CONCURRENT INFECTION WITH INFLUENZA VIRUS AND MUMPS VIRUS OR PNEUMONIA VIRUS OF MICE (PVM) AS BEARING ON THE INHIBITION OF VIRUS MULTIPLICATION BY BACTERIAL POLYSACCHARIDES

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(Received for publication, October 1, 1948)

The course of certain viral infections can be modified by the injection of an appropriate polysaccharide even though infection is well established (1, 2). Indeed, multiplication of pneumonia virus of mice (PVM) (1) or mumps virus (3) is inhibited by injection of small quantities of the capsular polysaccharide of Friedländer bacillus type B, even when the carbohydrate is given as late as 4 days after inoculation of either virus. Present evidence suggests that the polysaccharide inhibits multiplication of mumps virus or PVM by virtue of its capacity to block an intracellular metabolic system of the host which is essential for the multiplication of these viruses (3). The polysaccharide does not inactivate or demonstrably alter either virus as such, nor does the carbohydrate block the virus "receptors" of susceptible host cells; prevent adsorption of either virus by such cells (1, 4). So far as could be determined, combination between mumps virus and polysaccharide does not occur (5).

The type-specific polysaccharides of Friedländer bacilli do not inhibit multiplication of influenza A, influenza B, or Newcastle disease viruses (3). That these three viruses are capable of unrestricted multiplication in the presence of large quantities of polysaccharide, whereas multiplication of either mumps virus or PVM is inhibited by very small amounts of polysaccharide, suggested that the two groups of viruses might require different host metabolic systems for multiplication (3).

It is well established that influenza A, influenza B, and Newcastle disease viruses interfere with the multiplication of one another (6–9). If this group of viruses required metabolic systems within susceptible host cells which were not identical with those required by mumps virus and PVM, it seemed possible that neither mumps virus nor PVM would interfere with the multiplication of the viruses of the influenza-Newcastle group. It is the purpose of this paper to report the results of experiments which were carried out to test the validity of this hypothesis. A brief preliminary report has appeared elsewhere (10). Evidence will be presented to show that neither infection with PVM in the mouse nor mumps virus in the chick embryo prevents the multiplication of influenza A or influenza B virus and *vice versa*.

Materials and Methods

Viruses.—The following viruses were employed: mumps; influenza A, PR8 and FM1 strains; influenza B, Lee and B1103 strains; and PVM. For convenience these viruses will hereafter be referred to as mumps, PR8, FM1, Lee, and B1103, respectively. Except for PVM the viruses were maintained by passage in the allantoic sac of chick embryos. PVM was maintained by serial passage in the lungs of albino Swiss mice. The methods of cultivation and storage were identical to those previously described (3, 11).

The FM1 strain of influenza A virus was recovered in 1947 from a patient at Fort Monmouth, New Jersey, and was obtained from Dr. Joseph E. Smadel, The Army Medical Center. The B1103 strain of influenza B virus was recovered in 1945, and was obtained from Dr. Richard M. Taylor, International Health Division Laboratories of The Rockefeller Foundation. These strains had been passed only in the chick embryo. The other viruses employed were identified previously (3).

Virus Infectivity and Hemagglutination Titrations.—The methods employed in titrations were identical to those already described (3, 11, 12).

Immune Serum.—Rabbits were given an intravenous injection of 10 cc. of undiluted infected allantoic fluid which was followed in 2 weeks by an intraperitoneal injection of 10 cc. of similar infected fluid. Blood was withdrawn 2 weeks after the second injection, the serum was separated, and stored at 4°C. In the case of PVM rabbits were immunized by the intraperitoneal injection of 5 cc. of a suspension of infected mouse lungs.

In certain instances immune serum was absorbed with ground chick embryo tissue in order to remove antibodies against chick embryo antigens. All sera were diluted 1:2 in 0.85 per cent NaCl buffered at pH 7.2 (0.01 M phosphate) and then heated at 65.C. for 30 minutes in order to reduce non-specific inhibition of hemagglutination (5, 13).

Identification of Viruses.—

1. In Allantoic Fluid.—Following the determination of the hemagglutination titer of each allantoic fluid, the fluid was diluted so as to give final dilutions of 1:4, 1:20, 1:100, and 1:500, and the dilutions were distributed into each of three series of tubes with 0.2 cc. per tube. To one series was added 0.2 cc. of normal rabbit serum in a dilution equal to the lowest dilution of immune rabbit serum employed. To each of the other series was added 0.2 cc. of an immune serum corresponding to one of the viruses employed in the experiment. The immune serum was used in the highest dilution which completely inhibited hemagglutination of the homologous virus at a dilution of 1:4. To each tube was then added 0.4 cc. of a 1 per cent suspension of fresh chicken erythrocytes. The titrations were read after 1 hour at room temperature.

2. In Mouse Lungs.—A 10 per cent suspension of lungs from each group of 6 mice was prepared in buffered saline and divided into two aliquots: one was heated at 70°C. for 30 minutes, centrifuged at 8,000 R.P.M. for 10 minutes, and the supernate tested for the presence of PVM by the hemagglutination technique with mouse RBC (14); the other was heated at 37°C. for 3 hours, centrifuged at 8,000 R.P.M. for 10 minutes, and the supernate tested for the presence of influenza virus by the hemagglutination technique with a 0.5 per cent suspension of chicken RBC. In certain experiments identification of these viruses was confirmed by hemagglutination-inhibition titrations with homologous immune sera.

EXPERIMENTAL

Adsorption of Influenza A or Influenza B Virus by Chick Embryo Tissue Infected with the Heterologous Virus.—A number of hypotheses have been proposed concerning the mechanism of the interference phenomenon. Among

these are the following: (1) Blockade within the host cell of a "key enzyme," necessary for the multiplication of a virus (15); (2) alteration of the cell surface by the penetration of one virus so that a second virus is unable to enter (16); and (3) blockade of cell "receptors" by one virus which prevents adsorption upon them of a second virus (8). In the present study it seemed of importance to determine by as direct means as possible whether the presence of infection with one virus simply prevented the requisite first step in infection with a second virus whatever its nature, namely, combination between virus and host cell. If this were the case, it would appear that blockade of "receptors" would provide a reasonable explanation for interference. Moreover, the finding, under these circumstances, that mumps and influenza viruses or PVM and influenza viruses multiplied concurrently in the same host tissue, whereas influenza A and influenza B viruses did not, could be interpreted as due merely to initial combination with different host cell "receptors" by the different viruses. This problem has been investigated previously by Henle and his coworkers with ultraviolet-irradiated influenza virus. Employing the hemagglutination titration technique, they disclosed the fact (17) that the membrane of the allantoic sac exposed to irradiated virus could still adsorb afterwards additional influenza virus of either the homologous or heterologous type. However, in another report they showed (18) by means of infectivity titrations that adsorption on embryo tissue of influenza virus in the allantoic fluid could be prevented or interrupted by the injection of irradiated virus. In order to obtain more evidence bearing on this problem, the following experiments were carried out in the present study.

One-half cc. of undiluted allantoic fluid infected with either PR8 or Lee was inoculated into the allantoic sac of 11 or 12 day old chick embryos. In certain experiments an aliquot of the allantoic fluid was heated at 65°C. for 30 minutes, and 0.5 cc. was inoculated into control embryos. In other experiments normal allantoic fluid from 12 to 14 day old embryos was employed as a control. After incubation of the embryos at 35°C. for 3 hours 1.0 cc. of allantoic fluid was removed from each by means of a needle and syringe, and 0.5 cc. of allantoic fluid infected with the heterologous virus, either PR8 or Lee, was then injected into the allantoic sac. The fluid was flushed back and forth in the syringe to aid in mixing. Specimens of allantoic fluid were subsequently removed from each embryo after 5 minutes, and at various intervals thereafter for as long as 20 hours in certain experiments. In order to reduce the possibility of bacterial contamination, 1,000 units of crystalline G penicillin and 0.05 mg. of streptomycin per embryo were injected intra-allantoically at the beginning of each experiment. In each instance the embryos remained alive throughout the experimental period. Allantoic fluid specimens were kept at 4°C. until the termination of the experiment, and hemagglutination titrations then were carried out in the presence of a constant quantity of anti-PR8 or anti-Lee immune serum as described above.

In every experiment PR8 or Lee was adsorbed by the allantoic membrane to the same extent in embryos previously inoculated with a large amount of infectious heterologous virus as in control embryos injected with either normal

allantoic fluid or heat-inactivated virus which was not capable of causing hemagglutination. The results of a typical experiment are shown in Fig. 1. It is evident that in the presence of infection with Lee virus, PR8 was adsorbed from the allantoic fluid in a manner similar to its adsorption by the control embryo which had received normal allantoic fluid. It should be noted that multiplication of PR8 occurred in the control embryo whereas in the embryo which initially received Lee virus there was interference with the multiplication of PR8 despite the fact that it was adsorbed from the allantoic fluid. It

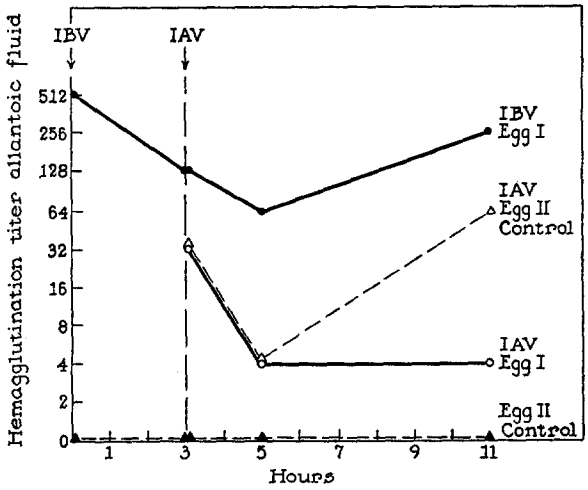

FIG. 1. Adsorption of influenza A virus (PR8) from the allantoic fluid in a chick embryo previously infected with a large quantity of influenza B virus (Lee). Egg I was given 0.5 cc. of undiluted allantoic fluid infected with Lee (IBV), and 3 hours later was given 0.5 cc. of undiluted fluid infected with PR8 (IAV). Egg II was given 0.5 cc. of normal allantoic fluid (NAF), and 3 hours later was given 0.5 cc. of undiluted fluid infected with PR8 (IAV).

appears, therefore, that allantoic membranes infected with one influenza virus and incapable of supporting multiplication of another are capable nonetheless of adsorbing the second virus in undiminished degree. In the light of these results and those obtained by Henle *et al.* (17) it appears very unlikely that interference is to be explained on the basis of blockade of host cell "receptors."

Concurrent Multiplication of Mumps Virus and Influenza A Virus in the Chick Embryo.—It has been shown (8) that influenza A and influenza B viruses prevent the multiplication of each other within definite quantitative limits of dosage and time. In order to determine whether mumps and PR8 viruses could multiply concurrently in the allantoic sac of the chick embryo, dosages and time intervals analogous to those previously employed were used in the experiments which follow.

Groups of 4 embryos, 7 to 9 days old, were inoculated intra-allantoically with from 10 to 10^4 embryo infectious doses (E.I.D.) of mumps virus. After a period of 4 days of incubation at 35°C. the embryos were reinoculated at the same site with from 1 to 10^3 E.I.D. of influenza A virus (PR8). In each experiment control groups were inoculated with either virus alone in each dilution employed, and 10 per cent normal horse serum broth was employed for reinoculation in place of the second virus. After a further incubation period of 2 days the eggs

TABLE I

Multiplication of Influenza A Virus (PR8) in the Allantoic Sac of Chick Embryos Infected with Mumps Virus

1st inoculation Intra-allantoic	Interval at 35°C.	2nd inoculation Intra-allantoic	Interval at 35°C.	Hemagglutination titer of allantoic fluids* in presence of rabbit serum‡ in a dilution of 1:16			Viruses present in allantoic fluids	
							Score§	
				Normal	Anti-mumps	Anti-PR8	Mumps	PR8
E.I.D.‖	*days*	*E.I.D.*‖	*days*					
MV¶ 10	4	Broth	2	100	0	100	3/3	—
				4	0	4		
				20	0	20		
" "	"	PR8 10	"	>500	>500	100	4/4	4/4
				>500	>500	20		
				>500	>500	20		
				>500	>500	4		
Broth	"	" "	"	>500	>500	0	—	4/4
				>500	>500	0		
				>500	>500	0		
				>500	>500	0		

* Expressed as the reciprocal of the final dilution.

‡ Serum absorbed with chicken embryo material; diluted 1:2 in saline and heated at 65°C. for 30 minutes.

§ Numerator represents the number of allantoic fluids in which virus was demonstrated to be present; denominator represents the number of embryos inoculated which survived the incubation period.

‖ E.I.D. = embryo infectious doses.

¶ MV = Mumps virus.

were chilled at 4°C., and the allantoic fluids removed individually. The hemagglutination titer of each fluid was determined, and identification of the viruses present was carried out as described above.

The results of one experiment are presented in detail in Table I for purposes of clarity. When 10 E.I.D. of PR8 was inoculated intra-allantoically into embryos 4 days after the intra-allantoic inoculation of 10 E.I.D. of mumps virus, multiplication of both viruses occurred. The hemagglutination titer of

either virus was not appreciably different from that found in control embryos. It should be pointed out that 4 days after the intra-allantoic inoculation of mumps virus in the doses employed in these experiments multiplication of virus had progressed sufficiently to be detectable by the hemagglutination technique (3), that is the virus infectivity was greater than $10^{-4.3}$, and therefore it is probable that most if not all susceptible cells in the allantoic sac were already infected.

The results of several such experiments are summarized in Table II. It will be noted that when as little as 1 E.I.D. of PR8 was inoculated into the allantoic

TABLE II

Concurrent Multiplication of Mumps Virus and Influenza A Virus (PR8) in the Allantoic Sac of the Chick Embryo

1st inoculation Intra-allantoic	Interval at 35°C.	2nd inoculation Intra-allantoic	Interval at 35°C.	Geometric mean of hemagglutination titer of allantoic fluids in presence of rabbit serum			Viruses present in allantoic fluids	
							Score	
				Normal	Anti-mumps	Anti-PR8	Mumps	PR8
E.I.D.	*days*	*E.I.D.*	*days*	*log*	*log*	*log*		
MV 10	4	PR8 1	2	−2.18	−0.95	−1.83	4/4	4/4
" "	"	" 10	"	−2.71	−2.71	−1.30	4/4	4/4
" "	"	" 10^2	"	−2.53	−2.35	−0.98	3/4	4/4
" 10^3	"	" 10	"	−2.03	−1.32	−1.52	7/8	6/8
" "	"	" 10^3	"	−2.53	−1.83	−2.00	4/4	4/4
" 10^4	"	" 10	"	−2.00	0	−2.00	4/4	0/4
" "	"	" 10^3	"	−2.00	−0.95	−1.65	4/4	4/4
" 10	"	Broth	"	−0.83	0	−1.07	4/4	—
" 10^3	"	"	"	−1.65	0	−1.65	8/8	—
" 10^4	"	"	"	−1.65	0	−1.65	8/8	—
Broth	"	PR8 1	"	−2.02	−2.02	0	—	5/6
"	"	" 10	"	−2.25	−2.25	0	—	10/12
"	"	" 10^2	"	−2.03	−2.03	0	—	3/4
"	"	" 10^3	"	−2.70	−2.70	0	—	8/8

sac as long as 4 days after the inoculation of mumps virus, multiplication of both viruses occurred. It was only when as much as 10^4 E.I.D. of mumps virus was inoculated prior to the inoculation of 10 E.I.D. of PR8 that multiplication of PR8 was not demonstrable by means of the hemagglutination technique. It should be pointed out that in embryos infected with mumps virus the titer reached by PR8 was not regularly as high as that found in control embryos. However, that multiplication of PR8 took place in embryos previously infected with mumps virus is unquestionable.

It was desirable also to determine whether PR8 and mumps virus could

multiply in the same embryo if PR8 was inoculated before mumps. In order to carry out such experiments, it was necessary to alter the procedure because intra-allantoic inoculation with PR8 resulted in death of the embryos in about 3 days. In order to circumvent this difficulty, very large amounts of each virus were employed; in this manner the entire experiment could be carried out within about 72 hours.

Approximately 10^6 E.I.D. of PR8 was inoculated into the allantoic sac of 9 to 10 day old chick embryos in groups of 6. After either 3 or 9 hours of incubation at 35°C., 10^6 E.I.D. of mumps virus was inoculated at the same site. In each experiment control groups of 6 embryos were inoculated with 10^6 E.I.D. of one or the other virus. Due to the low dilution of allantoic fluid employed as inoculum, each embryo also was given intra-allantoically 1,000 units of

TABLE III

Multiplication of Mumps Virus in the Allantoic Sac of the Chick Embryo Infected with Influenza A Virus (PR8)

1st inoculation Intra-allantoic	Interval at 35°C.	2nd inoculation Intra-allantoic	Interval at 35°C.	Geometric mean of hemagglutination titer of allantoic fluids in presence of rabbit serum			Viruses present in allantoic fluids	
							Score	
				Normal	Anti-mumps	Anti-PR8	PR8	Mumps
E.I.D.	*hrs.*	*E.I.D.*	*hrs.*	*log*	*log*	*log*		
PR8 10^6	3	Broth	60–65	−1.88	−1.53	0	6/6	—
" "	"	MV 10^6	"	−2.00	−1.18	−1.53	6/6	6/6
Broth	"	" "	"	−0.73	0	−0.73	—	5/6
PR8 10^6	9	Broth	"	−1.55	−1.60	0	12/12	—
" "	"	MV 10^6	"	−1.75	−1.48	−1.26	10/10	8/10
Broth	"	" "	"	−1.16	0	−1.22	—	9/11

crystalline G penicillin and 0.05 mg. of streptomycin to assure sterility. After a further period of incubation of 60 to 65 hours, the live embryos were chilled at 4°C., and the allantoic fluid of each removed. The identification of the viruses present was carried out as previously described above.

The results of these experiments are summarized in Table III. It is evident that when mumps virus was inoculated, as long as 9 hours after the inoculation of PR8, multiplication of both viruses occurred in the allantoic sac. It is of interest to note that in each instance the hemagglutination titer of mumps virus was of the same order of magnitude whether it multiplied in embryos infected with large amounts of PR8 or in control embryos. It has been found (18) that PR8 virus when multiplying in the allantoic membrane of the chick embryo is first released into the allantoic fluid about 6 hours after inoculation. On this basis it is probable that when mumps virus was inoculated 9 hours after inoculation with PR8 a high titer of PR8 was already present. The somewhat

lower than usual titers obtained with PR8 probably resulted from "auto-interference" which occurs commonly when very large amounts of influenza virus are employed as the inoculum (7).

In these experiments as well as in other similar experiments in which Lee virus was employed as the first inoculum, mumps virus, even though 10^6 E.I.D. was inoculated, could not be detected at 24 hours in the allantoic fluid by the hemagglutination technique. Therefore, the mumps virus demonstrable after 60 hours was present undoubtedly as a result of active multiplication.

TABLE IV

Multiplication of Influenza B Virus (Lee) in the Allantoic Sac of the Chick Embryo Infected with Mumps Virus

1st inoculation Intra-allantoic	Interval at 35°C.	2nd inoculation Intra-allantoic	Interval at 35°C.	Geometric mean of hemagglutination titer of allantoic fluids in presence of rabbit serum			Viruses present in allantoic fluids	
							Score	
				Normal	Anti-mumps	Anti-Lee	Mumps	Lee
E.I.D.	*days*	*E.I.D.*	*days*	*log*	*log*	*log*		
MV 10	4	Lee 10	2	−2.10	−0.90	−1.42	4/6	4/6
" "	"	" 10^2	"	−2.47	−2.23	−1.53	6/6	6/6
" 10^4	"	" 10	"	−1.96	−0.41	−1.73	6/7	2/7
" "	"	" 10^2	"	−1.86	−0.66	−1.60	10/10	5/10
" "	"	" 10^3	"	−2.18	−0.93	−1.78	11/12	7/12
" "	"	" 10^4	"	−2.53	−2.27	−1.65	5/5	5/5
" 10	"	Broth	"	−1.75	0	−1.40	7/8	—
" 10^4	"	"	"	−1.58	0	−1.48	12/13	—
Broth	"	Lee 10	"	−2.38	−2.38	0	—	15/17
"	"	" 10^2	"	−2.70	−2.70	0	—	18/18
"	"	" 10^3	"	−2.70	−2.70	0	—	12/12
"	"	" 10^4	"	−2.70	−2.70	0	—	8/8

Concurrent Multiplication of Mumps Virus and Influenza B Virus in the Chick Embryo.—It was of interest also to determine whether the viruses of influenza B and mumps could multiply simultaneously in the allantoic sac of the chick embryo.

These experiments were carried out in a manner identical to that described above. Ten to 10^4 E.I.D. of mumps and Lee viruses was employed, the former being the first virus inoculated.

The results of several experiments are summarized in Table IV. In these experiments, as in those in which PR8 was employed as the second inoculum, there was evidence of multiplication of both viruses in the allantoic sac.

Indeed, when either 10 or 10^4 E.I.D. of both viruses was inoculated, multiplication of each agent occurred despite the fact that mumps virus had been increasing in concentration for 4 days before Lee virus was inoculated. Even when as little as 10 E.I.D. of Lee was inoculated after inoculation of 10^4 E.I.D. of mumps virus, there was evidence of multiplication of both viruses in 2 of 7 embryos. In most instances the hemagglutination titer of Lee virus in doubly infected embryos was significantly lower than that found in control embryos.

To demonstrate concomitant multiplication of mumps and Lee viruses when the latter agent was used as the first inoculum, it was again necessary to alter the experimental procedure because of the lethal effect of Lee virus on chick embryos. These experiments were carried out in an identical manner to those

TABLE V

Multiplication of Mumps Virus in the Allantoic Sac of the Chick Embryo Infected with Influenza B Virus (Lee)

1st inoculation Intra-allantoic	Interval at 35°C.	2nd inoculation Intra-allantoic	Interval at 35°C.	Geometric mean of hemagglutination titer of allantoic fluids in presence of rabbit serum			Viruses present in allantoic fluids, Score	
				Normal	Anti-mumps	Anti-Lee	Lee	Mumps
E.I.D.	*hrs.*	*E.I.D.*	*hrs.*	*log*	*log*	*log*		
Lee 10^6	3	Broth	60	−2.21	−2.21	0	10/10	—
" "	"	MV 10^6	"	−2.18	−2.00	−0.53	8/8	7/8
Broth	"	" "	"	−1.19	0	−1.19	—	11/12
Lee 10^6	9	Broth	"	−1.51	−1.42	0	7/7	—
" "	"	MV 10^6	"	−2.00	−2.00	−0.40	4/4	2/4
Broth	"	" "	"	−1.09	0	−1.15	—	9/11

described above in which PR8 virus was inoculated before mumps virus. In the large dose employed, Lee virus proved to be very toxic for chick embryos; many died after 48 hours of incubation, and those which survived the 72 hours' experimental period were moribund.

A summary of the results obtained is presented in Table V. It is seen that an increase in the concentration of both viruses occurred. It will be noted that in most instances the titer of the second virus inoculated (*i.e.*, mumps) was significantly lower than in the controls. It seems probable that this decrease in the multiplication of mumps virus may, to some extent, have been due to the severity of the infection induced in the embryo by Lee virus.

Concurrent Multiplication of PVM and Influenza Viruses in the Mouse Lung.— Earlier work (7, 19) has shown that influenza A and influenza B viruses mutually interfere in the mouse lung; under appropriate conditions the intranasal inocula-

tion of one virus prevents the multiplication of the second. If the hypothesis proposed above were valid, then PVM and influenza viruses should not interfere with the multiplication of each other in the lung of the mouse.

PVM and Influenza A Virus.—

Groups of mice were inoculated intranasally with 10^2 M.S.50 of PVM. After an interval of 4 days the mice were again inoculated intranasally with varying quantities of PR8. In each experiment control groups of mice received an intranasal inoculation of either PVM or an appropriate dilution of PR8, and were given 10 per cent normal horse serum broth in lieu of the second viral inoculum. After an additional interval of 2 days the mice were killed, the

TABLE VI

Multiplication of Influenza A Virus (PR8) in the Mouse Lung Infected with Pneumonia Virus of Mice (PVM)

1st inoculation Intranasal	Interval	2nd inoculation Intranasal	Interval	Hemagglutination titer* of mouse lung suspension‡	
				Heated at 70°C. for 30 min.	Heated at 37°C. for 180 min.
				vs. 0.8 per cent mouse RBC (PVM)	*vs.* 0.5 per cent chicken RBC (PR8)
M.S.50§	*days*	*M.S.50*§	*days*		
PVM 10^2	4	Broth	2	128	0
" "	"	PR8 10	"	128	0
Broth	"	" "	"	0	16
PVM 10^2	"	" 10^2	"	128	32
Broth	"	" "	"	0	256
PVM 10^2	"	" 10^3	"	64	128
Broth	"	" "	"	0	256

* Expressed as the reciprocal of the hemagglutination titer.

‡ 10 per cent suspension in saline.

§ M.S.50 = 50 per cent maximum score.

lungs of each group were pooled, and 10 per cent suspensions were made in 0.85 per cent saline buffered at pH 7.2 (0.01 M phosphate). The viruses present in the lung suspensions were identified as described above.

The results of a typical experiment are presented in Table VI. When 10^2 or 10^3 M.S.50 of PR8 was inoculated intranasally into mice 4 days after the intranasal inoculation of 10^2 M.S.50 of PVM, multiplication of PR8 occurred despite the preexisting infection with PVM. It was only when 10 M.S.50 of PR8 was employed that multiplication of this virus in mice infected with PVM could not be demonstrated by means of the hemagglutination technique. It will be noted that in these experiments, as in certain of those described above, the titer of the second virus inoculated was lower than that in control mice.

Mice infected with PR8 died 3 to 4 days after inoculation when doses which

were useful for these experiments were employed. Therefore, in order that influenza A virus might be used as the initial inoculum, it was necessary to employ a strain which had not been "adapted" to the mouse lung. It has been shown recently (20) that such strains, even though unable to cause pulmonary lesions, are capable of active multiplication in the mouse lung.

The FM1 strain of influenza A virus which had been maintained by chick embryo passage was employed. A 0.05 cc. volume of a 10^{-2} dilution of allantoic fluid infected with this virus was inoculated intranasally into groups of 6 mice, and followed in 2 days by 10^2 M.S.50 of PVM. Suitable controls for each virus were included. After an additional interval of 6 days the mice were killed, and 10 per cent lung suspensions in saline were prepared. The mouse lung suspensions were each divided into three aliquots, two of which were used to determine the hemagglutination titers of PVM and FM1, respectively, as described above. It was soon found that FM1 did not multiply sufficiently to give positive hemagglutination reactions, and therefore the titer of this virus was determined with the third aliquot by carrying out infectivity titrations in the allantoic sac of the chick embryo. To insure sterility 1,000 units of penicillin and 0.05 mg. of streptomycin were given per embryo.

The results of a typical experiment are shown in Table VII. When FM1 was employed as the first inoculum, PVM, although inoculated 2 days later, was still capable of multiplication in the mouse lung. It should be noted, however, that the titer of the second virus inoculated, *i.e.* PVM, was lower than that obtained in the lungs of control mice.

PVM and Influenza B Virus.—Experiments identical to those described above were carried out in mice with PVM and Lee viruses. Table VIII shows the results of an experiment in which both PVM and Lee multiplied in the mouse lung even though no more than 10 M.S.50 of Lee virus was inoculated 4 days after inoculation of PVM.

Due to the fact that Lee virus, when inoculated intranasally, caused death of mice in 3 to 4 days, it was necessary to employ the B1103 strain in order that influenza B virus might be used as the first inoculum. These experiments were carried out in a manner identical to those described above in which FM1 and PVM were employed. As is shown in Table IX, when the B1103 strain which had not been "adapted" to the mouse lung was inoculated 2 days before inoculation of PVM, the latter virus was still capable of multiplication in the mouse lung.

Interference between Unadapted and Adapted Influenza Viruses in the Mouse Lung.—In light of the results described above it was desirable to determine whether strains of influenza virus which were unable to cause pulmonary lesions in the mouse could interfere with the multiplication of mouse-"adapted" strains.

Dilutions of 10^{-2} of allantoic fluid infected with FM1 or B1103 were inoculated intranasally in groups of 6 mice. After an interval of 24 hours 10^2 M.S.50 of Lee or PR8 was inoculated by the same route. Following an additional interval of 2 days the mice were killed, a 10 per

cent suspension of the lungs from each group was prepared in saline, and divided into two portions. One aliquot was employed for hemagglutination titrations of the adapted influ-

TABLE VII

Multiplication of Pneumonia Virus of Mice (PVM) in the Mouse Lung Infected with Influenza A Virus (FM1)

1st inoculation Intranasal	Interval	2nd inoculation Intranasal	Interval	Mouse lung suspension: Hemagglutination titer: Heated at 70°C. for 30 min. vs. 0.85 per cent mouse RBC (PVM)	Mouse lung suspension: Hemagglutination titer: Heated at 37°C. for 180 min. vs. 0.5 per cent chicken RBC (FM1)	Mouse lung suspension: Embryo infectivity titer (FM1): Dilution mouse lungs inoculated intra-allantoically*	Mouse lung suspension: Embryo infectivity titer (FM1): Allantoic fluids producing hemagglutination‡
dilution	*days*	*M.S.50*	*days*			*0.1 cc.*	
FM1 10^{-2}	2	Broth	6	0	0	10^{-2}	3/3
						10^{-4}	3/3
						10^{-6}	0/3
" "	"	PVM 10^{2}	"	16	0	10^{-2}	3/3
						10^{-4}	2/3
						10^{-6}	0/3
Broth	"	" "	"	512	0		

* Embryos incubated at 35°C. for 40 to 48 hours.

‡ Numerator represents number of allantoic fluids producing hemagglutination; denominator denotes the number of embryos inoculated which survived the experimental period.

TABLE VIII

Multiplication of Influenza B Virus (Lee) in the Mouse Lung Infected with Pneumonia Virus of Mice (PVM)

1st inoculation Intranasal	Interval	2nd inoculation Intranasal	Interval	Hemagglutination titer of mouse lung suspension: Heated at 70°C. for 30 min. vs. 0.85 per cent mouse RBC (PVM)	Hemagglutination titer of mouse lung suspension: Heated at 37°C. for 180 min. vs. 0.5 per cent chicken RBC (Lee)
M.S.50	*days*	*M.S.50*	*days*		
PVM 10^{2}	4	Broth	2	256	0
" "	"	Lee 10	"	256	4
Broth	"	" "	"	0	32

enza virus, and the other was used to determine the infectivity titer in chick embryos of either FM1 or B1103. It was essential to carry out the infectivity titrations in the presence of immune serum against the adapted virus, *e.g.* when a titration of FM1 was carried out,

TABLE IX

Multiplication of Pneumonia Virus in Mice (PVM) in the Mouse Lung Infected with Influenza B Virus (B1103)

1st inoculation Intranasal	Interval	2nd inoculation Intranasal	Interval	Mouse lung suspension	
				Embryo infectivity titer (B1103)	Hemagglutination titer
					Heated at 70°C. for 30 min.
					vs. 0.85 per cent mouse RBC (PVM)
dilution	*days*	*M.S.50*	*days*		
B1103 10^{-2}	2	Broth	6	$10^{-2.3}$	0
" "	"	PVM 10^{2}	"	$>10^{-3.0}$	32
Broth	"	" "	"	—	64

TABLE X

Interference with the Multiplication of Influenza B Virus (Lee) in the Mouse Lung Infected with an Unadapted Strain of Influenza A Virus (FM1)

1st inoculation Intranasal	Interval	2nd inoculation Intranasal	Interval	Mouse lung suspension*		
				Hemagglutination titer	Embryo infectivity titer (FM1)	
				Heated at 37°C. for 180 min.	Dilution suspension inoculated intra-allantoic + anti-Lee serum‡	Allantoic fluids producing hemagglutination
				vs. 0.5 per cent chicken RBC (Lee)		
dilution	*days*	*M.S.50*	*days*		*0.1 cc./embryo*	
FM1 10^{-2}	1	Broth	2	0	10^{-2}	3/4
					10^{-3}	4/4
					10^{-4}	4/4
					10^{-5}	4/4
FM1 10^{-2}	"	Lee 10^{2}	"	0	10^{-2}	4/4
					10^{-3}	4/4
					10^{-4}	4/4
					10^{-5}	4/4
Broth	"	" "	"	128	—	—

* 10 per cent suspension in saline.

‡ Equal volume of anti-Lee immune serum diluted 1:2 and heated at 65°C. for 30 minutes

each embryo was injected with 0.1 cc. of a 1:2 dilution of anti-Lee serum which had been heated at 65°C. for 30 minutes. In each instance identification of the virus was performed as described above.

The results of one such experiment are presented in Table X. It will be seen that no Lee virus demonstrable by the hemagglutination technique was present in the lungs of the mice previously infected with FM1, despite the fact that the FM1 strain caused no pulmonary lesions or other manifest evidence of infection. Similar results were obtained when B1103 was inoculated and followed in 24 hours by the inoculation of PR8. It is of considerable interest that strains of influenza virus which cause neither demonstrable lesions nor illness in the host are still able to interfere with the multiplication of other influenza virus strains which possess both of these properties.

DISCUSSION

The available information concerning the interference phenomenon with viruses supports the idea that intracellular metabolic systems of the host, not merely cell surface receptors, are the major factors upon which the phenomenon depends (6, 15, 17). Additional support for this concept is provided by the results obtained in adsorption experiments with influenza viruses in the present study. It is well established that numerous pairs of viruses which may or may not be related to one another show the interference phenomenon. Also, it is well known that many other pairs of viruses do not show the phenomenon; indeed, it has been demonstrated that two or more viruses may infect a single cell (21).

The present study was not undertaken with a view to add further examples to the long list of viruses which have been investigated with respect to interference. Its purpose was to determine whether the effects of type-specific Friedländer bacillus polysaccharides on the multiplication of different viruses were correlated with the capacities of the same viruses to cause interference with each other. It appears that such a correlation has been demonstrated by the results obtained.

For a number of reasons it is probable that the different viruses employed multiply in the same types of cells if not in identical cells. Both PVM and the influenza viruses are strictly pneumotropic in the mouse, and induce manifest infection of the lung only when inoculated into the respiratory passages. Moreover, the pathological alterations which result from pulmonary infections with PVM or the influenza viruses are so similar as to be indistinguishable. Both mumps virus and the influenza viruses multiply in the allantoic sac of the chick embryo, presumably in the cells lining this structure, and thereafter are released into the allantoic fluid.

It is unfortunate that all attempts to obtain multiplication of PVM in the chick embryo (11, 22) or mumps virus in the mouse lung (5) have been entirely unsuccessful. Because of this it was not possible to carry out experiments to determine whether PVM and mumps virus could reciprocally interfere with the multiplication of each other in either host. In terms of the hypothesis proposed above it is to be expected that they would.

There is no information available as to the nature of the metabolic systems which animal viruses require in the host cells in which they multiply. That such metabolic requirements may not be identical even for viruses which multiply in the same host cells is suggested by the results of the present experiments. Similarly, the fact that a few micrograms of capsular polysaccharide derived from Friedländer bacilli inhibits multiplication of either mumps virus (3) or PVM (1), whereas a large amount of the same polysaccharide does not influence the multiplication of the viruses of influenza A, influenza B, and Newcastle disease, suggests that these two groups of viruses require the presence of different factors within the host cells in which they multiply, as has been indicated previously (3). It appears likely that the polysaccharide may compete for a metabolic system or substance which both mumps virus and PVM require in order that they may multiply, and also that those viruses which do not require this system or substance multiply in an uninhibited manner in the presence of the same polysaccharide. That multiplication of the viruses of influenza A, influenza B, and Newcastle disease is dependent upon intracellular host factors different from those required by PVM or mumps virus seems a probable explanation for the fact that the multiplication of the latter group of viruses is inhibited by the capsular polysaccharides of Friedländer bacilli whereas the multiplication of the former group is unaffected by the same carbohydrates.

SUMMARY

Preexisting infection with PVM or mumps virus does not prevent multiplication of the virus of influenza A or B in the same tissue. Similarly, preexisting infection with one or another of the influenza viruses does not prevent multiplication of either PVM or mumps virus in the same tissue. The failure of these two groups of viruses to interfere with the multiplication of each other is discussed in relation to the mechanism of inhibition of multiplication of mumps virus and PVM by the capsular polysaccharides of Friedländer bacilli, and the ability of influenza A and influenza B viruses to multiply in the presence of large quantities of these carbohydrates. It is suggested that differences in the requirements of the two groups of viruses for host cell metabolic systems may serve to explain both the lack of interference between them and the differing effect of polysaccharides upon their multiplication.

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