantoic fluids obtained from chick embryos at intervals after intra-allantoic inoculation with 1,000 E.I.D. of mumps virus. Each end point represents the mean titer of at least 4 allantoic fluids.

Hemagglutination titrations, in duplicate, were performed with the uncentrifuged pool, supernate, and resuspended sediment.

The results of this experiment are shown in Table II. Both the virus and hemagglutination titers of the supernate were markedly lower than the corresponding titers obtained with the uncentrifuged pool. Conversely, both titers of the resuspended sediment were closely similar to those of the uncentrifuged pool. These results indicate that within the limits of the experimental techniques employed the effect of high gravitational fields upon both virus

and hemagglutination titers was similar if not identical. Because sedimentation of the virus was accompanied by proportional sedimentation of the component responsible for hemagglutination, it is evident that the two were not separable by gravitational force under the conditions of the experiment. This indicates that the property of infectiousness and the capacity to cause hemagglutination are associated with particles which, if not identical, are closely

TABLE II

Effect of High Speed Centrifugation on Infectivity and Hemagglutination Titers of Mumps Virus

Mumps virus	Hemagglutina-tion titer*	Infectivity titration		
Pooled allantoic fluids		Dilution of material inoculated intra-allantoi-cally	Infectivity score‡	Virus titration end point E.I.50§ Log
Before centrifugation	512	10^{-5}	4/4	
		10^{-6}	4/4	
		10^{-7}	3/4	-7.33
		10^{-8}	0/4	
Supernate after centrifugation at 15,000 R.P.M. for 30 min.	4	10^{-2}	4/4	
		10^{-3}	3/3	
		10^{-4}	4/4	-4.67
		10^{-5}	1/4	
		10^{-6}	0/4	
Sediment resuspended in original volume	512	10^{-4}	4/4	
		10^{-5}	4/4	
		10^{-6}	3/4	-6.50
		10^{-7}	1/4	
		10^{-8}	0/4	

* Expressed as the reciprocal.

‡ Numerator = number of allantoic fluids which caused hemagglutination. Denominator = number chick embryos inoculated.

§ E.I.50 = 50 per cent embryo infectivity end point.

similar in size and density. These results, as well as those dealing with the rate of increase in virus and hemagglutination titers, strongly suggest that mumps virus hemagglutination is caused by the virus particle itself and that the hemagglutination technique may indeed be used to measure virus concentration. It will be recalled that similar evidence has been obtained with influenza virus (29) and PVM (30).

In several experiments amniotic fluids, as well as 10 per cent suspensions of ground embryos which had been inoculated intra-allantoically, were tested for hemagglutination. In every instance virus could not be demonstrated by the

hemagglutination technique despite the fact that high titers were obtained with the allantoic fluids. Moreover, a virus infectivity titration carried out with an amniotic fluid pool yielded a titer of only $10^{-3.00}$. These results suggest that the chief site of multiplication of mumps virus after inoculation into the allantoic sac is the allantoic membrane.

III. Effect of Capsular Polysaccharide of Friedländer Bacillus Type B on the Susceptibility of Chick Embryos to Infection with Mumps Virus.—The results of preliminary experiments (3) indicated that the injection of capsular polysaccharide of Friedländer bacillus type B (Fr.B) caused inhibition of multiplication of mumps virus when as much as 10^4 E.I.D. was inoculated in the allantoic sac. Experiments were carried out to determine the extent to which the injection of Fr.B modified the susceptibility of the chick embryo to infection with mumps virus.

Each of a number of embryos was injected intra-allantoically with 1 mg. of Fr.B dissolved in 0.1 cc. of saline. Other embryos, which served as controls, were given 0.1 cc. of saline by the same route. After an interval of 3 hours groups of 4 embryos each were inoculated intra-allantoically with mumps virus in dilutions from 10^{-3} to 10^{-7}. In certain experiments the order of the injections was reversed and dilutions of virus were given 3 hours before the polysaccharide. Allantoic fluid was obtained from each embryo 6 days after inoculation and the hemagglutination titer determined. The virus titration end point was calculated as described above.

For purposes of clarity the results of one such experiment are presented in detail in Table III. It will be noted that, even though mumps virus was inoculated into the allantoic sac 3 hours before polysaccharide was given, the virus titration end point ($10^{-3.66}$) was strikingly lower than that obtained in control embryos ($10^{-6.33}$). It is also evident that the mean hemagglutination titer of allantoic fluids from each group of embryos which had received Fr.B polysaccharide was significantly lower than that of comparable controls. In many instances fluids from the former embryos failed to cause demonstrable hemagglutination. That the low hemagglutination titers observed were not attributable merely to the presence of polysaccharide in the allantoic fluids is evident from the findings presented in the accompanying paper (10); concentrations of Fr.B 10 times greater than those employed in this study did not lower the hemagglutination titer of mumps virus even when mixtures were held at 35°C. for as long as 48 hours.

In Table IV the results of such experiments are presented in summary form. In each instance the virus titration end point was definitely lower when determined in embryos which had been injected with Fr.B. The geometric mean of the differences between the titration end points obtained in control embryos and in those which were given polysaccharide is log -2.00. This indicates that 100 times more virus was required to initiate infection in Fr.B-treated embryos than in controls; that the susceptibility of such embryos to infection

with mumps virus was 100 times less than that of the controls. It is of interest to note that the effect of polysaccharide on susceptibility was equally as striking whether it was injected 3 hours before or after inoculation of virus.

The results of hemagglutination titrations carried out on allantoic fluids obtained from control and polysaccharide-treated embryos inoculated with varying amounts of mumps virus are shown in Table V. When amounts of virus from 10 to 10^4 E.I.D. were inoculated into embryos which had been given 1.0 mg. of Fr.B 3 hours previously, the hemagglutination titers obtained were in each instance significantly lower than those found with comparable control embryos. Only when as much as 10^5 E.I.D. of virus was given were similar titers obtained with allantoic fluids from polysaccharide-treated and control

TABLE III

Results of a Titration of Mumps Virus in Chick Embryos Injected with the Capsular Polysaccharide of Friedländer Bacillus Type B

1st injection 0.1 cc. intra-allantoic	Inter-val	2nd injection 0.1 cc. intra-allantoic	Incu-bation at 35°C.	Hemagglutination titer*					Infec-tivity score	Virus titration end point E.I.50 Log
				Individual allantoic fluids				Mean		
	hrs.		days							
MV 10^{-4}	3	Saline	6	64	512	8	32	154	4/4	
" 10^{-5}	"	"	"	64	32	512	8	154	4/4	
" 10^{-6}	"	"	"	16	128	16	0	40	3/4	−6.33
" 10^{-7}	"	"	"	0	0	0	0	0	0/4	
" 10^{-3}	"	Fr.B 1.0 mg.‡	"	0	16	4	8	7	3/4	
" 10^{-4}	"	" " "	"	0	4	0	0	1	1/4	−3.66
" 10^{-5}	"	" " "	"	0	0	8	0	2	1/4	

* Expressed as the reciprocal.

‡ Each embryo was given 1.0 mg. of polysaccharide intra-allantoically.

embryos. In view of the results obtained in the experiments described above, it appears that embryos given Fr.B are not only less susceptible to infection with but also are less capable of supporting multiplication of mumps virus than are control embryos.

IV. Toxicity of Fr.B Polysaccharide.—Embryos which were given Fr.B polysaccharide intra-allantoically showed no gross evidence of deleterious effects. They grew and developed at the same rate as controls and died no more frequently than embryos given saline by the same route. This was true even when 10 mg. of Fr.B per embryo was injected which is 5 times the quantity used in any experiment. The chorioallantoic membranes of 8 day embryos injected with 1 mg. of Fr.B were examined microscopically after the embryos had been incubated for either 2 or 6 days. The membranes were fixed in

Zenker's solution and stained with hematoxylin and eosin. There were no demonstrable microscopic lesions in any of the embryonic tissues examined. It appears, therefore, that Fr.B polysaccharide in the quantities given is innocuous for and has no evident toxic effects upon the chick embryo.

V. Effect of Fr.B Polysaccharide on Virus Titer after Injection with Mumps Virus.—It was necessary to determine directly whether or not allantoic fluid

TABLE IV

Titrations of Mumps Virus in Chick Embryos Injected with the Capsular Polysaccharide of Friedländer Bacillus Type B

1st injection 0.1 cc. intra-allantoic	Interval	2nd Injection 0.1 cc. intra-allantoic	Incubation at 35°C.	Virus titration end point E.I.50 Log	Difference from control Log
	hrs.		*days*		
Saline	3	MV dilutions*	6	−6.17	−2.17
Fr.B‡	"	" "	"	−4.00	
Saline	"	" "	"	−5.63	−1.63
Fr.B‡	"	" "	"	−4.00	
Saline	"	" "	"	−6.17	−1.67
Fr.B‡	"	" "	"	−4.50	
Saline	"	" "	"	−6.60	−1.85
Fr.B‡	"	" "	"	−4.75	
MV dilutions*	"	Saline	"	−6.33	−2.67
" "	"	Fr.B‡	"	−3.66	
Geometric mean		Saline		−6.18	−2.00
		Fr.B		−4.18	

* Serial tenfold dilutions from 10⁻³ to 10⁻⁷ were employed in each titration. A group of at least 4 embryos was inoculated with each dilution.

‡ 1.0 mg. of polysaccharide per embryo.

from embryos infected with mumps virus and also given Fr.B polysaccharide showed a reduction in virus infectivity titer which corresponded to the observed reduction in hemagglutination titer.

Four 9 day old embryos were inoculated intra-allantoically with 10² E.I.D. of mumps virus. Three hours later each of 2 of the embryos was given 1.0 mg. of Fr.B by the same route; the remaining 2 were given 0.1 cc. of saline. They were then incubated at 35°C. for 6 days after which allantoic fluids were removed and two pools prepared. The hemagglutination titer and, in addition, the virus infectivity titer of the pools were determined.

The results of this experiment are shown in Table VI. The allantoic fluid pool obtained from control embryos had a hemagglutination titer of 1:256 and a virus titer of $10^{-7.00}$, whereas that obtained from embryos which received Fr.B caused no hemagglutination and had a virus infectivity titer of only $10^{-4.25}$. It is evident that the differences between the hemagglutination titers (*i.e.*, log -2.41) and virus infectivity titers (*i.e.*, log -2.75), respectively, of the allantoic fluid pools were closely similar. It appears, therefore, that the actual concentration of mumps virus present in the allantoic fluid of polysaccharide-

TABLE V

Reduction in Hemagglutination Titer of Mumps Virus in the Allantoic Fluid of Chick Embryos Injected with the Capsular Polysaccharide of Friedländer Bacillus Type B

1st injection 0.1 cc. intra-allantoic	Interval	2nd injection Intra-allantoic mumps virus	Incubation at 35°C.	Hemagglutination titer of allantoic fluids* Log	Difference from controls Log
	hrs.	*E.I.D.*	*days*		
Saline	3	10	6	-2.01	-1.53
Fr.B‡	"	"	"	-0.48	
Saline	"	10^2	"	-2.27	-1.93
Fr.B‡	"	"	"	-0.34	
Saline	"	10^3	"	-2.16	-1.53
Fr.B‡	"	"	"	-0.63	
Saline	"	10^4	"	-2.25	-1.18
Fr.B‡	"	"	"	-1.07	
Saline	"	10^5	"	-2.48	-0.37
Fr.B‡	"	"	"	-2.11	

* Geometric mean of the hemagglutination titers of allantoic fluids from at least 2 groups of embryos (4 embryos per group).

‡ 1 mg./embryo.

treated embryos was markedly lower than that obtained with controls. Moreover, it appears that this difference can be measured with as much precision by means of the hemagglutination technique as by virus infectivity titrations.

VI. *Effect of Fr.B Polysaccharide upon Infectivity of Mumps Virus.*—It was of importance to determine whether the polysaccharide (Fr.B) employed in the preceding experiments caused inactivation of mumps virus *in vitro*. If this occurred, it appeared probable that an explanation for the effects observed *in vivo* might be obtained.

Allantoic fluid from embryos infected with mumps virus was diluted 10^{-1} in sterile normal horse serum. To one aliquot was added an equal volume of Fr.B in a concentration of 10

mg. per cc. in saline.   To another aliquot an equal volume of saline was added.   The mixtures
were held at 4°C. for 30 minutes after which serial dilutions were prepared.   Virus infectivity
titrations were then carried out.

The results of this experiment are presented in Table VII.   It will be noted
that, despite the presence of a relatively high concentration of polysaccharide
in the initial mixture, the infectivity titer of the virus following treatment with
Fr.B was identical with that of the control.   These findings indicate clearly
that under the conditions of the experiment there was no demonstrable inac-
tivation of virus in the presence of a large quantity of polysaccharide.   More-

TABLE VI

*Reduction of Both Hemagglutination and Infectivity Titers of Mumps Virus in the Allantoic
Fluid of Chick Embryos Injected with the Capsular Polysaccharide of Friedländer
Bacillus Type B*

1st injection Intra-allantoic	Interval	2nd injection Intra-allantoic	Incubation at 35°C.	Hemagglutination titer of allantoic fluid	Infectivity titrations of allantoic fluid		
					Dilution	Infectivity score	Virus titration end point E.I.50 Log
E.I.D.	*hrs.*		*days*				
MV 10²	3	Saline	6	1:256	10⁻⁵	3/3	
					10⁻⁶	3/4	
					10⁻⁷	2/4	−7.00
					10⁻⁸	1/3	
" "	"	Fr.B 1.0 mg./embryo	"	0	10⁻³	3/3	
					10⁻⁴	2/4	−4.25
					10⁻⁵	1/3	
					10⁻⁶	0/4	

over, they suggest that the effects observed *in vivo* are not to be explained on
the basis of direct action by Fr.B on mumps virus.

*VII.   Quantity of Fr.B Polysaccharide Required to Inhibit Multiplication of
Mumps Virus.*—It appeared of interest to determine the smallest amount of
Fr.B polysaccharide which could cause a significant reduction in the multipli-
cation of mumps virus in the allantoic sac.

Approximately 100 E.I.D. of virus was used in these experiments and embryos were in-
oculated intra-allantoically with virus 3 hours before the polysaccharide was injected.   A
number of experiments were performed; in each instance two control groups of 4 embryos
given 0.1 cc. of saline 3 hours after inoculation of virus were included.   Allantoic fluids were
removed 6 days after inoculation and their hemagglutination titers determined.

The results obtained are presented graphically in Fig. 2 in which the loga-
rithm of the difference in the hemagglutination titers of polysaccharide-treated

and control embryos is plotted against the quantity of Fr.B injected. It will be noted that Fr.B polysaccharide was capable of inhibiting mumps virus multiplication to a significant degree when as little as 5 μg. per embryo was injected into the allantoic sac. When relatively large amounts of Fr.B (*i.e.*, 0.5 to 1.0 mg. per embryo) were given, the extent to which virus multiplication was inhibited was definitely greater than when smaller amounts were injected. However, the degree of inhibition obtained after the injection of 200 μg. was not strikingly different from that obtained when only 5 μg. per embryo was given. It will be recalled that in similar experiments with PVM it was found

TABLE VII

The Effect of the Capsular Polysaccharide of Friedländer Bacillus Type B on Mumps Virus in Vitro

Mixture held at 4°C. 30 min.		Dilution of mixture inoculated intra-allan-toically	Constituents of inoculum		Hemag-glutination titer of allantoic fluids*	Infectivity score	Virus titration end point E.I.50 Log
Mumps virus dilution	Diluent		Mumps virus	Fr.B			
				$\mu g./embryo$			
$10^{-1.3}$	Saline	10^{0}	$10^{-1.3}$	0	171	3/3	
		$10^{-2.7}$	10^{-4}	"	224	4/4	
		$10^{-3.7}$	10^{-5}	"	144	4/4	
		$10^{-4.7}$	10^{-6}	"	171	3/3	
		$10^{-5.7}$	10^{-7}	"	21	1/3	-6.75
$10^{-1.3}$	Fr.B 5 mg./cc.	10^{0}	$10^{-1.3}$	500	96	2/4	
		$10^{-2.7}$	10^{-4}	1.0	20	3/4	
		$10^{-3.7}$	10^{-5}	0.1	156	4/4	
		$10^{-4.7}$	10^{-6}	0.01	149	3/3	
		$10^{-5.7}$	10^{-7}	0.001	4	1/4	-6.67

* Expressed as the reciprocal of the mean of the hemagglutination titers obtained 6 days after inoculation.

that 1.6 μg. of Fr.B per mouse was capable of causing inhibition of virus multiplication (1).

VIII. Effect of the Time Interval between Injections of Virus and Polysaccharide.—Experiments were carried out to determine the duration of the interval after inoculation with mumps during which injection of Fr.B polysaccharide would cause inhibition of virus multiplication.

In these experiments embryos were inoculated intra-allantoically with 100 E.I.D. of mumps virus. Three hours before and after the inoculation of virus, as well as at intervals of 24 hours thereafter, each of 4 embryos was given a single injection of 1.0 mg. of Fr.B by the same route. The eggs were incubated at 35°C. for 6 days following inoculation with mumps virus, and the hemagglutination titer of each allantoic fluid was then determined.

The results obtained are presented in Fig. 3.   The logarithm of the difference between the hemagglutination titers of the allantoic fluids obtained from poly-saccharide-treated and control embryos is plotted against the time interval which elapsed between inoculation of virus and injection of Fr.B.   It is important to point out that all allantoic fluids were obtained 6 days after inoculation with virus.   It is evident that a single injection of 1.0 mg. of polysaccharide,
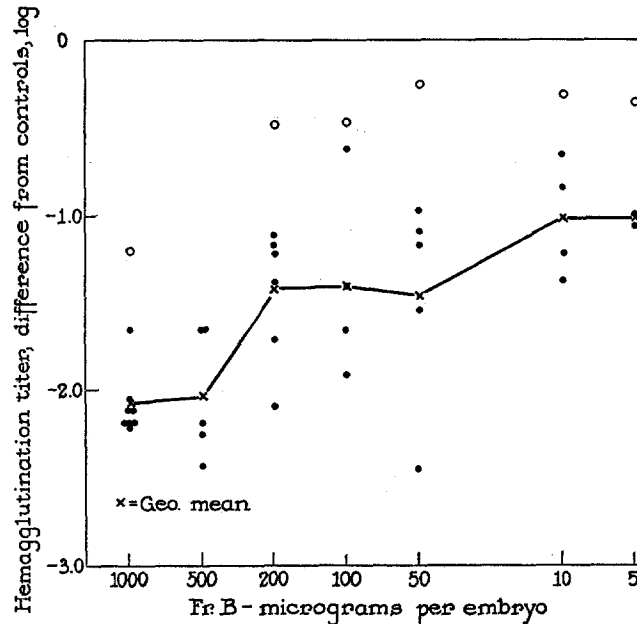

Fig. 2. Effect of different quantities of capsular polysaccharide of Friedländer bacillus type B (Fr.B) on the hemagglutination titer of allantoic fluids obtained from chick embryos inoculated with 100 E.I.D. of mumps virus.   Polysaccharide was injected intra-allantoically 3 hours after virus.   Groups of 4 embryos were employed; each end point represents the mean difference in titer between one experimental and two control groups.   Results indicated by open circles were obtained in a single experiment and, because they deviate systematically from the other results, they were not included in the calculation of geometric mean.

whether given 3 hours before or as long as 48 hours after inoculation with mumps virus, markedly inhibited viral multiplication.   Although inhibition became progressively less striking as the time interval was further increased, a significant degree of inhibition was demonstrated when the interval was as long as 96 hours, though not when it was 120 hours.   For purposes of comparison the rate of multiplication of mumps virus in the allantoic sac of control embryos is also shown in Fig. 3.   It will be seen that the effect of the polysaccharide was most marked when multiplication of virus had not yet reached the minimal threshold (*i.e.*, virus titer of $10^{-4.30}$) at which it can be demonstrated by means of the

hemagglutination technique. As further multiplication occurred with increasing time, the effect of the polysaccharide became less and less evident. When nearly maximal multiplication had taken place (*i.e.*, 5 days after inoculation), injection of polysaccharide caused no significant effect. It is of importance to emphasize that at no time following inoculation of the virus did injection of polysaccharide cause a *reduction* in the concentration of virus already present.
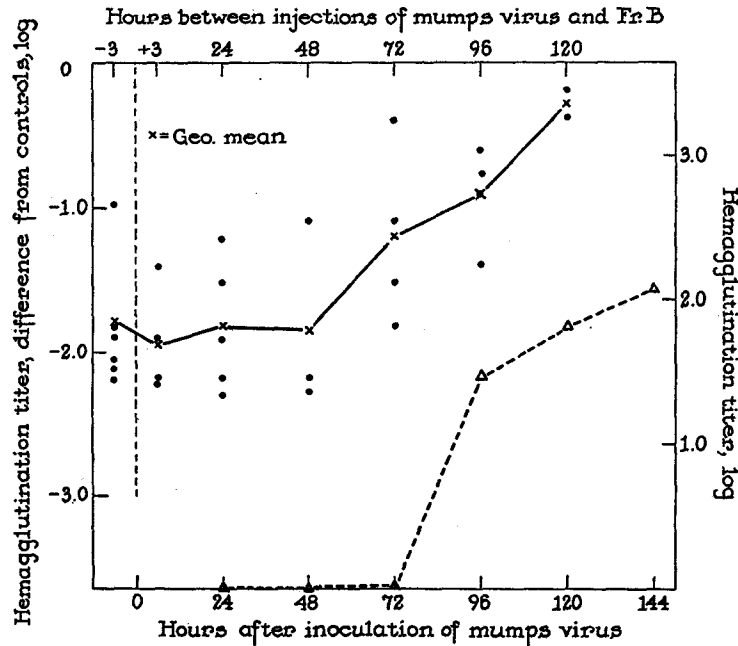

FIG. 3. Upper graph: The effect of time between inoculation with mumps virus and injection of Fr.B polysaccharide on the hemagglutination titer of allantoic fluids obtained 6 days after viral infection. Groups of 4 embryos were employed; each end point represents the mean difference in titer between one experimental and two control groups.

Lower graph: The rate of increase in the hemagglutination titer of the allantoic fluid of control embryos inoculated with mumps virus.

It appears that in each instance the effect observed is to be explained entirely on the basis of inhibition of further viral multiplication.

IX. Effect of Injection of Fr.B Polysaccharide into the Yolk Sac.—A number of experiments were carried out to determine whether injection of Fr.B polysaccharide by a route other than the allantoic would also cause inhibition of multiplication of mumps virus in the allantoic sac. It was found that among 45 embryos, each of which was given 5 mg. of polysaccharide into the yolk sac and then inoculated with 100 E.I.D. of virus intra-allantoically, 24 (53.2 per

cent) yielded allantoic fluids with significantly lower hemagglutination titers than were obtained in appropriate controls. When precipitin tests were performed with anti-Fr.B serum, it was found that the polysaccharide was present in the allantoic fluid of some embryos, but was not demonstrable in that from others despite the fact that all had been given the same amount of Fr.B in the yolk sac. It was found also that allantoic fluids in which Fr.B was demonstrable gave in almost every instance low hemagglutination titers, whereas those in which Fr.B was not demonstrable showed titers similar to those obtained with controls. That the low hemagglutination titers were not caused by a direct effect of Fr.B on the virus can be concluded from the finding that mixtures of polysaccharide and virus gave theoretical titers as is shown in the accompanying paper (10). It appears, therefore, that when polysaccharide was transported from the yolk sac to the allantoic sac, and appeared at the latter site in demonstrable amounts, inhibition of virus multiplication in the allantoic sac was obtained. Why polysaccharide failed to be so transported in approximately 50 per cent of embryos is not known.

X. Effect of Various Polysaccharides upon Multiplication of Mumps Virus.— It has been shown that a variety of polysaccharides are effective in inhibiting multiplication of PVM in the mouse lung (1). In view of the fact that Fr.B polysaccharide exhibited this property and, in addition, inhibited multiplication of mumps virus, it was of interest to study other carbohydrates which actively inhibit multiplication of PVM as well as others which show no such activity.

It will be seen from the results shown in Table VIII that, with the exception of Fr.B polysaccharide, none of the preparations tested which were effective in inhibiting multiplication of PVM in the mouse lung had a similar effect upon infection with mumps virus in the allantoic sac of the chick embryo. The capsular polysaccharide of streptococcus MG and blood group A substance inhibit multiplication of PVM (1), but were entirely ineffective when tested with mumps virus. Recent studies in this laboratory have shown that dextran synthesized from sucrose by means of cell-free enzyme obtained from *Leuconostoc mesenteroides*[1] was capable of inhibiting multiplication of PVM. However, this carbohydrate also was ineffective with mumps virus. It is of considerable interest that capsular polysaccharide of pneumococcus type II, although immunologically related to capsular polysaccharide of Friedländer bacillus type B, likewise had no significant effect on the multiplication of mumps virus. Moreover, type II capsular polysaccharide has been found to have no effect upon the multiplication of PVM.[1]

Because of their serological relationship, experiments were carried out to determine whether capsular polysaccharide of pneumococcus type II was ca-

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[1] Unpublished observations.

pable of blocking the effect of Fr.B polysaccharide.   Two mg. of type II capsular polysaccharide was injected into the allantoic sac of embryos 3 hours before

TABLE VIII

The Effect of Various Polysaccharides upon the Multiplication of Mumps Virus in the Allantoic Sac of the Chick Embryo

1st injection 0.1 cc. intra-allantoic		Inter-val	2nd injection Intra-allantoic mumps virus	Incuba-tion at 35°C.	Hemagglutination titer* of allantoic fluids of embryos injected with		Difference from controls Log
Polysaccharide	Amount				Saline Log	Polysac-charide Log	
	$mg./embryo$	$hrs.$	$E.I.D.$	$days$			
Fr.B	1	3	10^2	6	−2.27	−0.34	−1.93
Pneumococcus type II SSS‡	1	"	10	"	−1.61	−1.38	−0.23
" "	2	"	10^2	"	−2.08	−1.43	−0.65
Pneumococcus type III SSS‡	1	"	10	"	−2.10	−1.36	−0.74
" "	1	"	10^2	"	−2.30	−1.57	−0.73
Apple pectin	1	"	10	"	−2.62	−2.48	−0.14
" "	1	"	10^2	"	−2.37	−2.98	+0.61
" "	2	"	10	"	−1.52	−2.05	+0.53
Streptococcus MG, SSS‡	1	"	10	"	−1.61	−1.66	+0.05
Blood group A	1	"	10	"	−1.61	−1.85	+0.24
Dextran§	1	"	10	"	−2.62	−2.48	−0.14
"	1	"	10^2	"	−2.37	−2.33	−0.04
Levan‖	1	"	10^2	"	−1.94	−1.90	−0.04
Corn starch	1	"	10^2	"	−1.79	−2.11	+0.32

* Expressed as the geometric mean.
‡ SSS = capsular polysaccharide.
§ Produced *in vitro* from sucrose by cell-free enzymes from *Leuconostoc mesenteroides*.
‖ " " " " " " " " " " *Streptococcus salivarius* type II.

0.2 mg. of Fr.B was given; 100 E.I.D. of mumps virus was then inoculated 3 hours later.   In each instance Fr.B polysaccharide inhibited the multiplication of mumps virus in undiminished degree.   It appears, therefore, that type II

capsular polysaccharide does not act as an antagonist with respect to Fr.B. The striking differences noted in the biological activities of these two serologically related polysaccharides suggest that this function is not necessarily related to immunological similarity. In this connection it is of considerable interest that a small quantity of Fr.B polysaccharide neutralized with an amount of specific immune serum sufficient to provide an excess of antibody caused as marked inhibition of multiplication of mumps virus as did the same quantity

TABLE IX

Effect of the Capsular Polysaccharides of Friedländer Bacillus Types A, B, and C, Respectively, on Multiplication of Mumps Virus in the Chick Embryo

1st injection Intra-allantoic	Interval	2nd injection Intra-allantoic	Amount of polysaccharide	Hemagglutination titer of allantoic fluids* Log	Difference from controls Log
	hrs.		*mg./embryo*		
Fr.A‡	3	MV 10^2 E.I.D.	1.0	−0.90	−1.21
MV 10^2 E.I.D.	"	Fr.A‡	0.2	−0.70	−1.36
" " "	"	"	0.05	−1.23	−0.73
" " "	"	"	0.01	−0.70	−1.36
Fr.B‡	"	MV 10^2 E.I.D.	1.0	0	−2.11
MV 10^2 E.I.D.	"	Fr.B‡	0.2	−0.90	−1.16
" " "	"	"	0.05	−0.48	−1.58
" " "	"	"	0.01	−0.70	−1.36
Fr.C‡	"	MV 10^2 E.I.D.	1.0	0	−2.11
MV 10^2 E.I.D.	"	Fr.C‡	0.2	0	−2.06
" " "	"	"	0.05	0	−2.06
" " "	"	"	0.01	0	−2.06

* Geometric mean of the hemagglutination titers of allantoic fluids obtained 6 days after inoculation.

‡ Fr.A = capsular polysaccharide of type A Friedländer bacillus.
Fr.B =   "   "   "   " B   "   "
Fr.C =   "   "   "   " C   "   "

of Fr.B in the absence of serum. Chemical evidence bearing on this point is presented below.

The capsular polysaccharides of Friedländer bacillus types A and C, respectively, were also tested to determine whether they were capable of inhibiting the multiplication of mumps virus in the allantoic sac of the chick embryo. As is shown in Table IX, not only were both these polysaccharides strikingly effective as inhibitors of the multiplication of mumps virus, but also they significantly inhibited multiplication when quantities as small as 10 μg. were given. It is of interest that both Fr.A and Fr.C polysaccharides have been found to act as inhibitors with respect to PVM.[1]

XI. The Effect of Fr.B Polysaccharide on the Multiplication of Other Viruses.—
The inhibitory effect of Fr.B polysaccharide upon viruses other than mumps
also was investigated.   In Table X the results of these experiments are shown.
It will be observed that even when very small amounts of influenza A, influenza
B, or Newcastle disease viruses were inoculated 3 hours after relatively large
amounts of polysaccharide had been injected intra-allantoically, no evidence
of inhibition of multiplication of any of these viruses was obtained.   It appears
evident that Fr.B polysaccharide, when used in amounts which are strikingly
effective against mumps virus, was not capable of affecting significantly the

TABLE X

*Effect of the Capsular Polysaccharide of Friedländer Bacillus Type B on the Multiplication
of Influenza A, Influenza B, and Newcastle Disease Viruses in the Chick Embryo*

Virus inoculated	No. E.I.D. injected intra-allantoically	Amount Fr.B	Incuba-tion at 35°C.	Hemagglutination titer of allantoic fluids* of embryos injected with		Difference from control Log
				Saline‡ Log	Fr.B‡ Log	
		mg./ embryo	*days*			
Influenza A	10	1.0	2	−2.53	−2.60	+0.07
" "	10²	1.0	"	−2.85	−2.33	−0.52
" "	10	5.0	"	−2.53	−2.41	−0.12
Influenza B	10	0.6	"	−2.28	−1.88	−0.40
" "	10²	0.6	"	−2.01	−1.86	−0.15
Newcastle disease	1	0.6	"	−1.90	−2.53	+0.63
" "	10	0.6	"	−2.58	−2.58	0
" "	10²	0.6	"	−2.65	−2.58	−0.07

* Expressed as the mean of the hemagglutination titers.

‡ Saline or polysaccharide was injected intra-allantoically 3 hours before inoculation of
virus.

hemagglutination titers of the allantoic fluids of embryos infected with influenza
A, influenza B, or Newcastle disease viruses.   Moreover, it was found that the
infectivity titers of each of these viruses were closely similar both in polysac-
charide-treated and in control embryos.   Other experiments have demonstrated
that Fr.B polysaccharide given intranasally does not lower the virus infectivity
titration end point (M.S.50) in mice when these animals are infected with either
influenza A or influenza B virus.

*XII. Effects of Various Chemical Procedures on the Inhibitory Capacity of
Fr.B Polysaccharide.—*Polysaccharides can be subjected to a variety of pro-
cedures which will bring about alterations in their chemical structures.   In the

present investigation the effects of oxidation by periodic acid and of hydroxyl ions on the activity of Fr.B polysaccharide with respect to mumps virus were studied.

Oxidation of the polysaccharide with periodic acid was carried out as follows: A solution of carbohydrate containing 0.4 gm. in 20 cc. was diluted with an equal volume of 0.5 M acetate buffer at pH 5.0. 30 cc. of 0.1 M HIO$_4$ was added, and the mixture diluted to 100 cc. At 10, 40, and 160 minutes, as well as at 24 hours, 25 cc. of solution was removed and 0.5 cc. of 50 per cent glycerol added to decompose the excess periodate. The solutions were thoroughly
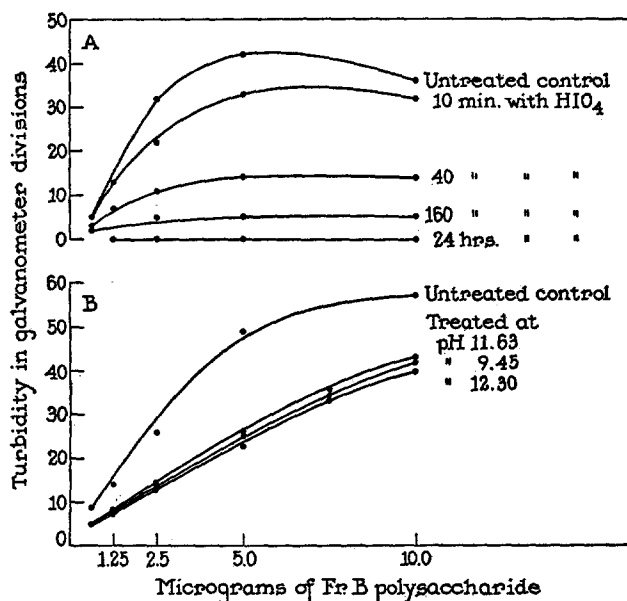

FIG. 4 A. Effect of treatment with 0.03 M HIO$_4$ at pH 5.0 on the specific serological activity of Fr.B polysaccharide. Solutions were mixed with immune rabbit serum diluted 1:20. Photometric observations were made 15 minutes after the mixtures were prepared.

FIG. 4 B. Effect of treatment with OH$^-$ on the specific serological activity of Fr.B polysaccharide. Neutral solutions were mixed with immune rabbit serum diluted 1:15. Photometric readings were made 15 minutes later.

dialyzed against distilled water and the solid material recovered from the frozen state. In each instance a quantitative recovery was obtained.

Treatment of the polysaccharide with OH$^-$ was performed by dissolving 20 mg. amounts of carbohydrate in 0.1 M borate buffers at pH 9.45, 11.63, and 12.30, respectively. After standing at 37°C. for 24 hours the borate was removed by dialysis against 0.9 per cent NaCl solution. Each solution was made to a final concentration of 5.0 mg. per cc. A further specimen was prepared by allowing a solution of carbohydrate to stand at pH 12.3 in an appropriate concentration of NaOH for 24 hours at 37°C. This solution was then neutralized with HCl but was not dialyzed.

Quantitative serological precipitation tests on solutions of the polysaccharide treated with periodate for various time intervals, and with OH^- at varying values of pH, were carried out photometrically as described above. The antiserum used was prepared by the immunization of rabbits with Friedländer bacillus type B.

The activity of these same solutions of polysaccharide with respect to mumps virus was determined in the following manner: Approximately 100 E.I.D. of virus was inoculated into the allantoic sac of each of a number of embryos and 3 hours later either unaltered Fr. B or a preparation of polysaccharide which had been subjected to chemical treatment was injected by the same route. Following the usual incubation period of 6 days the hemagglutination titer of each allantoic fluid was determined.

The results of quantitative precipitation tests with immune rabbit serum which are presented graphically in Fig. 4 A demonstrate that oxidation of Fr.B polysaccharide by 0.03 M HIO_4 at pH 5.0 brings about a rapid and progressive reduction in the specific serological activity of the carbohydrate. Indeed, at the end of a 24 hour period of contact with the reagent not only was there no serological activity demonstrable by the precipitation technique, but also the product of oxidation failed completely to inhibit specific precipitation by unaltered polysaccharide with homologous antiserum. This loss in serological activity, however, was not accompanied by any demonstrable reduction in biological activity with respect to mumps virus, as is shown below. In contrast to these results the effect of hydroxyl ions did not bring about destruction of serological activity ($cf.$ Fig. 4 B), but completely abolished the biological activity of the carbohydrate as will be seen from the evidence presented below.

In Table XI are shown the results of experiments with chemically treated polysaccharide preparations and mumps virus in the chick embryo. It is evident that even after treatment with periodic acid for 24 hours the product of oxidation of the Fr.B polysaccharide was still capable of inhibiting multiplication of virus. It is also seen that, whereas treatment with OH^- at pH 9.45 did not affect the inhibitory activity of the polysaccharide, similar treatment at pH 11.63 completely inactivated the inhibitory effect of the carbohydrate. Polysaccharide treated with NaOH at pH 12.30 for 24 hours, but not dialyzed thereafter, was also found to have lost all capacity to inhibit multiplication of mumps virus. As is also shown in Table XI the aldobionic acid derived from Fr.B polysaccharide by acid hydrolysis had no effect upon multiplication of virus in the embryo even when as much as 5.0 mg. was injected.

The Fr.B polysaccharide preparation which was used throughout these experiments, although highly purified by repeated alcoholic precipitations, still contained 0.7 per cent nitrogen. It was of interest to determine what effect further purification would have upon the activity of the polysaccharide. The carbohydrate was therefore precipitated as its copper salt and after dissolving in a high concentration of acetate buffer was repeatedly precipitated with alcohol. The solution was then shaken with octyl alcohol-chloroform and finally electrodialyzed. The carbohydrate was recovered by freezing and drying and

its nitrogen content determined. The final product contained only 0.25 per cent nitrogen. When this more thoroughly purified preparation was tested in the chick embryo with mumps virus in the usual manner, it was found that its effectiveness in causing inhibition of virus multiplication was not changed. The fact that further purification did not diminish activity is important evidence indicating that the active substance is the polysaccharide itself and not an impurity associated with it.

TABLE XI

The Effect of Chemical Alterations of the Capsular Polysaccharide of Friedländer Bacillus Type B on Its Capacity to Cause Inhibition of Multiplication of Mumps Virus

1st injection Intra-allantoic	Inter-val	2nd injection Intra-allantoic	Specific sero-logical activity	Amount of material injected	Hemagglu-tination titer of allantoic fluids* Log	Difference from control Log
E.I.D.	hrs.		per cent‡	mg./ embryo		
MV 10²	3	Fr.B	100	0.05	−0.48	−1.58
" "	"	Fr.B treated with HIO₄ 160 min.	10	1.0	−0.59	−1.58
" "	"	" "		0.05	−1.43	−0.82
" "	"	Fr.B treated with HIO₄ 24 hrs.	0	1.0	−0.39	−1.78
" "	"	" "		0.05	−1.28	−0.97
" "	"	Fr.B treated with OH⁻ pH 9.45 24 hrs.	50	0.1	−0.30	−2.09
" "	"	Fr.B treated with OH⁻ pH 11.63 24 hrs.	50	0.5	−2.25	−0.14
" "	"	" "		0.1	−2.53	+0.14
" "	"	Aldobionic acid (from Fr.B)	0	5.0	−2.41	+0.16

* Geometric mean of the hemagglutination titers.
‡ Approximate values (*cf*. Fig. 4).

DISCUSSION

The evidence obtained in this study indicates that capsular polysaccharides derived from type-specific Friedländer bacilli possess the capacity to cause inhibition of the multiplication of mumps virus in the allantoic sac of the chick embryo. These same polysaccharides are also capable of inhibiting the multiplication of pneumonia virus of mice (PVM) in the mouse lung (1,[1]). However, at least one of these carbohydrates, the capsular polysaccharide of Friedländer bacillus type B, shows no such inhibitory effect on the multiplication of Newcastle disease virus in the chick embryo or on the multiplication of influenza A and influenza B viruses either in the chick embryo or in the mouse lung.

Despite the fact that each of the five different viruses employed possesses the capacity to cause hemagglutination with appropriate erythrocytes, each

possesses in addition to immunological specificity other properties which serve sharply to differentiate one agent from another. It appears pertinent to point out that the viruses enumerated above can be separated into two groups on the basis of their rates of multiplication in susceptible hosts. Mumps virus and PVM (30) multiply at relatively slow rates in the chick embryo and the mouse, respectively, and both reach maximal titers in approximately 6 days. Influenza A (31), influenza B (32), and Newcastle disease (33) viruses multiply at relatively rapid rates in the chick embryo, as too do influenza A (34) and B viruses in the mouse, and each reaches maximal titers in approximately 2 days. It is of suggestive interest that among the viruses studied there appears to be a close correlation between the rate of multiplication and the capacity of the capsular polysaccharide of Friedländer bacillus type B to inhibit multiplication: viruses which multiply slowly are inhibited; those which multiply rapidly are not. It might be thought that the rapidity with which viruses in the latter group multiply is an adequate explanation for the fact that their multiplication is not inhibited by Friedländer polysaccharide. There is reason to think, however, that some other explanation is required. Even the injection of relatively large quantities of polysaccharide some hours before the inoculation of very small amounts of these viruses does not cause inhibition of their multiplication. These agents, in contrast to those which multiply slowly, appear to be capable of increasing in titer in a susceptible host regardless of the presence of a considerable concentration of Friedländer polysaccharide. It does not follow, however, that the viruses which multiply rapidly will necessarily continue to do so in the presence of other polysaccharides. Indeed, there is evidence indicating that with one of them (influenza A virus) apple pectin is effective as an inhibitor of multiplication when 25 to 50 mg. is injected into the allantoic sac (2). It will be recalled that this latter polysaccharide, in the quantity employed (2 mg.), showed no inhibitory activity even against very small amounts of mumps virus, which multiplies slowly.

In the light of all of these findings it seems reasonable to consider the possibility that both mumps virus and PVM require, in order that they may multiply, the presence of cellular metabolic systems which are not identical with those required for the multiplication of influenza A, influenza B, and Newcastle disease viruses. Were but a single chain of metabolic steps necessary for the multiplication of all of these agents, it would be expected that a polysaccharide which is capable of blocking multiplication of one should also prevent multiplication of the others. It appears evident that this is not the case.

The available evidence indicates that Friedländer polysaccharide does not induce its inhibitory effects as a result of direct action on mumps virus *per se*. Additional experimental evidence in support of this conclusion is presented in the accompanying paper (10). Previously it was shown that inhibition of multiplication of PVM (1) could not reasonably be explained by postulating a

direct effect of the same polysaccharide on the virus itself. It appears that with both agents the mechanisms of inhibition may be similar and dependent entirely upon metabolic alterations induced in the susceptible cells of appropriate hosts   As earlier with PVM (1), so too with mumps virus (10) evidence has been obtained which indicates that the so called virus receptor of tissues susceptible to infection is not blocked by Friedländer polysaccharide. As a consequence, the first step in the initiation of infection, that is, the establishment of intimate contact between virus particles and susceptible cells, is undoubtedly accomplished even when much polysaccharide is present. It is, apparently, some later step in the process resulting in an increase in the number of virus particles which fails to function normally if Friedländer polysaccharide is present. That this step is one which is associated with the metabolic activities of the host cell, not the virus particle, seems very probable. Because the same polysaccharides which inhibit the multiplication of mumps virus in the allantoic sac also inhibit the multiplication of PVM in the mouse lung, it appears possible that but a single mechanism is involved. It is suggested that these polysaccharides block a metabolic step in the cells of both hosts, which step is required for the multiplication of both viruses.

The results obtained following chemical treatment of polysaccharide by the procedures described provide evidence indicating that the structural configuration which endows the Fr.B polysaccharide with specific serological activity is distinct from that which brings about inhibition of the multiplication of mumps virus and PVM (1). It should be pointed out that oxidation of the carbohydrate by periodic acid alters the molecule in such a way that the linkage between the carbon atoms of the individual hexose and hexoseuronic acid units bearing adjacent hydroxyl groups is severed without destroying the glycosidic unions. The hydroxyl groups themselves are oxidized to aldehydic groups. The resulting derivative may therefore be considered as a polyaldehyde (35). That the derivative has suffered no gross degradation is evident from the fact that it does not diffuse through cellophane membranes. It is difficult to explain why drastic chemical change of the polysaccharide molecule, such as that brought about by oxidation with periodic acid, fails to impair biological activity, whereas treatment with hydroxyl ions causes complete loss of the latter function. Although the two chemical reactions are by no means comparable, the fact remains that profound alteration in chemical structure is not necessarily accompanied by a change in biological activity. That alkali impairs but slightly the serological activity of the polysaccharide, but destroys completely its biological activity with respect to mumps virus, may be due to either depolymerization of the carbohydrate, enolization of terminal aldehydic groups, or to chemical changes of those groups which have a specific affinity for the susceptible cells of the host.

It is obvious that the hypothesis presented regarding the mechanism of action of polysaccharide inhibitors with respect to these two viruses is superficially similar to present ideas on the mechanism of action of certain chemotherapeutic agents on various microbial species. There is, however, a very fundamental difference. In the latter case the active substance appears to exert its effect by affecting the metabolism of the microbe *per se*. With viruses the point of attack is one step removed and it is probably the metabolic systems of the host cell, not the infectious agent, which are affected by polysaccharide.

SUMMARY

Polysaccharides derived from type-specific Friedländer bacilli cause inhibition of the multiplication of mumps virus in the allantoic sac of the chick embryo. As little as 5 μg. of polysaccharide is effective as an inhibitor. Inhibition of multiplication is obtained when polysaccharide is injected as long as 4 days after inoculation of virus. Chemical studies have shown that the structural configurations of the polysaccharide responsible for specific serological activity are not identical with those which determine the inhibitory effect relative to mumps virus. The possible mechanisms of the inhibition of viral multiplication by means of polysaccharides are discussed.

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