A LATENT VIRUS IN NORMAL MICE CAPABLE OF PRODUCING PNEUMONIA IN ITS NATURAL HOST

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The presence of viruses in apparently healthy animals has not infrequently complicated experimental procedures. Previous investigators have discovered various viruses in mice from different highly inbred laboratory stocks. Marchal (1) described a natural disease which occurred in stock mice and was caused by infectious ectromelia virus. Traub (2) isolated a virus from normal white mice and subsequently found it to be identical with lymphocytic choriomeningitis virus described by Armstrong and Lillie (3). Theiler (4) observed spontaneously paralyzed mice in an otherwise normal stock and isolated the causal agent, mouse encephalomyelitis virus. Viruses capable of producing fatal pneumonia in mice have been found previously in normal mouse lungs by Dochez, Mills, and Mulliken (5) and Gordon, Freeman, and Clampit (6).

During the fall of 1938 attempts were made to isolate viruses from nasopharyngeal washings obtained from patients with various acute non-influenzal diseases of the respiratory tract. In the course of these experiments washings from patients with common cold, sporadic grippe, so called "primary atypical pneumonia," or pneumococcus pneumonia were inoculated intranasally in mice. Suspensions of the lungs of inoculated mice were passed in series to other mice in an attempt to determine whether or not viruses potentially pneumotropic for mice were present in any of the washings. In almost every mouse passage series transmissible pulmonary consolidations occurred after a few passages. To determine the significance of these results serial mouse lung passages were carried in normal stock mice, and in these series, too, transmissible pulmonary consolidations were encountered. By means of serial passage a virus capable of producing fatal pneumonia in mice was isolated from the lungs of apparently healthy mice. A preliminary note concerning this virus has already been published (7).

Mice are extensively used in studies of epidemic influenza and other acute infections of the respiratory tract. The frequency with which it is

necessary to resort to serial mouse lung passage in attempts to establish strains of human influenza virus in mice has made it imperative to learn as much as possible of the characteristics of this new virus found in normal mouse lungs. This information should assist in the recognition of lesions produced by this agent and may eliminate the possible confusion of results due to its presence.

It is the purpose of this paper to report the isolation of a number of strains of a pneumotropic virus from normal mouse lungs and to present the results of studies designed to determine some of its characteristics. Evidence that this virus is different from the viruses which have been found in normal mouse lungs by other investigators (5, 6) will be given.

Material and Methods

Mice.—Albino Swiss mice between $3\frac{1}{2}$ and 4 weeks of age were used throughout this study. These were obtained at weekly intervals from eight separate commercial breeders in the vicinity of New York City. It is known that three of the breeders obtained their original breeding stock from one source. The mice appeared to be perfectly healthy, and numerous groups held for observation failed to develop any signs of illness during periods of several weeks. Spontaneous pulmonary consolidation, which usually affected only a portion of one lobe, occurred with about equal frequency (1 to 2 per cent) in the mice from the various breeders.

Serial Mouse Lung Passages.—Groups of six mice were killed and their lungs removed aseptically. The lungs from each group were pooled and ground in a mortar with crystalline alundum; and sufficient beef infusion broth was added to make approximately 10 or 20 per cent suspensions. After the suspensions were centrifuged for 15 minutes at about 2500 R.P.M. the supernatant fluid was withdrawn. Groups of six mice were inoculated intranasally with the supernatant from each of the centrifuged lung suspensions. All intranasal inoculations were done under ether anesthesia using 0.05 cc. of the material. Passages to other groups of mice were made in some series at 4 to 5 day intervals and in others at 7 to 9 day intervals. Cultures of the lung suspensions were made regularly to determine sterility.

Interpretation of Results by Means of Numerical Infectivity Scores.—For the purpose of expressing in uniform terms the results obtained in a group of mice by the various experimental procedures, it has been found useful to determine the additive numerical infectivity score for each of the groups. The details of this scoring method as applied to the studies on epidemic influenza virus have already been described (8). This method was found to be satisfactory in the interpretation of results obtained with the pneumonia virus of mice. In the tables to be presented the numerical infectivity score is given as a fraction, the denominator indicating the number of mice in the group, and the numerator indicating the sum of the individual infectivity scores obtained for each mouse in the group. Thus, death of a mouse with 4/4 of lungs consolidated = 5; survival with 4/4, $\frac{3}{4}$, $\frac{1}{2}$, or $\frac{1}{4}$ of lungs consolidated = 4, 3, 2, and 1, respectively. The maximum possible infectivity score, caused by death of all mice in a group, therefore, = 5 × the denominator.

EXPERIMENTAL

Incidence of Virus Carriers among Normal Mice and Isolation of Virus.—
In the initial serial mouse lung passages no distinction was made between
the mice obtained from the eight different breeders, and groups of mice
from any one of the breeders were used in each successive passage. A total
of thirty-one separate series was carried through four or more serial pas-
sages made at 7 to 9 day intervals in these experiments. From twenty, or
65 per cent, of these thirty-one series a virus capable of producing trans-
missible pulmonary consolidation in mice was isolated. Definite evidence
of pneumonia was noted in these twenty series after an average of four
serial passages. In certain of the series definite pulmonary consolidation
occurred as soon as the second passage, while in some others it did not de-
velop until the seventh passage. Fatal pneumonia was produced as early
as the third passage, but on the average deaths did not occur until the
fifth passage.

The frequency with which the virus was encountered in these series made
it important to determine whether it was present in the mice of all or only
some of the individual breeders. Eight separate serial passages were ini-
tiated with suspensions of lungs from groups of mice obtained from each
of the eight breeders, and each series was subsequently carried in mice of
the same breeder. Transfers were made at 4 to 5 day intervals. The re-
sults were entirely negative. Although an average of nineteen serial
passages was carried out in each series, no evidence was obtained indicat-
ing the presence of a virus capable of producing pneumonia.

After a varying number of passages at 4 to 5 day intervals, parallel branch
series were initiated and were passed at 7 to 9 day intervals. The results
are shown in Table I. It will be noted that in mice obtained from breeders
1, 2, and 3, definite pulmonary consolidation occurred on the second, first,
and second passages, respectively, in the branch series maintained at the
7 to 9 day interval. From these three series the virus was readily isolated.
On the other hand, in mice from the remaining five breeders no definite
pulmonary consolidations were observed, even though an average of six
passages at the 7 to 9 day interval was carried out. The ease with which
the presence of the virus was demonstrated in mice from breeders 1, 2,
and 3 made it seem unlikely that the agent was present in mice of the
other breeders.

Experimental Disease in Mice.—Mice which were inoculated with the virus intra-
nasally appeared to remain perfectly healthy for 5 to 7 days; then they became less
active, and ate less. The usual increase in weight ceased, and many seemed to lose

weight until later, when moribund, they appeared thin and emaciated. Sick mice had ruffled, dirty fur and sat hunched up on their bedding. Respiration was slower and deeper than normal and sometimes labored. Often there was cyanosis of the ears and tail. Mice infected with small doses of the pneumonia virus often did not die until the 12th or 13th day, whereas those infected with influenza virus very rarely died later than the 9th day after inoculation (8).

Pathology.—Mice which died after the intranasal inoculation of the virus did not always show as extensive or regularly distributed pulmonary consolidations as those in mice infected with epidemic influenza virus. Not infrequently the consolidations were found to affect no more than ½ or ¾ of the lung. Occasionally mice which had been killed while moribund showed consolidation of less than ½ of the lung. Macroscopically the lesions appeared somewhat different from those produced by influenza virus. They

TABLE I

Results of Serial Mouse Lung Passages at 4 to 5 Day Intervals Followed by Branch Series at 7 to 9 Day Intervals with Mice from Eight Breeders

Mouse breeder	Serial passage						Pneumonia virus isolated
	Initial			Secondary			
	Interval	Number of passages	Pulmonary consolidation	Interval	Number of passages	Passage showing pulmonary consolidation	
	days			*days*			
1	4–5	8	0	7–9	2	10	+
2	4–5	9	0	7–9	1	10	+
3	4–5	7	0	7–9	2	9	+
4	4–5	13	0	7–9	6	0	0
5	4–5	7	0	7–9	6	0	0
6	4–5	4	0	7–9	11	0	0
7	4–5	5	0	7–9	3	0	0
8	4–5	6	0	7–9	5	0	0

were more hilar in distribution in the early stages and later tended to radiate outward along the bronchi. The lesions were less plum-colored, less dense, and less homogeneous than those observed in experimental influenza and often had a striated appearance. However, it was seldom possible to distinguish definitely between two individual lungs in which the respective consolidations had been produced by these two different viruses.

Microscopically the lesions were not strikingly different from those due to influenza virus. The bronchi and blood vessels were surrounded by dense and often quite bulky accumulations of cells which were largely mononuclear. The alveolar septa were thickened and contained cellular exudate. The alveoli contained fluid and varying numbers of mononuclear and red blood cells. Polymorphonuclear leucocytes were seldom seen except in the exudates in the bronchial lumena in which they were numerous. The bronchial epithelium was regularly well preserved and often appeared to be hyperplastic.

Bacteriology.—Repeated attempts were made to culture various aerobic and anaerobic

bacterial organisms from suspensions of mouse lungs infected with the virus. Although bacteria were isolated occasionally from these suspensions, none of those studied were found capable of producing transmissible pneumonia in mice after intranasal inoculation. Approximately 85 per cent of the suspensions of infected mouse lungs proved to be sterile in beef infusion broth and on blood-agar plates.

Pleuropneumonia-Like Organisms.—Suspensions of mouse lungs were also cultured on 30 per cent normal horse serum agar plates after the method of Klieneberger (9). The serum was filtered through a Berkefeld V candle and then through a Seitz pad before it was mixed with the agar. Only a small central portion of the surface of each serum agar plate was inoculated, and the remainder of the plate, which had been streaked with sterile broth, was used as a control. The plates were incubated at 37°C. and were observed for 6 days. When pleuropneumonia-like colonies were seen under the microscope, small blocks of serum agar carrying a few colonies were removed and transferred to 10 per cent normal rabbit serum broth which had been filtered in a manner identical with that described above. The serum broth cultures were transferred at 2 day intervals. Antisera against these organisms were prepared in rabbits. For this purpose the organisms contained in 30 cc. of serum broth culture were sedimented in the open-air angle centrifuge at 13,000 R.P.M. for 30 minutes, and were then resuspended in 3.0 cc. of 0.85 per cent NaCl. Suspensions prepared in this manner were injected intraperitoneally in rabbits, and 10 to 12 days later serum was obtained by intracardiac puncture.

The pleuropneumonia-like organisms were isolated from suspensions of consolidated mouse lungs with relative ease. At the same time no difficulty was experienced in isolating similar organisms from mouse lungs which did not contain the virus as well as from those infected with epidemic influenza virus. The number of colonies of pleuropneumonia-like organisms produced by these three different varieties of mouse lung suspensions was approximately the same. The organisms were readily subcultured in rabbit serum broth, and these cultures failed to produce any evidence of pulmonary consolidation after the intranasal inoculation of mice. The mixture of large numbers of pleuropneumonia-like organisms with small amounts of the virus did not increase the extent or the severity of the pneumonia produced in mice by the virus. Rabbit antisera which were capable of producing macroscopic agglutination of the mouse pleuropneumonia-like organisms failed to show evidence of neutralizing capacity when they were tested against 100 lethal doses of the pneumonia virus of mice. These antisera likewise failed to neutralize a similar amount of epidemic influenza virus.

Physical Characteristics of the Virus

Stability.—The virus was completely inactivated in suspensions of infected mouse lungs which were heated to 56°C. for 30 minutes. Even at room temperature the virus rapidly became inactivated, and in broth suspensions the infectious titer frequently decreased one hundredfold in 1 hour. The addition of 10 per cent normal horse serum to virus suspensions reduced the rate of inactivation at room temperature, and under these conditions the infectious titer seldom decreased more than fivefold in 1 hour. The virus was stored frozen for 4 months in a low temperature

cabinet (10) at −76°C. without noticeable decrease in infectious titer, and suspensions in 20 per cent normal horse serum were found to be active after desiccation in the frozen state.

Centrifugation.—

Suspensions of infected mouse lungs in 2 per cent normal horse serum broth were centrifuged both in the open-air angle centrifuge described by Bauer and Pickels (11) and in the high speed vacuum centrifuge developed by the same authors (12). Celluloid tubes containing 6 cc. of the suspensions were run at 13,000 R.P.M. for 30 minutes in the former apparatus and at 27,000 R.P.M. for 60 minutes in the latter. Samples were removed at various levels from the centrifuged specimens by means of the device described by Hughes, Pickels, and Horsfall (13). Serial decimal dilutions of these samples were tested for the presence of virus by the intranasal inoculation of groups of six mice.

The results indicated that after centrifugation at 13,000 R.P.M. for 30 minutes there was only partial sedimentation of the virus, whereas after centrifugation for 60 minutes at 27,000 R.P.M. it was almost completely sedimented.

Ultrafiltration.—The virus passed readily through Berkefeld V and N candles but not Seitz filters. A number of ultrafiltration experiments were carried out using graded collodion membranes of the Elford type as prepared by Bauer and Hughes (14).

As a source of virus, suspensions of infected mouse lungs in 10 per cent normal horse serum broth were centrifuged for 30 minutes at 2500 R.P.M., and the supernatant liquid was then diluted 1 to 10 with a similar diluent. This was quickly filtered through membranes with an average pore diameter of approximately 800 mμ. Portions of this stock filtrate were then passed through a series of collodion membranes of varying porosities and the infectivity of the filtrates was tested by intranasal inoculation into groups of five or six mice. In a number of instances duplicate groups of mice were used for each ultrafiltrate. Both groups were kept under observation for 14 days, at the end of which period mice of one group were killed and their lungs examined for the presence of pulmonary consolidation. The mice of the second group, however, were then reinoculated intranasally with 100 lethal doses of the virus to determine the presence of active immunity.

The results obtained with one of the strains of the virus are shown in Table II. It will be noted that a sufficient amount of virus passed through membranes with an A.P.D. of 400 mμ or larger not only to produce pneumonia in mice but also to render them actively immune to a second intranasal inoculation of unfiltered virus. The virus has passed through membranes having an A.P.D. of 348 mμ, but not through those with an A.P.D. of 300 mμ or smaller.

Partly because of the marked instability of the virus, but more par-

ticularly because of the relatively low titer of infected mouse lung suspensions, it has been very difficult to determine accurately the ultrafiltration end point. It is realized that the virus may actually be considerably smaller than these ultrafiltration results would indicate, but in the absence of suspensions of high titer it seems impossible to arrive at anything more than a rough approximation of particle size. Within the limits permitted by the present data the virus appears to have a diameter of approximately 100 to 150 mμ.

TABLE II

Results of Ultrafiltration of Pneumonia Virus

Average pore diameter of membranes	Infectivity test			Immunity test		
	Intranasal inoculation of ultrafiltrate			Intranasal inoculation of ultrafiltrate, reinoculated with 100 lethal doses after 14 days		
	Died	Survived	Infectivity score	Died	Survived	Infectivity score
mμ						
707	6	0	30/6			
612	1	5	7/6	2	4	15/6
528	0	6	12/6			
502	1	11	8/12	1	5	12/6
409	0	6	7/6	1	5	10/6
348	0	12	2/12			
301	0	6	0/6	5	1	26/6
230	0	11	0/11			
200	0	12	0/12	6	0	30/6
154	0	11	0/11			
106	0	12	0/12	6	0	30/6
Control, stock filtrate	6	0	30/6			

Biological Characteristics of the Virus

Virulence for Mice.—As may be assumed from the fact that the virus is harbored in apparently healthy mice, its initial virulence was low. The virulence increased gradually as passages were continued until eventually fatal pneumonia ensued. With some of the most virulent strains 5.0 × 10^{-6} gm. (or 0.05 cc. of a 10^{-4} dilution) of infected lung sufficed to produce death from pneumonia. However, the infectious titer of a single suspension of one strain of the virus depended to a considerable extent upon the source of the mice in which it was tested. In Table III are shown the results of a series of titrations of one strain of the virus done simultaneously in mice from each of the eight breeders. Titration end points were cal-

culated by the method of Reed and Muench (15). The titers were found to be practically identical in the three varieties of mice which had been shown to carry the virus, but varied widely in the mice from the other five sources. The 50 per cent mortality end point was $10^{-2.2}$ in mice from breeder 4, while it was $10^{-5.0}$ in mice from breeder 6. The amount of virus necessary to produce fatal pneumonia in mice from these two sources, therefore, differed by approximately 600 times.

Evidence that the virus was far more infectious for mice than was indicated by the production of pulmonary consolidation by various dilutions was obtained indirectly by testing for the presence of active immunity. Groups of mice were inoculated intranasally with decimal dilutions from 10^{-1} to 10^{-7} of one strain of the virus and were

TABLE III

Results of Simultaneous Titrations of Strain 15 Pneumonia Virus in Mice from Eight Breeders

Mouse breeder	Pneumonia virus carriers	Titration end points	
		50 per cent mortality	50 per cent pulmonary consolidation
		log	*log*
1	+	−3.9	−5.1
2	+	−3.8	−5.4
3	+	−3.8	−5.5
4	0	−2.2	−3.2
5	0	−2.4	−3.6
6	0	−5.0	−5.8
7	0	−3.1	−4.4
8	0	−3.8	−5.0

observed for an interval of 14 days. At the end of this period the mice which had survived in the groups that had received dilutions 10^{-2} to 10^{-4} were killed and their lungs examined for the presence of pulmonary consolidation. However, those groups which had been given 10^{-5} to 10^{-7} dilutions were not killed at the end of the observation period, but instead they were reinoculated intranasally with ten lethal doses of the virus. A group of normal mice from the same breeder and of the same age were inoculated simultaneously with an identical amount of virus to serve as controls. All were observed for an additional 14 day period. The results of this experiment are shown in Table IV. It will be noted that fatal pneumonia was not produced with a dilution greater than 10^{-2}, and that only two of five mice inoculated with the 10^{-4} dilution of the virus developed small areas of pulmonary consolidation. Nonetheless, mice which were inoculated with both the 10^{-5} and 10^{-6} dilutions of the virus were found to be solidly immune to the subsequent intranasal inoculation of ten lethal doses of the virus. On the basis of these observations 1/10,000 lethal dose or 1/100 infectious dose of this virus appears capable of conferring active immunity in mice after intranasal inoculation.

The virus failed entirely to infect mice except when given by the intranasal route. Repeated attempts to infect them by intracerebral, intraperitoneal, intravenous, intramuscular, and subcutaneous routes were regularly ineffective. Serial intranasal passages of the brains of mice that had received virus intracerebrally, or of the livers of mice that had been given virus intraperitoneally, failed to produce pulmonary consolidation in the test mice. The virus appears, therefore, to be strictly pneumotropic for mice.

Attempts to produce infection in normal mice by contact with those which had been given the virus intranasally were uniformly unsuccessful. A total of 64 normal mice were placed in the same jars with mice which

TABLE IV

Results of Reinoculation with Pneumonia Virus in Mice Which Had Received Varying Amounts of the Virus Intranasally

Pneumonia virus dilution	Intranasal inoculation			Reinoculation with 10 lethal doses of virus intranasally		
	Died	Survived	Infectivity score	Died	Survived	Infectivity score
10^{-1}	5	0	25/5	—	—	—
10^{-2}	4	1	23/5	—	—	—
10^{-3}	0	5	6/5	—	—	—
10^{-4}	0	5	2/5	—	—	—
10^{-5}	0	5	—	0	5	0/5
10^{-6}	0	5	—	0	5	0/5
10^{-7}	0	5	—	3	2	20/5
Control	—	—	—	5	0	25/5

had been inoculated intranasally with the pneumonia virus. Normal mice were exposed to those carrying infection at intervals varying from 12 hours to 4 days following the intranasal inoculation of the latter and contact was continued for an average of 48 hours. At the end of this time the contact mice were removed to clean jars and were observed for an additional 12 days. During this period none of the mice developed symptoms suggestive of infection by the virus, and at autopsy none showed any evidence of pulmonary consolidation.

Susceptibility Tests with Other Animal Species.—Ferrets inoculated intranasally with 10 per cent infected mouse lung suspensions did not develop fever or signs of respiratory infection. Serial passage of suspensions of turbinates and lungs of ferrets which had received the virus intranasally did not produce fever or signs of nasal infection in the passage animals. Ferrets which had been inoculated intranasally with this virus were

fully susceptible 3 weeks later to the intranasal inoculation of the PR8 strain of epidemic influenza virus. The virus has been inoculated intranasally in rabbits, guinea pigs, *rhesus* monkeys, voles, deer mice, skunks, woodchucks, opossums, and Syrian hamsters, all under ether anesthesia, and has not produced evidences of infection in any of these animals. It has been given intraperitoneally to both rabbits and guinea pigs with equally negative results. The results obtained thus far in ten species of animals indicated that this virus was pathogenic only for mice.

Cultivation in Vitro.—Attempts were made to cultivate the virus in the minced chick-embryo Tyrode medium described by Li and Rivers (16).

For this purpose either a small peripheral portion of consolidated mouse lung or 0.1 cc. of a 10 per cent suspension of infected lung was used as the inoculum for the first culture. Transfers to fresh culture media were made at 2 day and 4 day intervals.

Four strains of the virus have been successfully propagated in chick-embryo Tyrode tissue-culture medium. One strain has been maintained through ten successive transfers. All four strains showed a marked reduction in virulence under these conditions, and although they were still capable of producing pulmonary consolidation in mice, the pneumonia was seldom fatal.

Immunization of Mice.—Groups of mice were actively immunized against various strains of the virus by means of two intraperitoneal injections, separated by 14 days, of 0.5 cc. of a 10 per cent suspension of infected mouse lungs in 0.85 per cent NaCl. Other groups of mice were actively immunized in an identical manner against the PR8 strain of epidemic influenza virus. The immunized mice were held for a period of 2 weeks or more after the second intraperitoneal injection and were then tested for the presence of active immunity by the intranasal inoculation of various viruses. Groups of normal mice of the same age and from corresponding breeders were inoculated simultaneously with the immunized mice to serve as controls. Following the intranasal inoculation of the homologous or heterologous virus the mice were observed for a period of 14 days. At the end of this period all surviving mice were killed, and their lungs were examined for the presence of pulmonary consolidation. The results of these cross immunity tests, as shown in Table V, are presented as numerical infectivity scores which were derived by the method described above. It will be observed that all strains of the pneumonia virus tested were capable of inducing active immunity in mice against all other strains of this virus. The immunity resulting from two intraperitoneal injections of active virus has been found to persist for at least 4 months. None of the mice immunized with various strains of the pneumonia virus, however, were found to be immune to either the PR8 strain of epidemic influenza virus or swine influenza virus. Conversely, the mice which had been immunized with the PR8 strain of influenza virus were not immune to the pneumonia virus of mice.

Neutralization Tests with the Virus.—In order to detect the presence of specific neutralizing substances in the serum of normal mice, other animals, or man, neutralization tests were carried out in the following manner. Serial twofold or fourfold dilutions of serum to be tested, which had been inactivated at 56°C. for 30 minutes immediately before dilution, were mixed with constant amounts of the pneumonia virus. The mixtures were incubated at 37°C. for 30 minutes, and immediately thereafter each was given intranasally to a group of four to six mice. The mice were observed for 14 days, and the lungs of mice dying during this interval, as well as the lungs of those which survived and were killed at the end of the observation period, were examined for pulmonary consolidation. Neutralization of the virus was determined by means of the numerical infectivity score as described above.

TABLE V

Results of Cross Immunity Tests with Various Strains of Pneumonia Virus and the Viruses of Human and Swine Influenza

Immunized with virus Strain	Infectivity score						
	Pneumonia virus Strain					Influenza viruses	
	2	15	16	17	28	Human PR8 strain	Swine
2	1/6	0/5	—	—	—	25/5	22/5
5	—	0/5	—	0/6	—	22/5	23/5
15	—	1/5	0/5	—	—	21/5	15/5
16	0/6	1/5	0/6	1/5	—	—	—
28	—	3/5	2/5	—	3/6	25/5	—
PR8	—	25/5	18/6	—	—	0/5	—
Controls	21/6	23/6	30/6	30/6	27/6	25/5	23/5

Neutralization by Normal Mouse Serum.—A series of neutralization tests was carried out with sera of mice from four different breeders. Mice from three of these were found to carry virus, while those from one breeder were negative. Serum was obtained by intracardiac puncture, and the serum from groups of mice from each breeder was pooled separately. Serum of mice 3 and 8 weeks old from each breeder was tested separately. The results of these tests, as shown in Table VI, are expressed in terms of the numerical infectivity score. It will be seen that sera of 3 week old mice obtained from breeders 1, 2, and 3, which had been shown to carry the virus, were not capable of neutralizing the virus, nor was the serum from young mice obtained from breeder 4. However, at 8 weeks of age the serum of mice from breeder 2 did neutralize the virus, although the sera of mice from breeders 1 and 3 did not. On the other hand, the serum of

mice 8 weeks old from breeder 4 also neutralized the virus, even though it was shown that young mice from this breeder did not carry the virus.

These results seem to indicate that the presence or absence of neutralizing substances against the pneumonia virus in the serum of 8 week old mice bears no relation to the presence or absence of the virus in the lungs of 3 week old mice obtained from the same breeders.

Neutralization by Human Serum.—Sera from 67 apparently healthy human beings were tested for their capacity to neutralize the pneumonia virus of mice. Twenty-two of these sera, or 33 per cent, were found to neutralize 100 or more lethal doses of the virus, and a few of the sera were capable of neutralizing this quantity of virus in a dilution of 1:50. Many of these

TABLE VI

Results of Neutralization Tests with Pneumonia Virus and Normal Mouse Serum

Mouse breeder	Virus carriers	Age	Serum dilution	Infectivity score
		wks.		
1	+	3	1:2	21/5
		8	1:2	19/5
2	+	3	1:2	23/5
		8	1:2	0/5
3	+	3	1:2	25/5
		8	1:2	18/5
4	0	3	1:2	15/5
		8	1:2	3/5

sera also were tested for their capacity to neutralize the PR8 strain of influenza virus. No correlation was found between the serum neutralization titers against the pneumonia virus of mice and epidemic influenza virus respectively.

Acute and convalescent phase sera obtained from each of fifteen patients with various acute respiratory diseases were also tested for their capacity to neutralize the pneumonia virus of mice. The results of these tests are shown in Table VII, and in each group of mice are expressed as the numerical infectivity score. Six of the thirty sera, or 20 per cent, were found to neutralize 100 lethal doses of the virus. In thirteen persons the neutralization titer of the acute phase serum was the same as that of the convalescent phase serum. However, with the sera obtained from two patients with so called "atypical pneumonia" the results with the acute and convalescent

phase sera were different. The serum of one of these patients increased in titer during convalescence, while that of the other showed a decrease in titer during convalescence.

Neutralization by Specific Antiserum.—

Rabbits, from which normal serum had been obtained as a control, were immunized by a single intraperitoneal injection of 3.0 cc. of a 10 per cent suspension of infected mouse lungs in 0.85 per cent NaCl or 3.0 cc. of a tissue culture supernatant containing the active virus. 10 or 12 days later serum was again obtained by intracardiac puncture. Rabbit antisera prepared in this manner against each of eight strains of the pneumonia

TABLE VII

Results of Neutralization Tests with Pneumonia Virus and Human Sera Obtained during Acute and Convalescent Phases of Various Acute Respiratory Diseases

Acute respiratory disease Clinical diagnosis	Serum		Infectivity score		
	Phase	Dilution	Cases		
			1	2	3
Common cold	Acute	1:2	15/4	20/4	10/4
" "	Convalescent	1:2	12/4	12/4	14/4
Sporadic grippe	Acute	1:2	11/4	4/4	2/4
" "	Convalescent	1:2	20/4	0/4	2/4
Epidemic influenza	Acute	1:2	8/4	8/4	10/4
" "	Convalescent	1:2	4/4	11/4	7/4
Atypical pneumonia	Acute	1:2	15/4	13/4	3/4
" "	Convalescent	1:2	5/4	15/4	12/4
Pneumococcus pneumonia	Acute	1:2	20/4	18/4	15/4
" "	Convalescent	1:2	20/4	20/4	13/4

virus of mice were tested for their capacity to neutralize the homologous as well as various heterologous strains. Certain of these antisera were also tested for their capacity to neutralize human and swine influenza viruses. Rabbit antisera, similarly prepared, against the PR8 strain of epidemic influenza virus or swine influenza virus, and the serum of rabbits which had received 10 per cent suspensions of normal mouse lungs intraperitoneally 12 days previously, were also tested against various strains of the pneumonia virus of mice.

The results of these cross neutralization tests are presented in Table VIII and, in each group of mice, are expressed as numerical infectivity scores. It will be noted that each antiserum neutralized all strains of this virus against which it was tested, but not human or swine influenza viruses.

The antisera against the PR8 strain of influenza and swine influenza viruses failed to neutralize any of the strains of the pneumonia virus. The serum of rabbits injected with suspensions of normal mouse lungs also failed to neutralize the pneumonia virus.

Specific ferret and rabbit antisera against a large number of strains of epidemic influenza virus, including such well known strains as PR8, W. S., Talmey, and Gatenby, as well as certain strains isolated during 1939 (17), were also tested for their capacity to neutralize the pneumonia virus. None of these, when diluted 1:10 or more, was capable of neutralizing 100 lethal

TABLE VIII

Results of Cross Neutralization Tests with Various Strains of Pneumonia Virus and the Viruses of Human and Swine Influenza

Antiserum		Infectivity score						
		Pneumonia virus Strain					Influenza viruses	
Strain	Dilution	2	15	16	17	28	Human PR8 strain	Swine
2	1:2	0/5	2/5	0/5	1/5	—	—	20/5
7	1:2	0/5	2/5	1/5	0/5	—	—	—
15	1:2	2/5	2/5	0/5	0/5	—	—	—
16	1:2	0/5	3/5	0/5	1/5	—	25/5	—
17	1:2	0/5	2/5	0/5	0/5	—	—	22/5
25	1:2	0/5	0/5	0/5	0/5	0/5	25/5	—
27	1:2	0/5	5/5	0/5	1/5	—	—	—
28	1:2	2/5	4/5	0/5	1/5	0/5	25/5	—
PR8	1:2	13/5	25/5	25/5	—	25/5	0/5	—
Swine	1:2	—	20/4	23/5	—	—	—	0/5
Mouse lung	1:2	16/5	21/5	25/5	18/5	—	—	—
Control	1:2	17/5	25/5	25/5	22/5	25/5	25/5	25/5

doses of this virus. However, in a dilution of 1:2, some antisera produced against Brooklyn 15 strain (18) and strain 149 (17) of influenza virus were found to neutralize the pneumonia virus. The possibility that either of these strains was contaminated with the pneumonia virus was excluded since neither had been carried in mice.

These results seem to indicate that all strains of the pneumonia virus which have been tested were immunologically identical. They indicate also that the pneumonia virus of mice was not related antigenically to either swine influenza virus or to most strains of human influenza virus.

DISCUSSION

Besides their importance to natural history, the group of viruses now known to occur in the lungs of apparently healthy mice have definite practical significance. The fact that these agents are entirely avirulent as they occur naturally and develop virulence only after serial mouse passage adds considerably to the difficulty of determining their presence. The possibility that they may exist in almost any stock mice complicates the interpretation of pulmonary consolidations observed after serial mouse passage. Since serial passage is usually necessary before epidemic influenza virus can be established in mice (19, 20), there exists the possibility that a mouse lung virus may be encountered during the process. Although it has not been difficult to distinguish between this pneumonia virus of mice and epidemic influenza virus, there is evidence indicating that mouse lungs may contain other viruses which are less readily differentiated from influenza virus.

It seems probable that the virus isolated from the lungs of mice in this laboratory is quite different from the viruses which have been described previously by Dochez, Mills, and Mulliken (5) and Gordon, Freeman, and Clampit (6). These latter agents were readily encountered in rapid (4 to 5 day) serial mouse passages, whereas the virus described herein was recoverable only when slow (7 to 9 day) serial mouse passages were made. Furthermore, the course of the disease was relatively rapid with those viruses which have been described by other investigators (5, 6) and mice usually died in 2 to 4 days. The virus described in this communication seldom killed mice before the 8th day after inoculation irrespective of the quantity of virus given. Dochez, Mills, and Mulliken (5) reported that their virus was infectious for ferrets and produced high fever and nasal symptoms in these animals. Our virus, on the other hand, was entirely non-infectious for ferrets and did not become infectious for this species even after a number of serial ferret passages. Most significant, perhaps, as evidence of the differences is the fact that those viruses described by previous investigators (5, 6) were either non-antigenic or were very poor antigens and failed to immunize mice against themselves. They also failed to produce neutralizing antisera after injection into rabbits. The virus described in this report readily immunized mice after either intraperitoneal or intranasal inoculation and produced satisfactory neutralizing antisera when injected into rabbits.

The strict pneumotropism of the virus for mice and its complete avirulence for any other species of animals studied seems to distinguish this

virus from others known to occur in normal mice. On this basis its differences from infectious ectromelia virus (1), lymphocytic choriomeningitis virus (2, 3), and mouse encephalomyelitis virus (4) seem obvious. The agent can also readily be differentiated from meningopneumonitis virus (21), psittacosis virus (22), and lymphogranuloma inguinale virus (23) since all of these agents possess characteristics which are quite different from those of the pneumonia virus of mice.

If there are, as there seem to be, at least two different pneumotropic viruses present in the lungs of certain apparently healthy mice, the interpretation of pulmonary consolidation produced in mice by serial passage becomes hazardous unless the causal agent can be positively identified by means of specific neutralization tests.

How and from what source this virus gains access to the lungs of mice are unknown. However, there is some evidence which suggests that the original source of the virus was not mice themselves. Three of the eight breeders whose mice were studied during the course of this investigation received their original stock mice from one source. It is known that the progeny of mice from this common source do not now carry the virus. It has been shown, however, that the mice obtained from one of these three breeders regularly carried the virus and that those from the other two did not. This might be taken to indicate that those mice which now carry the virus became infected after they had been acquired by the breeder in question and that young mice from this stock either acquired the virus from their mothers or were regularly infected from another source. That this other source might actually be human beings is suggested by the fact that approximately 30 per cent of normal human sera are capable of neutralizing the virus. In view of the large body of evidence which has been assembled in support of the specificity of the neutralization test in many other virus infections, it seems unreasonable to overlook the possible significance of the results obtained with this virus. However, if the virus is derived from human beings, it probably does not have any etiological relationship to the five different acute respiratory infections which were studied since no definite evidence was obtained of an increase in neutralizing antibodies against the virus during convalescence from these infections.

SUMMARY

1. A virus capable of producing fatal pneumonia in mice has been isolated repeatedly from the lungs of certain apparently healthy mice. Not all mice carry the virus. It was obtained only from mice supplied by three breeders although mice from eight different sources were studied.

2. The virus was avirulent as it occurred in normal mouse lungs and became virulent only after serial mouse lung passage. It was strictly pneumotropic for mice and produced pneumonia when given intranasally but showed no evidence of infection when given by other routes. The virus was non-infectious for ferrets and did not become pathogenic for this species after numerous serial passages. It was also non-pathogenic for rabbits, guinea pigs, *rhesus* monkeys, voles, deer mice, skunks, woodchucks, opossums, and Syrian hamsters.

3. All strains of the virus which have been tested have been immunologically identical, as indicated both by cross immunity and cross neutralization tests in mice.

4. The virus was antigenic both in mice and in rabbits and was readily differentiated from viruses of human influenza and of swine influenza by means of either cross immunity or cross neutralization tests.

5. The virus was also neutralized by about 30 per cent of normal human sera tested.

6. The virus was extremely labile, and suspensions prepared in saline or broth became inactivated within a few hours at room temperature. The addition of normal horse serum to the virus suspensions, however, exerted a definite stabilizing effect.

7. Ultrafiltration results indicated that the virus particles have a diameter of about 100 to 150 millimicrons.

8. Evidence is presented which indicates that this virus is different from other viruses which various investigators have found in normal mouse lungs.

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