A RESISTANT VARIANT OF MUMPS VIRUS

MULTIPLICATION OF THE VARIANT IN THE PRESENCE OF INHIBITORY QUANTITIES OF FRIEDLÄNDER BACILLUS POLYSACCHARIDE

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Evidence obtained in previous studies (1–3) on the inhibition of multiplication of mumps virus by the capsular polysaccharides of Friedländer bacilli raised the possibility that the individual viral particles are not all entirely identical and suggested that some might differ from others as to mechanism of multiplication. The following findings are pertinent: When small quantities of virus (*i.e.*, 10^2 E.I.D.) were inoculated, the injection of polysaccharide caused quantitative inhibition in multiplication which was reflected in the production of only 0.5 to 10 per cent of the maximum concentration of virus but did not prevent completely the multiplication of the agent. Even when relatively large amounts of polysaccharide (*i.e.*, 1.0 mg. per embryo) were injected, slight multiplication of the virus occurred although the degree of inhibition was striking. When large quantities of virus (*i.e.*, 10^5 E.I.D.) were given, the injection of polysaccharide did not result in any significant inhibition of multiplication (2). The range of the variables studied was sufficiently wide, and the results reproducible enough to make it appear improbable that quantitative factors alone were to be held responsible for the findings. More probable seemed the possibility that qualitative variables were operative, and the idea arose that mumps virus preparations contained, in addition to the predominant typical viral particles, a numerically small proportion of variant particles which, unlike the typical virus, were capable of unrestricted multiplication in the presence of the polysaccharide. It seemed likely that, should such variant viral particles be present, they could be revealed readily because of the availability of a chemical agent which would preferentially select them from the viral populations under study.

The hypothesis was subjected to experimental test. The results obtained appear to indicate clearly that a variant is present in mumps virus preparations; that the variant is capable of unrestricted multiplication in the presence of large amounts of polysaccharide; that the variant is, as to other properties tested, indistinguishable from the original strain; that the only means at hand for demonstrating the presence of the variant is polysaccharide which is active as an inhibitor of mumps virus multiplication.

Materials and Methods

Viruses.—The following viruses were employed: mumps (Habel strain); influenza A, PR8 strain; influenza B, Lee strain. For convenience, they will be referred to hereafter as mumps, PR8, and Lee. They were cultivated in the allantoic sac of chick embryos which were 7 to 9 days of age for mumps and 9 to 11 days of age for PR8 or Lee. The methods of cultivation and the procedures for identification were identical to those previously described (3). Between passages each of the viruses was stored in a solid carbon dioxide cabinet at −70°C. In certain instances mumps virus during serial passages was stored for short periods in an electrically operated refrigerator at −30°C. As reported recently (4) the virus can be maintained without reduction in infectivity for at least 3 months at the latter temperature.

Virus Infectivity and Hemagglutination Titrations.—The methods employed were identical to those described in earlier reports from this laboratory (2). Infectivity titrations were carried out with serial tenfold dilutions which were inoculated into the allantoic sac of chick embryos. Hemagglutination titrations were carried out with serial twofold dilutions which were mixed with an equal volume of 1.0 per cent chicken RBC.

Polysaccharide Preparations.—The capsular polysaccharides of Friedländer bacillus type A (Fr.A), type B (Fr.B), and type C (Fr.C), respectively, (5, 6) were employed.[1] Polysaccharide solutions were prepared in 0.85 per cent sodium chloride solution buffered at pH 7.2 (0.05 M phosphate). Each solution was heated at 70°C. for 30 minutes before use.

Immune Serum.—Rabbits were injected intravenously with 10 cc. of undiluted infected allantoic fluid, and this was followed at 2 week intervals by two intraperitoneal injections of 10 cc. of similarly infected fluid. Two weeks after the last injection blood was withdrawn, the serum separated, and stored at 4°C. The immune serum was absorbed with normal embryo tissue and inactivated by heating at 65°C. as previously described (3).

Reproducibility of End Points.—In an earlier paper (2) the reproducibility of hemagglutination titration end points with mumps virus was determined in 33 groups of control infected embryos. In the present study similar computations were carried out with end points obtained in an additional series of 38 groups of control infected embryos. The standard deviation of the distribution of end points was 0.331 log unit (2.1-fold), a value slightly lower than that previously obtained. Thus, a difference of 0.94 log unit (8.7-fold) between the end points found in any two groups should occur by chance only once in 20 times (7). As in previous studies (1, 2) the arithmetic mean of the individual allantoic fluid end points was employed in the present investigation. Comparative computations with the available data indicate that the use of the geometric mean does not yield any statistical advantage, and does not significantly alter the probability values.

EXPERIMENTAL

Demonstration of a Variant of Mumps Virus by Serial Passage in the Presence of the Capsular Polysaccharide of Friedländer Bacillus Type B.—Earlier studies (1, 2) revealed that, despite the marked inhibition of multiplication of mumps virus which was caused by the capsular polysaccharides of Friedländer bacilli, multiplication was not prevented completely. It was also demonstrated that when very large inocula of virus were employed no significant inhibition of multiplication was demonstrable. As an explanation of these findings, it seemed probable that the multiplication observed might be initiated by viral particles

[1] The polysaccharides were obtained through the kindness of Dr. Walther F. Goebel, The Rockefeller Institute for Medical Research.

which differed from the predominant particles comprising the viral population under study. In an attempt to demonstrate the presence of a variant, resistant to the inhibitory activity of Friedländer type B polysaccharide (Fr. B), mumps virus was subjected to serial passage in the allantoic sac in the presence of small but inhibitory quantities of Fr.B. After each successive passage the capacity of the strain to multiply in the presence of Fr.B was determined.

Approximately 10^2 embryo infectious doses (E.I.D.) of mumps virus was inoculated intra-allantoically into two groups of 9 day chick embryos. After 1 hour one group was given Fr.B intra-allantoically, 0.1 mg. per embryo, and the embryos of the other group received 0.1 cc. of saline. After incubation at 35°C. for 6 days, the eggs were chilled at 4°C. overnight, the allantoic fluids removed, and their hemagglutination titers determined. The allantoic fluid with the highest titer from the group which had received Fr.B was used as inoculum for the next serial passage. It was diluted 10^{-5} in 10 per cent normal horse serum broth, and inoculated intra-allantoically in groups of embryos which were treated as above. From the control groups an allantoic fluid was selected which had a hemagglutination titer similar to that of the fluid selected from the polysaccharide-treated group. This fluid, diluted 10^{-5}, was used as inoculum for the next serial passage in the control line. In subsequent passages 0.05 mg. of Fr.B per embryo was employed. After serial passage 1.0 mg. of Fr.B per embryo was employed to determine the degree of polysaccharide resistance of the two passage strains. In most instances during serial passage 500 units of crystalline penicillin G and 5 mg. of streptomycin per embryo were injected along with the inoculum in order to insure sterility.

The results of significant portions of one such experiment are presented in Table I. It will be noted that following but two passages of mumps virus in the presence of relatively small quantities of polysaccharide, the multiplication of the virus was not significantly inhibited by Fr.B. After 10 passages in the presence of Fr.B quantities of the carbohydrate as large as 1.0 mg. per embryo did not prevent this strain from multiplying in an unaltered manner. In addition, it was found that equally large quantities of capsular polysaccharide from type A or type C Friedländer bacilli did not diminish the multiplication of this resistant strain. As is evident, marked inhibition of multiplication was demonstrable at every step in the control series with mumps virus which was passed in parallel, but in the absence of the polysaccharide.

In another experiment carried out in an identical manner, 10 passages in the presence of Fr.B were required before a stable resistant strain of mumps virus was obtained. The initial virus suspension which was used in the latter experiment was different from that employed in the experiment described above.

As is indicated below, resistant variants, once obtained, retained their capacity to multiply in unrestricted manner in the presence of polysaccharide after as many as 3 to 4 serial passages in the absence of the substance.

Demonstration of a Resistant Variant of Mumps Virus after a Single Passage in the Presence of Fr.B.—When it was found that a resistant variant could be obtained at will upon serial passage of mumps virus in the presence of Fr.B, attempts were made to obtain similar variants in a single passage. If, as seemed probable, the variant virus was present in a low concentration in suspensions

TABLE I

Demonstration of a Resistant Variant of Mumps Virus by Serial Passage in the Presence of the Capsular Polysaccharide of Friedländer Bacillus, Type B

Serial passage of mumps virus				Mean hemagglutination titer* of allantoic fluids‡ of embryos inoculated with virus and injected with			Difference in titers of NaCl and Fr.B groups	
In presence of		No. of passages	Dilution employed	NaCl§	Fr.B§			
	mg./embryo				*mg./embryo*		*fold*	*log*
—	—	Parent strain	10^{-5}	336	0.10	16	−21×	−1.33
Fr.B	0.10	1	"	597	0.05	48	−12×	−1.10
"	0.05	2	"	264	"	232	0	−0.05
"	"	4	"	853	"	544	"	−0.19
"	"	10	"	240	"	299	"	+0.10
"	"	"	"	"	1.00	213	"	−0.05
NaCl		1	"	64	0.05	3	−23×	−1.36
"		2	"	256	"	24	−12×	−1.10
"		4	"	469	"	42	−11×	−1.06
"		10	"	95	"	12	− 8×	−0.90
"		"	"	"	1.00	0	−95×	−1.98

* Expressed as the reciprocal of the hemagglutination titer.

‡ Allantoic fluids were harvested 6 days after inoculation.

§ Injection of NaCl or Fr.B was given 1 hour after the virus.

TABLE II

Demonstration of a Resistant Variant of Mumps Virus in a Single Passage in the Presence of the Capsular Polysaccharide of Friedländer Bacillus, Type B

Serial passage of mumps virus				Mean hemagglutination titer of allantoic fluids* of embryos inoculated with virus and injected with			Difference in titers of NaCl and Fr.B groups	
In presence of		No. of passages	Dilution employed	NaCl‡	Fr.B‡			
	mg./embryo				*mg./embryo*		*fold*	*log*
—		Parent strain	10^{-1}	420	1.00	384	0	−0.04
Fr.B	1.00	1	10^{-5}	128	0.05	65	−2×	−0.30
"	0.05	2	"	152	1.00	128	0	−0.07
NaCl		1	"	129	0.05	4	−33×	−1.52
—		Parent strain	"	512	0.05	"	−128×	−2.11

* Allantoic fluids were harvested 6 days after inoculation.

‡ Injection of NaCl or Fr.B was given 1 hour after virus.

of the parent strain, it should be possible by means of the inhibiting polysaccharide to demonstrate its presence in a single cycle of multiplication.

A large quantity of mumps virus, *i.e.* 10^6 E.I.D., was inoculated into the allantoic sac of each of two groups of embryos. After an interval of 1 hour one group was injected intra-allantoically with 1.0 mg. of Fr.B per embryo, whereas the other group was given 0.1 cc. saline. After incubation at 35°C. for 6 days, the allantoic fluids were removed separately and their hemagglutination titers determined. Two passages were carried out in a manner identical to that described above.

The results obtained are presented in Table II. It will be seen that the virus which was present after a single passage in the presence of Fr.B was capable of multiplication which was not inhibited by the polysaccharide. The variant strain obtained under these conditions in a single passage was found to be relatively stable, and retained its capacity to multiply in unrestricted fashion in the presence of Fr.B even after serial passage in the absence of the polysaccharide. It should be pointed out that a resistant variant

TABLE III

Effect of Capsular Polysaccharide of Friedländer Bacillus, Type B, on Multiplication of Large Inocula of Mumps Virus

1st injection Intra-allantoic	Interval	2nd injection Intra-allantoic	Interval at 35°C.	Mean hemagglutination titer of allantoic fluids	Difference in titers of NaCl and Fr.B groups	
*E.I.D.**	*hrs.*		*days*		*fold*	*log*
MV 10^6	3	Saline	3	162		
" "	"	Fr.B 1.0 mg./embryo	"	16	−10×	−1.01
" "	"	Saline	6	213		
" "	"	Fr.B 1.0 mg./embryo	"	192	0	−0.05

* E.I.D. = embryo infectious doses.

was obtained also from the Enders strain[2] of mumps virus in a single passage by means of an identical experimental procedure. Thus it appears that when an inoculum containing a large quantity of mumps virus is employed, there is present in it a sufficient number of variant particles so that they become clearly demonstrable during a single cycle of multiplication. Additional evidence in support of this view is shown in Table III. It will be observed that during the first 3 days after inoculation the virus multiplied less readily in the presence of polysaccharide, and reached a concentration equivalent to only 10 per cent of that found in control embryos. At 6 days, however, there was no difference in the titers. Because of the limitations of available techniques, it is not possible to estimate with any reliability the probable concentration of variant virus particles in the parent strain. However, without making unwarranted assumptions, it appears that a ratio between variant and typical viral particles of the order of $1:10^4$ or lower would conform with the available data. These re-

[2] Received through the courtesy of Dr. John F. Enders, Children's Hospital, Boston.

sults provide an adequate explanation for the earlier finding (2) that quantitative inhibition of multiplication was demonstrable 6 days after inoculation with 10^4 E.I.D. but not with 10^5 E.I.D. of mumps virus.

Disappearance of Variant on Serial Passage in the Absence of Polysaccharide.— It was of interest to determine whether (*a*) the resistant variant would retain its capacity to multiply in the presence of Fr.B after serial passage, or (*b*) upon several passages of the variant in the absence of Fr.B. it would disappear, and a strain of virus reappear which would be inhibited by polysaccharide.

TABLE IV

Reappearance of Sensitive Strain on Serial Passage of Resistant Variant of Mumps Virus in the Absence of Fr.B

Serial passage of mumps virus				Mean hemagglutination titer of allantoic fluids* of embryos inoculated with virus and injected with			Difference in titers of NaCl and Fr.B groups	
Strain	In presence of	No. of passages	Dilution employed	NaCl‡	Fr.B‡			
					mg./embryo		*fold*	*log*
Parent	—	—	10^{-5}	74	0.05	1	−74×	−1.87
Resistant variant§	Fr.B	13	"	704	1.00	672	0	−0.02
" "	NaCl	1	"	193	0.05	85	−2×	−0.36
" "	"	"	"	"	1.00	40	−5×	−0.69
" "	"	4	"	384	0.05	144	−3×	−0.42
" "	"	"	"	"	1.00	72	−5×	−0.72
" "	"	5	"	1024	0.05	25	−41×	−1.61
" "	"	"	"	"	1.00	18	−63×	−1.80
Parent	—	—	"	339	0.05	16	−21×	−1.33

* Allantoic fluids were harvested 6 days after inoculation.

‡ Injection of NaCl or Fr.B was given 1 hour after the virus.

§ Resistant variant employed after 13 serial passages in presence of Fr.B.

Approximately 10^2 E.I.D. of resistant variant strains, obtained as described above, was inoculated intra-allantoically in groups of embryos. Each embryo received 0.1 cc. of saline intra-allantoically 1 hour after inoculation. Serial passage was carried out with allantoic fluid obtained after 6 days' incubation. The procedure was identical to that employed in the selection of the variant virus except that polysaccharide was not used in the passage series. The individual allantoic fluid with the highest titer was employed as inoculum at each passage, and was diluted 10^{-5}. The ability of the virus to multiply in the presence of 0.05 and 1.0 mg., respectively, of Fr.B was determined after each passage.

The pertinent data from one such experiment are presented in Table IV. The variant virus strain employed was that derived from the passage series

recorded in Table I. This experiment was carried out after the virus had been through 13 serial passages in the presence of polysaccharide. It is evident that after 4 passages in the absence of Fr.B the strain was still resistant to the inhibitory effect of the polysaccharide; significant inhibition of multiplication was not obtained even when as much as 1.0 mg. of Fr.B per embryo was employed. However, after 5 passages in the absence of the carbohydrate, multiplication of the virus was markedly inhibited when as little as 0.05 mg. of Fr.B was used. Further passages showed that this strain remained sensitive to the inhibitory effects of Fr.B. In this respect it was indistinguishable from the parent strain.

The variant strain which had been selected in a single passage in the presence of Fr.B, as shown in Table II, regained sensitivity to the inhibitory effect of the polysaccharide somewhat more rapidly. After 3 serial passages in the absence of Fr.B the multiplication of this strain was inhibited by 0.05 mg. of Fr.B. It is of interest that in each instance the reappearance of sensitivity to the effect of the polysaccharide was as readily demonstrable with 0.05 mg. as with 1.0 mg. of Fr.B.

Rate of Multiplication of Mumps Virus and Its Resistant Variant.—In an earlier study (2) it was shown that mumps virus did not reach maximum concentration in the allantoic fluid until 6 days after the inoculation of 10^2 E.I.D. It appeared important to determine, as precisely as possible, the rate of multiplication of the virus in the allantoic sac, and to compare this with the rate of multiplication of the resistant variant derived from the parent strain. Experiments were carried out simultaneously with the parent and variant strains. With both strains very large, as well as relatively small, inocula were employed. It was expected that when large inocula were given a large number of cells would be infected almost simultaneously, and that this would result in a sharp increase in viral concentration following the so called latent period. In this manner, it was anticipated that information might be gained as to the approximate duration of the period required for the multiplication of mumps virus and the resistant variant in individual cells of the allantoic sac.

Two large groups of 8 day embryos were inoculated intra-allantoically with approximately 10^7 E.I.D. of virus; one group was given the parent strain, the other received the variant strain. After incubation at 35°C. for 24 hours, the hemagglutination titers of the allantoic fluids from 5 or 6 embryos of each group were determined. Similar titrations were carried out with groups of allantoic fluids removed at frequent intervals thereafter. In other experiments 10^2 E.I.D. of either the parent strain or the variant strain was inoculated intra-allantoically in a large group of embryos. After 1 hour the embryos which had received the resistant variant were given 0.05 mg. of Fr.B while those which had received the parent strain were given 0.1 cc. of saline. After 3 days' incubation at 35°C., the hemagglutination titers of the allantoic fluids from 5 embryos of each group were determined. Thereafter, at 24 hour intervals, similar titrations were carried out with groups of allantoic fluids. Two identical experiments were carried out, and the geometric means of the titers obtained at each time interval were computed.

The results of these experiments are shown in Fig. 1. It will be seen that when 10^7 E.I.D. of either the parent strain or the variant was inoculated, the virus

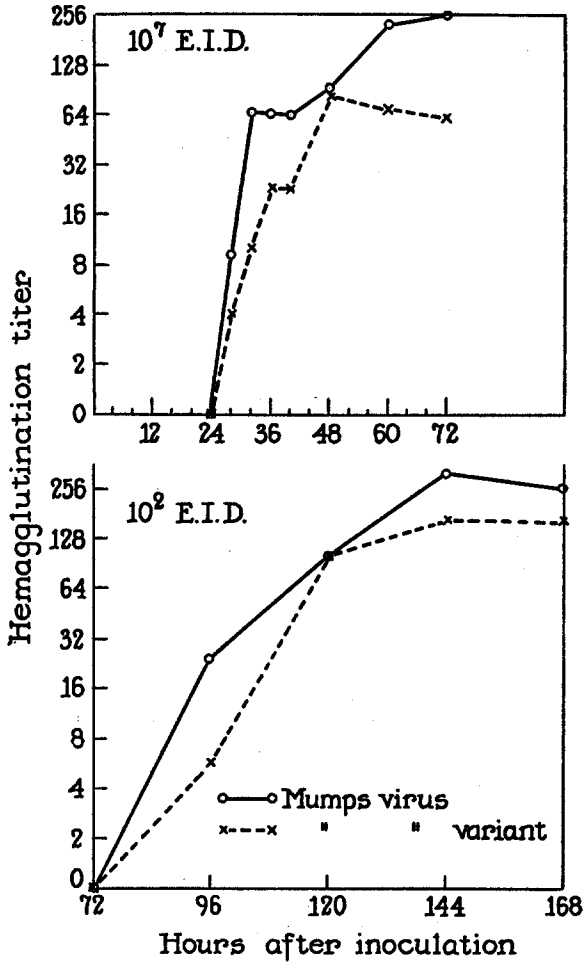

FIG. 1. Rate of multiplication of mumps virus and resistant variant in the allantoic sac. Mean hemagglutination titers of allantoic fluids are plotted against time after inoculation. Upper graph shows results obtained in groups of 5 to 6 embryos after inoculation of 10^7 E.I.D. Lower graph shows combined results of two experiments in groups of similar size after inoculation of 10^2 E.I.D.

was first demonstrable by means of the hemagglutination technique at 28 hours. At 32 hours all allantoic fluids contained detectable concentrations of virus. With the parent strain a concentration plateau appeared at 32 hours and persisted to 40 hours. However, with the resistant variant evidence of such a

plateau did not appear until 36 hours. With both strains evidence of a sharp increase in concentration appeared after 40 hours. It will be noted that when 10^2 E.I.D. was inoculated neither strain was demonstrable at 72 hours and that the parent strain showed a higher concentration than the resistant variant at 96 hours. As was to be expected, no evidence of a plateau was obtained under the experimental conditions employed with the smaller inoculum.

These results indicate that the rates of multiplication of the parent strain and the resistant variant, although not greatly dissimilar, probably are not identical. It appears that the variant strain undergoes multiplication at a slightly slower rate than the parent virus and, as would be expected, this is most evident when large inocula are employed and short time intervals are utilized. It should be pointed out that in a previous study (2) it was shown that hemagglutination is caused by the mumps virus particle itself and that the hemagglutination titer is a measure of viral concentration.

Infectivity of the Resistant Variant.—The infectivity titer of the variant strain was determined in the chick embryo in the presence of polysaccharide. For comparison, a virus titration of the parent strain was carried out simultaneously. Strains were employed which had been subjected to 9 serial passages in parallel; the variant in the presence of Fr.B, the parent in the absence of polysaccharide.

The infected allantoic fluids which were selected for titration experiments had similar hemagglutination titers. Infectivity titers were determined in 8 day embryos in the manner described above. One hour after inoculation of dilutions of the resistant variant 0.05 mg. of Fr.B was injected. The inhibitory effect of Fr.B on each strain was determined at the same time that the titrations were carried out.

The results of this experiment are shown in Table V. The virus infectivity titer of the resistant variant was found to be practically identical to that of the parent strain of mumps virus. It should be emphasized that despite the fact that the variant strain was titrated in the presence of Fr.B it appeared to be equally as infectious as the parent strain.

Immunological Properties of the Resistant Variant.—It was of interest to determine whether the variant strain, which had been obtained by means of a chemical inhibitor, was distinguishable in immunological properties from the parent mumps virus strain. The immunological relationship of the two strains was investigated by means of cross-neutralization and cross-hemagglutinaton–inhibition experiments with specific immune rabbit sera.

Antibody titrations were carried out simultaneously with the resistant variant and the parent strains. To reduce dilution errors, each strain was tested against aliquots from a single series of dilutions prepared from the desired immune serum. In hemagglutination-inhibition titrations final concentrations of 4 hemagglutinating units of virus and 0.5 per cent chick RBC were employed. Readings were made after 1 hour at room temperature; the end point was taken as the highest dilution which gave complete inhibition. In virus neutralization

TABLE V

Infectivity Titers of Resistant Variant of Mumps Virus and Parent Strain in the Chick Embryo

Virus	1st injection Intra-allantoic	2nd injection* Intra-allantoic	Mean hemagglutination titer of allantoic fluids‡	Difference from controls		Infectivity score	Infectivity titer
	dilution			*fold*	*log*	*positive/total*	*E.I.D.50*
Parent strain§	10^{-5}	NaCl	28			4/4	
" "	"	Fr.B 0.05 mg./embryo	2	−14×	−1.16	1/3	
" "	10^{-6}	NaCl				4/4	
" "	10^{-7}	"				2/4	$10^{-7.0}$
" "	10^{-8}	"				0/4	
" "	10^{-9}	"				0/4	
Resistant variant‖	10^{-5}	NaCl	256			3/3	
" "	"	Fr.B 0.05 mg./embryo	120	−2×	−0.33	4/4	
" "	10^{-6}	" "				4/4	
" "	10^{-7}	" "				3/4	$10^{-7.3}$
" "	10^{-8}	" "				0/4	
" "	10^{-9}	" "				0/4	

* 2nd injection given 1 hour after 1st injection.

‡ Allantoic fluids harvested 6 days after inoculation.

§ After 9 serial passages in presence of NaCl.

‖ After 9 serial passages in presence of Fr.B, 0.05 mg./embryo.

TABLE VI

Neutralization and Hemagglutination-Inhibition Titers of Sera from Rabbits Immunized with Parent Strain or Resistant Variant of Mumps Virus

Immune serum against	Hemagglutination-inhibition titer* *vs.*		Neutralization titer‡ *vs.*	
	Parent strain	Resistant variant	Parent strain	Resistant variant
Parent strain	1024	1024	600	300
Resistant variant	256	256	50	62

* Final concentration of 4 hemagglutinating units of virus employed.

‡ Approximately 10^3 embryo infectious doses of virus employed.

experiments a constant quantity of virus, *i.e.* 10^3 E.I.D., was used. Serum dilution-virus mixtures were incubated at 37°C. for 30 minutes. Each mixture was inoculated intra-allantoically in a group of 4 embryos. After incubation for 6 days at 35°C. the hemagglutination titers of the allantoic fluids were determined and the virus neutralization titer of the serum was calculated by the method of Reed and Muench (8).

The results of typical antibody titrations with immune rabbit sera against the parent strain and the resistant variant of mumps virus are shown in Table VI. It will be observed that similar antibody titers were obtained with each immune serum irrespective of the viral strain employed. Similar experiments were carried out with sera from other rabbits immunized either with the parent or the variant strain, and in every instance almost identical results were obtained. It appears evident that the resistant variant possesses immunological properties which are not dissimilar from those of the parent strain and it seems probable that the variant is antigenically indistinguishable from the parent strain.

Rate of Reaction of the Resistant Variant with Chicken Erythrocytes.—Björkman and Horsfall (9) demonstrated that stable variants could be obtained from influenza viruses by treatment with lanthanum acetate or by irradiation with ultraviolet light. Such variants differed from the parent virus strains in that their rates of elution from chicken RBC were slower than those of the viruses from which they were derived. Experiments were carried out to determine whether the resistant variant of mumps virus differed from the parent strain in a similar manner. It was found that there was no demonstrable difference between the parent strain and the variant as to the rate or degree of adsorption by chicken RBC as well as in the rate or extent of elution from such erythrocytes.

Concurrent Multiplication of the Resistant Variant and Influenza Virus in the Chick Embryo.—The results of an earlier study (3) indicated that viruses which are affected by the inhibitory action of Friedländer polysaccharides do not show the interference phenomenon with viruses which multiply in an unrestricted manner in the presence of such carbohydrates. As an example, it was demonstrated that multiplication of influenza A or B virus occurred in the allantoic sac when either virus was injected as long as 4 days after infection with mumps virus. Because the multiplication of the resistant variant of mumps virus was as unaffected by the presence of polysaccharide as is the multiplication of influenza viruses (2), the possibility that they might show interference was investigated.

Experiments were carried out in a manner identical to that employed in previous studies (3). The resistant variant was inoculated into the allantoic sac in groups of embryos and allowed to multiply for 4 days. Either the PR8 or Lee strain of influenza virus was then inoculated and incubation was continued for 2 additional days. The concentration of each of the viruses present in the allantoic fluids was determined by the hemagglutination technique in the presence of appropriate specific immune sera as described previously (3).

It was found that preexisting infection of the chick embryo with the variant of mumps virus did not prevent infection with either influenza A or B virus. In this respect, the resistant variant is similar to the parent strain; both are capable of concurrent multiplication with influenza viruses in the allantoic sac.

It has not been possible to devise a procedure which would be satisfactory to test the possibility that the resistant variant and the parent strain of mumps virus cause reciprocal interference.

DISCUSSION

That a variant can be obtained from mumps virus appears clear from the results of this study. Demonstration of the presence of the variant depended upon the availability of a chemical inhibitor of mumps virus multiplication. The property which clearly distinguishes the variant from the parent strain is its capacity to multiply as readily in the presence as in the absence of Friedländer capsular polysaccharides. It seems probable that this property is highly significant, and reflects an important difference in the biochemical mechanism by which the variant undergoes multiplication in the host cell as compared to that required by the parent strain.

Because the variant is consistently demonstrable after a single cycle of multiplication in the presence of polysaccharide, it appears that it is commonly present; that it is a naturally occurring variant which arises spontaneously, and does not develop as a result of a stimulus provided by the presence of polysaccharide. If this is the case, then it follows that mumps virus is not represented by individual viral particles, all of which are identical. A small proportion of the viral population, possibly of the order of 0.01 per cent, appears to possess at least one property which sharply distinguishes it from the bulk of the population. This property, the capacity to multiply readily in the presence of Friedländer polysaccharide, makes possible both the demonstration of the presence of the variant and its separation from the typical viral population.

The probable existence of inhomogeneity among animal virus populations was called to attention previously (9) in a study which showed that variants could be separated from influenza viruses by means of chemical or physical agents. In the case of influenza viruses the property which distinguished the variants, slow rate of elution from RBC, could not have been predicted on the basis of the agents, lanthanum acetate or ultraviolet light, employed to separate them. On the other hand, in the case of the resistant variant of mumps virus disclosed in this study, the distinguishing property of the variant is predictable, although undirected, and appears to be related in a specific manner to the chemical inhibitor, Friedländer polysaccharide, used to separate it.

There is as yet no means by which an entirely "pure line" strain of an animal virus can be obtained with certainty. With available techniques it is not possible to develop a viral population known to have originated from a single particle of an animal virus. As a consequence, variant strains derived from animal viruses probably cannot be considered to be composed solely of variant particles. In all likelihood, they represent mixtures, are inhomogeneous, and contain both variant and parent particles in different proportions. Serial passage

of such a mixture in the absence of the substance which favors the appearance of the variant would be expected to result in the reappearance of the parent strain if the parent possesses a slight advantage over the variant. The finding that the parent strain of mumps virus has a slightly more rapid rate of multiplication than the resistant variant indicates that it possesses such an advantage, and provides a probable explanation for the reappearance of the parent strain on serial passage of the variant in the absence of polysaccharide. However, if it is assumed that prolonged serial passage in the presence of inhibitory polysaccharide results in complete elimination of the parent virus, *i.e.* in the development of a "pure line" variant strain, it is necessary to consider an alternative hypothesis. Under these circumstances, so called reverse variation, with reappearance of the parent strain, would provide an explanation for the findings. Because of the rapidity with which the parent strain reappeared, it seems improbable that reverse variation occurred.

Variation with viruses, plant, bacterial, or animal, is a well known phenomenon which has been studied extensively. A large body of experimental data indicates that viruses, like other infectious agents, may show variation with respect to almost any property. Some of the most clearly established examples of variation are concerned with differences in pathogenicity, host range, and antigenic composition. It may be pointed out that these are the properties of viruses which, because of the limitations of available techniques, have been subjected to most intensive study. It should be emphasized that variation relative to these properties is unforeseeable and, although readily demonstrable, not subject to precise experimental control.

The resistant variant of mumps virus revealed in this study appears to represent a new type of viral variant, obtainable at will, with properties which can be anticipated in terms of the chemical inhibitor of multiplication used for selection of the variant. So far as we can discover, the only other example of this type of variation is that recorded by Foster (10) who found that "mutants" selected from T_4 and T_6 bacteriophage with proflavine showed somewhat higher tolerance to the inhibitory effects of the chemical than the parent viruses. It is obvious that the resistant variant of mumps virus is, in a formal sense, analogous to bacterial mutants which are resistant to antimicrobial agents. There is, however, an important difference which should be stressed. The available evidence (2, 11) indicates that Friedländer polysaccharide has no direct effect upon mumps virus itself, and suggests that the inhibition of multiplication obtained with the carbohydrate is the result of an effect upon the host cell.

That a variant of mumps virus is capable of unrestricted multiplication in the presence of inhibitory quantities of polysaccharide has implications bearing on the mechanism of inhibition. In terms of present views on the manner of viral multiplication there appear to be four steps in the process which in theory could be affected: (1) Polysaccharide might prevent virus-cell combination;

i.e., block or compete with "receptors" at the cell membrane. Previous studies with both PVM (12) and mumps virus (2, 11) indicate that this hypothesis is untenable. However, Green and Woolley (13) suggested that apple pectin inhibited influenza A virus by such a mechanism. In the case of the resistant variant of mumps virus it is evident that virus-cell combination occurs whether or not polysaccharide is present and there appears to be no reason to raise the unlikely possibility that some different cell "receptor" is utilized by the variant. (2) Polysaccharide might prevent invasion or penetration of the cell by virus. Earlier studies make this theory improbable; with mumps virus (2), as too with PVM (12), inhibition is obtained after the viral concentration has reached a level high enough to indicate that almost all susceptible cells are already infected. Other workers (14, 15) have considered that the results they obtained on inhibition of bacteriophage and influenza A virus with pectins could be explained in this manner. With the resistant variant of mumps virus it is apparent that invasion of the cell is accomplished despite the presence of polysaccharide, and it seems unnecessary to assume that the variant penetrates the cell in a manner different than the parent virus. (3) Polysaccharide might prevent release of virus from the infected cell after multiplication occurs. This hypothesis could explain the reduced concentration of mumps virus in allantoic fluid but would not explain the fact that finely ground tissues show, with either PVM (12) or mumps virus (16), a similarly reduced viral concentration when polysaccharide is employed. Moreover, the resistant variant is released from infected cells into the allantoic fluid as completely when polysaccharide is present as when it is absent. (4) Polysaccharide may, as was suggested previously (2, 3, 12), block or compete with an intracellular process which is required by the virus during multiplication. This hypothesis, in contrast to those discussed above, appears to conform with available information and is supported by the results obtained in the present study. To bring the resistant variant into line with this theory it is only necessary to assume that it has certain biochemical requirements different from the parent virus. Such an assumption is not at odds with well established facts bearing on the mechanism of variation with other infectious agents. If inhibition of mumps virus multplication is in fact due to blockage of an essential cellular metabolic step by polysaccharide, then it would be predicted that the resistant variant either makes use of some metabolic step other than that affected by the polysaccharides of Friedländer bacilli or does not require that step.

SUMMARY

Serial passage of mumps virus in the presence of inhibitory quantities of the capsular polysaccharide of Friedländer bacillus type B results in the appearance of a variant strain of the virus. Multiplication of the variant virus is not inhibited by the polysaccharide. A similar resistant variant is obtained with

polysaccharide in a single cycle of multiplication when very large inocula of mumps virus are employed. The resistant variant is indistinguishable from the parent strain as to infectivity, reactivity with erythrocytes, and immunological properties, but appears to have a somewhat slower rate of multiplication. Serial passage of the resistant variant in the absence of polysaccharide results in the reappearance of a sensitive strain. It is suggested that mumps virus populations are inhomogeneous; that naturally occurring variants are present in such populations and possess distinctive properties; that the use of a chemical inhibitor of mumps virus multiplication makes possible the selection of a variant possessing a predictable property. The findings are discussed in relation to the mechanism of inhibition of mumps virus multiplication by polysaccharide.

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