ANTIGENIC VARIANTS OF INFLUENZA A VIRUS (PR8 STRAIN)

I. Their Development during Serial Passage in the Lungs of Partially Immune Mice*

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Plates 44 and 45

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The survival of human influenza viruses in nature in the presence of a relatively high herd immunity due to prior infection or immunization has been attributed to the immunological instability of these agents (1–3). The partially immune hosts provide a selective environment for the emergence of antigenic variants capable of resisting neutralization by specific antibodies. This hypothesis, first advanced by Taylor (1) in 1949, found experimental support by the work of Archetti and Horsfall (4) who demonstrated the occurrence of antigenic changes during serial passage of influenza A viruses in fertile eggs in the presence of neutralizing antibodies to a related strain. This led to the present investigation carried out in the murine host which allowed correlation of serological data with immunity and virulence since it is well established (5) that serological tests alone are insufficient for the evaluation of the significance of antigenic changes among influenza viruses.

It has been shown previously that pulmonary influenza A virus infections can be produced in mice immunized with inactivated homologous virus (6, 7). The procedure employed in this study consisted of serial passage of mouse-adapted influenza A virus (PR8 strain) in the lungs of mice previously vaccinated with formalin-inactivated homologous virus. This report describes the development of antigenic variants during this process. Although antigenic variation could be detected in earlier passage strains, the 17th and 21st strains were selected for a detailed study of their serological, immunological and growth characteristics.

Materials and Methods

Viruses.—The mouse-adapted PR8 strain of influenza A virus was employed. This strain, originally obtained from Dr. Thomas Francis, Jr., of the University of Michigan, Ann Arbor,

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in 1941, has been maintained through continuous mouse lung passages in this laboratory and is highly pathogenic for mice both by the air-borne and intranasal routes of infection. For the purpose of identification the laboratory stock of PR8 virus is referred to as PR8-S, the control passage strain as PR8-C and the strains passed serially in vaccinated mice are designated as PR8-T strains. The figure following the "T" refers to the passage number.

Stock Passage Virus.—Stock passage virus (PR8-S) was prepared as previously described (8) by placing thirty mice in a 170 liter chamber and exposing them to dense lethal clouds of virus for 40 minutes. From 5 to 6 ml. of a 10 per cent mouse lung virus suspension was nebulized during the first 20 minutes, and the mice were allowed to breathe the infectious atmosphere for another 20 minutes. After 48 hours the animals were sacrificed, the lungs removed aseptically and ground with sterile alundum. Antibiotic broth (beef heart infusion broth containing penicillin and dihydrostreptomycin, 1,000 units and 1 mg. per ml., respectively, was added to make a 10 per cent suspension. Following centrifugation at 2,000 R.P.M. for 10 minutes the supernatant fluid was divided into aliquots quickly frozen and stored at −50°C. in an electric deep freeze. Such virus preparations usually had an EID_{50} titer of $10^{9.5}$ and a mouse LD_{50} titer of $10^{7.0}$ to $10^{7.5}$.

Serial Passage Viruses.—Parallel serial passages of PR8 virus were made in immunized (PR8-T) and unimmunized (PR8-C) mice. In these series the mice were inoculated intranasally under light ether anesthesia with 0.05 ml. of a 10 per cent mouse lung suspension of the respective strains. The animals were then sacrificed 2 days after inoculation. The preparation of a suspension from a given passage was similar to that employed for preparing stock virus.

Preparation of Virus Antigens for Hemagglutination (H.I.), Neutralization, and Absorption Tests.—Virus antigens employed in these tests were prepared by inoculating intra-allantoically groups of 11-day-old fertile eggs with 0.2 ml. of a 10^{-3} dilution of mouse lung suspension of the different passage strains. Only first egg passage fluids were used in all the experiments to avoid possible antigenic changes which may have resulted from repeated egg passages.

Virus Titrations.—For both mouse and egg infectivity tests serial tenfold dilutions of virus in antibiotic broth were prepared. For egg infectivity titrations, 0.1 ml. from each dilution was inoculated into the allantoic cavity of a group of four 11-day-old fertile eggs which were incubated at 36°C. for 48 hours. The eggs were then chilled and the allantoic fluid from each egg was tested for agglutination of chicken red cells (9). Presence of hemagglutination indicated infection. The titers are expressed as the reciprocal of the dilutions calculated as the EID_{50} by the method of Reed and Muench (10). Mouse infectivity tests were performed by intranasal inoculation under ether anesthesia of the 3- to 4- week-old Swiss mice. Six mice were used per dilution each animal receiving 0.05 ml. of the virus suspension. Deaths were recorded for 10 days and the 50 per cent lethal dose (LD_{50}) was calculated by the method of Reed and Muench (10).

In the air-borne experiments groups of mice were exposed to 100 and 1,000 50 per cent lethal air-borne doses of nebulized mouse lung virus suspension in a 170 liter cloud chamber. For the purpose of this study one 50 per cent lethal air-borne dose, hereafter referred to as LAD_{50}, is considered the smallest amount of nebulized mouse lung virus suspension which produces a fatal infection in 50 per cent of normal mice exposed to the infectious atmosphere for 40 minutes.

Immunization of Mice.—Vaccines were prepared from first egg passage allantoic fluid virus treated with a 1:2000 dilution of formalin for 24 to 48 hours at 4°C. and tested for the loss of viability by egg inoculation. Each mouse received 0.1, 0.25, or 0.5 ml. of vaccine intraperitoneally depending on the degree of immunity desired. For the production of high antibody titers a second inoculation of 0.5 ml. was given a week later. All animals were challenged 10 days after the last inoculation. This procedure was employed for both passage experiments and cross-immunity tests.

Preparation and Testing of Antisera.—Groups of mice, hamsters, and ferrets were employed in the production of antisera against the different virus strains under study. Mice weighing 25 to 28 gm. were inoculated intraperitoneally with 0.5 ml. of a 10^{-2} dilution of infected mouse lung suspension. Adult Syrian hamsters were infected intranasally under light ether anesthesia with 0.2 to 0.3 ml. of a 10^{-2} dilution of the same virus suspension. Adult ferrets were exposed to nebulized virus in a cloud chamber. Four to 5 ml. of a 10^{-2} dilution of mouse lung virus suspension were atomized for a period of 15 minutes and the animals were allowed to remain in this atmosphere for another 15 minute period. All animals were bled 2 to 3 weeks after inoculation or exposure and the sera kept frozen until used.

The pattern test was employed to determine the titer of hemagglutinin-inhibiting antibody (11). The tests were read after 60 minutes at 4°C. and the titers were expressed as the reciprocal of the highest initial serum dilution showing complete inhibition. Although nonspecific inhibition of hemagglutination occurred only with ferret sera all sera were treated as routine with receptor-destroying enzyme (RDE) prepared according to the method of Tyrrell and Horsfall (12).

The H.I. titer of individual immunized mice was determined from tail vein blood collected in white blood cell–counting pipettes previously rinsed with 0.1 per cent heparin and dried before use. The whole blood sample was diluted 1:10 with buffered saline and the cells removed by centrifugation. Since the average hematocrit value of adult mouse blood is about 50 per cent the plasma dilution was considered to be 1:20.

For the titration of virus neutralizing antibodies the *in ovo* test of Hilleman and Horsfall (13) was used. The sera were diluted in threefold steps and mixed with an equal volume of virus dilution containing approximately 1,000 EID_{50}.

Antibody absorption.—The method described by Jensen and Francis (14) was employed. It consists of coating formalinized, washed human red blood cells with periodate-treated allantoic fluid virus. Heterologous antibody was removed by single absorption with two absorbing units of virus-coated erythrocytes at 4°C. for 16 to 18 hours. One absorbing unit is defined as the minimal volume of cell-virus complex capable of removing 64 units of homologous H.I. antibody (14).

Serial Passage of PR8 Virus in Immunized Mice.—Ten days following vaccination the mice were bled from the tail vein and the individual H.I. antibody titers determined as described above. Starting with animals with low H.I. antibody titers (1:40), a group of mice under light ether anesthesia were inoculated intranasally each with 0.05 ml. of a 10^{-1} dilution of stock (PR8-S) mouse lung virus suspension. Mice with gradually increasing immune levels were selected for subsequent passages until virus growth could be maintained in the presence of high antibody titers (640–1280). At the same time the original stock virus was passaged serially in unvaccinated, control mice (PR8-C) of the same age and lot as the test animals. After each passage, the animals were sacrificed 48 hours after challenge. The lungs from each group were perfused, pooled, ground, and suspended in antibiotic broth. Following determination of the virus content by egg infectivity titration the material was again inoculated undiluted (10^{-1}) intranasally into the next group of selected mice. This process was continued for 22 consecutive passages.

RESULTS

The procedures for evaluating antigenic variation consisted of (*a*) cross-hemagglutination–inhibition tests, (*b*) antibody absorption, (*c*) *in ovo* neutralization tests, (*d*) cross-protection tests in mice, and (*e*) comparison of growth curves of the variant and stock virus strains in immune and non-immune mice, and microscopic study of the mouse lungs.

H.I. Tests.—The results of a cross-hemagglutination–inhibition test with the 17th passage test virus strain (PR8-T_{17}) are presented in Table I. It is evident that the control (PR8-C_{17}) and stock strain (PR8-S) are serologically identical which indicates that 17 serial passages of PR8 virus in non-immune mice had no effect on its antigenic character. On the other hand, 17 serial passages of PR8 in vaccinated mice resulted in the selection of an antigenic variant which was only slightly inhibited by antisera to the control or stock virus strains while the mouse, hamster, and ferret antisera to PR8-T_{17} virus inhibited to a much lesser extent the control and stock virus strains. This observation may be considered to indicate the extent to which the variant strain continues to share antigenic components with the original stock virus.

TABLE I

Cross-Hemagglutination-Inhibition Test with the Parent (PR8-S) Strain and Strains of Influenza A Virus Serially Passed in Vaccinated (PR8-T_{17}) and Normal Mice (PR8-C)

Virus antigen	Mouse antisera			Hamster antisera			Ferret antisera		
	PR8-T_{17}	PR8-C_{17}	PR8-S	PR8-T_{17}	PR8-C_{17}	PR8-S	PR8-T_{17}	PR8-C_{17}	PR8-S
PR8-T_{17}	256	8	8	256	16	16	512	64	64
PR8-C_{17}	64	256	512	64	128	256	256	512	512
PR8-S	64	256	512	64	128	256	256	512	512

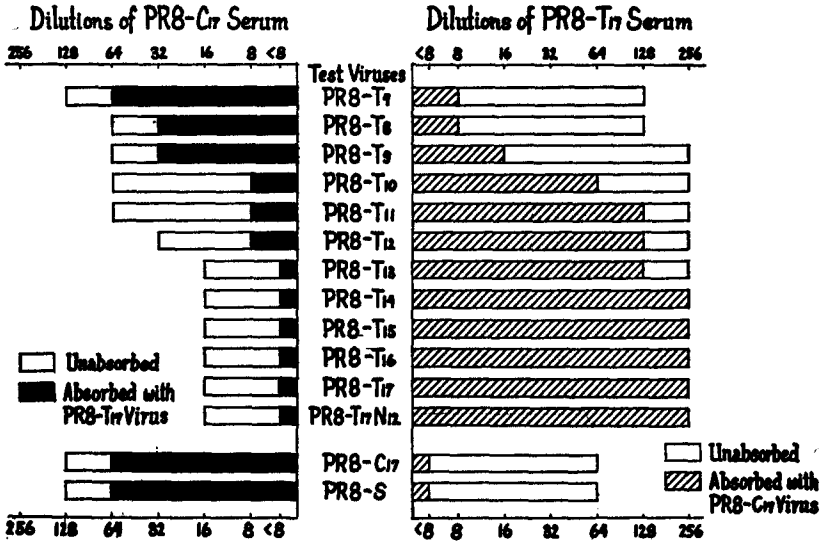
Titers are expressed as the reciprocal of the initial serum dilution.

In order to determine whether the emergence of an apparently new antigenic character was a one step or multiple step phenomenon, strain-specific antisera were employed. These were readily prepared from convalescent hamster sera by the absorption technique of Jensen and Francis (14). H.I. tests using absorbed and unabsorbed sera were then carried out simultaneously with the virus strains obtained at each passage of PR8 virus in vaccinated mice (PR8-T series), with the 17th control passage strain (PR8-C_{17}) and the original stock virus (PR8-S). The results are shown in Text-fig. 1. The first 6 test passages are omitted since their antigenic character was identical with the 7th passage virus (PR8-T_7). The right side of Text-fig. 1 shows the antibody titers against PR8-T_{17} antiserum before and after absorption with the control passage virus (PR8-C_{17}). The shaded areas represent an antigenic component present at first in minor amounts and gradually increasing in quantity with continued passage of PR8 virus in vaccinated mice. The converse situation is shown at the left half of Text-fig. 1 giving the H.I. titers against PR8-C_{17} serum before and after absorption with PR8-T_{17} virus. Here the shaded areas indicate the gradually decreasing amounts of the PR8 component in the antigenic mosaic of the virus.

The stepwise selection of antigenic variants reached a maximum between the 13th and 14th serial passage following which no further changes were detectable

with the procedures employed. That this variant is stable and independent of the presence of a selective environment is shown by the persistence of the antigenic character of PR8-T_{17} virus following 12 serial passages in the lungs of non-immunized mice (PR8-$T_{17}N_{12}$).

In Ovo Neutralization Tests.—Antigenic analyses based entirely on *in vitro* procedures are inadequate since neutralizing and hemagglutinin-inhibiting antibodies appear to be distinct (15). Cross-neutralization tests *in ovo* were,



TEXT-FIG. 1. Comparison of H.I. titers of PR8-S virus with antigenic variants (PR8-T) using unabsorbed and absorbed convalescent hamster sera.

therefore, carried out and the results are shown in Table II. As can be seen, antisera to PR8-C_{17} prepared in the three species of animals employed had considerably lower neutralizing antibodies against the test strain PR8-T_{17} which had undergone 17 passages in immunized mice than to the homologous (PR8-C_{17}) virus. The persistence of a portion of the original PR8 component in the antigenic complex of the variant is again indicated by the extent of the cross-reactions between PR8-T_{17} antisera and the control virus strain. These results confirm the data shown in Table I.

Cross-Protection Test.—Two groups of forty mice each were vaccinated intraperitoneally with two doses of 0.5 ml. of formalin-inactivated PR8-S and PR8-T_{17} virus (allantoic fluid) respectively. Prior to challenge the uniformity and level of the antibody response were ascertained in a random sample of two-thirds of the population by determining the H.I. titers of individual mice. These ranged from 256 to 512. Groups of ten mice in each vaccine group and

the same number of control mice were then challenged intranasally with 100 and 1,000 LD_{50} doses of the variant and stock viruses. These virus suspensions had an LD_{50} titer of $10^{7.0}$ and $10^{7.7}$ respectively. 48 hours following challenge three mice of each group were sacrificed, their lungs perfused with saline, pooled, ground, 10 per cent suspensions prepared in antibiotic broth and frozen at −50°C. The virus titer of the respective lung suspensions was then

TABLE II

Cross-Neutralization Test in Eggs with Strains of Influenza A (PR8) Virus Serially Passed in Vaccinated ($PR8\text{-}T_{17}$) and Normal Mice (PR8-C)

Virus antigen	Mouse antisera		Hamster antisera		Ferret antisera	
	$PR8\text{-}T_{17}$	$PR8\text{-}C_{17}$	$PR8\text{-}T_{17}$	$PR8\text{-}C_{17}$	$PR8\text{-}T_{17}$	$PR8\text{-}C_{17}$
$PR8\text{-}T_{17}$	288	9	452	64	270	129
$PR8\text{-}C_{17}$	47	266	126	>905	156	4208

50 per cent serum neutralizing titers are expressed as the reciprocal of the initial serum dilution.

TABLE III

The Virus Content of the Lungs at 48 Hours in Immunized and Control Mice Challenged with the Stock and Passage T_{17} Strains of PR8 Virus

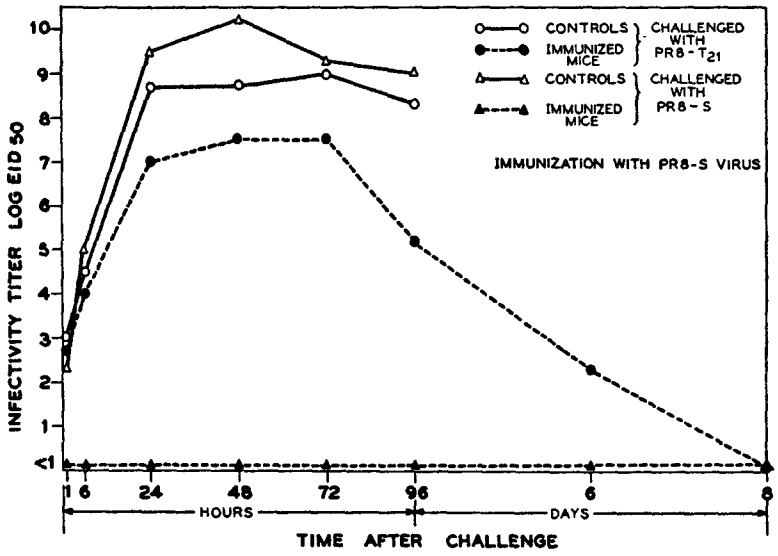
Challenge virus	Challenge dose LD_{50} intranasally	EID_{50} titer of lungs		
		Mice vaccinated with		Unvaccinated mice*
		PR8-S	$PR8\text{-}T_{17}$	
PR8-S	100	<1	<1	8.8
PR8-S	1000	<1	3.7	9.5
$PR8\text{-}T_{17}$	100	<1	<1	8.5
$PR8\text{-}T_{17}$	1000	7.5	<1	8.7

* All remaining control mice died by the 6th day after challenge.

determined by egg infectivity titration. The remaining mice were observed for a period of 12 days after which time the survivors were sacrificed and their lungs examined for lesions. The results of this experiment are recorded in Table III.

It can be seen that all non-vaccinated control mice developed high virus titers in their lungs at 48 hours, and the remaining mice all died by the 6th day with extensive pulmonary consolidation. There were no deaths, however, in the vaccinated groups of mice challenged with either homologous or heterologous virus and no gross pulmonary changes were seen 12 days after challenge. No virus growth was detected when 100 LD_{50} doses of virus were employed in the homologous or heterologous challenges. With the higher dose (1000 LD_{50})

solid immunity was indicated by the lack of virus growth following challenge with the respective homologous virus. As would be expected from the serological studies (Tables I and II, and Text-fig. 1) only limited virus growth ($10^{3.7}$ EID_{50}) occurred in mice vaccinated with the variant PR8-T_{17} and challenged with 1000 LD_{50} of PR8-S. On the other hand, in spite of the absence of gross pulmonary changes at 48 hours, virus was present in significantly higher titer ($10^{7.5}$ EID_{50}) in the lungs of mice vaccinated with PR8-S and challenged with 1000 LD_{50} of PR8-T_{17} virus (Table III).



TEXT-FIG. 2. Growth curves of PR8-S virus and its antigenic variant PR8-T_{21} in the lungs of immunized mice (PR8-S vaccine) and non-immune controls.

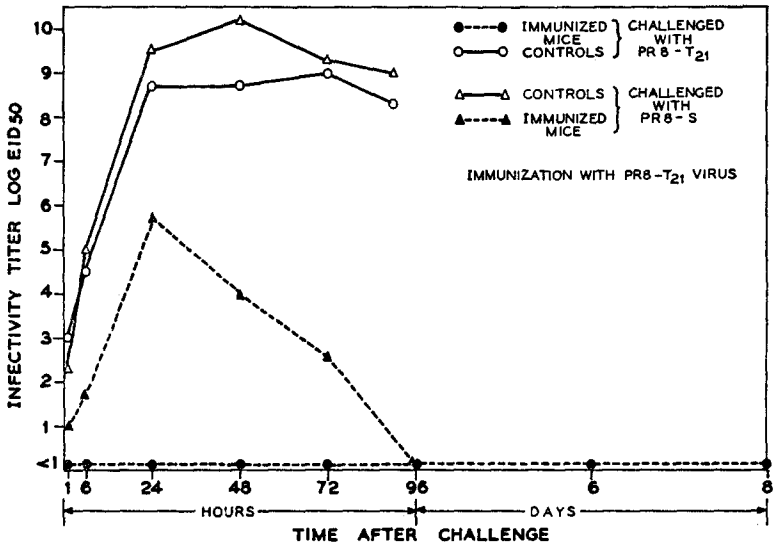
Comparison of Growth Curves and Pathology.—In order to demonstrate the actual virus growth and the associated gross and microscopic changes in the lungs of immunized and control mice resulting from infection with influenza virus the following experiments were designed.

The air-borne route was chosen since it has been shown previously to yield a more uniform infection (8) and because it also facilitates the use of a large number of animals. Two groups of 120 mice each were vaccinated intraperitoneally with two doses of 0.25 ml. of formalin-inactivated PR8-S and PR8-T_{21} allantoic fluid virus respectively. 120 control mice received the same volume of formalin-treated normal allantoic fluid. Ten days following vaccination the degree and uniformity of immune response were determined in a random sample of one-third of the total vaccinated group by testing the H.I. titers of individual mice. The titers ranged from 256 to 512.

Half of each vaccinated and control group was then exposed in a chamber to approximately 1,000 lethal air-borne doses of PR8-T_{21} and PR8-S respectively. At increasing intervals

following challenge, five mice in each group were sacrificed, their lungs perfused with saline, pooled, ground, and 10 per cent suspensions prepared with antibiotic broth which were then stored at −50°C. At the same time two additional animals in each group were sacrificed and their lungs fixed for histopathological studies by intratracheal injection of Zenker-formol solution (16). Ten mice in each group were set aside to observe death and the surviving animals were sacrificed after 12 days. The virus content of the lungs was determined by egg infectivity titration.

The comparative growth curves of the stock and variant virus strains in immune and non-immune mice are shown in Text-figs. 2 and 3. Both the stock



TEXT-FIG. 3. Growth curves of PR8-S virus and its antigenic variant PR8-T_{21} in the lungs of immunized mice (PR8-T_{21} vaccine) and non-immune controls.

and variant strains of PR8 virus grew rapidly in the lungs of non-immune mice during the first 24 hours. The rates of multiplication of both strains during this period are almost identical as indicated by the slopes of the curves shown in Text-figs. 2 and 3. The variant strain, however, differed from the stock virus by failing to reach the same peak titer.

All the non-immune animals infected with either virus died 4 to 5 days after exposure to the virus clouds. Extensive pulmonary consolidation was present in the gross. Microscopically there was no difference in the appearance of pulmonary lesions produced by the PR8-S virus or its variant PR8-T_{21}. As seen in Figs. 1 and 2 both produced at 4 days characteristic and extensive destruction of the bronchial lining cells and extensive pulmonary exudation of edema fluid and leucocytes. The peribronchial connective tissue was also

infiltrated with cells. In the inflammatory areas no bacteria were seen microscopically or demonstrated on culture.

Text-fig. 2 also shows the growth curves of PR8-S and PR8-T_{21} virus in the lungs of mice immunized with PR8-S vaccine. The solid homologous immunity is reflected by the absence of detectable virus in the lungs of vaccinated mice exposed to PR8-S virus. The variant strain, on the other hand, multiplied rapidly and reached a maximum titer of $10^{7.5}$ EID_{50} at 48 hours. The rate of virus multiplication during the first 24 hour period, as indicated by the slope of the curves, is somewhat lower than that of the corresponding control. The virus content of the lungs decreased gradually after 72 hours and disappeared by the 8th day.

None of the immunized animals which were challenged with either virus died. Likewise, no gross or microscopic lesions could be seen in the lungs of the mice exposed to the air-borne cloud of homologous PR8-S virus. On the other hand, spotted areas of consolidation were seen in the lungs of the PR8-S—immunized animals infected with the variant PR8-T_{21} strain. These were noted in the lungs of mice killed at 3, 4, 5, 6, and 7 days after exposure to the nebulized virus. Microscopically the pulmonary lesions were extensive but focal in distribution. At 4 days as seen in Fig. 3, there was marked destruction of the bronchial lining cells and focal areas of exudation of leucocytes into the bronchial lumina and alveolar spaces about the bronchi. Only occasional alveoli, however, showed a serous exudate.

The results of the parallel experiment in mice vaccinated with the variant PR8-T_{21} influenza virus and challenged with PR8-S are illustrated in Text-fig. 3. No homologous challenge virus (PR8-T_{21}) was detectable in the lungs of immunized mice, whereas PR8-S virus after reaching a relatively low peak titer of $10^{5.6}$ EID_{50} at 24 hours decreased rapidly and disappeared by the 4th day. None of these immunized animals which were challenged with either virus died, nor could gross or microscopic lesions be seen in the immunized mice challenged with the homologous (PR8-T_{21}) virus. Gross pulmonary lesions also were not detected in the lungs of the mice infected with the heterologous (PR8-S) virus, and as seen in Fig. 4 only minimal changes were noted in the bronchial lining cells in the animals killed at 2, 3, and 4 days after exposure to the virus cloud. There was no involvement of the alveolar walls and spaces.

DISCUSSION

Antigenic analyses by Hilleman (18) and Magill and Jotz (19) of influenza A strains isolated during the period of 1933–51 revealed the occurrence of a progressive change of antigenic characteristics over the 18 year period. They showed that antigenic complexes prevalent in older strains were replaced or obscured by antigenic components appearing in the more recently isolated

strains which reacted only weakly with antisera against the earlier ones, nevertheless retaining the capacity to elicit antibodies to them. The use of the antibody absorption technique in the study of antigenic complexes of influenza A viruses enabled Jensen and Francis (14) to demonstrate clearly the quantitative and qualitative differences in antigenic components of strains isolated in different years. The data presented in the present paper furnish experimental evidence for these epidemiological findings.

Successful transmission of the PR8 virus in the immunized mice was dependent on the initial selection of animals with uniformally low H.I. antibody titers, determined from tail vein blood, and the intranasal instillation of sufficient virus to provide a survival advantage for those virus particles whose antigenic character corresponded least with the antibodies present. With serial lung passage of the PR8 virus in immunized mice with increasingly higher antibody titers, variant strains emerged after 17 passages which differed markedly from the parent strain. This is shown by the fact that following homologous challenge with either the parent (PR8-S) or variant (PR8-T_{21}) strain no growth of virus occurred while following heterologous challenge virus growth occurred in both groups but was much greater in the lungs of the immunized (PR8-S) mice which were challenged with the PR8-T_{21} strain. The extent of cross-protection afforded by vaccination with the parent or variant strains against infection was predictable from the comparative serological and immunological data. That these variant strains were stable and independent of the selective environment was shown by the persistence of their antigenic character following 12 serial passages in the lungs of normal mice.

Of epidemiological interest is the observation that mice vaccinated with the PR8-S vaccine which resulted in high antibody levels were not protected from marked pulmonary infections as indicated by virus growth and pulmonary consolidation when allowed to breathe a cloud of variant PR8-T_{21} virus. This approach of the immunological selection of antigenic variants in the presence of homologous antibody is being continued. Variants derived from variants are under study in an effort to gain a better understanding of the mechanism and significance of antigenic changes in influenza A viruses. Comparative serological studies of variants of variants with the original PR8 virus indicate still further deviation of their antigenic make-up.

In a study of this nature the possible danger of a "laboratory pick-up" of a variant strain of influenza virus, as emphasized by Andrewes (17), always must be kept in mind. The following facts and observations, in our opinion, rule out any possible laboratory contamination, either from stock viruses or human sources: (*a*) during the period of investigation only the mouse-adapted PR8 strain of influenza A virus was handled in this laboratory; (*b*) there was a stepwise increase of a "new" antigenic component during serial passage of

PR8 in immunized mice; and (*c*) the passage strains remained highly virulent for mice whereas freshly isolated strains from human sources are usually not pathogenic for mice.

SUMMARY

Antigenically different strains of mouse-adapted PR8 influenza A virus have been produced by 17 serial passages of the virus in the lungs of mice immunized with the homologous agent. Comparative serological tests show that the variant strains share antigenic components with the parent strain but the dominant antigen is different. By means of antibody absorption it was shown that the "new" antigenic component of the variant was already present in minor amounts up to the eighth passage and thereafter gained prominence with continued passage in vaccinated mice.

Groups of mice vaccinated with either the PR8-S or T_{21} virus and having comparable antibody titers showed no growth of virus in the lungs following aid-borne challenge with homologous strains. On the other hand, following heterologous air-borne challenge no deaths occurred, but virus grew in the lungs of both groups of vaccinated mice. Almost unrestricted virus multiplication took place in the lungs of mice vaccinated with the parent strain and challenged with the PR8-T_{21} virus which resulted in extensive consolidation. Less virus grew in the lungs of the mice vaccinated with the variant strains and challenged with the PR8-S virus. In these animals only microscopic evidence of changes due to virus growth in the lungs was observed.

The successful serial passage of PR8 influenza A virus in immunized animals was dependent on the initial selection of mice with uniformly low H.I. antibody titers as determined on tail blood, and the intranasal instillation of sufficient virus to favor the survival of those virus particles least related to the antibodies present. The epidemiological implications of these observations are discussed briefly.

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EXPLANATION OF PLATES

PLATE 44

FIG. 1. Photomicrograph of a section of lung of a mouse (unvaccinated control) sacrificed 4 days after exposure to a lethal cloud of PR8-S virus. The bronchial lining cells show extensive destruction. There is also widespread exudation of edema fluid and leucocytes in the alveolar spaces and marked perivascular and peribronchial cellular infiltration. Hematoxylin-eosin-azure II stain. × 110.

FIG. 2. Photomicrograph of a section of lung of a mouse (unvaccinated control) sacrificed 4 days after exposure to a lethal cloud of PR8-T_{21} virus. The microscopic findings are the same as those described in Fig. 1. Hematoxylin-eosin-azure II stain. × 110.

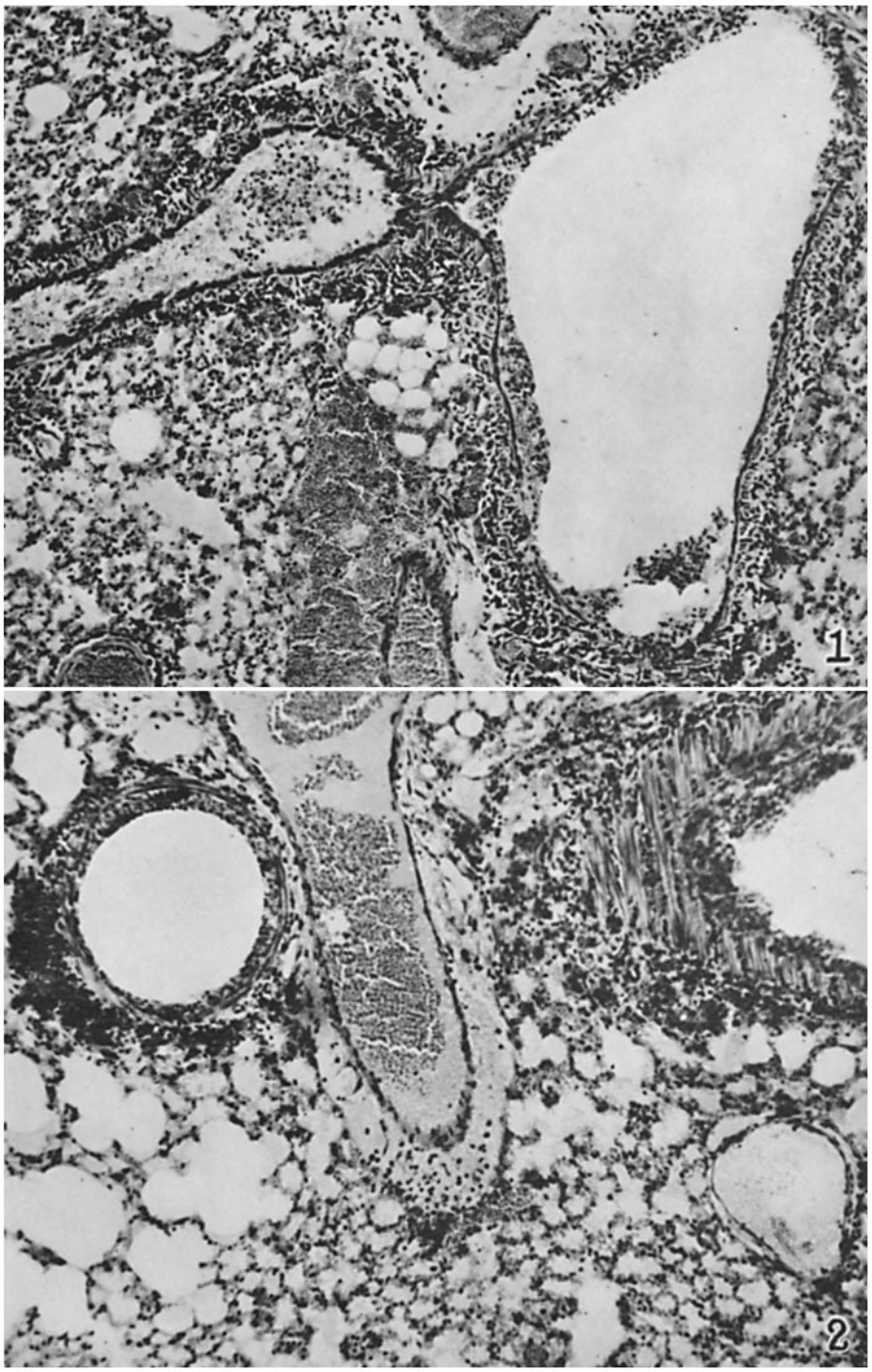

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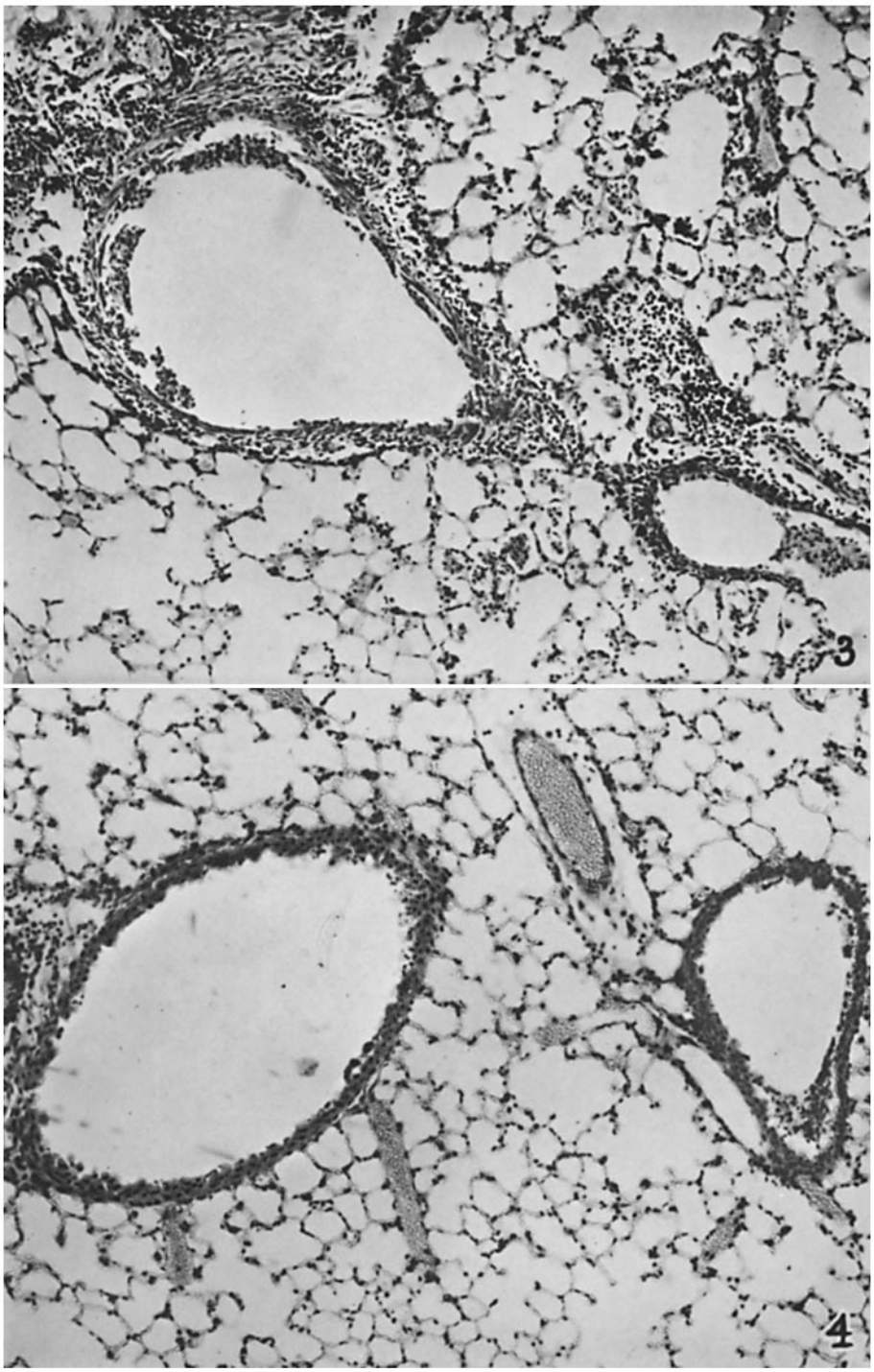
PLATE 45

FIG. 3. Photomicrograph of a section of lung of a mouse immunized with PR8-S vaccine and killed 4 days after air-borne challenge with PR8-T_{21} virus. Note the marked destruction of the bronchial lining cells and the focal distribution of exudate in the peribronchial alveoli. Hematoxylin-eosin-azure II stain. × 110.

FIG. 4. Photomicrograph of a section of lung of a mouse immunized with PR8-T_{21} vaccine and killed 4 days after air-borne challenge with PR8-S virus. The inflammatory process is restricted to minor changes in the bronchial lining cells, the alveolar portion remaining unaffected. Hematoxylin-eosin-azure II stain. × 110.



(Gerber *et al.*: Antigenic variants of influenza A virus)