QUALITATIVE DIFFERENCES IN THE ANTIGENIC COMPOSITION OF INFLUENZA A VIRUS STRAINS

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Antigenic differences among strains of influenza A virus have been demonstrated by means of cross neutralization, immunity (1–7), agglutination inhibition (8–12), and complement fixation (13) tests. These studies indicate that there are antigens which are common to all of the strains, but that certain strains contain very different proportions of the antigenic components. The present paper reports a study of the antigenic composition of several strains of influenza A virus by means of specific antibody absorption tests. Evidence will be presented which indicates that each contains qualitatively distinct antigens.

Antibody absorption tests with influenza virus have been reported by several groups of workers. Smorodintsev *et al.* (14) showed the relationship of the Leningrad strain of influenza virus to the W.S. virus by absorbing neutralizing antibodies from a W.S. immune serum with a mouse lung emulsion containing the Leningrad strain of virus. Stuart-Harris *et al.* (4) could demonstrate absorption of antibody from a W.S. serum with homologous virus suspensions only if the serum was first diluted almost to the limit of its efficacy. Burnet (2) found that serum antibody demonstrable by the egg membrane technique could be readily absorbed with influenza virus, but usually no significant reduction was apparent by mouse titration. Titrated by the egg membrane technique strains Melbourne, Philadelphia, and PR8 absorbed antibody from a Melbourne antiserum, whereas strains swine, W.S., Moscow, and Leningrad showed no detectable absorption. Smith and Andrewes (7) reported absorption of W.S. serum antibody (horse, ferret, or rabbit sera) with the very potent W.S. virus. They were much less successful with swine, Talmey, Philadelphia, or Bur. viruses and their homologous rabbit or ferret antisera. They did succeed, however, in absorbing the antibodies against W.S. virus contained in Talmey, Philadelphia, and Bur. antisera with W.S. virus.

Methods

Virus Suspensions.—Six strains of influenza A virus—PR8 (15), W.S. (16), swine[1], Talmey, Christie (7), and Dra. 2 (8)—and the Lee strain (17) of influenza B virus were used in this work. Suspensions of the virus strains were prepared by inoculating infected allantoic fluid in dilutions of 10^{-3} or 10^{-4} into the allantoic sac of 11 day old

[1] Strain 1976 obtained from Dr. R. E. Shope.

chick embryos. After incubation at 37°C. for 48 hours, the eggs were placed at 4°C. overnight, and blood-free allantoic fluid was removed the following morning. The fluids were cleared by a short centrifugation at 2,000 R.P.M. and stored at −72°C. until used.

Immune Sera.—Ferrets were inoculated intranasally with a 10^{-3} dilution of allantoic suspensions of the virus strains. The animals were bled before inoculation and 2 weeks after infection. Human sera were obtained before and 2 weeks after infection or following vaccination. The sera were stored at 4°C., and all were inactivated at 56°C. for 30 minutes before use in the tests.

Absorption of Antibody.—Immune serum diluted 1:16 to 1:32 with 0.9 per cent saline solution buffered at pH 6.9 with M/50 phosphate, was mixed with undiluted allantoic fluid suspensions of virus or with concentrated virus suspensions obtained from allantoic fluid by high-speed centrifugation or by the red cell adsorption and elution technique (18). The serum-virus mixtures were incubated at 37°C. for 30 minutes and kept at 4°C. overnight. They were then spun at 11,000 R.P.M. for 30 minutes in an angle centrifuge to remove most of the virus, and the supernatant fluid was filtered through graded collodion membranes of 600 mμ average pore diameter. The membranes were provided by Dr. J. H. Bauer of this laboratory. The filtrates were finally cleared of virus by passage through 100 mμ membranes. As previously reported (19), filtrates obtained in this way contained no virus demonstrable by infectivity or agglutination tests. Control mixtures of immune serum and buffered saline were concurrently treated in identical manner. The control mixtures showed a small loss in serum antibody as a result of the filtration, but the decrease was usually not more than 15 per cent of the original concentration. The absorbed sera were kept at −72°C. until used. They were then tested for residual antibody by means of the standard agglutination inhibition and complement fixation tests. In some experiments the mouse neutralization test was also done.

Agglutination Inhibition Test.—Serum antibody titrations by this method were made by a standard technique already fully described (20). A final concentration of 4 agglutinating units of virus was used in each test by performing preliminary hemagglutinin titrations with the same chicken red blood cells to be employed in the final test. The virus was again titrated in the final test, and any deviation from 4 units was compensated by correcting the final antibody titers obtained as described by Hirst (8). The titers are expressed as the reciprocal of the serum dilution (final concentration after adding all reagents) inhibiting agglutination of 50 per cent of the red cells under the standard conditions of the test.

Complement Fixation Test.—Dilutions of serum were mixed with 2 fifty per cent hemolytic units of complement (titrated in the presence of antigen used in the test) and allantoic fluid virus suspensions in dilution of 1:5 as antigens, according to a technique previously described (13). The titers are expressed as the reciprocal of the initial serum dilution producing hemolysis of 50 per cent of the sheep red cells as recorded by a photoelectric densitometer. The final dilution of serum after addition of antigen and hemolytic system would be 6 times that given.

Neutralization Test.—Serum dilutions in steps of $10^{-0.5}$ were mixed with allantoic fluid containing about 31 M. L. D. of virus. All dilutions were made with 10 per cent

horse serum broth. The mixtures were kept at 4°C. overnight[2] and the following day 0.05 cc. samples of each dilution were instilled intranasally into groups of 6 albino Swiss mice under light ether anesthesia. The 50 per cent mortality titer was calculated from the number of mice which died within 10 days after inoculation.

EXPERIMENTAL

Absorption of Homologous Ferret Serum Antibodies with Influenza Virus.— In a first experiment to learn whether serum antibody could be removed by absorption with allantoic fluid containing influenza virus, a PR8 and a Lee ferret serum in dilution of 1:20 was mixed in equal parts with buffer solution, PR8, Lee, and normal allantoic fluids. The mixtures were incubated, centrifuged, and filtered as described under Methods. Fig. 1 shows the amount of residual antibody as determined by the agglutination inhibition test in which the homologous virus strain was used on the test virus. The PR8 allantoic fluid absorbed 79 per cent of the antibody in the PR8 ferret serum, whereas the Lee and normal allantoic fluids failed to remove any detectable amount of antibody. Conversely, only the Lee allantoic fluid absorbed antibody (85 per cent) from the Lee ferret serum.

To demonstrate further the specific absorption of antibodies with influenza virus, PR8, W.S., and swine-ferret antisera were absorbed with large amounts of PR8, W.S., swine, and Lee viruses concentrated from allantoic fluid by high-speed centrifugation.

Forty-five cc. portions of PR8, W.S., swine, Lee, and normal allantoic fluids were centrifuged at 30,000 R.P.M. for 45 minutes in the vacuum centrifuge, and the supernatant fluids were discarded. The pellets of sediment, which contained most of the virus, were resuspended in 15 cc. aliquots of PR8, W.S., and swine-ferret sera, all in dilution of 1:32. The mixtures were incubated, centrifuged, and filtered as described under Methods. A second absorption of a portion of the sera (8 cc.) was carried out as before with the sediment from 45 cc. of the various allantoic fluids. All of the filtrates were then tested for residual antibody by means of neutralization, agglutination inhibition, and complement fixation tests. The serum specimens were tested against the homologous virus in each instance.

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[2] In unpublished experiments in collaboration with Dr. G. K. Hirst it was found that the neutralization of allantoic fluid suspensions of influenza virus with immune serum was more complete when the mixtures were left overnight at 4°C. than after incubation at 37°C. for 1 hour. With short incubation at 37°C. a given serum became less efficient in its capacity to neutralize virus as it was used in progressively greater dilution, just as reported by Horsfall in tests with mouse lung extracts (21). When the same serum-virus mixtures were kept overnight at 4°C., this effect of dilution on the combining capacity of antibody was greatly reduced, and the relation between the quantity of virus neutralized and quantity of serum used closely approximated one of multiple proportions over a wide range of virus and serum concentrations.

The results of the neutralization test are shown in Fig. 2. In general it will be seen that the antibody in each serum was completely removed only by the

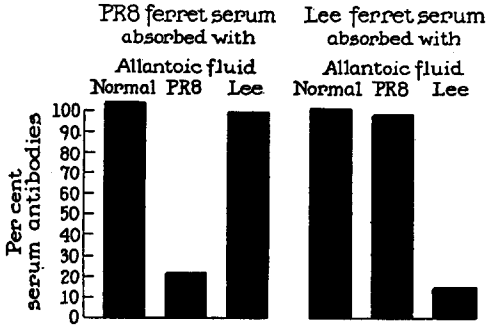

FIG. 1. Agglutination inhibition tests with PR8 and Lee ferret antisera after absorption with normal, PR8, and Lee allantoic fluid. The amount of antibody remaining after absorption is shown in terms of per cent of the antibody content of the control serum buffer mixtures (see text). The PR8 serum was tested for inhibition of agglutination of PR8 virus and the Lee serum was tested against Lee virus.

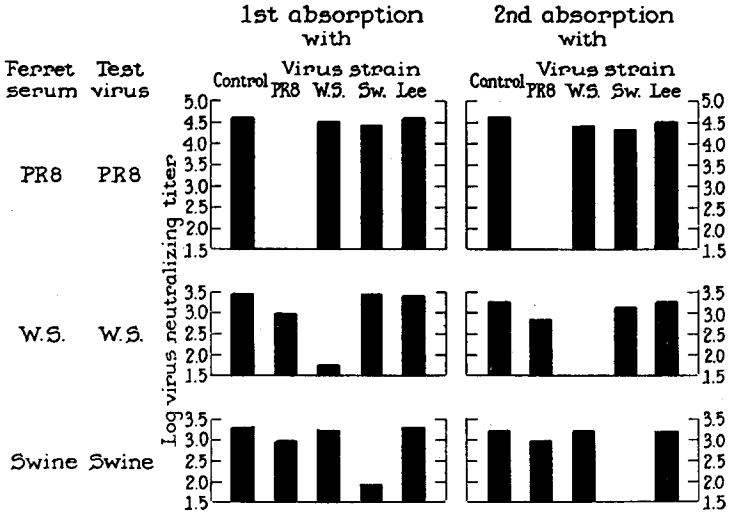

FIG. 2 Neutralization tests with ferret antisera absorbed twice with the sediment from normal (control) and virus-infected allantoic fluids (see text). The antisera were tested for capacity to neutralize about 31 M. L. D. of the homologous virus.

homologous virus strain, even though 2 absorptions were carried out with large amounts of heterologous virus strains. For example, most of the W.S. antibodies were removed on the first absorption with W.S. virus, whereas PR8 virus caused only partial absorption and the swine and Lee viruses had no detectable

effect on the antibody level. The second absorption with W.S. virus removed all of the remaining antibody from the W.S. serum, but the heterologous strains had no further detectable effect. The PR8 virus removed all of the detectable antibody from the PR8 serum on the first absorption. The agglutination inhibition and complement fixation tests gave results similar to those obtained with the neutralization test. These tests are more sensitive to small changes in antibody concentration than the neutralization test, and partial absorption of antibody with the heterologous A strains was more apparent as shown later in Fig. 4. However, the significance of small changes in antibody level following absorption is somewhat uncertain because of the necessity of using a different collodion membrane for filtration of each serum-virus mixture. Nevertheless, the failure of the second absorption with heterologous virus strains to remove further antibody from the ferret sera strongly suggests complete absence of virus antigen related to the unabsorbed antibody.

Manifestly the absorption tests revealed a high degree of specificity and qualitative individuality of the PR8, W.S., and swine strains of influenza virus. Each virus completely absorbed the neutralizing, agglutination-inhibiting, and complement-fixing antibodies from the homologous ferret serum, but 2 absorptions with large amounts of virus failed to remove the antibodies in heterologous sera.

Quantitative Relations of the Absorption of Neutralizing, Agglutination-Inhibiting, and Complement-Fixing Antibodies.—Evidence already obtained has indicated that the infective activity and the property of influenza virus to agglutinate red blood cells are closely associated (8, 18). The complement-fixing antigen has been found to consist of a fraction intimately associated with the virus particle and another fraction of a smaller size which is separable from the virus particle—the so called soluble antigen (13). It seemed possible that absorption of the antibodies directed against influenza virus suspensions might throw further light on the relationship of these properties of the virus particle. In the next experiment a serum from a ferret convalescent from PR8 virus infection was absorbed with varying amounts of PR8 virus obtained by the red cell adsorption and elution technique.

Three hundred cc. of PR8 allantoic fluid was mixed with 3 cc. of chicken red cells which had been washed 3 times and packed by light centrifugation. The mixture was kept at 4°C. for 30 minutes to permit adsorption of the virus, and the cells were then removed by centrifugation. The cells were resuspended in 100 cc. of buffered saline solution, and elution of the virus was carried out at 37°C. for 2 hours. The red cells were then discarded after centrifugation. One hundred cc. of the eluate was again adsorbed with 1 per cent red cells and the virus eluted into 10 cc. of buffered saline solution as before. The original allantoic fluid had an agglutination titer of 1:479 and an infectivity titer of $10^{-6.0}$, whereas the final eluate had an agglutination titer of 1:5420 and infectivity titer of $10^{-6.9}$. These findings indicate that only 38 per cent of the original hemagglutinin and 26 per cent of the infectivity were recovered

by this method. The loss in virus could not be accounted for in the supernatant fluids following adsorption and hence it appeared to be due to failure of much of the virus to elute from the red cells.

Four cc. aliquots of a PR8 ferret serum in dilution of 1:16 was mixed in equal parts with the above eluate undiluted and in dilutions of 1:2, 1:4, 1:8, and 1:16. The mixtures were incubated, centrifuged, and filtered as in the preceding experiment. In addition each filtrate was again passed through another 100 mμ membrane. The filtrates were then tested for residual antibody by means of neutralization, agglutina-

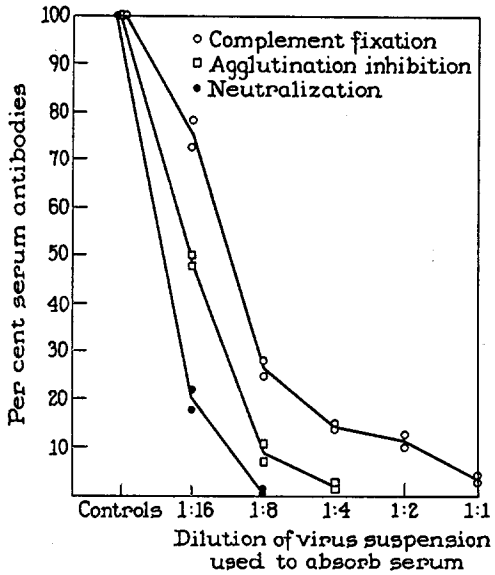

FIG. 3. Absorption of a PR8 ferret serum with varying amounts of a concentrated suspension of PR8 virus prepared by the red cell adsorption and elution technique (see text).

tion, and complement fixation tests. Each test was done in duplicate at different times.

Fig. 3 shows the results of the experiment in terms of per cent of the concentration of antibody in the control serum mixture. The amount of antibody remaining in the serum, as determined by all 3 tests, varied inversely as the concentration of virus used for absorption. A given concentration of virus, however, removed more neutralizing antibody than either agglutination-inhibiting or complement-fixing antibodies. This was particularly striking when the dilute concentration of virus (1:16) was used for absorption—80 per cent of the neutralizing antibody was removed, whereas 50 per cent of the agglutination-inhibiting and only 25 per cent of the complement-fixing antibody were

absorbed by the virus. These differences were somewhat smaller when more concentrated virus was used for absorption. The possible significance of these

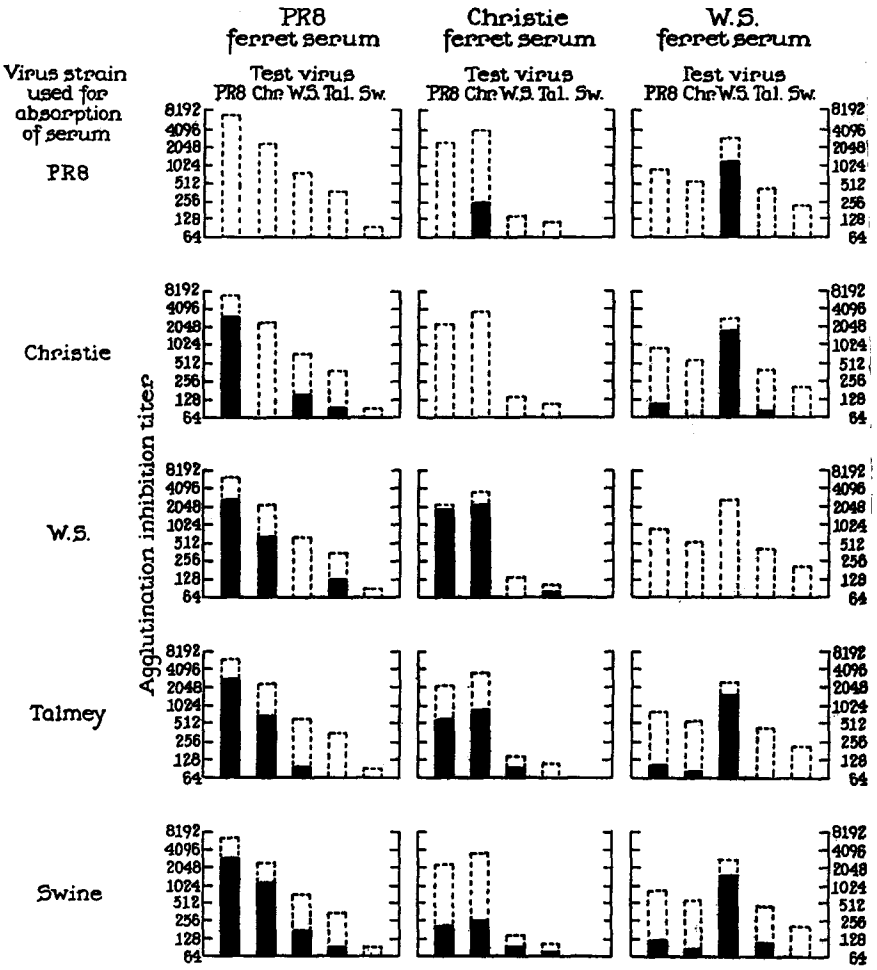

FIG. 4. Absorption of homologous and heterologous antibodies from PR8, Christie, and W.S. ferret antisera with various virus strains.

differences in the rate of absorption of the antibodies detectable by the 3 tests will be considered in the discussion.

Absorption of Homologous and Heterologous Ferret Serum Antibodies.—The preceding experiments have dealt primarily with absorption of the antibodies in ferret sera which react with the homologous virus strain used to elicit the

antibodies. Absorption of the antibodies in these sera which react with heterologous virus strains was next investigated.

PR8, Christie, and W.S. ferret antisera were diluted to 1:16 with buffered saline. Ten cc. aliquots were mixed with the virus sediment, obtained by centrifugation at 13,000 R.P.M. for 45 minutes, from W.S., swine, Talmey, and Christie allantoic fluids. The mixtures were incubated, centrifuged, and filtered as in the preceding experiments. A control serum sample containing buffered saline instead of virus sediment was treated in identical manner. The filtrates were then tested for residual antibody by means of agglutination inhibition and complement fixation tests using PR8, W.S., swine, Talmey, and Christie allantoic fluids as the test viruses.

The results of the complement fixation and agglutination tests were similar, and hence only the latter are shown in Fig. 4. In each instance the antibodies in the PR8, Christie, and W.S. sera were completely absorbed only by the homologous virus strain. PR8 virus absorbed all of the heterologous antibodies from the Christie and W.S. sera but failed to remove completely the homologous antibodies. The other virus strains differed from PR8 virus in that each absorbed completely only those heterologous antibodies which reacted with the particular strain used for absorption. A possible exception to this were the swine antibodies which were absorbed by all of the virus strains, but the initial titers were so low in each serum that the extent of reduction by absorption could not be determined.

The experiment illustrates the complex antigenic composition of the influenza virus strains. Absorption of a serum with large amounts of a heterologous virus usually left antibodies which reacted with several other heterologous as well as the homologous virus strain. PR8 virus appeared to have the broadest antigenic pattern of the strains tested, yet it lacked certain components possessed by the Christie and W.S. viruses.

Absorption Tests with Human Sera

It has been repeatedly shown that the specificity of strains of influenza virus is much less apparent in their cross reactions with human sera than with animal sera obtained after a single injection of a strain of virus. Horsfall and Rickard (22) could demonstrate no strain specificity in the sera of 5 persons infected with influenza A. Bodily and Eaton (23), however, studied a larger number of sera from vaccinated and convalescent persons and found a limited amount of antibody specificity for strains of influenza virus. It was of interest, therefore, to utilize absorption tests in the study of the specificity of the antibody response of human beings following vaccination and following infection with influenza virus.

Postvaccination Sera.—Human sera taken before and 2 weeks after vaccination with influenza virus were available for study. The vaccine used consisted

TABLE I

Human Serum Antibody Response to Vaccination with PR8 Virus

Patient No.	Test virus strain	Serum antibody titer*				Antibody rise‡	
		Agglutination inhibition		Complement fixation		Agglutination inhibition	Complement fixation
		Pre-	Post-	Pre-	Post-		
	PR8	135	3,520	14	388	**3,385**	**374**
	Christie	156	2,684	16	223	2,528	207
1	W.S.	124	2,164	15	194	2,040	179
	Talmey	66	1,053	15	208	987	193
	Swine	97	718	13	120	621	107
	PR8	102	1,845	11	208	**1,743**	**197**
	Christie	223	1,092	14	128	869	114
2	W.S.	100	863	12	42	763	30
	Talmey	76	651	16	147	575	131
	Swine	167	359	23	104	192	81
	PR8	90	2,408	6	223	**2,318**	**216**
	Christie	97	1,614	4	112	1,517	108
3	W.S.	70	1,060	5	60	990	55
	Talmey	14	695	4	158	681	154
	Swine	32	236	3	24	204	21
	PR8	310	2,864	28	388	**2,554**	**360**
	Christie	312	1,337	26	128	1,025	102
4	W.S.	187	568	22	60	381	38
	Talmey	100	1,388	28	338	1,288	310
	Swine	254	441	42	64	187	22
	PR8	178	2,200	15	223	**2,022**	**208**
	Christie	136	1,092	13	91	956	78
5	W.S.	100	530	13	60	430	47
	Talmey	26	608	11	194	582	183
	Swine	73	507	10	97	434	87
	PR8	166	1,336	14	120	**1,170**	**106**
	Christie	146	670	12	28	524	16
6	W.S.	132	1,060	15	91	828	76
	Talmey	32	282	11	49	250	38
	Swine	254	668	28	104	414	76
	PR8	72	1,080	7	104	**1,008**	**97**
	Christie	78	545	6	32	467	26
7	W.S.	38	748	6	74	710	68
	Talmey	25	151	6	49	126	43
	Swine	84	312	7	39	228	32
	PR8	72	940	8	97	**868**	**89**
	Christie	78	545	11	22	467	11
8	W.S.	47	326	8	24	279	16
	Talmey	13	246	7	64	233	57
	Swine	39	118	7	16	79	9

* Titers are expressed as the reciprocal of the serum dilution end point (see Methods).

‡ Obtained by subtracting the pre- from the postvaccination titer.

of formolinized PR8 and Lee influenza viruses which had been concentrated from freshly thawed allantoic fluid according to the method described by Hirst, Rickard, and Whitman (24). Sera from 8 patients were taken at random and tested for antibody response by means of cross agglutination inhibition and complement fixation tests against PR8, Christie, W.S., Talmey, and swine viruses. The results are summarized in Table I. It will be seen that the

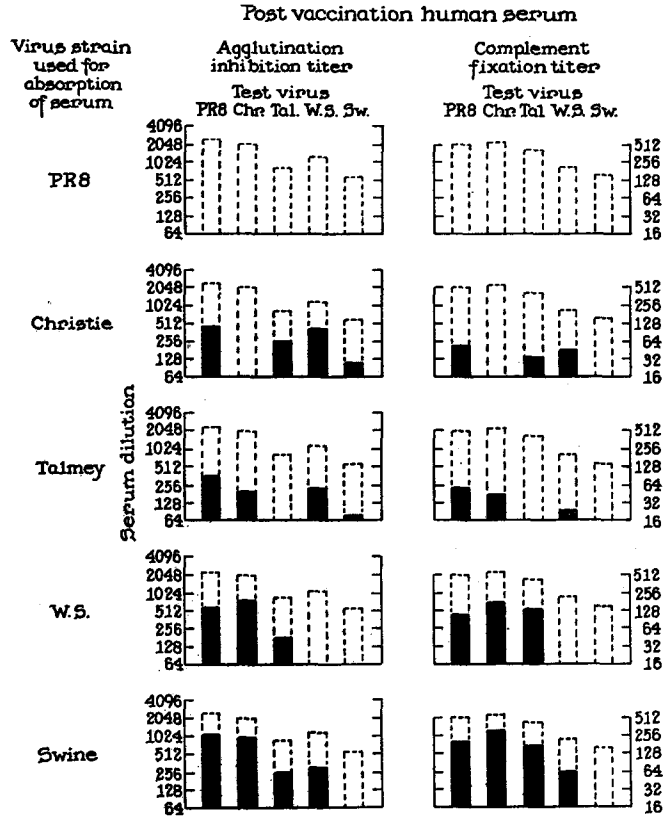

FIG. 5. Absorption of a human serum obtained 2 weeks after vaccination with PR8 virus.

vaccine elicited a rise in serum antibodies against all of the virus strains tested. The antibody rise in each individual, obtained by subtracting the pre- from the postvaccination titers, was consistently highest against PR8 virus, although the amount of antibody elicited by some of the other strains was only slightly less in some instances. The cross tests, therefore, indicated a limited degree of specificity in the total antibody response to vaccination. In general the results obtained by the agglutination inhibition and the complement fixation test showed a close parallelism.

The postvaccination serum from patient 1 (Table I) was selected for absorption because of the small differences in antibody titer against PR8, Christie, and W.S. viruses. The serum in dilution of 1:32 was absorbed with the sedimented virus from 45 cc. lots of PR8, Christie, Talmey, W.S., and swine allantoic fluids as described in the preceding experiment. The results of the

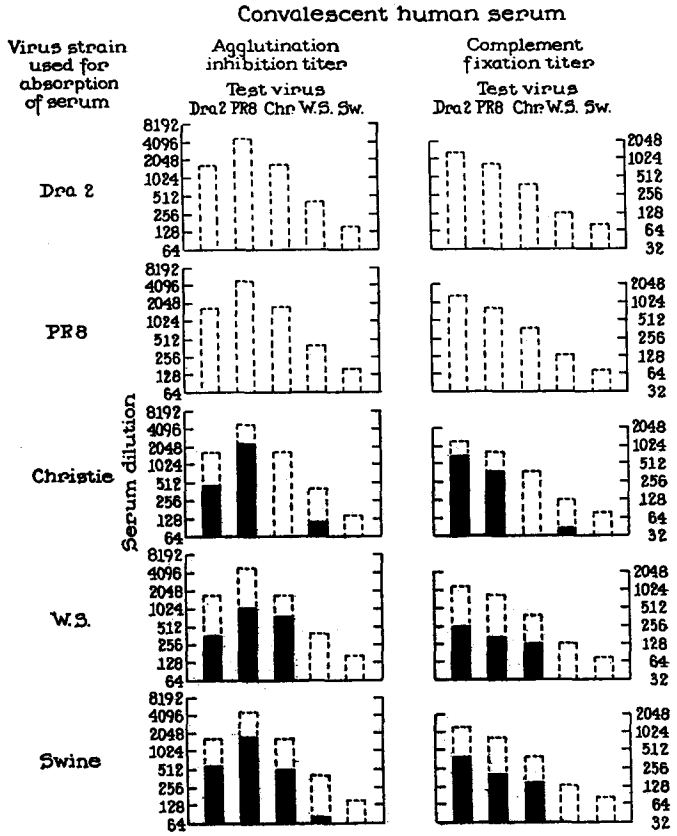

FIG. 6. Absorption of a human serum obtained 2 weeks after an attack of influenza. The virus strain Dra. 2 was isolated from the patient during the acute illness.

experiment (Fig. 5) were similar to those obtained on absorption of PR8 ferret serum (Fig. 4). PR8 virus removed all of the antibodies which reacted with the homologous and the heterologous virus strains, whereas the other virus strains absorbed only the antibodies directed against the virus used for absorption and in most instances the antibodies which reacted with swine virus. Similar results were obtained on absorption of the postvaccination serum from patient 4 (Table I), and the findings need not be given in detail.

Convalescent Serum.—In the next experiment a serum obtained from a

patient convalescing from an attack of influenza was absorbed as described in the preceding experiment. In addition the virus strain (Dra. 2), which was obtained from the patient's throat washing during the acute illness, was also used for absorption of the serum. Fig. 6 shows the result of the test. The virus strain isolated from the patient (Dra. 2) and PR8 virus absorbed all of the antibodies against the test viruses employed. Christie, W.S., and swine viruses absorbed only the swine antibodies and the antibodies which reacted with the virus used for absorption. Although the PR8 and Dra. 2 virus strains are closely related, as shown in this experiment, further tests with ferret antisera indicated minor antigenic differences.

DISCUSSION

The foregoing experiments clearly show that antibody absorption tests provide a valuable tool for studying the antigenic composition of influenza viruses. While much more remains to be done to obtain a clear picture of the complex antigenic interrelationship of the different virus strains, some new facts have already emerged which permit a more precise evaluation of this problem. Both by absorption and by the previously employed techniques the influenza A and B groups of viruses stand out as distinct entities with no overlapping or related antigens. In respect to the antigenic comparisons within the influenza A group, the cross reactions between the virus strains previously reported brought out the fact that the quantitative distribution of antigens common to various strains was more or less different. On the basis of cross absorption tests we may now further conclude that there are qualitative antigenic distinctions as well. The PR8 virus strain, for example, contains an antigen (or antigens) which is not possessed by the Christie, Talmey, W.S., or swine virus strains.

Whether or not the distinctive antibodies, demonstrable after 1 or 2 absorptions of a serum with a heterologous virus, represent the response to a "strain specific" antigen (*i.e.*, an antigen possessed only by the particular strain) cannot be deduced from the available data. The fact that absorption of a serum with large amounts of a heterologous virus usually left polyvalent antibody (*i.e.*, able to react with other heterologous as well as the homologous strains) would suggest that at least a portion of the residual unabsorbed antibody may have been produced by antigens common to other virus strains. PR8 virus provided an exception for it absorbed all of the heterologous antibodies from Christie and W.S. antisera and left only those which reacted with the respective homologous strains. In order to demonstrate true strain specificity of these unabsorbed antibodies, however, it would be necessary to show that successive absorptions with large amounts of a number of heterologous strains failed to remove completely the antibodies directed against the homologous strain.

Smith and Andrewes (7) found that 4 strains of influenza A virus (W.S., Christie, Talmey, and Gatenby) exhibited large quantitative differences in the homologous and heterologous antibody response demonstrable by means of

cross neutralization tests. PR8 virus was found to be an "intermediate" strain reacting in high titer with both Christie and Talmey viruses. The present absorption tests revealed a higher degree of specificity and individuality of the virus strains than could have been anticipated by their cross reactions. PR8 and Christie viruses, for example, appear to be very closely related in cross tests (neutralization, agglutination inhibition, complement fixation) yet absorption tests clearly show that each contains antigens which are not shared by the other. The absorption tests confirmed the finding of Smith and Andrewes (7) that the Christie and Talmey strains are antigenically distinct, although Burnet and Clark (9) could demonstrate no difference between the 2 strains.

The specificity of the PR8 virus strain was equally manifest in absorption tests with ferret sera and with human sera following vaccination. The latter finding is particularly significant because human sera show much less difference in antibody levels against homologous and heterologous virus strains than animal sera. If the quality of the antibodies called forth by different virus strains proved to be an important factor in immunity to human infection with influenza virus, it would seem desirable to classify influenza virus strains on the basis of the number and kind of available antigenic components. Of the virus strains studied in the present work PR8 virus would appear to be the strain of choice in evoking immunity, for it has the broadest range of specific antigenic components (Figs. 4 and 5) and elicits high titers of antibodies against a variety of different strains.

The absorption tests provide further evidence that the hemagglutinin, complement-fixing antigen, and infective activity of influenza virus are closely related properties of the virus particle *per se*, though not necessarily identical. A suspension of PR8 virus particles, obtained by the red cell adsorption and elution technique, concurrently absorbed the neutralizing, agglutination-inhibiting, and complement-fixing antibodies from a PR8 ferret serum (Fig. 3), and the amount of antibody removed in each instance was inversely proportional to the concentration of virus used for absorption. However, a greater proportion of neutralizing antibodies was absorbed by a given concentration of virus than either agglutination-inhibiting or complement-fixing antibodies. It seems possible that the neutralizing antibodies are elicited chiefly by the surface components of the intact virus particle and hence are more readily absorbed by a suspension of virus particles than the agglutination-inhibiting or complement-fixing antibodies which appear to be directed not only against the primary virus particle but also against disintegration products of the virus (19).

SUMMARY

A study of the PR8, Christie, Talmey, W.S., and swine strains of influenza A virus by means of antibody absorption tests revealed the following findings:

1. Serum antibody could be specifically absorbed with allantoic fluid contain-

ing influenza virus or, more effectively, with concentrated suspensions of virus obtained from allantoic fluid by high-speed centrifugation or by the red cell adsorption and elution technique. Normal allantoic fluid, or the centrifugalized sediment therefrom, failed to absorb antibodies. Influenza B virus (Lee) caused no detectable absorption of antibody from antisera directed against influenza A virus strains, but it specifically absorbed antibody from Lee antisera.

2. The neutralizing, agglutination-inhibiting, and complement-fixing antibodies in ferret antisera were completely absorbed only by the homologous virus strain, even though 2 absorptions were carried out with large amounts of heterologous virus strains.

3. PR8 virus appeared to have the broadest range of specific antigenic components for it completely absorbed the heterologous antibodies in Christie and W.S. antisera and left only those antibodies which reacted with the respective homologous strains. The other virus strains (Christie, Talmey, W.S., swine) were more specific in the absorption of heterologous antibodies and completely removed only those antibodies which reacted with the absorbing virus.

4. The absorption tests revealed a higher degree of specificity and individuality of the virus strains than the various cross reactions previously reported. The strain specificity of PR8 virus was equally manifest in absorption tests with ferret sera and with human sera following vaccination.

5. The amount of homologous antibody remaining in a PR8 ferret serum after absorption with PR8 virus, obained by the red cell adsorption and elution method, varied inversely as the concentration of virus used for absorption. A given concentration of virus, however, absorbed a greater percentage of neutralizing antibodies than either agglutination-inhibiting or complement-fixing antibodies.

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