ANTIGENIC VARIANTS OF INFLUENZA A VIRUS (PR8 STRAIN)

V. Virulence, Antigenic Potency, and Cross-Protection Tests in Mice of the Original and Second Series*

By CLAYTON G. LOOSLI, M.D., DOROTHY HAMRE, Ph.D., and PAUL GERBER,‡ Ph.D.

(From the Section of Preventive Medicine, Department of Medicine, University of Chicago School of Medicine, Chicago)

(Received for publication, February 6, 1958)

Two series of antigenic variants of influenza virus type A, PR8 strain, have been characterized serologically (1, 2). In the original series, seven variants derived in succession in mice immunized with the homologous virus showed a progressive deviation from the parent PR8-S virus when tested with PR8-S antiserum. However, all variants continued to produce some antibody reacting with PR8-S virus. The fifth and sixth variants produced antibody with significantly less cross-reaction to PR8-S virus, but antiserum of the seventh variant showed somewhat higher levels of PR8-S antibody resembling in this respect the antisera of the first four variants. By cross-absorption tests, it was shown that the variants contained new antigenic components which were shared in different amounts.

The second series of four variants were derived from the third variant of the original series by passage in mice immunized with PR8-S virus. Subsequent variants were developed in mice given polyvalent vaccine composed of PR8-S virus and the preceding variant. In this series the first variant was not serologically different from its parent.

However, the three subsequent variants showed marked serological deviation from the variants of the original line. They reacted only slightly with PR8-antiserum and provoked only small amounts of antibody which reacted with the PR8-S virus. The results of cross-protection tests in mice also reflect the marked antigenic differences noted in the H.I. and neutralization tests. These along with the results of experiments to show virulence, antigenicity and immunogenicity of some of the variants of both series compared to the original PR8-S virus will be given in this report.

* These investigations were conducted under the sponsorship of the Commission on Influenza, Armed Forces Epidemiological Board and were supported (in part) by the Office of the Surgeon General, Department of the Army; and (in part) by the Seymour Coman Fellowship Fund of the University of Chicago.

‡ Present address: Squibb Institute for Medical Research, New Brunswick, New Jersey.

Materials and Methods

The methods employed in the production of the two series of variants of PR8-S virus and the serological procedures used to characterize them serologically have been described in previous reports (1, 2). The details of the tests for demonstrating comparative virulence antigenicity, immunogenicity, and cross-protection will be given in the appropriate sections under Results.

RESULTS

Pathogenicity (Virulence) of the Variant Viruses for Mice and Chick Embryos.—It was anticipated that the method employed in the production

TABLE I

Pathogenicity of PR8-S and Its Variant Viruses for Chick Embryos and Mice

Viruses	Log titers		$\frac{EID_{50}}{LD_{50}}$
	EID_{50}	LD_{50}	
First series			
PR8-S	8.3	6.5	1.8
As22N6	8.6	7.2	1.4
Ba25N20	8.5	6.8	1.7
Cb17N13	8.6	7.5	1.1
Dc26N4	8.0	6.4	1.6
Fd19N5	7.6	6.0	1.6
Gf33N8	8.4	6.8	1.6
Hg33N5	8.6	6.5	2.1
Second series			
Cb17N13	8.6	7.5	1.1
D/s45N5	7.6	6.2	1.4
Fd/s20N12	8.5	6.8	1.7
Gf/s25N5	8.4	7.0	1.4
Hg/s30N5	8.5	6.5	2.0

of the variants would not favor the selection of non-virulent strains. However, while there was no obvious evidence that the PR8-S variants differed in their ability to infect and kill mice and eggs, tests were carried out to determine their comparable pathogenicity.

Individual mouse lung virus suspensions with PR8-S or its variant were prepared. Groups of five normal mice each weighing 19 to 22 gm. were inoculated intranasally under light ether anesthesia with 0.05 ml. of a 10^{-2} dilution of each lung-virus suspension. At 48 hours after inoculation the mice of each group were killed, their lungs pooled, and ground in sterile sand. Ten per cent suspensions of stock viruses were prepared. Each lot of sterile virus was then divided into small aliquots, quickly frozen, and stored at −50°C. EID_{50} and LD_{50} titers were then determined on each lung virus suspension. Five 10 day fertile eggs and five mice were inoculated respectively with 0.2 ml. intraallantoically and 0.05 ml. intranasally with each tenfold dilution of a given virus suspension.

The results are shown in Table I. As can be seen the EID_{50} and LD_{50} titers of the original PR8-S virus and the variant viruses of both series were essentially the same. This is also seen on inspection of ratios of the EID_{50} and LD_{50} titers. Furthermore, examination of the time of death of mice inoculated intranasally with tenfold dilutions of PR8-S or variant viruses failed to show any significant difference.

Antigenic Potency of PR8-S and Variant Viruses.—During the development of the two series of PR8-S variants it was noted that while all variants appeared to retain the same degree of virulence or pathogenicity for mice and chick

TABLE II

Antigenic Potency of PR8-S Virus and Its Variants

Virus	Homologous H.I. titers with HA units/dose of vaccine		Recovered mice
	8 HA	128 HA	
First series			
PR8-S	128	512	440
As22N6	48	384	64
Ba25N2	32	128	96
Cb17N24	60	120	96
Dc26N7	64	192	64
Fd19N5	8	32	45
Gf33N8	16	48	8
Hg33N5	12	64	48
Second series			
Cb17N24	60	120	96
D/s45N2	8	32	64
Fd/s20N2	<8	16	22
Gf/s25N5	<8	8	8
Hg/s30N5	<8	12	8

embryos there was a progressive loss in the ability of each successive variant to produce homologous antibody. In order to evaluate on a comparative basis the ability of the different variants to produce antibody the following experiments were carried out.

Monovalent formalin-inactivated allantoic fluid vaccines, one with 128 HA units and the other with 8 HA units of virus, were prepared with the PR8-S virus and each variant. A group of five mice each weighing 19 to 22 gm. was given intraperitoneally 0.5 ml. each of the high titer vaccine and another group of mice the same amount of the low titer vaccine.

Fourteen days after inoculation the five mice from each group were bled from the axillary vein. The blood was pooled and serum H.I. and *in ovo* neutralizing antibody titers for homologous antibody were determined. Also to demonstrate the loss of antigenic potency of the variant viruses, sera from mice surviving the virulence tests and showing pulmonary lesions were tested for homologous antibody. The results are shown in Table II.

It can be seen that the PR8-S virus produced the greatest antibody response following both vaccination and infection. Among the variant viruses the antibody response progressively decreased to the point where no antibody could be detected in the sera of mice vaccinated with 8 HA units of the final three variants of the second series, and only small amounts following vaccination with 128 HA units or after infection. *In ovo* neutralizing antibody titers, not

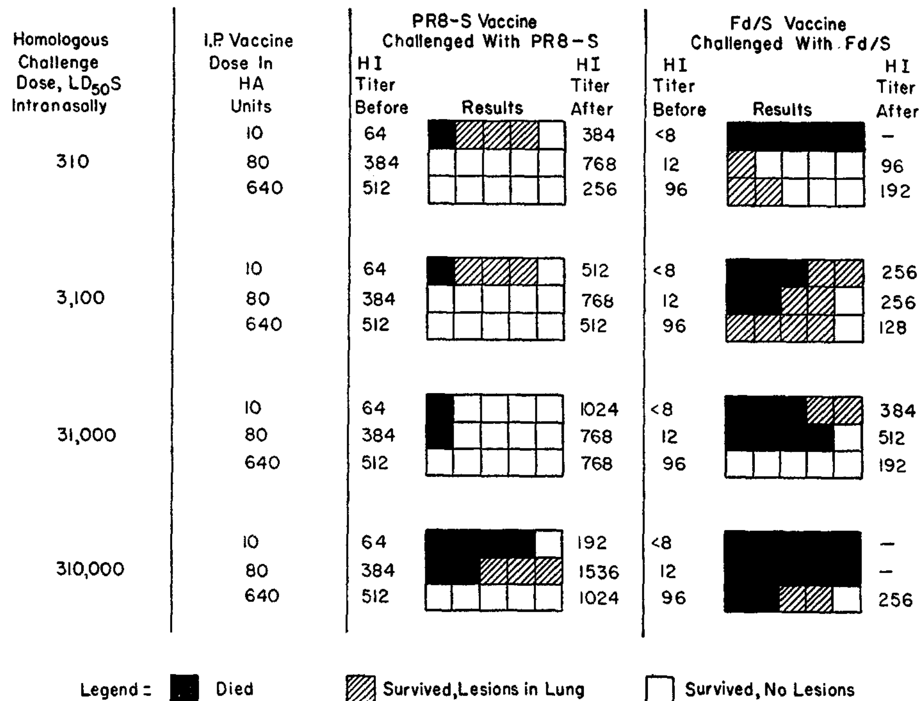

FIG. 1. Comparison of the immunogenicity of PR8-S virus and Fd/s variant virus in mice.

shown in Table II, also showed a similar decrease with each succeeding variant.

Immunogenicity of Low and High Potency Antigens.—To demonstrate the effect of different antigenic responses upon resistance to homologous challenge with equally pathogenic viruses, Fd/s variant, a poor antigen, was compared with PR8-S, a good antigen.

A first egg passage allantoic fluid harvest vaccine was prepared with each strain and inactivated with formalin. From each vaccine three lots each respectively containing 640, 80, and 10 HA units were prepared. Groups of 25 mice were then inoculated with a given lot, each mouse receiving 0.5 ml. intraperitoneally. Fourteen days after vaccination each

group of mice receiving a lot of vaccine were divided into groups of five. Five mice (one group) were bled and the sera pooled for antibody determination. A group of five mice receiving each lot of vaccine and a control group of unvaccinated mice were then inoculated intranasally with increasing amounts (tenfold increases) of mouse lung passage influenza virus having an LD_{50} titer of $10^{6.5}$. The mice were then observed for death or survival over

TABLE III

Cross-Protection Tests with PR8-S and Variants Fd, Gf, and Hg of the Original Line

Experiment No.	Challenge virus 100 LAD_{50}	Vaccine	H.I. titer*		Results of challenge		
			PR8-S	Variant	Dead/Total	Lesions/Total	Lung titer‡ EID_{50}
1	PR8-S	Control	—	—	20/20	20/20	9.0
		PR8-S	**256**	<16	0/20	1/20	<1.0
		Fd19	64	**192**	0/20	0/20	<1.0
	Fd19	Control	—	—	20/20	20/20	9.2
		PR8-S	**256**	<16	7/19	18/19	7.9
		Fd19	64	**192**	0/20	0/20	<1.0
2	PR8-S	Control	—	—	10/10	10/10	9.5
		PR8-S	**290**	<8	0/10	1/10	<1.0
		Gf33	<8	**91**	0/10	0/10	7.0
	Gf33	Control	—	—	10/10	10/10	9.0
		PR8-S	**290**	<8	2/10	10/10	7.3
		Gf33	<8	**91**	0/10	0/10	<1.0
3	PR8-S	Control	—	—	10/10	10/10	9.3
		PR8-S	**630**	<8	0/10	0/10	<1.0
		Hg33	49	**178**	0/10	1/10	6.0
	Hg33	Control	—	—	10/10	10/10	8.7
		PR8-S	**630**	<8	1/10	5/10	6.7
		Hg33	49	**178**	0/10	0/10	<1.0

* Geometric mean titers of 5 mice 12 to 14 days after last dose of vaccine. Homologous titers in bold type.

‡ Log EID_{50} 48 hours after challenge.

a 14 day period. Those dying were examined for pulmonary consolidation and those surviving were sacrificed at 14 days and examined for lung lesions.

The results are shown in Fig. 1. As can be seen, the three PR8-S virus vaccines provoked considerably more antibody than did the Fd/s variant vaccines of comparable strength. When the two vaccine groups of mice were challenged with the same amounts of respective homologous virus, in both groups as would be expected, the greatest number of deaths occurred in the group with the lowest antibody titers. However, more Fd/s-vaccinated mice died or

showed lesions in all four challenge groups than did PR8-S vaccinated animals. Fd/s-vaccinated mice which survived challenge showed proportionally a greater boost in antibody titer than was found in the surviving PR8-S mice.

Cross-Protection Tests.—Cross-challenge protection tests were carried out as follows.

Mice of the same age and weight were employed. Three groups of mice were employed in each cross-challenge test. One served as controls, one was vaccinated intraperitoneally with the PR8-S virus vaccine and the other with a given variant vaccine. The group receiving the PR8-S vaccine were given two 0.5 ml. doses at 5 to 7 day intervals while those receiving the variant vaccines were given three 0.5 ml. doses in an attempt to produce comparable antibody levels. The viruses in the Fd/s, Gf/s, and Hg/s vaccines were concentrated tenfold by high speed centrifugation in order to produce reasonably comparable antibody titers to those elicited by the diluted PR8-S vaccines. Sufficient mice were vaccinated with each vaccine so that the prechallenge antibody titers would be determined on the pooled blood of five mice, another three were sacrificed at 48 hours after challenge to determine lung virus titers and at least ten mice used to determine deaths or survival rates.

TABLE IV

Comparison of Growth of Cb17, Ds45 and PR8-S in the Lungs of Mice Recovered from Infection with PR8-S

Virus strain	Mouse LD_{50} doses inoculated intranasally	Lung titer 48 hours EID_{50}
PR8-S	10,000	<1.0
	1,000	<1.0
Cb17	10,000	6.5
	1,000	6.5
	100	4.2
Ds45	10,000	6.5
	1,000	6.0
	100	6.3

The challenge tests were carried out from 10 to 14 days after the last vaccine inoculation. In the tests all challenge doses of virus were given by aerosol with one exception. The amount was 100 lethal air-borne doses$_{50}$ (LAD_{50}) given in a closed chamber. The LAD_{50} of each virus was previously determined by titration of tenfold dilutions nebulized in the closed chamber using ten mice for each dilution. Forty-eight hours after challenge three mice in each group were sacrificed and 10 per cent lung virus suspension prepared. The EID_{50} of these suspensions were determined. The mice were observed for 14 days after challenge for death or survival. Those alive at 14 days were sacrificed and pulmonary lesions noted.

The low challenge dose of virus was employed in order to avoid a break-through of homologous immunity and thus to increase the sensitivity of the cross-protection test. This along with the above observation of lung virus titer at 48 hours, death or survival and presence or absence of pulmonary lesions in surviving mice made it possible to detect differences in degrees of cross-protection among the variants and the original PR8-S virus.

In Table III are shown the results of cross-protection tests employing the fifth, sixth, and seventh variants of the first series and the PR8-S virus. In all three experiments all control mice challenged with the PR8-S virus or a variant showed comparable high titers in the lungs at 48 hours while mice challenged with homologous virus showed no virus at this time. The Fd-vaccinated mice was the only group which appeared to be completely resistant to challenge with the PR8-S virus since none died and no virus

TABLE V

Cross-Protection Tests with PR8-S and Cb and D/s Variants

Experiment No.	Challenge virus 100 LAD_{50}	Vaccine group	H.I. titer		Results of challenge		
			PR8-S	Variant	Dead/Total	Lesions/Total	Lung titer EID_{50}
1	PR8-S	Control	—	—	10/10	10/10	10.0
		PR8-S	**640**	10	0/10	0/10	<1.0
		Cb	320	**480**	0/10	0/10	2.3
	Cb	Control	—	—	10/10	10/10	9.5
		PR8-S	**640**	10	0/10	5/10	6.5
		Cb	320	**480**	0/10	0/10	<1.0
2	PR8-S	Control	—	—	10/10	10/10	9.7
		PR8-S	**768**	<16	0/10	0/10	<1.0
		D/s	192	**256**	0/10	1/10	3.7
	D/s	Control	—	—	10/10	10/10	9.0
		PR8-S	**768**	<16	0/10	10/10	8.0
		D/s	192	**256**	0/10	0/10	<1.0

* Geometric mean antibody titer of five mice, 2 to 14 days after last dose. Log EID_{50} 48 hours after challenge. Homologous titers in bold type.

could be detected in the lungs at 48 hours. The mice vaccinated with Gf and Hg variants and challenged with PR8-S virus showed moderately high lung virus titers but two deaths and only one Hg-vaccinated mouse showed pulmonary lesions. On the other hand all PR8-S–vaccinated mice challenged with the variants showed high lung virus titers at 48 hours. Some in each group died and many surviving mice showed pulmonary lesions.

The differences in the cross-immunity tests can be explained on the basis of the relative amounts of antibody provoked by the heterologous virus. PR8-S produced little or no antibody to the variants while the variants, with the exception of Gf, provoked considerable antibody to PR8-S. The degree of cross-protection between PR8-S and the Gf variant was greater than would be expected on the basis of antibody titers in the mice.

Cross-Protection Tests with PR8-S and the Variant Viruses of the Second Series.—Cross-H.I. and *in ovo* neutralization and antibody absorption tests demonstrated a close antigenic similarity between Cb and its D/s variant.

In Table IV are shown the results of an experiment to compare the growth of Cb and D/s variants in the lungs of mice recovered from infections with PR8-S virus 6 weeks previously. Homologous challenge showed the mice to be solidly immune to growth in the lungs at 48 hours even at the highest challenge dose while Cb and D/s grew about equally well in the PR8-S–recovered mice. In the mice challenged with 100 LD_{50} doses the titer of D/s virus was significantly higher than the Cb virus titers.

Cross-challenge tests with the Cb and its D/s variant and the PR8-S virus are shown

TABLE VI

Cross-Protection Test with PR8-S Virus and Fd/s Variant

Challenge virus 100 LAD_{50}	Vaccine group	H. I. titer*		Results of challenge							
		PR8-S	Fd/s	Lung titers‡ at time after challenge						Dead/Total	Lesions/Total
				24 hr.	48 hr.	3 days	4 days	6 day	8 days		
PR8-S	Control	—	—	9.0	9.5	9.3	d			10/10	10/10
	PR8-S	**384**	<8	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0	0/10	0/10
	Fd/s	16	**96**	5.3	5.7	<1.0	2.7	<1.0	<1.0	0/10	1/10
Fd/s20	Control	—	—	8.5	7.3	8.5	8.0	d		10/10	10/10
	PR8-S	**384**	<8	4.0	5.7	7.0	5.7	5.7	<1.0	2/10	8/10
	Fd/s	16	**96**	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0	0/10	0/10

* Titer at time of challenge 12 to 14 days after last vaccine dose.

‡ EID_{50}.

in Table V. In these experiments all the unvaccinated control mice died and showed high lung virus titers at 48 hours. Solid, homologous immunity was also present. On the other hand, challenge of Cb- and D/s-vaccinated mice with PR8-S virus produced lung virus titers of 2.3 and 3.7 respectively. PR8-S–vaccinated mice challenged with Cb and D/s variants had virus titers of 6.5 and 8.0 respectively. These results also show the close similarity of these two variants as demonstrated previously by serological procedures. Both reacted in small amounts with the PR8-S antiserum and both produced antibody to the PR8-S virus almost or as great as the respective homologous titers.

In Table VI are given the results of a cross-protection test with the second variant Fd/s of the second series and the PR8-S virus. Larger numbers of mice were employed so that virus titers in the lungs at six intervals after challenge could be determined. It can be seen that the two antisera possessed only a small amount of H.I. antibody for the respective heterologous virus. A small amount of demonstrable antibody was present for the PR8-S virus in Fd/s antiserum while there was little or no antibody to the Fd/s virus in the PR8-S antiserum. On challenge with the two viruses all the control mice died and showed high virus titers in the lungs. Homologous challenge demonstrated solid immunity with no virus being demonstrated at any of the post-inoculation time intervals. Heterologous infection was produced with both the PR8-S and Fd/s viruses. The infection was less severe in the Fd/s-vaccinated mice as indicated by the lower titer of virus in the lungs, no deaths, and

only one lung lesion in the ten surviving mice. In the PR8-S vaccinated mice challenged with the Fd/s virus the virus titer persisted in the lungs at moderately high titer for 6 days. Two of ten mice died and eight of ten showed lesions in the lungs. Again it is demonstrated that the extent of cross-protection between these viruses appears to be greater than is reflected in their serological relationships.

The results of cross-protection tests with the third and fourth variants (Gf/s and Hg/s) the second series and the PR8-S virus are shown in Table

TABLE VII

Cross-Protection Tests with PR8-S Virus and Gf/s Variant; and with PR8-S and Hg/s

Experiment No.	Challenge virus 100 LAD_{50}	Vaccine group	H.I. titer‡		Results of challenge		
			PR8-S	Variant	Dead/Total	Lesions/Total	Lung titer* EID_{50}
1	PR8-S	Control	—	—	10/10	10/10	9.6
		PR8-S	**450**	<8	0/10	0/10	<1.0
		Gf/s25	<8	**57**	0/10	0/10	5.5
	Gf/s25	Control	—	—	10/10	10/10	9.3
		PR8-S	**450**	<8	1/10	3/10	6.5
		Gf/s25	<8	**57**	0/10	0/10	<1.0
2	PR8-S	Control	—	—	10/10	10/10	9.7
		PR8-S	**93**	<8	0/10	0/10	<1.0
		Hg/s30	<8	**27**	0/10	0/10	7.0
	Hg/s30	Control	—	—	10/10	10/10	9.7
		PR8-S	**93**	<8	0/10	5/10	7.6
		Hg/s30	<8	**27**	0/10	0/10	<1.0

* 48 hours after challenge, pooled lungs three mice.
‡ Titer at time of challenge 12 to 14 days after last vaccine doses.

VII. These variants produced no antibody to the PR8-S virus and the PR8-S antiserum contained no antibody to the variants. The control mice in both experiments died and showed equally high virus titers in the lungs at 48 hours. Homologous challenge showed solid immunity as indicated by no virus being demonstrated in the lungs at 48 hours and no deaths or lesions in the mice. Heterologous challenge showed somewhat greater protection in the variant vaccinated mice infected with the PR8-S virus than occurred when the PR8-S–vaccinated mice were challenged with the Gf/s and Hg/s variants. This is indicated by the higher virus titers in the lungs of the PR8-S–vaccinated mice and noted with the presence of pulmonary lesions in the surviving animals. Again the cross-protection tests reveal a much closer relationship than the serological tests would indicate.

DISCUSSION

Although the pathogenicity of the variants of the first and second series was similar to that of the original PR8-S virus there was a progressive loss with each succeeding variant in the ability to provoke antibodies. This loss of antigenicity may be the result of the methods employed in the development of the variant viruses. As they were all derived from their passage in the lungs of homologously immune mice, it might be expected that some of the more potent antigens in the virus complex would be selectively neutralized or suppressed by their specific antibody in the immune environment. Thus, the antigenic complex of each succeeding variant would be expected to contain less and less potent antigens. This appears to be the case for when the antigenic potency is compared in relation to the sequential deviation of the variants, a marked and progressive decline in the ability of the variants to provoke an antibody response in mice and ferrets can be seen.

The loss of antigenicity without a significant decrease in pathogenicity among the variants of influenza PR8-S virus does not appear to correspond to P-Q variation as described by Van der Veen and Mulder (3) and studied by Fiset and Depoux (4). The variants described here have been derived entirely by mouse passage. Q strains of influenza virus appear to arise mainly among egg-adapted viruses, after passage of P strains in eggs in the presence of homologous antiserum. Furthermore, Q strains can be converted to P strains by mouse passage, but all antigenic variants described in this and preceding reports appear to be stable on passage in normal mice.

In all cross-protection tests with PR8-S parent virus and its variants, mice vaccinated with PR8-S virus were less resistant to challenge with variant viruses than mice vaccinated with variant viruses and challenged with PR8-S. This reflected the serological relationships shown between the variant viruses and PR8-S. All variants produced some antibody reacting with PR8-S but the variant viruses reacted slightly or not at all with the PR8-S antibody. Although Fd and Gf variants of the original line, and Fd/s and Gf/s variants of the Bar-S line produced significantly less PR8-S antibody than other variants of these two lines, mice vaccinated with these variants were not significantly less resistant to challenge with PR8-S than mice vaccinated with other variants. Although the serological procedures showed marked antigenic differences between the PR8-S virus and its variants these differences were not as well demonstrated in the cross-protection tests.

For instance, Fd and subsequent variants of the original line, and Fd/s variant and subsequent variants of the Bar-S line reacted little if at all with PR8-S antiserum, but mice vaccinated with PR8-S nevertheless showed some degree of resistance to challenge with these variants. Less than 50 per cent of these mice died. However, lung lesions and titer of virus in lungs showed that infection did occur.

In recent years little attention has been paid to correlation of serological differences with degree of cross-immunity in mice. In 1938 Francis and Magill (5) and Smith and Andrewes (6) concluded that serological differences among the type A strains of that time could be correlated in a general way with cross-immunity in mice. However, Francis and Magill (5) pointed out that single doses of vaccine often failed to immunize mice to homologous challenge, while multiple doses of vaccine increased the degree of cross-protection among strains. More recently Francis (7) reported that mice given two doses of Keffer '47 strain produced little antibody to Rhodes '47 virus, yet were resistant to log 5.0 LD_{50} intranasal doses of Rhodes virus. Also, Herzberg, May, and Beck (8) recently reported that mice immunized with PR8-S influenza virus which produced no antibody to FM1 virus, did not all die when challenged intranasally with FM1 strain. In their experiments most of the surviving mice showed lung lesions indicating a high degree of infection. It appears from these studies that use of death or survival alone in cross-protection tests with influenza type A or A′ strains is not sufficiently sensitive to correlate with even wide serological differences in the corresponding strains. The antigenic variants described in this report are more closely related to PR8-S than is FM1 virus. Therefore, it is hardly surprising that mice vaccinated with PR8-S and challenged with the most serologically different variants did not all die. By using virus titer of the lungs at 48 hours, as well as deaths and lung lesions, it is possible to demonstrate lack of complete reciprocal immunity among the variants and PR8-S virus. Thus, the results of cross-protection tests were in general agreement with serological findings, that is PR8-S vaccine protected least against challenge with those variants which reacted least with PR8-S antisera in the serological tests.

These studies demonstrate clearly the antigenic instability of influenza viruses when subjected to passage through a partially immune host. Whether the immune state of the human population has operated in a similar fashion to bring about the progressive antigenic changes in the group A influenza viruses since their first isolation in 1933 can only be surmised. These studies suggest that the alteration of antigenic components, in such a manner that older strains fail to provoke antibody against more recent ones, is essential to the survival of influenza virus in man. In recent years, it has been noted that influenza viruses have also shown a decrease in their capacity to provoke homologous as well as heterologous antibody but no decline in their ability to infect man in widespread epidemics. These observations have their counterpart in our studies of the production of variant influenza A (PR8) viruses following passage through immunized animals.

SUMMARY

Two series of variants of influenza PR8-S virus have been described. While all retain the same degree of pathogenicity for mice and fertile eggs, there was

a progressive loss in the ability of the variants to provoke antibody following vaccination or infection of mice and ferrets. The immunogenicity of the variants was, therefore, less than that of the original strain. Although little or no serological relationship could be demonstrated between some of the variants and the PR8-S virus a considerable degree of cross-immunity could be demonstrated in the cross-protection tests with these viruses if observations were based solely on death or survival of the mice. By employing the occurrence of lesions in the lung and the titer of virus in the lung 48 hours after challenge, the amount of cross-protection in mice could be related to the amount of serological cross-reaction. In general mice vaccinated with PR8-S virus were less resistant to infection with the variant viruses than mice vaccinated with variants and challenged with the PR8-S parent virus.

The role of the immune environment of the host in the production of the variant influenza viruses with their serological differences, decreasing antigenicity, and persisting pathogenicity as well as the epidemiological implications of these findings with respect to epidemic influenza in man are discussed.

BIBLIOGRAPHY

1. Hamre, D., Loosli, C. G., and Gerber, P., Antigenic variants of influenza A virus (PR8 strain). III. Serological relationships of a line of variants derived in sequence in mice given homologous vaccine, *J. Exp. Med.*, 1958, **107,** 829.
2. Hamre, D., Loosli, C. G., and Gerber, P., Antigenic variants of influenza A virus (PR8 strain). IV. Serological characteristics of a second line of variants developed in mice given polyvalent vaccine, *J. Exp. Med.*, 1958, **107,** 845.
3. Van der Veen, J., and Mulder, J., Studies on the Antigenic Composition of Human Influenza Virus A Strain, Leiden, Stenfert Kroese, 1950.
4. Fiset, P., and Depoux, R., Serological behaviour of influenza viruses. 2. Patterns of antigenic relationship, *Bull. World Health Organ.*, 1954, **11,** 987.
5. Francis, T., Jr., and Magill, T. P., Antigenic differences in strains of epidemic influenza virus. II. Cross immunization tests in mice, *Brit. J. Exp. Path.*, 1938, **19,** 284.
6. Smith, W., and Andrewes, C. H., Serological races of influenza virus, *Brit. J. Exp. Path.*, 1938, **19,** 293.
7. Francis, T., Jr., Significance of antigenic variation of influenza viruses in relation to vaccination in man, *Fed. Proc.*, 1952, **11,** 808.
8. Herzberg, K., May, G., and Beck, H., Versuche uber influenza-immunitat. I. Mitteilung: verhalten der Stamme PR8 and FM1, Z. Immunitätsforsch, 1955, **112,** 409.