THE ASSOCIATIVE REACTIONS OF PNEUMONIA VIRUS OF MICE (PVM) AND INFLUENZA VIRUSES: THE EFFECTS OF pH AND ELECTROLYTES UPON VIRUS-HOST CELL COMBINATIONS

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Previous studies (1–4) on pneumonia virus of mice (PVM) have shown that the virus combines either with erythrocytes or with lung particles obtained from species susceptible to infection with the agent but not with other tissues. Moreover, it has been demonstrated (1–5) that, unlike influenza (6), mumps (7), and Newcastle disease (8) viruses, spontaneous dissociation from the combined state does not occur with PVM. Release of the virus from combination can be achieved by heat (1–3) or treatment with alkali (5) but either procedure destroys the infectivity of the virus, as well as the combining capacity of the tissue component, although neither demonstrably alters the combining capacity of the virus itself.

In the course of the present investigation a technique was developed by means of which dissociation of PVM from the combined state can be accomplished without destroying either the infectivity of the virus, the combining capacity of the virus, or the combining capacity of the tissue component. This technique provided a means suitable for a study of some of the factors which influence virus–tissue component combinations.

In the present investigation the effects of electrolyte concentration, ionic composition, and pH, as well as virus concentration upon combination have been studied. It will be shown that dissociation of PVM from the combined state is strikingly dependent upon electrolyte concentration and pH; that the effect of electrolytes upon combination between the virus and tissue components is independent of their ionic composition but is dependent upon their concentration; that with respect to combination there is an inverse relationship between virus concentration and electrolyte concentration. In addition, it will be shown that the reactions between influenza viruses and erythrocytes also are strikingly influenced by the ionic environment and certain of the similarities and differences between the reactions of PVM and the influenza viruses with erythrocytes will be presented.

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Materials and Methods

PVM.—Strain 15 of the pneumonia virus of mice (PVM) (9) was used exclusively. It was maintained by occasional passage in albino Swiss mice and stored as a 10 per cent suspension of infected mouse lungs at −70°C. Either *combined virus* or *heat-released virus* suspensions were prepared as described in previous communications (5, 10). *Dissociated* PVM preparations were obtained from 10 per cent suspensions of perfused infected lungs in non-electrolyte solutions (*i.e.*, distilled H_2O, 0.25 M dextrose, or 0.25 M sucrose) and were centrifuged at 12,000 R.P.M. for 30 minutes in order to sediment the tissue-combining component (4).

Influenza Viruses.—The PR8 and FM1 strains of influenza A virus, and the Lee and B1103[1] strains of influenza B virus were employed. The viruses were passed in the allantoic sac of chick embryos according to the technique previously described (11).

Hemagglutination Titrations.—(*1*) *PVM:* The technique of hemagglutination titrations with mouse RBC, utilizing serial twofold dilutions, and the method of estimating end points were identical with those previously employed (3). (*2*) *Influenza virus:* The technique of hemagglutination titrations with 1 per cent chicken RBC has been described elsewhere (11).

RBC Suspensions.—Blood was obtained from either mice or chickens and was mixed with 2.5 per cent sodium citrate. The erythrocytes were washed 3 times in the desired diluent, as indicated in the text, and made up by volume to the desired concentration.

Normal Mouse Lung Suspensions.—The lungs of normal Swiss mice were perfused *in situ* with buffered saline. After removal the lungs were ground to a 10 per cent suspension in a modified blendor for 2 minutes at 4°C. As indicated in the text, the diluent employed varied in accordance with the experiment. Suspensions were stored at −30°C. until used.

Virus-Combining Capacity of Mouse Lung Suspensions.—As described previously (4) the virus-combining capacity of mouse lung suspensions was taken as the highest dilution which completely combined with either 8 or 16 hemagglutinating units of PVM.

Solutions.—The solutions used frequently in this investigation are designated as follows in the text: Saline = 0.15 M NaCl solution buffered at pH 7.2 with 0.01 M phosphate. Dextrose or sucrose = 0.25 M dextrose or sucrose solution buffered as above. Water = distilled water which was used exclusively.

EXPERIMENTAL

Dissociation of PVM–Lung Particle Combination.—Previous studies have shown that, although PVM cannot be demonstrated by the hemagglutination technique in suspensions of infected mouse lungs unless they are subjected to treatment which releases the virus from combination with tissue particles (1–5), the virus can be so demonstrated with slices of unground lungs (5) as well as in tracheal washings from infected lungs (4). This indicates that in the intact infected lung some virus is present in an uncombined state, and that combination between free PVM and lung particles occurs when the lungs are ground.

In the course of studies on the mechanism of combination between PVM and lung particles, it was observed that as the NaCl concentration of the mixture was reduced the quantity of virus that combined with tissue particles also was reduced. This observation suggested that combination between

[1] The B1103 strain was obtained through the courtesy of Dr. R. M. Taylor, the International Health Division, The Rockefeller Foundation, New York, N. Y.

PVM and lung particles could be prevented, or that such combination once formed could be dissociated, by grinding infected lungs either in water or in a non-electrolyte solution so that the electrolyte concentration of the suspension would be low. To test this hypothesis a number of different experiments were carried out.

The lungs of mice infected with PVM were perfused with saline to remove both erythrocytes and antibodies against the virus which, if allowed to combine with PVM during grinding, would reduce the amount of virus obtained. Ten per cent suspensions then were made in either saline, water, 0.25 M dextrose, or 0.25 M sucrose. Aliquots of each of the suspensions were heated at 70°C. for 30 minutes and clarified by centrifugation. Additional aliquots were

TABLE I

The Dissociation of PVM from Infected Mouse Lung Suspensions in Distilled H_2O or Solutions of Non-Electrolytes

PVM suspension: 10 per cent perfused infected mouse lung prepared in	Treatment of suspension		Hemagglutination titer of supernate *vs.* mouse RBC
	70°C.	12,000 R.P.M.	
	min.	*min.*	
0.15 M NaCl	0	0	0
" " "	30	10	256
Distilled H_2O	0	30	256
" "	30	10	1024
0.25 M dextrose	0	30	512
" " "	30	10	2048
0.25 M sucrose	0	30	512
" " "	30	10	2048

immediately centrifuged. Each aliquot then was tested by the hemagglutination technique and the titer of free virus was determined.

The results of certain typical experiments are shown in Table I. As has been recorded previously (1–3) no virus was detectable by hemagglutination with unheated PVM suspensions prepared in saline but the agent was demonstrable when such suspensions were heated appropriately. On the other hand, virus was readily demonstrated by hemagglutination with suspensions prepared in either water, dextrose, or sucrose. Suspensions of normal mouse lungs, prepared in identical manners, did not cause hemagglutination reactions similar to those obtained with infected lungs. In some instances, with low dilutions of normal lung suspensions, slight aggregation or clumping of erythrocytes was observed; both qualitatively and quantitatively such clumping was readily distinguishable from that caused by the virus. Moreover, specific

serological tests (3) clearly demonstrated that the hemagglutination reactions obtained with suspensions of infected lungs, containing but little electrolyte, were attributable to PVM. A suspension of the virus in 0.25 M sucrose, which gave a hemagglutination titer of 1:512, caused no hemagglutination after it was mixed with PVM immune hamster serum.

The results of these experiments suggest that when the electrolyte concentration of the suspension is maintained at a low level PVM either does not combine with or dissociates from tissue particles formed during grinding of mouse lungs. With the techniques now available it is impossible to determine how much of the virus in intact infected lungs is combined and how much is uncombined. Nor is it possible to determine directly whether virus first combines with and then dissociates from particles formed when infected lungs are ground in non-electrolyte solutions, or whether such combination is prevented. However, when combination between the virus and lung particles is caused to occur by grinding infected lungs in saline, the combined virus can be dissociated readily by reducing the electrolyte concentration to a low level. A distinction between the alternatives, therefore, would appear to be unimportant and the term *dissociated virus* will be used to designate the uncombined virus present in the supernate of preparations ground in solutions of low electrolyte concentration.

Table I shows, in addition, that the hemagglutination titer of virus obtained with preparations of dissociated PVM is usually equal to or greater than the titer obtained with heat-released virus in saline. However, the titer of suspensions heated in non-electrolyte solutions is in general fourfold greater than the titer obtained with either heat-released virus in saline or dissociated virus. It is evident that the hemagglutination titer of heat-released virus in saline, or dissociated virus in non-electrolyte solutions, reveals but a small proportion (*i.e.*, of the order of 25 per cent) of the virus present in infected mouse lungs as determined by hemagglutination with suspensions heated in non-electrolyte solutions.

Infectivity of Dissociated PVM.—Either heat or alkali treatment, procedures used previously to release PVM from combination in infected lung suspensions, renders the virus non-infectious (1–5). On the other hand, *free infectious PVM*, prepared by extracting unground lung slices with cold saline, has been shown to be infective (5). It was important to determine if dissociated virus preparations also were infectious.

Tests for infectivity were carried out in the following manner. A suspension of dissociated PVM was prepared in 0.25 M dextrose. In order not to cause inactivation of the very unstable property of infectivity, the dextrose solution was buffered at pH 7.2 with 0.01 M phosphate, and centrifugation was carried out at 4°C. The supernate was diluted in cold buffered dextrose and the virus titered in mice as described elsewhere (5).

It was found that the dissociated PVM preparation employed, which had a hemagglutination titer of $10^{-3.1}$, had an M.S.50 virus titration end point of $10^{-2.2}$. The latter figure is comparable to the infectivity titers obtained previously with free infectious virus of similar hemagglutination titer (5). It is evident that following dissociation in the presence of dextrose PVM retained the property of infectivity.

The Effect of Centrifugation on Dissociated PVM.—The effects of centrifugation and of heat upon dissociated virus were studied in order to provide a basis for the interpretation of experiments reported in subsequent sections.

TABLE II

Effect of Centrifugation on Dissociated PVM in Distilled H_2O

PVM suspension: 10 per cent perfused infected mouse lung prepared in	Treatment of suspension				Hemagglutination titer of supernate *vs.* mouse RBC
	NaCl added to	70°C.	Centrifugation		
	M	*min.*	*R.P.M.*	*min.*	
Distilled H_2O	0	0	12,000*	30	256
" "	"	"	"	60	512
" "	"	"	18,000‡	166	32
" "	"	30	8,000	5	512
" "	"	"	12,000	30	"
" "	0.15	"	8,000	5	256
" "	"	"	12,000	30	"
0.15 M NaCl	0	"	8,000	5	128
" " "	"	"	12,000	30	"

* Rotor diameter = 19.8 cm.

‡ Rotor diameter = 30.1 cm.

A suspension of infected lungs in water was divided into aliquots which were centrifuged at high speed under various conditions. In certain instances the specimens were heated at 70°C. for 30 minutes; in others NaCl to 0.15 M was added before heating; in still others neither the addition of NaCl nor heating was employed. A suspension of heat-released virus in saline served as a control preparation. The hemagglutination titer of the supernate from each aliquot was determined after it had been centrifuged at 8,000, 12,000, or 18,000 R.P.M. for various periods. When the latter speed was desired, a high speed vacuum centrifuge (12) with a rotor about 1.5 times the diameter (*i.e.*, 30.1 cm.) of that of the open air high speed centrifuge (13) was employed. The greatest centrifugal force used was, on the basis of previous studies on the sedimentation of uncombined PVM (10), sufficient to sediment approximately 90 per cent of the free virus.

The results of these experiments are presented in Table II. It will be seen that, as was found previously (10), the centrifugal force used did not cause sedimentation of a significant amount of heat-released virus. Similarly, it

was found that PVM, dissociated from combination in water, showed no increased sedimentation in high gravitational fields and, moreover, was unaffected by heating in the presence or absence of added NaCl. Finally, approximately 90 per cent of the dissociated virus was sedimented by a gravitational force calculated to sediment the uncombined virus. These results indicate that the procedures employed did not lead to aggregation of the virus particles and suggest that dissociated PVM is not significantly larger than either heat-released virus or free infectious virus obtained from intact lungs (10). The effect of heating dissociated virus was also studied. It was found that dissociated virus showed heat stability similar to that of combined PVM.

The Effect of Centrifugation and Heat on Lung Particles in Water.—For reasons mentioned in the preceding section, studies were made on the effect of centrifugation and of heat on tissue particles present in suspensions of normal mouse lungs in either saline or water.

Suspensions in these diluents were centrifuged at 12,000 R.P.M. for 15, 30, and 60 minutes, respectively, and the virus-combining capacity of the supernates was determined in the presence of 0.15 M NaCl as described above.

No significant difference in the combining capacity of the water and saline supernates was observed. These results indicate that grinding mouse lungs in water does not yield tissue particles which are less readily sedimented than those present in lungs ground in saline.

Studies on the heat stability of lung particles in saline and in water were carried out. Suspensions of normal mouse lungs in these diluents were heated at 70°C. for 30 minutes and their virus-combining capacity then was determined. It was found with both types of suspension that the capacity to combine with PVM was destroyed by this treatment. These results indicate that the combining capacity of particles suspended in water is not more heat-stable than that of lung particles in saline.

Dialysis of Dissociated PVM.—In the preceding experiment with dissociated PVM, although virus suspensions were prepared in water or in solutions of non-electrolytes, they still contained the electrolytes present in the infected lung tissue itself. In order to remove the diffusible portion of such electrolytes, suspensions of infected lungs in water were dialyzed in cellophane against a large volume of water for 24 hours at 4°C. The properties of dialyzed suspensions of dissociated PVM were then investigated.

It was found that a greater proportion (*i.e.*, of the order of 50 per cent) of the virus present in dialyzed suspensions was demonstrable by hemagglutination than in undialyzed suspensions prepared in water. Combination and dissociation between dialyzed virus and dialyzed lung particles, obtained either from infected or normal lungs, can be caused to occur at will by appropriate manipulation of the NaCl concentration of the mixture as is indi-

cated below. The increased sedimentation of heat-released PVM following dialysis (3) also was observed to occur with dialyzed dissociated PVM. An unexpected finding was the observation that dialyzed suspensions lost the greater proportion of their hemagglutinating capacity if heated in the absence of salt. This property remained unaffected if NaCl were added to the dialyzed suspension prior to heating.

Recombination of Dissociated PVM and Lung Particles.—The finding that dissociation of PVM–lung particle complexes can be accomplished by lowering the electrolyte concentration raised questions as to whether combination and subsequent dissociation had altered the combining capacity of either the virus or the tissue particles, and whether recombination could be caused to occur

TABLE III

Recombination between Dissociated PVM and Mouse Lung Particles in the Presence of NaCl

PVM suspension: 10 per cent perfused infected mouse lung prepared in	Treatment of suspension			Treatment of supernate		Hemagglutination titer of supernate *vs.* mouse RBC
	70°C.	NaCl added	12,000 R.P.M.	Normal mouse lung particles added in	12,000 R.P.M.	
	min.	*M*	*min.*		*min.*	
Distilled H_2O	0	0	30	0	0	128
" "	"	0.15	"	"	"	0
" "	30	0	10	"	"	128
" "	"	"	"	Distilled H_2O	30	"
0.15 M NaCl	"	"	"	0	0	"
" " "	"	"	"	0.15 M NaCl	30	0

by raising the electrolyte concentration of the mixture. The following experiments were carried out.

A suspension of infected lungs in water was divided into aliquots. One was centrifuged in the usual manner. To the other was added NaCl to 0.15 M, after which it was centrifuged similarly. To a heat-released PVM preparation in water was added an equal volume of a suspension of normal mouse lungs in water. The mixture was held at 37°C. for 30 minutes and then centrifuged to remove the lung particles. As a control, a heat-released PVM suspension in saline was employed. To one volume was added an equal volume of a suspension of normal lungs in saline. The mixture was incubated and then centrifuged as above. Each of the supernates was tested for the presence of PVM by the hemagglutination technique.

The results of these experiments are presented in Table III. As has been demonstrated previously (2, 3), heat-released virus combined with lung particles in saline and was not demonstrable by hemagglutination. It is evident that in low concentrations of NaCl dissociated PVM did not recombine with lung particles to an appreciable extent. However, when NaCl was added in sufficient concentration dissociated virus and lung particles did recombine.

This demonstrates clearly that the combining capacity of neither the virus nor lung particles is irreversibly altered after dissociation has been effected.

Effect of NaCl Concentration upon Recombination between Dissociated PVM and Lung Particles.—The finding that dissociated PVM recombined with lung particles when NaCl was added suggested that the reaction could be used to determine the relationship between NaCl concentration and recombination of the virus and lung particles.

To aliquots of a suspension of infected lungs in water NaCl was added to yield concentrations ranging from 0.145 to 0.018 M. The mixtures were held at room temperature for 20 minutes, centrifuged at 12,000 R.P.M. for 30 minutes, and the supernates tested by the hemagglutination technique.

The results of this experiment are shown in Table IV. Under the experimental conditions employed, recombination between dissociated PVM and

TABLE IV

The Effect of NaCl Concentration on Recombination between Dissociated PVM and Mouse Lung Particles

PVM suspension: 10 per cent perfused infected mouse lung prepared in	Treatment of suspension		Hemagglutination titer of supernate *vs.* mouse RBC
	NaCl added	12,000 R.P.M.	
	M	*min.*	
Distilled H_2O	0	30	128
" "	0.018	"	32
" "	0.036	"	"
" "	0.072	"	0
" "	0.145	"	"

lung particles was complete at NaCl concentrations of 0.072 M or more. With 0.036 M NaCl or less not all of the dissociated virus present in the suspension was bound by the lung particles. It is evident that the amount of dissociated PVM bound by lung particles is directly related to the electrolyte concentration.

Effect of NaCl Concentration on Recombination between Heat-Released PVM and Lung Particles.—Because the combining capacity of dissociated PVM appeared to be identical with that of heat-released virus, a study of the relationship between NaCl concentration and recombination between heat-released PVM and lung particles also was undertaken.

Mixtures of heated virus in water and lung particles in water were prepared and NaCl was added to concentrations ranging from 0.145 M to 0.018 M. The quantity of virus which combined with the lung particles was determined in the usual manner.

The results of this experiment were similar to those described in the preceding section. At NaCl concentrations of 0.072 M or more at least 50 per cent of the heated virus present combined with the lung particles, while at NaCl

concentrations of 0.036 M or less no evidence of combination between the virus and lung particles was observed.

Virus-Combining Capacity of Normal Lung Suspensions.—The experiments described in a preceding section show that as the NaCl concentration was reduced the amount of PVM bound by lung particles was also reduced. In the present experiments the virus-combining capacity of normal lung suspensions in varying concentrations of NaCl was studied.

To each of a series of twofold dilutions of a suspension of perfused normal lungs in water or in 0.15 M NaCl, 16 hemagglutinating units of PVM in 0.15 M NaCl was added. An equal quantity of PVM in 0.68 M NaCl was added to each of a series of twofold dilutions of the lung suspension in 0.68 M NaCl. Following incubation at 37°C. for 30 minutes, the combined virus was sedimented in the centrifuge and the supernates were tested for free virus by the hemagglutination technique.

TABLE V

The Effect of NaCl Concentration upon PVM-Combining Capacity of Normal Mouse Lung Suspensions

Suspension: 10 per cent perfused normal mouse lung diluted in	Virus added to each dilution	Final NaCl concentration of PVM–mouse lung particle mixture	Hemagglutination-inhibition titer of suspension
	Hemagglutinating units		
		M	
Distilled H_2O	16	0.07	8
0.15 M NaCl	"	0.15	64
0.68 " "	"	0.68	512

The results of a typical experiment are shown in Table V. It is evident that as the NaCl concentration of the normal lung suspension was increased, the hemagglutination-inhibition titer of the suspension also increased. It will be recalled that the inhibition titer of a lung suspension is taken as the highest dilution that completely combines with a constant amount of virus; this is a measure of the virus-combining capacity of the suspension (4).

As an explanation for the unexpected result obtained with high salt concentration, it seemed possible that in 0.68 M NaCl solution the lung particles were in a more dispersed state or, indeed, that some of the combining component might have become soluble. As another explanation it seemed possible that the state of the virus might be altered in hypertonic NaCl. If the virus particles became aggregated, the net effect might be to increase, falsely, the apparent virus-combining capacity of the lung suspension. However, the hemagglutination titer of a PVM suspension at NaCl concentrations ranging from 0.07 to 0.62 M did not vary and therefore it appeared that the effect of NaCl on the virus-combining capacity of lung suspensions was not to be explained on this basis. The results of these various experiments indicate

clearly that the amount of virus which is bound by a lung suspension is directly related to the NaCl concentration over the range studied.

Effect of Various Electrolytes on Combination between PVM and Lung Particles.—To determine whether the effect of NaCl upon combination between PVM and lung particles was due to either Na^+ or Cl^- ions or could be duplicated with other electrolytes, additional experiments were performed. The effect of KCl, $MgCl_2$, NH_4Cl, and Na_2SO_4, respectively, on the virus-combining capacity of lung suspensions also was studied.

To each of a series of twofold dilutions of perfused normal lung suspension prepared in one or another of the salts mentioned above, 8 hemagglutinating units of PVM in the corresponding electrolyte was added. Because of the finding that the virus-combining capacity of lung particles is greater in hypertonic solution, the electrolyte concentration in each mixture was maintained at 0.3 M in order to increase the probability of detecting small differences in the

TABLE VI

The Effect of Various Electrolytes on Combination between PVM and Mouse Lung Particles

Mixture			Hemagglutination-inhibition titer of suspension
PVM hemagglutinating units	Suspension: 10 per cent perfused normal mouse lung	Diluent	
8	Serial dilutions	0.3 M NaCl	512
"	" "	0.3 M KCl	"
"	" "	0.3 M $MgCl_2$	"
"	" "	0.3 M NH_4Cl	"
"	" "	0.3 M Na_2SO_4	"
"	" "	Distilled H_2O	0

effect of various electrolytes upon the combination reaction. The virus-combining capacity of the suspension was then determined in the usual manner.

The results of these experiments, as recorded in Table VI, demonstrate that the virus-combining capacity of a lung suspension is independent of the ionic composition of the mixture. Obviously, either mono- or bivalent ions can be substituted for NaCl without influencing the capacity of lung particles to bind the virus. These results indicate that the effect of electrolytes on PVM–lung particle combination is not specific for any single ion or pair of ions, and that the effect is due to a property shared by one, at least, of each ion pair tested.

Effect of NaCl on Combination between PVM and Mouse Erythrocytes.—Sufficient evidence has been accumulated to support the concept that infection by PVM is initiated by combination between the free virus and cells of the respiratory tract (4). It appeared that a study of the influence of electrolytes upon combination of the virus with intact cells rather than with cell fragments

might provide information about some of the factors concerned in this reaction and might yield a clue concerning the mechanism. With suspensions of erythrocytes the external electrolyte environment can be readily controlled, but it would be technically difficult to control this variable with intact cells of the excised lung. Present evidence indicates that, as regards combination with PVM, mouse RBC do not differ significantly from respiratory tract cells (4). The effect of NaCl upon combination between PVM and mouse RBC was therefore studied.

A suspension of dissociated PVM in 0.25 M dextrose was added to a suspension of 50 per cent mouse RBC which had been washed in 0.25 M dextrose. The final concentration of RBC was 5 per cent. The mixture was held at room temperature; at 30 and 240 minutes aliquots were removed, the cells sedimented, and the free virus content of the supernate determined. After 240 minutes sufficient NaCl was added to raise the concentration of the mixture to 0.15 M. After an additional 30 minutes the virus content of the supernate was measured. A control consisted of a similar mixture to which 0.15 M NaCl was added immediately.

TABLE VII

The Effect of NaCl on Combination between PVM and Mouse Erythrocytes

Mixture	Treatment			Hemagglutination titer of supernate *vs.* mouse RBC
	NaCl added	Held at room temperature	2,000 R.P.M.	
	M	*min.*	*min.*	
PVM + RBC + 0.25 M dextrose	0	30	5	512
" " " " "	"	240	"	"
" " " " "	0.15	240 + 30	"	32
" + 0.15 M NaCl	0	0	0	512
" + RBC + 0.15 M NaCl	"	30	5	16

As shown in Table VII, combination between PVM and mouse RBC in dextrose solution did not occur even though the mixture was held for 4 hours. Yet, when NaCl was added, the virus promptly united with the erythrocytes. In other experiments the virus-combining capacity of mouse erythrocytes in hypertonic NaCl solution was compared to that in isotonic NaCl. The technique employed was similar to that described above in the section on the effect of NaCl concentration upon the combining capacity of lung particles. It was found that the combining capacity of mouse RBC in 0.73 M saline was two- to fourfold greater than that in 0.15 M NaCl.

Combination between PVM and Erythrocytes in Various Electrolyte Solutions.— Since the effect of NaCl upon virus-RBC combination appeared to be the same as upon virus–lung particle combination, the effect of various electrolytes upon the former combination was determined.

Serial twofold dilutions of a dissociated PVM suspension in water were made either in various electrolyte solutions at 0.15 M or in non-electrolyte solutions at 0.25 M. To each dilution was added an equal volume of mouse RBC suspended in a solution of corresponding composition. The hemagglutination titers were then determined.

The results are recorded in Table VIII. When hemagglutination titrations were performed in non-electrolyte solutions, agglutination of RBC did not occur. Because combination between virus and erythrocytes is the first step in the hemagglutination reaction, the absence of agglutination in non-electrolyte solutions may be attributed to failure of the virus and RBC to unite. In each of the various electrolyte solutions, on the other hand, the hemaglutination titer was identical with that obtained in the presence of NaCl. It appears that the effect of electrolytes upon combination between PVM and erythrocytes

TABLE VIII

Hemagglutination Titer of PVM in Various Electrolyte or Non-Electrolyte Solutions

Diluent	Concentration	Hemagglutination titer *vs.* mouse RBC	Diluent	Concentration	Hemagglutination titer *vs.* mouse RBC
	M			M	
Dextrose	0.25*	0	$MgCl_2$	0.15	512
Sucrose	" *	"	Na_2SO_4	"	"
NaCl	0.15	512	NaH_2PO_4	"	"
KCl	"	"	Na_2HPO_4	"	"
$CaCl_2$	"	"	$NaNO_2$	"	"

* 0.01 M phosphate added.

is a property which is shared by all of the electrolytes studied, and is not dependent upon the presence of any particular anion or cation combination. These results parallel those obtained with various electrolytes and PVM–lung particle mixtures. The similarity of the reactions suggests that the combining components in lung particles and in RBC are either the same or closely related substances. The hemagglutination titer of a PVM suspension was determined in the presence of 0.15 M NaCl in buffers of 0.1 ionic strength which ranged from pH 5 to pH 9. No significant difference in titer was observed at any of the pH levels studied. This indicates that in the presence of a sufficient concentration of electrolytes combination between the virus and RBC occurs over a wide range independently of the pH of the suspension.

The Effects of pH and Electrolyte Concentration upon Dissociation of PVM from Combination with RBC.—The release of PVM from combination with erythrocytes by heat, as previously described (1–3), destroys the combining capacity of the RBC. Release of PVM combined with erythrocytes can be achieved readily by sedimenting the RBC and causing hemolysis with water. Dissocia-

tion of combined PVM without disruption of the RBC can be effected by resuspending the virus-RBC complex in 0.25 M dextrose or sucrose buffered at pH 7.2 with 0.01 M phosphate. In general, the degree of dissociation obtained appeared to be complete.

Although the PVM–RBC complex readily dissociated in 0.25 M dextrose buffered at pH 7.2 with 0.01 M phosphate, no dissociation occurred in unbuffered 0.25 M dextrose. This observation suggested that dissociation was influenced by either the pH or the electrolyte concentration, or, possibly, by both factors

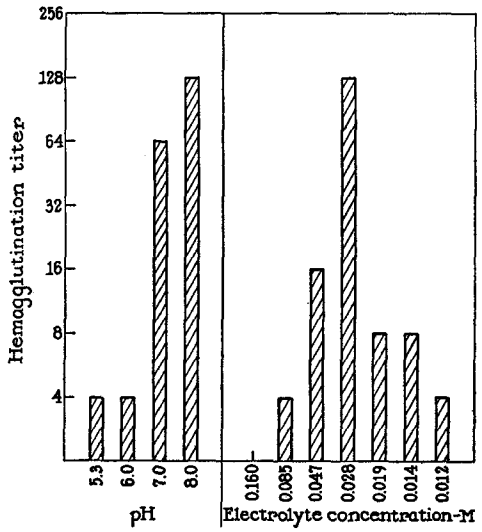

FIG. 1. The effects of varying pH and electrolyte concentration relative to each other upon dissociation of PVM from combination with mouse RBC. The pH was varied with phosphate buffers at 0.01 M. The electrolyte concentration was varied with NaCl at pH 6.0 in 0.01 M phosphate buffer.

The effect of varying pH at constant electrolyte concentration was first studied. To aliquots of a PVM preparation in saline sufficient mouse RBC were added to yield 5 per cent suspensions. After 10 minutes the mixtures were centrifuged, and the RBC washed twice in unbuffered 0.25 M dextrose. The sedimented cells were resuspended to volume in 0.25 M dextrose solutions buffered with 0.01 M phosphate at pH 5.3, 6.0, 7.0, and 8.0, respectively. After 30 minutes the cells were again sedimented, and the amount of virus in the supernates was measured by the hemagglutination technique.

The results of a typical experiment are shown in Fig. 1. It will be seen that a significant amount of the virus dissociated from the erythrocytes only when the cells were resuspended in dextrose buffered at pH 7 or 8. Therefore, it appears that at constant electrolyte concentration dissociation is a function of the pH of the suspension.

In order to investigate the effect of varying electrolyte concentration at constant pH upon dissociation, a modification of the procedure described above was employed.

Virus-RBC complexes were formed in saline. The erythrocytes then were washed twice in unbuffered dextrose and resuspended to volume in 0.25 M dextrose buffered at pH 6.0 with 0.01 M phosphate. NaCl was added to the suspension in concentrations ranging from 0.15 M to 0.01 M. After 30 minutes the cells were again sedimented, and the amount of virus present in the supernate was measured in the usual manner.

The results of a typical experiment also are shown in Fig. 1. It is evident that at pH 6.0 dissociation was maximal at 0.028 M electrolyte concentration and that either lower or higher concentrations markedly reduced dissociation. These results indicate that dissociation of PVM from combination with RBC is a function of both the electrolyte concentration and the pH of the suspension. A study of the behavior of PVM–RBC stromata complexes with respect to the effect of electrolyte concentration showed that combination and dissociation also could be caused to occur by appropriate regulation of this variable.

Sequential Cycles of Combination and Dissociation of PVM and Mouse Erythrocytes.—It is well established that mouse erythrocytes contain a substance with which PVM combines and that spontaneous dissociation of the virus from combination does not occur (1–5). Nonetheless, dissociation and recombination can be achieved by varying the electrolyte concentration. It appeared probable that additional information about the PVM–RBC reaction could be obtained by determining whether or not either the virus or the red blood cell was altered as a result of combination and dissociation.

To a suspension of dissociated PVM in 0.25 M sucrose was added sufficient NaCl to yield a 0.15 M solution. Packed mouse erythrocytes, washed in saline, were then added to a concentration of 5 per cent. Preliminary experiments indicated that this amount of RBC would combine with all of the virus present in the preparation used. The mixture was held 15 minutes, the cells sedimented, the supernate removed, and its hemagglutination titer determined. The packed RBC were resuspended to volume in 0.25 M sucrose, buffered at pH 7.2 with 0.01 M phosphate. After 15 minutes the RBC were again sedimented, an aliquot of the supernate removed, and its hemagglutination titer measured. To cause another cycle of combination and dissociation between virus and RBC, the mixture was shaken, sufficient NaCl was added to restore the concentration to 0.15 M, and the cells were again sedimented after 15 minutes. The supernate was removed and titered as before. Additional cycles were performed by a repetition of these steps.

The results of a typical experiment in which 3 cycles of combination and dissociation were carried out are shown in Table IX. They demonstrate clearly that in the presence of sucrose and NaCl combination between PVM and RBC occurs, while in the presence of sucrose alone dissociation of the complex results. This cycle can be repeated at will by appropriate regulation of the NaCl concentration and the pH of the mixture. These results indicate

that PVM, unlike the influenza group of viruses (6), does not inactivate or destroy the "receptor" of erythrocytes, and repeated cycles of combination and dissociation can occur without causing demonstrable alteration of the combining capacity of either the virus or the RBC.

The Effect of Low Electrolyte Concentration upon Hemagglutination with Influenza Viruses.—It was reported recently (14) that the concentration of NaCl markedly affects the hemagglutination reaction between the PR8 strain and human erythrocytes. In the present study preliminary observations demonstrated that, whereas hemagglutination with PVM and mouse RBC could be completely inhibited at electrolyte concentrations of 0.01 M or less, to achieve the same result with influenza viruses and chicken RBC it was necessary to reduce the electrolyte concentration to 0.001 M. This finding raised two

TABLE IX

Sequential Cycles of Combination and Dissociation between PVM and Mouse Erythrocytes

Step No.	PVM suspension in 0.25 M sucrose	Treatment of sedimented RBC			Hemagglutination titer of supernate vs. mouse RBC
		Resuspended in sucrose*	NaCl added	2,000 R.P.M.	
		M	M	min.	
1	Supernate	0	0	0	256
2	" + RBC	"	0.15	5	0
3	Resuspended sediment from No. 2	0.25	0	"	256
4	" " " " "	"	0.15	"	0
5	" " " " 4	"	0	"	256
6	" " " " "	"	0.15	"	0
7	" " " " 6	"	0	"	256

* Buffered at pH 7.2 with 0.01 M phosphate.

technical difficulties. Firstly, certain lots of chicken RBC are unstable and spontaneously agglutinate in 0.25 M dextrose containing 0.001 M phosphate. However, it was found that by holding the RBC in dextrose solution overnight at 4°C. this undesirable effect could be eliminated. Secondly, the pH of 10 per cent suspensions of chicken RBC in 0.001 M phosphate–buffered dextrose is only slightly above 6, whereas the pH of such cells in dextrose buffered with 0.01 M phosphate is 7.2. Therefore, in order to interpret the experiments to be carried out, it was necessary first to study the effect of pH upon the hemagglutination reaction with influenza viruses and chicken RBC. It was readily demonstrated that in saline, buffered at pH 6, 7, and 8, respectively, with phosphate buffer (0.1 ionic strength) the hemagglutination titer and the rate of elution of virus were not appreciably affected. In order to determine the effect of electrolyte concentration upon the hemagglutination titer of influenza virus, the following experiment was performed.

Pools of infected allantoic fluid were dialyzed at 4°C. against large volumes of water buffered at pH 7.2 with 0.001 M phosphate. Serial twofold dilutions were made in 0.25 M dextrose similarly buffered. To each dilution was added an equal volume of a 1 per cent suspension of chicken RBC in 0.25 M dextrose containing 0.001 M phosphate. Simultaneously, aliquots of the dialyzed virus preparations were titered in saline in the usual manner.

The results of typical experiments are recorded in Table X. It will be noted that in each instance hemagglutination was completely suppressed in solutions of low electrolyte concentration. That this result was not due to an irreversible effect of the dextrose solution upon either the virus or the RBC was readily established. When the NaCl concentration of the virus and RBC suspension in dextrose was raised to 0.15 M, the hemagglutination titer of the virus was undiminished, and both adsorption and elution of the virus occurred in the usual manner. These experiments demonstrate that hemagglutination with influenza viruses, like that with PVM, can be completely inhibited if the electrolyte concentration is sufficiently low.

TABLE X

The Effect of Various Electrolytes upon the Hemagglutination Titer of Influenza Viruses

Virus dilutions and RBC prepared in solutions of*	Concentration	Hemagglutination titer *vs.* chicken RBC			
		PR8	FM1	Lee	B1103
	M				
Dextrose	0.25	0	0	0	0
Sucrose	"	"	"	"	"
NaCl	0.15	4096	512	2048	1024
KCl	"	2048	"	"	"
$MgCl_2$	"	1024	128	1024	2048
$LiNO_3$	"	"	512	"	"
Na_2HPO_4	"	512	256	4096	"
NaH_2PO_4	"	1024	4	4	0
Na_2SO_4	"	"	256	2048	2048

* Each solution contained 0.001 M phosphate.

The Effect of Various Electrolytes upon Hemagglutination with Influenza Viruses.—Because hemagglutination with influenza viruses was completely inhibited if the electrolyte concentration was held at 0.001 M but was demonstrable in unaltered degree upon the addition of 0.15 M NaCl, it was of interest to determine the effect of various other electrolytes upon the reaction.

Pools of infected allantoic fluid were dialyzed against water buffered at pH 7.2 with 0.001 M phosphate. Serial twofold dilutions were made in a solution of the desired electrolyte at 0.15 M and 0.001 M phosphate. To each dilution was added an equal volume of chicken RBC suspended in a solution of corresponding composition. The hemagglutination titers were then determined. Spontaneous agglutination of RBC in the solutions used was not observed.

The results of these experiments, as shown in Table X, demonstrate that, as in the case of PVM, hemagglutination with influenza viruses is not dependent upon the presence of any single ion or ion pair investigated but is a function of the electrolyte concentration. No detailed investigation of the variability shown in the hemagglutination titer in various electrolytes was undertaken. The unusually low titer of the Lee, FM1, and B1103 strains in 0.15 M NaH_2PO_4 is attributable to the fact that the hemagglutination titer of each of these strains was greatly diminished at pH 5.0, and the pH of the NaH_2PO_4 solution employed was 4.7.

The Effect of Low Electrolyte Concentration upon Combination between Influenza Viruses and RBC.—It was important to determine whether the results described above were due to a failure of the virus to combine with erythrocytes, or to a failure of erythrocytes which had combined with virus to agglutinate.

Infected allantoic fluids were dialyzed at 4°C. against distilled water buffered at pH 7.2 with 0.001 M phosphate. Dextrose was added to a concentration of 0.25 M. Packed RBC, washed in 0.25 M dextrose containing 0.001 M phosphate, were added to yield a 5 per cent suspension. The mixtures were held at room temperature and at intervals aliquots were removed, centrifuged, and the hemagglutination titer of the supernate measured. As a control, 0.15 M NaCl and sufficient RBC to yield a 5 per cent suspension were added to an aliquot of dialyzed virus.

The results of a typical experiment are recorded in Table XI. At 10 minutes all of the virus present in the control mixture had combined with the erythrocytes. In contrast, in dextrose solution, there was no significant reduction in the hemagglutination titer of aliquots removed at the indicated intervals. Similar experiments with dialyzed allantoic fluid infected with the PR8 strain gave identical results. Because the virus concentration was measured in twofold dilution series, as much as 50 per cent of the virus could have combined with the RBC without causing more than a one tube reduction in titer. In addition, it should be emphasized that the two reactions, *i.e.* combination and elution, occur simultaneously and at different rates.

In order to estimate more precisely the amount of virus which might have combined with chicken erythrocytes in dextrose, the following experiment was performed.

To a Lee virus preparation which had been dialyzed, as described above, was added dextrose to 0.25 M. Packed RBC, washed in buffered 0.25 M dextrose, were added to yield a 5 per cent suspension. The mixture was held at room temperature and at intervals of 10, 30, 60, and 120 minutes aliquots were removed and the RBC sedimented. The sediments were washed twice in dextrose and resuspended to volume in saline. The resuspended RBC were held at room temperature for 2 hours to allow dissocation of the virus to occur. The RBC were again sedimented and the hemagglutination titers of the supernates determined.

It was found that approximately 10 per cent of the virus originally present in the dextrose suspension was recovered from the RBC by spontaneous dis-

sociation in saline at each of the time intervals tested. Therefore, it appears evident that at low electrolyte concentrations influenza virus combines with erythrocytes in greatly diminished amount.

The effect of electrolyte concentration upon combination between RBC and influenza virus which had been treated so as to eliminate the phenomenon of elution (15) also was investigated.

Allantoic fluid, infected with the Lee strain, was heated at 56°C. for 30 minutes. The preparation then was dialyzed as in previous experiments. Dextrose was added to 0.25 M.

TABLE XI

The Effect of Non-Electrolyte Solutions on Combination between Influenza Virus and Chicken RBC

Mixture			Treatment of mixture		Hemagglutination titer of supernate *vs.* chicken RBC
Virus dialyzed *vs.* phosphate 0.001 M	Chicken RBC	Diluent	Held at room temperature for	2,000 R.P.M.	
	per cent		*min.*	*min.*	
Lee	0	NaCl 0.15 M	0	0	512
"	5	" " "	10	5	0
"	"	Dextrose 0.25 M, phosphate 0.001 M	"	"	512
"	"	" "	30	"	256
"	"	" "	60	"	512
"	"	" "	120	"	"
Lee heated*	0	" "	0	0	256
" "	5‡	" "	10	5	128
" "	"	" "	30	"	"
" "	"	" "	60	"	"
" "	"	NaCl added to 0.15 M	60 + 10	"	0

* 56°C. for 30 minutes after dialysis.

‡ Dialyzed *vs.* 0.25 M dextrose containing 0.001 M phosphate.

In order to maintain the pH as close to physiological limits as possible, chicken RBC were dialyzed at 4°C. against large volumes of 0.25 M dextrose buffered at pH 7.2 with 0.001 M phosphate. The pH of the resultant suspension was 6.8. The dialyzed RBC were then added to the virus preparation to yield a 5 per cent suspension. The mixture was held at room temperature and at intervals aliquots were removed, centrifuged, and the hemagglutination titer of the supernate measured. After 60 minutes NaCl was added to 0.15 M and the concentration of uncombined virus in the supernate measured after an additional 10 minutes.

The results of a typical experiment are shown in Table XI. It will be seen that influenza virus treated so as to eliminate elution also combined in greatly diminished amounts with chicken RBC at low electrolyte concentration. However, prompt combination resulted when 0.15 M NaCl was added to the mixture.

Influenza Virus–Erythrocyte Dissociation in Non-Electrolyte Solutions.—The effect of isotonic non-electrolyte solutions upon spontaneous dissociation of influenza virus from erythrocytes was determined. In two recent papers (14, 16) certain effects of various electrolytes and of electrolyte concentration upon the elution of influenza viruses from human RBC were reported.

To pools of allantoic fluid infected with the Lee strain were added RBC in buffered saline to yield a 5 per cent suspension. After 5 minutes the suspensions were centrifuged and the cells washed in unbuffered 0.25 M dextrose. One aliquot was resuspended to volume in a solution containing 0.15 M NaCl, 0.25 M dextrose, and 0.001 M phosphate. The other was resuspended to volume in 0.25 M dextrose containing 0.001 M phosphate. At intervals samples were withdrawn and after centrifugation the hemagglutination titer of the supernates was determined.

TABLE XII

The Effect of Low Electrolyte Concentration upon Dissociation of Influenza Virus from Chicken RBC

Mixture		Treatment of sediment			Hemagglutination titer of supernate *vs.* chicken RBC
Virus strain	Chicken RBC	Resuspended in	Held at room temperature for	2,000 R.P.M.	
	per cent	*M*	*min.*	*min.*	
Lee	5	0.15 NaCl 0.25 dextrose 0.001 phosphate	15	5	64
"	"	" "	45	"	128
"	"	" "	90	"	256
"	"	" "	120	"	"
"	"	0.25 dextrose 0.001 phosphate	15	"	16
"	"	" "	45	"	32
"	"	" "	90	"	64
"	"	" "	120	"	"
"	0	0	0	0	512

The results of typical experiments are presented in Table XII. It was found that, unlike PVM, influenza virus did not rapidly dissociate from erythrocytes upon reduction of the electrolyte concentration of the suspension. On the contrary the rate of spontaneous dissociation was considerably retarded at low salt concentrations. The results of similar experiments with the B1103 strain were identical. It is of interest that high salt concentrations have been found to increase the elution rate (17). A comparable reduction in the rate of elution of the Lee strain from human RBC in low electrolyte concentration

was also demonstrated. In order to determine whether or not the different effects of low electrolyte concentrations upon PVM- and influenza virus-RBC combinations were due to a difference in the properties of the virus strains or species of erythrocytes employed in these experiments, attempts were made to induce rapid dissociation at low electrolyte concentrations with PR8–chicken and human RBC combinations. In addition, the effect of low electrolyte concentrations upon combinations between human, chicken, and mouse RBC with Lee virus, heated at 56°C. for 30 minutes, was investigated. In each instance rapid dissociation comparable to that obtained with PVM–mouse RBC combinations, was not demonstrated. Moreover, with heated Lee virus low electrolyte concentrations caused no dissociation even though the mixture was held for 2 hours at room temperature. These findings support the concept that the marked differences in the reactions of PVM and influenza

TABLE XIII

The Relationship between Influenza Virus Concentration, Electrolyte Concentration, and Hemagglutination

Hemagglutinating units added	Electrolyte concentration, M									
	0.146	0.073	0.037	0.019	0.010	0.005	0.003	0.002	0.0015	0.0012
8	4*	3	3	1	±	0	0	0	0	0
32	4	3	3	3	3	3	2	0	0	0
128	4	4	3	3	3	3	3	3	2	0
0	0	0	0	0	0	0	0	0	0	0

* Degree of hemagglutination.

viruses with erythrocytes at low electrolyte concentrations are attributable to fundamental differences in the viruses themselves.

Relationship between Concentrations of Influenza Viruses and Electrolytes and Combination with RBC.—In order to assess the effects of varying the concentration of both influenza viruses and electrolytes relative to each other upon combination with RBC, the following experiment was performed.

Replicate serial twofold dilutions of a 1.2 M solution of NaCl were made and to each dilution series was added either 8, 32, or 128 hemagglutinating units of dialyzed Lee virus. Chicken RBC were then added to give 0.5 per cent suspensions. The RBC and virus suspensions, as well as the NaCl dilutions, were prepared in 0.25 M dextrose containing 0.001 M phosphate. After 1 hour the degree of hemagglutination in each mixture was recorded.

The results of a typical experiment are shown in Table XIII. It will be noted that, as indicated by the absence of hemagglutination, the electrolyte concentration at which influenza virus–RBC combination was inhibited was inversely related to the amount of virus present in the mixture. Similar

experiments were carried out with the PR8 and B1103 strains and in both instances an identical relationship was found. In experiments of this type with PVM the effect of virus concentration was much less striking. Whereas with influenza viruses a fourfold increase in the amount of virus allowed approximately a fourfold decrease in the electrolyte concentration, with PVM a 32-fold increase in the amount of virus permitted only about a twofold decrease in electrolyte concentration. It seems probable that the inverse relationship between virus and electrolyte concentration can be attributed to the effects of these variables upon the combination equilibrium with erythrocytes. However, it is possible that it reflects an inhomogeneity in the virus population itself. Recent evidence (17) suggests that such an inhomogeneity in one property of influenza virus particles may exist.

DISCUSSION

Combination between viruses and host cell components is a well known phenomenon (18) which has been studied by numerous investigators. It is well established that such combinations occur with bacterial (19), plant (20), and animal viruses (3, 21). One example of combination between certain animal viruses and host cells is provided by the hemagglutination reaction which during recent years has received intensive study (1, 6, 22–26).

That variation of electrolyte concentration causes striking effects upon the reactions between pneumotropic viruses and cell components appears evident from the results of this study. In earlier studies (14, 16) the effects of electrolytes on reactions between influenza viruses and human erythrocytes were reported. Under conditions of constant osmotic pressure, not only is hemagglutination with either PVM or the influenza viruses completely inhibited at low electrolyte concentration, but also adsorption of the viruses on erythrocytes is greatly diminished. Of more interest is the fact that dissociation of PVM from combination with either lung particles or erythrocytes is brought about at low electrolyte concentration (*i.e.*, 0.01 M). This occurs with infectious virus and also with virus which has been heated at 70°C. It is apparent, therefore, that in the case of PVM dissociation is not dependent upon the integrity of heat-labile components of the virus. In contrast, dissociation of influenza virus from erythrocytes is adversely affected even by very low concentrations of electrolytes (*i.e.*, 0.001 M); with infectious virus the rate of elution is definitely decreased; with virus heated at 56°C. no dissociation occurs. Because of this it seems probable that the forces which hold PVM in combination with cell components are different from those responsible for combination between influenza virus and cell components and it is evident that they are oppositely influenced by alterations in electrolyte concentration.

It should be emphasized that heretofore it has not been possible to cause dissociation of either PVM–lung particle or PVM–erythrocyte combinations

by any procedure other than those which both inactivate the virus and irreversibly alter the combining component (5). As a consequence, it was not possible previously to determine whether PVM shares with influenza and certain other viruses the peculiar capacity to alter the components with which it combines. That PVM does not alter the combining component of erythrocytes seems apparent from the results obtained. Free infectious virus can be caused to combine with and dissociate from erythrocytes repeatedly without measurably affecting their combining capacity. Thus, it appears that the enzyme-like activity of influenza viruses (6) finds no counterpart in the activities of PVM.

In this connection it seems pertinent to point out that in nature PVM is a latent agent which, it appears, does not induce manifest disease unless the equilibrium between virus and host is upset by experimental manipulation (27). It may be that the capacity of the agent to remain latent and to induce only inapparent infection under normal environmental conditions is related to the fact that the virus is incapable of dissociating spontaneously from combination with cell components. It seems evident that an agent which could not readily become separated from an intact cell might find difficulty in migrating to and initiating infection of another cell. The fact that uncombined PVM can be obtained from the intact lungs of experimentally infected animals (4, 5) is not evidence against this hypothesis. Not only does manifest infection with the virus lead to necrosis of lung tissue cells which undoubtedly results in the release of intracellular enzymes, but also the combining component of such cells is more readily destroyed by proteolytic enzymes than is the virus (4). Under these circumstances it would be expected that some free virus could be demonstrated in heavily infected intact lungs.

Both in the case of PVM (5) and the influenza viruses (28, 29) it appears that hemagglutination titrations provide a measure of the concentration of free or uncombined virus but give no direct indication of the amount of combined virus which may be present. In both instances also the hemagglutination reaction may be markedly affected by the composition of the medium in which it occurs. As regards the effect of low electrolyte concentrations, the combining reactions of PVM are approximately 10 times more sensitive than are those of the influenza viruses.

It is obvious that the low electrolyte concentrations which are required to cause inhibition of the reactions between the viruses and cell components under study are far outside of the physiological range and undoubtedly would not be encountered in living tissues. Nevertheless, by exceeding physiological limits, it has been possible to learn more of the factors which are operative in the physiological range. The results obtained suggest that the attractive forces which cause either PVM or influenza viruses to combine with particular cell components are highly active at physiological concentrations of electrolytes.

Moreover, they suggest that in the case of PVM such forces appear to be ionic in nature inasmuch as it is evident that they depend upon the ionic concentration and the pH. The findings are consistent with the supposition that the PVM–cell component complex is of the nature of a weak salt.

SUMMARY

Combination between PVM and erythrocytes as well as between the influenza viruses and erythrocytes is inhibited at low electrolyte concentrations. Combination between PVM and lung particles as well as between the virus and erythrocytes can be dissociated in solutions of low electrolyte concentration. The rate of elution of influenza virus is decreased under similar conditions. PVM can combine with and be dissociated from erythrocytes repeatedly without affecting the combining capacity of the cells and does not possess an enzyme-like activity similar to that of the influenza viruses. Because dissociation depends on electrolyte concentration and pH, it appears that the PVM–cell component complex may be in the nature of a weak salt.

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