FURTHER STUDIES ON THE ASSOCIATIVE REACTIONS OF PNEUMONIA VIRUS OF MICE (PVM) AND INFLUENZA VIRUSES

Combination with Various Animal Tissues and Adsorbents

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In earlier studies (1–6) it was demonstrated that pneumonia virus of mice (PVM) combines with erythrocytes of the mouse or hamster as well as with tissue components present in the lungs of certain mammalian species. With the methods employed a similar component capable of combining with the virus could not be demonstrated in any tissue of avian species studied nor in organs, other than the lungs, of mammals susceptible to infection with (PVM) (5). Moreover, although multiplication of the virus occurs in the lung tissues of susceptible mammalian species (7–9), attempts to infect avian embryos or extraembryonic tissues with PVM as well as to demonstrate multiplication of the virus in non-pulmonary tissues of infected mammals have been uniformly unsuccessful (2, 4, 5, 7). These findings indicated that, with the notable exception of mouse or hamster erythrocytes, there was an almost complete correlation between the presence of components which combine with the virus in a given tissue and the capacity to support multiplication of the agent. On this basis the hypothesis was considered (2, 4, 5) that the lung tissue component may be an important factor in the pathogenesis of pulmonary infection with PVM and that susceptibility to infection with the virus may be in part dependent upon the presence of a tissue component with which the virus can combine.

During the course of an investigation on the nature of the virus-combining component in lung tissue, methods were developed which were more delicate than those employed previously to measure the concentration of the component. When other tissues were reexamined with the aid of these techniques, it was found that a wide variety of organs contain tissue components which combine with PVM. Moreover, it was found that the virus can combine with certain adsorbents and then can be eluted from them. Adsorption and elution of influenza viruses also were demonstrated with the same adsorbents.

Materials and Methods

PVM.—Strain 15 of pneumonia virus of mice (PVM) (7) was used exclusively. It was maintained by occasional lung passage in albino Swiss mice and was stored as a 10 per cent

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suspension of infected mouse lungs at −70°C. Heat-released virus suspensions in distilled water were prepared as previously described (6) and were stored at 4°C. Prior to use, sufficient NaCl to yield a concentration of 0.15 M was added.

Influenza Viruses.—The PR8 strain of influenza A virus and the Lee strain of influenza B virus were used. They were maintained by passage in the allantoic sac of chick embryos.

Hemagglutination Titrations.—The technique of hemagglutination titrations with mouse or chicken RBC, utilizing serial twofold dilutions, and the method of estimating end points were identical to those previously employed (6).

RBC Suspensions.—Blood from mice or chickens was mixed with an acid-citrate dextrose solution (10) to yield a final concentration of 75 per cent. The mixtures were stored at 4°C. for periods not exceeding 1 week. As needed, the erythrocytes were washed 3 times in buffered saline and made up by volume to the desired concentration.

Normal Tissue Suspensions.—Normal mice, rabbits, or chickens were selected which possessed no demonstrable antibodies against PVM in their serum. After anesthetization the vascular system of mice was perfused with saline to free the tissues of as much blood as possible. Perfusion was not carried out with rabbits or chickens. Various organs were removed from each animal at autopsy and separate organs were ground in buffered saline to 10 per cent suspensions by weight in a modified Waring blendor. Grinding was continued for 2 minutes and the blendor was constantly cooled in an ice bath. Tissue suspensions were stored at −28°C. until used.

Adsorbents.—Various commercial adsorbents, enumerated below, were employed. Each adsorbent, in a concentration of 10 per cent by weight, was suspended in buffered saline and washed 3 times by centrifugation.

Solutions.—Throughout this paper buffered saline refers to 0.15 M NaCl solution buffered at pH 7.2 with 0.01 M phosphate, and water refers to distilled water. All pH values were determined with a Beckman pH meter.

EXPERIMENTAL

Virus-Combining Capacity of Lung Tissue.—In early studies (1, 2) the capacity of certain tissues to combine with PVM was demonstrated in mixtures containing relatively large amounts of both tissue and virus. Determination of the amounts of virus in the supernate and in the sediment from such a mixture gave some indication of the combining capacity of the tissue but did not permit satisfactory quantitation. Subsequently (5) a more precise procedure was devised; serial dilutions of a tissue suspension were mixed with constant small amounts of virus and the presence of free virus in the supernates was determined. The virus-combining titer of a tissue suspension could be determined in this manner. It was found (5) that, among various tissues studied with this procedure, only mammalian lung tissue suspensions showed a demonstrable capacity to combine with PVM. Employing the same procedure (5) evidence was obtained that the virus and mouse lung tissue combined in stoichiometric proportions. It should be pointed out that these studies of the capacity of various tissues to combine with PVM were carried out under definite experimental conditions; all mixtures contained 0.15 M NaCl; all supernates were obtained after centrifugation at 10,900 *g.* for 10 minutes (equivalent to 109,000 *g.* minutes).

Recently it was shown (6) that the virus-combining titer of normal mouse lung suspensions was directly related to the NaCl concentration of the mixtures. The results of various experiments carried out during the present study raised the possibility that the amount of centrifugation also might influence the result. This hypothesis was tested in the following manner:

To each of a series of twofold dilutions of a suspension of perfused normal mouse lungs in 0.15 M NaCl, 16 hemagglutinating units of PVM in 0.15 M NaCl was added. Following incubation at 37°C. for 30 minutes the mixtures were centrifuged and the supernates were tested for free virus by the hemagglutination technique with mouse RBC. The amount of centrifugation was varied over a wide range but other variables were kept constant. Mixtures of virus and 0.15 M NaCl solution were centrifuged simultaneously to control sedimentation of uncombined virus. With the amounts of centrifugation used there was no significant sedimentation of free PVM.

TABLE I

Effect of Centrifugation on PVM-Combining Titer of Normal Mouse Lung Suspension

Mixtures		Centrifugation			Agglutination with supernates *vs.* mouse RBC										PVM-combining titer of suspension
Normal mouse lung suspension in 0.15 M NaCl	Virus added to each dilution	Gravitational force	Time	Amount of centrifugation	Dilution of mouse lung suspension										
					16	32	64	128	256	512	1024	2048	4096	8192	
	units	*g.*	*min.*	*g. min.*											
Serial dilutions	16	10,900	10	109,000	0	1	2	3	3						16
" "	16	10,900	15	163,000	0	0	0	2	3						64
" "	16	15,500	10	155,000	0	0	0	0	2	3	3	3			128
" "	16	15,500	30	465,000	0	0	0	0	±	3	3	3	3	3	256
" "	16	15,500	60	930,000	0	0	0	0	0	0	0	±	3	3	2048

The results of a typical experiment are shown in Table I. It is evident that as the amount of centrifugation (the product of the gravitational force and time which is expressed in *g.* minutes) was increased, the apparent virus-combining titer of the suspension also increased. Because it is obvious that the actual capacity of the tissue to combine with virus could not be influenced by the amount of centrifugation, it is apparent that other factors were responsible for the results.

It will be recalled that in quantitative sedimentation studies (3) with mixtures of normal mouse lung suspensions and PVM, it was shown that when 92,100 *g.* minutes of centrifugation was employed approximately 30 per cent of the combined virus remained in the supernate. The addition of mouse RBC to such a supernate provides another component with which the virus can combine. If the attractive forces between lung tissue and virus are of the same order as those between RBC and virus, as studies (6) with varying electrolyte

concentrations suggest, it would be expected that when all three components are present RBC-virus combination, *i.e.* hemagglutination, should occur when RBC concentration exceeds lung tissue concentration. In other words, if RBC and lung tissue compete for virus, the result will be determined by the ratio of the concentrations of the combining components.

On this basis, the effect of the amount of centrifugation on the virus-combining titer is readily understood. Increasing amounts of centrifugation result in the sedimentation of increasing quantities of combined virus. As progressively less combined virus remains in the supernate, less virus is available for transfer to added RBC and hemagglutination fails to be demonstrable. The net effect is an apparent increase in virus-combining titer which is dependent upon a decrease in combined virus in the supernate. It is apparent from these results and those obtained previously (6) that both the demonstration of PVM-tissue combination and the measurement of virus-combining capacity are markedly influenced by the experimental conditions employed.

Virus-Combining Capacity of Various Tissues.—Because of the findings discussed in the preceding section, it appeared important to devise a more satisfactory technique for the study of PVM-tissue combination and to employ it in an investigation on a variety of tissues from various animal species. The procedure which was developed for this study takes advantage of the recently established facts (6) that PVM-tissue component complexes can be washed repeatedly in the presence of 0.15 M NaCl without undergoing dissociation and that dissociation occurs when the electrolyte concentration is reduced to 0.01 M or less.

Ten per cent suspensions in 0.15 M NaCl were prepared from a variety of tissues obtained from normal mice, rabbits, and chickens as described above. One cc. of each suspension was centrifuged at 4,850 *g.* for 5 minutes (24,250 *g.* minutes). The supernate was poured off and the sedimented tissue particles were resuspended in 1 cc. of a 10 per cent suspension of heat-released PVM. The mixture was held at 37°C. for 30 minutes and then centrifuged at 4,850 *g.* for 10 minutes (48,500 *g.* minutes). The supernate was removed, and the concentration of free virus was measured by the hemagglutination technique. The sediment was resuspended and washed twice in 5 cc. of buffered saline with centrifugation of 48,500 *g.* minutes at each step. The supernate from the second washing was tested by the hemagglutination technique to determine the presence of any free virus. Almost without exception there was no demonstrable virus in this supernate; in occasional instances titers of 1:4 were obtained. The washed sediment was resuspended in 1 cc. of distilled water, held for 30 minutes at 23°C., and centrifuged 327,000 *g.* minutes. Two-tenths cc. of the supernate was removed and the concentration of free virus was measured by the hemagglutination technique. The sediment was then resuspended in the supernate, heated at 70°C. for 30 minutes, and centrifuged 72,800 *g.* minutes. The hemagglutination titer of this supernate was also determined. In each experiment a suspension of heat-released PVM and saline was carried through all steps in the procedure as a control. In addition, a suspension of each normal tissue in saline was carried through all steps of the procedure.

The results of typical experiments are recorded in Table II. It will be seen that with perfused tissues of normal mice, sedimentable particles derived from

either lung or heart muscle combined with large amounts of PVM. Sedimentable particles obtained from all other mouse tissues tested were also capable of combining with the virus although in smaller amount. Particles obtained from ground mouse RBC, in contrast to intact RBC (2), combined with relatively small amounts of virus. With tissues of normal rabbits, particles

TABLE II

Combination of PVM with Sedimentable Particles Derived from Various Animal Tissues

Mixture of PVM and sedimented particles from		Hemagglutination titer of supernate	Amount of virus* adsorbed from supernate	Hemagglutination titer of supernate from washed particles resuspended in H_2O	Hemagglutination titer of supernate from washed particles heated 70°C. for 30 min.	Amount of virus* released from washed particles
Species	Tissue					
			units			*units*
Control mouse	Saline	512	0	0	0	0
	Lung	8	1260	128	128	320
	Heart	64	1120	128	256	640
	Liver	256	640	32	64	160
	Spleen	512	0	32	64	160
	RBC	512	0	8	16	40
	Kidney	512	0	16	32	80
	Muscle	512	0	4	16	40
Control rabbit	Saline	256	0	0	0	0
	Lung	32	560	256	256	640
	Heart	64	480	128	128	320
	Liver	64	480	256	256	640
	Spleen	256	0	64	128	320
	RBC	128	320	64	64	160
	Kidney	256	0	16	32	80
	Muscle	256	0	0	0	0
Control chicken	Saline	256	0	0	0	0
	Lung	256	0	0	0	0
	Heart	256	0	16	32	80
	RBC	256	0	0	0	0
	Muscle	256	0	0	0	0

* Computed on the basis that 1.0 cc. of virus was added to each preparation of tissue particles.

derived from lung, heart muscle, and liver combined with considerable quantities of the agent. Rabbit skeletal muscle was the only tissue tested which failed to yield particles with some virus-combining capacity. With the exception of particles from chicken heart muscle which showed virus combination in minor degree, tissue particles obtained from normal chickens possessed no demonstrable combining capacity.

It should be emphasized that in every instance tissues were obtained only

from animals which possessed no demonstrable antibodies against PVM in their serum. Moreover, in the case of mice the vascular system was perfused with large amounts of saline to remove as much blood as possible from the tissues. That the small quantity of RBC which remained in the tissues could account for the combination which occurred with various tissues is highly improbable in view of the results obtained with ground RBC themselves. Although stromata of mouse RBC combine with PVM (2), the technique employed in this study does not effectively sediment such stromata and during successive washings of the sediment they are discarded with the supernates.

That the hemagglutinating component which was released from the washed and resuspended tissue particles was in fact the virus is evident for a number of reasons. The component was not demonstrable in the supernates until the sediments were resuspended in distilled water, a procedure which results in dissociation of PVM from combination (6). Heating the released component at 70°C. for 30 minutes followed by 72,800 *g.* minutes of centrifugation caused no reduction in the hemagglutination titer which is consistent with earlier results obtained with PVM (2, 3, 6). In no instance was a hemagglutinating component released from control tissue particles which were mixed with saline and carried through the entire procedure.

The results of these experiments are, in a number of instances, different from those obtained in previous studies (2, 5) which indicated that among mammalian organs only the lung contained a component capable of combining with PVM. The development of a more delicate technique for determining the occurrence of combination appears to have been responsible for the present demonstration that tissue particles derived from numerous organs of the mouse and rabbit are capable of combining with the virus in some degree.

Combination of PVM with Adsorbents.—Because PVM can combine and be sedimented with particles derived from a wide variety of animal tissues, it appeared unlikely that some special tissue component is necessary for such combination to occur. More probable seemed the possibility that the virus was adsorbed by tissue particles in a physicochemical sense, and was eluted from them when the environment was altered, as by reducing the electrolyte concentration (6), increasing the pH (4), or upon heating (2). If this hypothesis were correct, it should be possible to demonstrate adsorption of the agent with one or another of the adsorbents commonly used in physicochemical studies. A number of experiments were carried out as follows:—

Adsorbents which are available from commercial sources, such as potato starch, various grades of celite and amberlite IRA-400, an ion exchange resin, were employed. The adsorbent was washed 3 times in buffered saline in the centrifuge and the final supernate was discarded. Two cc. of a suspension of heat-released PVM in 0.15 M NaCl at pH 6.8 was added to the packed adsorbent. The mixture was agitated by tilting to and fro for 10 minutes. The adsorbent then was sedimented in the centrifuge and the concentration of virus remaining in the supernate was measured by the hemagglutination technique.

As is shown in Table III, definite adsorption of PVM by celite 503, hyflo-super cel, potato starch, or amberlite IRA-400 was not demonstrable. However, adsorption of 90 per cent or more of the virus by super-cel, celite 505, filter cel, or analytical filter-aid was readily demonstrated. It is noteworthy that adsorption of PVM by various celites was obtained only with those grades which give slow liquid flow rates, a finding which is correlated with reduced particle size and increased total surface area of the adsorbent.

It was to be expected that the attractive forces which bind PVM to celite adsorbents could be influenced by changing the ionic environment and the pH. It was found that partial elution of the virus from celite could be achieved by raising the pH of the mixture to 9 or more with buffers.

TABLE III

Adsorption of PVM on Various Adsorbents

Mixture PVM and adsorbent		Hemagglutination titer of supernate
	gm.	
Saline	—	512
Celite 503	0.5	256
Hyflo super-cel	0.5	256
Super-cel	0.5	32
Celite 505	0.5	64
Filter-cel	0.5	0
Analytical filter-aid	0.5	8
Saline	—	256
Potato starch	1.0	128
Amberlite IRA-400	1.0	128

One-half gm. aliquots of celite analytical filter-aid were washed 3 times in buffered saline in the centrifuge, and the final supernates discarded. Two cc. of a suspension of heat-released PVM in 0.15 M NaCl at pH 6.8 was added to the packed adsorbent. The mixture was agitated by tilting to and fro for 10 minutes. The adsorbent was sedimented in the centrifuge and the concentration of virus in the supernate measured by the hemagglutination technique. The adsorbent was then washed twice in 5 cc. of buffered saline and resuspended in 0.1 M buffer solution of the desired pH. In no instance was virus demonstrable in the supernate from the last washing. The mixture was equilibrated by agitation for 10 minutes and then centrifuged. The concentration of virus in the buffer supernate was measured as before. Each buffer solution used was shown to be incapable of agglutinating mouse cells.

The results are recorded in Table IV. It will be seen that, although approximately 90 per cent of the virus was adsorbed by the grade of celite employed, no elution was demonstrable at pH 8 or below. At pH 9 and above, elution of approximately 10 per cent of the adsorbed virus was obtained. That elution was due to the alkaline pH and not to an ionic effect of the NH_3-NH_4Cl buffer

is indicated by the fact that glycine-NaOH buffers at pH 9 or 10 also caused elution of the virus in similar amount. That the hemagglutinating component released with alkaline buffers was actually the virus was established by specific serological identification of the agent with anti-PVM immune mouse serum. It is of interest that elution of PVM from celite at alkaline pH is analogous to dissociation of combined virus from lung tissue particles at pH levels of 10 or higher (4).

Combination of Influenza Viruses with Adsorbents.—Adsorption of influenza virus (PR8) on celite has been reported previously (11). Recovery of the virus was achieved by treatment with a concentrated solution of isinglass (1.2 to 6 per cent) which resulted in displacement of virus from the adsorbent.

TABLE IV

Elution of PVM from Celite

Mixture	Hemagglutination titer of supernate	Buffer		Hemagglutination titer of supernate from adsorbent resuspended in buffer
		Composition	pH	
PVM and saline................	512	—	—	—
PVM and celite,* 0.5 gm........	64	0.1 M NaH_2PO_4 + Na_2HPO_4	7	0
" " " " "........	64	" "	8	0
" " " " "........	64	0.1 M NH_3 + NH_4Cl	9	32
" " " " "........	64	" "	10	64

* Celite analytical filter-aid.

It appeared of interest to determine whether adsorption and elution of influenza viruses could be achieved by procedures comparable to those employed with PVM.

The technique employed was almost identical to that described above. One-half gm. aliquots of the desired adsorbent were washed 3 times in buffered saline in the centrifuge and the final supernates decanted. To the packed adsorbent was added 2 cc. of influenza virus-infected allantoic fluid which, although not dialyzed, had been adjusted to pH 7 with N/10 HCl prior to use. The mixture was agitated for 10 minutes, the adsorbent sedimented, and the amount of unadsorbed virus in the supernate measured by the hemagglutination technique. The sediment was washed 3 times in buffered saline, and elution of the virus produced by the addition of 2 cc. of 0.1 M NH_3–NH_4Cl buffer at pH 10 to the packed adsorbent. The mixture was agitated for an additional 10 minutes, sedimented as before, and the supernate tested in the usual manner.

As is shown in Table V, adsorption of PR8 by all grades of celite tested was readily demonstrated. Elution of as much as 50 per cent of the adsorbed virus was obtained by the technique employed. It will be noted that the amount of virus which was eluted under these conditions varied considerably depending

upon the grade of celite used. In studies on the pH range over which PR8 was eluted from celite analytical filter-aid, by methods similar to those described above, it was found that with 0.1 M buffers of NH_3-NH_4Cl or glycine-NaOH at pH 9, no elution of virus was obtained, while at pH 10 approximately 25 per cent of the adsorbed virus was eluted. Serological identification of the hemagglutinating agent eluted from celite analytical filter-aid with glycine-NaOH buffer was carried out with specific anti-PR8 sera. It should be noted that PR8 did not elute from celite analytical filter-aid at pH 9, whereas PVM was eluted at this pH. This difference is probably attributable to differences in the viruses themselves. Adsorption of Lee virus and similar partial elution

TABLE V

Adsorption and Elution of Influenza Virus (PR8) with Various Adsorbents

Mixture PR8 and adsorbent		Hemagglutination titer of supernate	Hemagglutination titer of eluate*
	gm.		
Saline	—	512	—
Celite 545	0.5	32	256
Celite 503	0.5	8	256
Hyflo-Super-cel	0.5	0	64
Super-cel	0.5	0	32
Celite 505	0.5	4	128
Filter-cel	0.5	0	32
Analytical filter-aid	0.5	0	128

* 0.1 M NH_3-NH_4Cl buffer pH 10.

at pH 10 from celite analytical filter-aid also were demonstrated by the same procedure.

In the experiments described above, the recovery of PVM or of influenza viruses by elution at alkaline pH was considerably less than theoretical. Attempts were made to obtain the release of more virus by the successive addition of fresh alkaline buffer.

Experiments were carried out as described above employing 0.5 gm. aliquots of celite analytical filter-aid and 2 cc. of the desired virus suspension. After washing the virus-celite combination 3 times in buffered saline, virus was eluted in 2 cc. of 0.1 M NH_3-NH_4Cl buffer at pH 10 in the usual manner. The celite was sedimented, the alkaline supernate decanted, and the eluted virus in the supernate measured by the hemagglutination technique. An additional 2 cc. of alkaline buffer was then added to the packed adsorbent and the elution procedure repeated. This process was repeated a third time.

The results are recorded in Table VI. It will be seen that with PVM and the influenza viruses employed each additional aliquot of pH 10 buffer which was equilibrated with the celite adsorbent appeared to contain approximately 50

per cent as much eluted virus as the preceding aliquot. Within the limits of precision of the titration techniques used, these results suggest that the adsorbed viruses tended to be distributed between the solid phase and the liquid phase in relatively fixed proportions; of the order of 10 to 25 per cent of adsorbed virus was eluted at each successive step.

TABLE VI

Successive Elution of PVM and Influenza Viruses from Celite

Material tested	Dilution of supernate								
	4	8	16	32	64	128	256	512	1024
PVM and saline	4*	4	3	3	3	3	3	2	0
Supernate after adsorption with 0.5 gm. celite‡	4	3	3	3	2	0			
Supernate after washing adsorbent	0	0	0	0					
First eluate, pH 10§	4	3	3	3	2	0			
Second " " "	4	3	3	2	0	0			
Third " " "	3	2	0	0	0	0			
PR8 and saline	4	3	3	3	3	3	3	2	0
Supernate after adsorption with 0.5 gm. celite	0	0	0	0					
Supernate after washing adsorbent	0	0	0	0					
First eluate, pH 10	4	3	3	3	3	2	0		
Second " " "	4	3	3	3	2	1	0		
Third " " "	4	3	3	2	0	0	0		
Lee and saline	4	4	4	3	3	3	3	2	0
Supernate after adsorption with 0.5 gm. celite	4	3	3	3	3	2	0		
Supernate after washing adsorbent	1	0	0	0					
First eluate, pH 10	4	3	3	3	3	2	0		
Second " " "	4	3	3	3	2	0	0		
Third " " "	4	3	3	2	0	0	0		

* Degree of hemagglutination.

‡ Celite analytical filter-aid.

§ 0.1 M NH_3-NH_4Cl buffer.

DISCUSSION

The results of experiments described in this paper demonstrate that sedimentable particles derived from a wide variety of animal tissues possess the capacity to combine with PVM, though in varying degree. The virus can be dissociated in almost theoretical amount from combination with tissue particles of each sort merely by reducing the electrolyte concentration of the mixture, as was shown previously with mouse lung particles and RBC (6). Adsorption of PVM on and elution from various grades of celite also can be achieved by appropriate control of the ionic environment of the mixture. These findings

suggest that combination of PVM with tissue particles is the result of adsorptive forces operative at the surface of such particles, and may not be attributable to union in a chemical sense between the virus and some specific "receptor" substance present only in the lungs and RBC of susceptible species, as was postulated previously (2, 4, 5). That viruses may combine with one or more substances present in susceptible cells and that such cellular components may play an important rôle in virus synthesis or multiplication is a concept which is not challenged by the results obtained in this study. However, it seems apparent that the combination reaction between PVM and substances derived from tissues does not give indications of yielding important information as to the identity of the substance which may be essential for the multiplication of the virus.

The demonstration that both PVM and influenza viruses are adsorbed on and can be eluted from various adsorbents raises the possibility that under appropriately controlled conditions considerable purification of these agents could be achieved by taking advantage of the extraordinary properties of adsorbents in separative procedures. In preliminary experiments, not reported in detail in this paper, it has been found that not all the numerous substances present in a virus suspension were adsorbed under the conditions described in the experimental section. Moreover, only part of the substances which were adsorbed on celite were eluted at pH 9 or 10. The eluate, which in the case of influenza viruses contained between 25 and 50 per cent of the virus originally present, gave evidence of definite purification especially as regards reduction in non-virus protein. In other experiments in which either PVM or PR8 was adsorbed on celite packed in a column in a manner similar to that previously reported (12), elution of virus through the column was achieved with alkaline buffers. The extent to which either virus could be purified on a flowing chromatogram under practical conditions remains to be determined.

SUMMARY

Tissue particles from a wide variety of animal tissues possess the capacity to combine with PVM. Various adsorbents also combine with the virus. Elution of PVM from combination with either tissue particles or adsorbents can be achieved by appropriately altering the ionic environment. Influenza viruses also are adsorbed on and eluted from adsorbents under similar conditions. Reasons are presented for thinking that combination of PVM with tissue particles results from the action of adsorptive forces between virus and tissue particles.

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