THE RECOVERY FROM PATIENTS WITH ACUTE PNEUMONITIS OF A VIRUS CAUSING PNEUMONIA IN THE MONGOOSE*

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In the course of the last few years a number of reports have appeared concerning an acute infectious disease of the respiratory tract in man which has been associated with unusual pathological changes in the lungs. Bowen (1) described an epidemic disease which he termed "acute influenza pneumonitis." Allen (2) published an account of 68 cases of a disease which was called "acute pneumonitis." Smiley, Showacre, Lee, and Ferris (3) reported their clinical findings in 86 patients with so called "acute interstitial pneumonitis" and suggested that the disease described by them was probably the same as that studied by Bowen (1) and Allen (2). Reimann (4) reported his observations in eight patients with a similar disease which he designated "atypical pneumonia," and suggested on clinical grounds that this disease was probably caused by a virus. More recently Reimann and Havens (5) described a large epidemic of a disease which they considered similar to that reported by the authors mentioned above, and they also reviewed the literature pertaining to this disease.

Attempts by various investigators to determine the etiology of this disease have not been successful. Stokes, Kenney, and Shaw (6) reported the isolation of a filterable agent from two patients with this disease. This agent was infectious for mice, guinea pigs, and possibly for ferrets. Before serological tests could be done, the agent was lost, and consequently evidence was not obtained regarding any etiological relationship.

The frequency with which this clinical syndrome has been observed in the past 5 years (5), the apparent recent increase in the number of persons who contract the disease (7, 8), and the infectious nature of the illness (3, 5, 8) have given this disease sufficient importance to warrant a vigorous attempt to determine its etiology. Accordingly, the purpose of this paper

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is to report the isolation of a pneumotropic virus from the throat washings of patients ill with acute pneumonitis and, through the use of mongooses as experimental animals, to present evidence that the agent is etiologically related to this disease.

Material and Methods

During the latter part of December, 1938, a patient exhibiting symptoms similar to those described by the authors quoted above was admitted to the Hospital of The Rockefeller Institute. Throat washings and serum obtained from this patient through the kindness of Dr. Colin M. MacLeod served as the initial materials for this study. Throat washings and serum from other patients with a clinically similar disease at the New York State Hospital at Ray Brook, were obtained through the kindness of Dr. Edward S. Rogers, and from the Cornell University Infirmary, Ithaca, New York, through the kindness of Dr. D. F. Smiley.

Throat Washings.—Nutrient broth, or a broth-saline mixture, was used to obtain washings of the throats of patients during the first few days of the disease. These washings in some instances had been mixed with equal parts of glycerin and shipped to the laboratory by mail; in others they were frozen solid with carbon dioxide and transported in the frozen state. The glycerinated specimens were stored at 4°C., while the frozen washings were kept in a low temperature storage cabinet (9) at −76°C.

Serum.—Serum for immunological studies was obtained from the patients during the first few days of the illness and again during convalescence, or approximately 1 month after the onset of fever. All serum specimens were stored at 4°C.

Attempts to Infect Various Animals with Throat Washings.—Among the animals inoculated intranasally with throat washings from a number of clinically typical cases were ferrets, mice, guinea pigs, rabbits, monkeys, opossums, skunks, woodchucks, voles, deer mice, and Syrian hamsters. Serial lung passages by intranasal inoculation were also made in ferrets and mice. In no instance did the inoculated animals develop evidence of infection, and at autopsy no lesions were found in their respiratory tracts.

In view of the clear-cut negative results obtained in the attempts to infect common laboratory animals, as well as various wild animals obtainable in the vicinity of New York City, with the throat washings from patients with acute pneumonitis, the possibility of testing animals from other geographical regions was considered. In the search for a new experimental animal the desirability of having the animal available in relatively large numbers was kept in mind. It was finally decided to attempt to transmit the infection to the mongoose, the chief reasons for this decision being that the mongoose is available in abundance on some of the Caribbean islands and that in appearance it resembles somewhat the ferret which is susceptible to virus influenza, another respiratory infection. Inasmuch as the importation of mongooses to the United States is prohibited, it was decided to take throat washings and other potentially infectious material to Kingston, Jamaica, where the work on the mongoose described below was carried out. Laboratory and animal house facilities were available in Kingston at the Tuberculosis Research Station which is maintained jointly by The Rockefeller Foundation and the Government of Jamaica.

Mongooses.—The mongoose (*Herpestes griseus*) is somewhat similar in appearance and habit to the ferret and the weasel. Being carnivorous, it feeds mostly on wild and do-

mestic fowl, rodents, and other small animals. The mongooses used in the experiments described below were trapped in three different localities in Jamaica. Only adult animals were used, and while the weight of individual mongooses was not determined, it was estimated to vary between 600 and 1000 gm. Considerable difficulty was experienced in handling the animals because of their vicious natures. It was impossible to handle them manually even with heavy leather gloves, and consequently special metal tongs and metal tube traps were constructed for catching and holding the animals during various experimental procedures.

Inoculation of Mongooses and Serial Passage.—2 cc. of throat washing or other material were inoculated intranasally in mongooses anesthetized with ether. For this purpose material which had been frozen and dried in vacuum by the method of Bauer and Pickels (10) was rehydrated by the addition of 2 cc. of sterile distilled water. When glycerinated material was used, 2 cc. of the glycerin mixture were inoculated intranasally. Control animals were given 2 cc. of sterile 0.85 per cent NaCl solution intranasally as the initial inoculum. At the end of a 10- to 12-day observation period the animals were anesthetized, bled by cardiac puncture, and killed. Their lungs were removed aseptically and the turbinates and abdominal viscera examined. Serial passages were made with centrifuged suspensions of mongoose lung in 0.85 per cent NaCl or in 10 per cent normal horse serum in 0.85 per cent NaCl solution unless otherwise stated. Whenever possible, two mongooses were used for each passage.

EXPERIMENTAL

Susceptibility of Mongooses to a Virus Present in the Throat Washings of Patients with Acute Pneumonitis.—

Two mongooses were inoculated intranasally with each of four throat washings, Nos. 109, 440, 441, and 525, from cases of acute pneumonitis. Three were broth-saline washings which had been frozen and dried in vacuum and one, No. 525, was a glycerinated specimen. One of the broth-saline washings, No. 109, had been filtered through a Berkefeld V candle prior to freezing and drying. The inoculated mongooses were observed for abnormalities in temperature, respiration, and appetite, and for the presence or absence of diarrhea for 10 or 12 days. They were then killed, and 20 per cent suspensions were prepared from the lungs of each group. Each of these suspensions was passed to two other normal mongooses by intranasal inoculation. Similar serial passages were made in each case subsequently at 10-day intervals until the experiment was terminated. The results of these serial passages in the mongoose are recorded in Table I.

It will be observed that each of these four throat washings caused the development of definite pulmonary lesions in the mongoose upon serial intranasal passage. Two distinct types of lesions were observed. These consisted of circumscribed areas of consolidation and generalized pulmonary hyperemia. The consolidations were plum-colored and of a gelatinous consistency. They varied in size from small areas 1 mm. or more in diameter to large lesions filling an entire lobe. The distribution of the consolidations varied from single large lesions in one lobe to multiple small lesions in a

number of lobes. Pulmonary hyperemia was usually generalized and was found regardless of the presence or absence of consolidations. Hyperemic lungs were of a deep red color in striking contrast to normal mongoose lungs which were light gray in color with a pinkish tinge. In 10 serial passages of throat washing No. 109, which had been passed through a Berkefeld V filter, 11 of 19 inoculated animals exhibited lung lesions. In 6 serial passages of throat washing No. 440, lung lesions were produced in 6 of 12 inoculated animals. In 4 serial passages of throat washing No. 441, lung lesions were found in 3 of 8 inoculated animals. In 5 serial passages of throat washing No. 525, 38 of 51 inoculated animals showed lung lesions. Thus, in a total of 25 serial passages with the four throat washings, 64 per

TABLE I

Results of Serial Passages in the Mongoose of Throat Washings Obtained from Patients with Acute Pneumonitis

Throat washing	No. of mongooses	Serial passages	No. dead before 6th day	No. showing normal lungs	No. showing pulmonary hyperemia	No. showing pulmonary consolidations	Total pulmonary lesions
							per cent
109*	19	10	1	7	2	9	57.9
440	12	6	0	6	2	4	50.0
441	8	4	0	5	2	1	37.5
525	51	5	4	9	12	26	74.4
Total.....	90	25	5	27	18	40	64.4

* Throat washing No. 109 was filtered through a Berkefeld V candle prior to inoculation of mongooses.

cent of 90 inoculated animals exhibited lung lesions, and in 44 per cent of instances there was pulmonary consolidation.

Five animals died between 2 and 4 days after inoculation without any evident cause for death. As will be indicated below, a similar percentage of control animals died within the same period after inoculation.

It might be mentioned that no normal temperature curve could be determined for the mongoose. The rectal temperature of individual mongooses varied from 99° to 107°F. on different days (depending to a large extent upon the excitement produced in the animals when they were caught for temperature determinations). Nasal symptoms were unreliable owing to the development of a nasal discharge by animals which became angered upon the approach of the observer. Severe diarrheas developed in normal animals as often as in inoculated animals. Respiratory rates were also

highly variable in all mongooses and became quite irregular with the excitement produced by handling or observing the animals in their cages.

Serial Passages of Normal Mongoose Lungs.—It is well known that normal animals may carry latent viruses, the virulence of which becomes enhanced on serial passage (11). Since no previous experimental work with the wild mongoose appears to have been reported, it was essential to exclude the possibility that the pulmonary lesions observed were not due to a virus present in mongoose lung itself.

Groups of mongooses from the same stock used for the throat washing passages were inoculated intranasally with 2 cc. of sterile 0.85 per cent NaCl solution. These animals were carefully isolated from any possible contact with the infected animals and were observed for 10 days. At the end of this period they were killed and their lungs examined for lesions. Suspensions were prepared from each pair of mongoose lungs as before and

TABLE II

Results of Serial Passages in the Mongoose of Suspensions of Normal Mongoose Lungs

Group	No. of mongooses	Serial passages	No. dead before 6th day	No. showing normal lungs	No. showing pulmonary hyperemia	No. showing pulmonary consolidations	Total pulmonary lesions
							per cent
I	11	6	0	8	2	1	27.2
II	6	3	0	6	0	0	0
III	10	5	1	9	0	0	0
IV	32	16	3	29	0	0	0
Total.....	59	30	4	52	2	1	5.1

were passed to two other mongooses. Serial passages were continued in this manner until the experiment was terminated. The results of these serial passages are recorded in Table II.

It will be noted that the serial passages of lungs from saline-inoculated mongooses failed consistently to produce lung lesions. In 30 serial passages from 4 groups of control animals only 3 of 59 inoculated mongooses exhibited lung lesions, and of these only 1 showed a consolidation. Cultures of this single consolidated lung yielded a heavy growth of Gram-positive cocci after 24 hours' incubation.

It seems evident, therefore, that the normal mongoose lung did not contain a latent pneumotropic virus which became activated upon serial passage.

Contact Experiments.—The results of the serial passages in the mongoose of throat washings obtained from patients with acute pneumonitis indicated that lung lesions could be produced under these conditions. The

control experiments in which serial passages of saline-inoculated mongoose lungs were made indicated that similar lung lesions were not produced under these circumstances. It seemed of importance to determine whether the virus responsible for the lung lesions was capable of producing pulmonary lesions in uninoculated animals placed in contact with inoculated mongooses.

Five mongooses were inoculated intranasally with a 5 per cent lung suspension obtained during serial passage of throat washing No. 525. Each inoculated mongoose was placed in one side of a double cage, and an uninoculated mongoose was placed in the other compartment. The animals were kept in contact for 10 days, at which time the inoculated animals were killed and their lungs examined for lesions. The uninoculated animals were killed 13 days after the beginning of the contact experiment and their lungs examined likewise.

All of the inoculated animals were found to have pulmonary lesions, and 4 of the 5 contact animals also had lung lesions which were identical in character with those observed in the inoculated animals.

These and the previous experiments suggested that a pneumotropic virus had been isolated from the throat washings studied, that this virus was capable of infecting mongooses by contact, and that it was not present in normal mongoose lungs.

Bacteriology and Pathology of Mongoose Lung Lesions.—The pulmonary lesions which resulted from the serial passages in the mongoose of throat washings obtained from cases of acute pneumonitis were studied in order to determine whether they contained bacteria and whether they possessed pathological characteristics which would serve to distinguish the nature of the agent.

Four mongoose lungs containing extensive consolidations were cultured both in nutrient broth and on nutrient agar plates. The cultures were incubated at 37°C. and observed for bacterial growth for 72 hours; all proved to be sterile. One agar plate showed a few scattered colonies at 72 hours which were obviously due to a contaminant.

Pulmonary consolidations resulting from the serial passage of throat washings Nos. 109 and 525 were examined histologically. The pathological changes produced by the passage of both strains were identical. The lungs showed an extensive edema which filled and distended the alveolar spaces and caused thickening of the alveolar walls. There was a sparse cellular exudate composed almost entirely of mononuclear cells. The bronchial epithelium was well preserved and the bronchial lumina contained small amounts of protein-rich edema fluid. Neither perivascular nor peribronchial cellular infiltrations were seen.

Inoculation of Mongooses with Other Known Viruses.—The susceptibility of the mongoose to three different known viruses was tested. The PR8 strain (12) of influenza A virus (13) and strain 15 of the pneumonia virus

of mice (14) were inoculated intranasally in groups of mongooses, and serial passages were carried out using suspensions of both turbinates and lungs. In the case of mongooses inoculated with influenza A virus, passages were made at 4-day intervals. No pulmonary lesions were observed in the inoculated or the passage animals. Furthermore, attempts to demonstrate the presence of either virus in mongoose tissues by the subinoculation of mice were unsuccessful. Sera obtained from mongooses 10 to 12 days after inoculation with these viruses did not contain any demonstrable neutralizing antibodies against either virus.

Lymphocytic choriomeningitis virus (15)[1] was inoculated intracerebrally in mongooses, and serial passages were made with suspensions of brain. Although the inoculated mongooses became obviously ill, it seems probable that the symptoms may have resulted from the intracerebral inoculations themselves since the subinoculation of guinea pigs and mice with mongoose brain suspensions failed to reveal the presence of lymphocytic choriomeningitis virus.

Characteristics of the Virus

Filtration Experiments.—Efforts were made to determine whether the virus responsible for the production of lung lesions in the mongoose would pass through Berkefeld V and N filters.

It will be recalled that throat washing No. 109 had been filtered through a Berkefeld V candle prior to the inoculation of mongooses and that, as shown in Table I, this filtered throat washing was equally as active as the three unfiltered specimens in producing lung lesions in the mongoose. Suspensions of five consolidated mongoose lungs were passed through either Berkefeld V or N filters and 2 cc. of each filtrate were inoculated intranasally into each of two mongooses. After the usual observation period the mongooses were killed, and suspensions of their lungs were passed serially to other mongooses in the manner described above. The results of the serial passages of these filtrates are shown in Table III.

It will be seen that all five filtered suspensions produced lung lesions when passed serially in the mongoose. The percentage of animals exhibiting definite pulmonary lesions varied from 50 to 75 per cent in the five groups. In 27 serial passages 34 of 53 inoculated animals showed lung lesions, and of these 21 had consolidations. These results indicated clearly that the active agent was filterable.

Preservation.—The virus was studied to determine its ability to withstand storage in glycerin and freezing and drying in vacuum.

[1] This virus was kindly supplied by Dr. L. T. Coggeshall, Laboratories of the International Health Division of The Rockefeller Foundation, New York.

Three consolidated mongoose lungs were placed in 50 per cent glycerin and shipped to the New York laboratory without refrigeration. These three specimens remained in glycerin for 3 days after which they were removed, washed, and ground to form suspensions. These suspensions were then frozen and dried in vacuum and returned to the Jamaica laboratory. Throat washing No. 525 was glycerinated and remained in glycerin for 13 days before inoculation in the mongoose. For approximately 10 days of this period the glycerinated washing was kept in a refrigerator at −4°C. During the remaining 3 days the material stood without refrigeration during shipment to Jamaica. The frozen and dried suspensions of glycerinated lung and the glycerinated throat washing were passed serially in the mongoose by the method described above.

It was found that the virus remained active after these various procedures and in each instance was capable of producing lung lesions in the mongoose.

TABLE III

Results of Serial Passages in the Mongoose of Filtrates of Infected Suspensions of Mongoose Lungs

Strain of virus	Berkefeld filter	Serial passage of filtrate	No. of mongooses	No. dead before 6th day	No. showing normal lungs	No. showing pulmonary hyperemia	No. showing pulmonary consolidations	Total pulmonary lesions
								per cent
109	V	3	6	1	2	2	1	50.0
109	V	12	24	0	6	2	16	75.0
109	N	3	5	0	2	1	2	60.0
109	V	6	12	2	3	5	2	58.1
525	N	3	6	1	2	3	0	50.0
Total.....		27	53	4	15	13	21	64.1

Cultivation.—The virus was also studied to determine its ability to propagate on the chorio-allantoic membrane of the developing chick embryo.

Throat washings Nos. 109 and 440 were inoculated on the egg membrane by the method of Burnet (16) and were passed serially in chick embryos. Specimens of chick embryo tissue were taken at the 10th, 16th, 20th, and 30th egg membrane passages of throat washing No. 109, as well as from the 2nd and 5th egg membrane passages of throat washing No. 440; suspensions were prepared and frozen and dried in vacuum. They were then shipped to Jamaica and passed serially in the mongoose by the procedure described above. A suspension of the lung from the 12th serial mongoose passage of throat washing No. 109 was passed in a similar manner on the egg membrane. Material obtained from the 10th egg membrane passage was frozen and dried in vacuum and shipped to Jamaica where it was also tested in the mongoose. The results of the serial passage in the mongoose of the materials obtained from egg membrane passages are recorded in Table IV.

Pathogenicity.—It will be seen that the virus present in throat washing No. 109 retained its pathogenicity for the mongoose for at least 30 serial

passages on the egg membrane. It was also found that the virus could be propagated on the egg membrane directly from an infected mongoose lung. The various egg membrane passage materials produced lung lesions in 50 to 83 per cent of the inoculated animals. In 33 serial passages with all specimens 38 of 63 inoculated animals exhibited lung lesions, and in the lungs of 21 of the animals there were consolidations.

To test the infectiousness of the virus, falling fivefold dilutions were made from a 5 per cent suspension of a consolidated lung obtained from the 3rd serial mongoose passage of throat washing No. 525. 2 cc. of each dilution were inoculated intranasally in each of two mongooses. The animals were

TABLE IV

Results of Serial Passages in the Mongoose of Infected Chick Embryos

Strain of virus	Serial egg membrane passages	Serial passages in the mongoose	No. of mongooses	No. dead before 6th day	No. showing normal lungs	No. showing pulmonary hyperemia	No. showing pulmonary consolidations	Total pulmonary lesions
								per cent
109	16	13	23	1	10	3	9	52.1
109	21	3	6	0	3	1	2	50.0
109	30	5	10	0	4	2	4	60.0
109*	10	3	6	1	0	4	1	83.4
440	2	3	6	1	1	1	3	66.6
440	5	3	6	1	1	3	1	66.6
440	5	3	6	1	1	3	1	66.6
Total.....		33	63	5	20	17	21	60.3

* Original inoculum for egg membrane obtained from mongoose lung suspension of 12th serial mongoose passage of throat washing No. 109.

observed for 10 days and were then killed and their lungs examined. It was found that a $10^{-4.1}$ dilution of consolidated lung was sufficiently infectious to produce consolidations in the 1st passage but that higher dilutions failed to produce lung lesions on a single passage.

Although, as was stated above, attempts to establish the virus directly from throat washings in various animal species other than the mongoose had been entirely unsuccessful, it was of importance to restudy the pathogenicity of the virus for these species following serial passage in the mongoose and upon the chorio-allantoic membrane of the developing chick embryo. Suspensions of consolidated mongoose lungs from the 5th and 3rd serial mongoose passages of throat washings Nos. 109 and 525 respectively were inoculated intranasally in groups of Swiss mice and passed in series for from 3 to 7 passages at intervals varying from 5 to 14 days.

In no instance were lung lesions produced in the mice, which appeared healthy throughout the period of observation. Similar suspensions of consolidated mongoose lungs were tested by intracerebral inoculation of mice, as well as by the intranasal inoculation of a ferret. No evidences of infection occurred in either species. Furthermore, the ferret was shown subsequently to be fully susceptible to infection by influenza A virus. Suspensions prepared from various egg membrane passages of strains 109 and 525, which had been shown to be active in the mongoose, were tested by serial intranasal passage in Swiss mice, deer mice, voles, woodchucks, opossums, and monkeys. In no instance was there any indication of infection in the inoculated animals.

TABLE V

Results of Neutralization Tests with Normal and Convalescent Mongoose Sera and Acute Pneumonitis Virus

Mongoose serum	No. of mongooses	Serial passages	Serum dilution	Strain of acute pneumonitis virus	No. dead before 6th day	No. showing normal lungs	No. showing pulmonary hyperemia	No. showing pulmonary consolidations	Total pulmonary lesions
									per cent
Normal	14	7	1:5	525	1	2	4	7	78.5
Convalescent	8	4	1:5	525	0	7	1	0	12.5
	6	3	1:10	525	0	3	3	0	50.0

Neutralization of the Virus by Convalescent Mongoose Serum.—To determine whether the infection of the mongoose resulted in the production of antibodies capable of neutralizing the virus, animals were inoculated with 2 cc. of a 5 per cent suspension of consolidated mongoose lung. Blood specimens were obtained by cardiac puncture before and 7 weeks after inoculation.

5 per cent suspensions of consolidated mongoose lung were prepared and mixed with the various samples of serum in the proportion of 2 cc. of suspension to 0.5 cc. of serum. The serum-virus mixtures were allowed to stand at room temperature for 30 minutes, after which each mixture was inoculated intranasally in two mongooses. The mongooses were observed for 10 days and were then killed. Their lungs were examined and suspensions were made and passed serially to other mongooses for 2 to 4 passages. Serum was used only with the initial virus suspension and was not added to the suspensions used for subsequent passages. This laborious and time-consuming technique was considered necessary since it had been found that individual mongooses varied in their response to infection by the virus and that approximately 30 per cent of animals in any series did not show visible lung lesions in the gross.

The results of these experiments are shown in Table V. It will be seen that normal mongoose serum failed to neutralize the virus and that with a 1:5 dilution of serum 11 of 14 inoculated animals developed lung lesions of which 7 were consolidations. On the other hand, serum obtained from convalescent mongooses 7 weeks after inoculation did neutralize the virus. With a 1:5 dilution of serum only 1 of 8 inoculated animals developed pulmonary hyperemia, and the remaining 7 animals had normal lungs. However, a 1:10 dilution of convalescent mongoose serum was less effective in neutralizing the virus and 3 of 6 inoculated animals developed pulmonary hyperemia, although the other 3 were normal.

Neutralization of the Virus by Serum from Patients Convalescent from Acute Pneumonitis.—To determine if an etiological relationship existed between the virus isolated from the throat washings of patients with acute pneumonitis and the disease itself, attempts were made to discover if specific neutralizing antibodies against the virus were produced during convalescence from the disease.

Acute-phase and convalescent sera were obtained from four cases of acute pneumonitis occurring in widely separated areas. In addition, two convalescent sera were used from other cases from which no acute-phase serum was available. These sera were tested against strains 109, 440, and 525 to determine their capacity to neutralize the virus. The results obtained with acute-phase serum-virus mixtures are compared with those obtained with convalescent serum-virus mixtures in Table VI. The neutralization technique was identical with that described above. These sera were also tested for their capacity to neutralize the PR8 strain of influenza A virus.

It will be seen that the virus was not neutralized by the addition of acute-phase sera to virus suspensions. Each series of acute-phase serum-virus mixtures produced lung lesions on serial passage in the mongoose that were comparable to those obtained with serial passages in the absence of serum. The convalescent sera, however, did neutralize the virus, and in no case was the incidence of lung lesions comparable to that obtained either with the acute-phase sera or with passages of virus in the absence of serum. In 4 of the serial passages of convalescent serum-virus mixtures no lung lesions were encountered. In the other 4 serial passages some of the animals exhibited pulmonary hyperemia, but in no instance did consolidation develop. Serum 6 was obtained from an individual previously in contact with a case of acute pneumonitis but who did not have a clinical attack of the disease. In the 1st passage of this serum-virus mixture there were no lung lesions in the mongoose. A consolidation did, however, appear in the 2nd serial passage. This may be suggestive evidence of partial neutraliza-

tion of the virus by this serum, although similar normal passages have occurred in series receiving active virus. It seems likely, nonetheless, that there was partial neutralization by this serum since in these experiments

TABLE VI

Results of Neutralization Tests with Acute-Phase and Convalescent Human Sera and Acute Pneumonitis Virus

Case	No. of mongooses	Serial passages	Serum	Standard neutralizing titer of serum with PR8 influenza A virus	Strain of acute pneumonitis virus	No. dead before 6th day	No. showing normal lungs	No. showing pulmonary hyperemia	No. showing pulmonary consolidations	Total pulmonary lesions
										per cent
1	10	5	Acute	1:4	109	1	3	2	4	60.0
	10	5	Convalescent	1:4		0	8	2	0	20.0
1	4	2	Acute	1:4	525	1	1	1	1	50.0
	4	2	Convalescent	1:4		0	3	1	0	25.0
2	7	4	Acute	1:18	109	1	1	1	4	71.3
	8	4	Convalescent	1:18		0	7	1	0	12.5
2(*a*)	7	4	Acute	1:18	109	1	1	1	4	71.3
	7	4	Convalescent	1:20		0	6	1	0	14.3
3	4	2	Acute	1:4	440	0	3	0	1	25.0
	4	2	Convalescent	1:4		0	4	0	0	0
4	4	2	Acute	—	525	2	0	0	2	50.0
	4	2	Convalescent	1:35		0	4	0	0	0
5	4	2	Convalescent	1:8	525	0	4	0	0	0
6	4	2	Convalescent	1:2	525	0	4	0	0	0
7	4	2	Contact	1:10	525	0	2	1	1	50.0
Total...	36	19	Acute	—	—	5	8	4	12	55.2
Total...	49	25	Convalescent	—	—	0	42	6	1	14.2

all of the acute-phase serum-virus mixtures produced lung lesions in the 1st passage. It will also be observed that there was no increase in neutralizing antibodies against the PR8 strain of influenza A virus following an attack of acute pneumonitis.

Cross Neutralization Tests with Acute Pneumonitis and Influenza Viruses.—Despite the fact that it has been shown previously (17) that acute

pneumonitis does not produce an increase in antibodies against influenza A virus, it seemed of interest to determine whether or not any antigenic relationship existed between these two viruses since both are capable of causing acute respiratory infections in human beings.

Normal ferret serum and the serum of ferrets convalescent from infection with the PR8 strain of influenza A virus were mixed with suspensions of consolidated mongoose lung and tested in the mongoose according to the procedure used above. Similarly, normal mongoose serum and the serum of mongooses convalescent from infection with acute pneumonitis virus were mixed with the PR8 strain of influenza A virus and tested

TABLE VII

Results of Cross Neutralization Tests with Acute Pneumonitis Virus and the PR8 Strain of Influenza A Virus

Serum	Strain 525—acute pneumonitis virus								Standard neutralizing titer of serum with PR8 influenza A virus
	No. of mongooses	Serial passages	Serum dilution	No. dead before 6th day	No. showing normal lungs	No. showing pulmonary hyperemia	No. showing pulmonary consolidations	Total pulmonary lesions	
								per cent	
Normal mongoose	14	7	1:5	1	2	4	7	78.5	0
Convalescent mongoose	8	4	1:5	0	7	1	0	12.5	0
Normal ferret	8	4	1:5	1	2	2	3	62.5	0
PR8-immune ferret	12	6	1:5	0	3	5	4	75.0	1:404
Normal ferret	2	1	1:5	0	0	2	0	100.0	0
PR8-immune ferret	2	1	1:5	0	0	1	1	100.0	1:512

in mice according to the technique previously described (17). The results of these experiments are shown in Table VII.

It will be noted that neither the normal ferret serum nor the convalescent ferret serum was capable of neutralizing acute pneumonitis virus and that lung lesions were produced in from 62 to 100 per cent of the test animals. Similarly, it was found that neither normal nor convalescent mongoose sera possessed any neutralizing antibodies against the PR8 strain of influenza A virus. It should be emphasized that both of the convalescent ferret sera possessed high titers of neutralizing antibodies against the homologous virus and also that, as indicated in Table V, the convalescent mongoose serum was capable of neutralizing at least 250 infectious doses of acute pneumonitis virus.

DISCUSSION

By the use of the wild mongoose as an experimental animal it has been possible to recover a pneumotropic virus from the throat washings of patients with the clinical syndrome termed acute pneumonitis. After intranasal inoculation this virus caused pulmonary hyperemia and consolidation in the mongoose and could be maintained by the serial passage of infected mongoose lungs.

The evidence which has been presented indicates that this virus was present in the throat washings obtained from each of the four patients with acute pneumonitis. It was recovered both from filtered and from unfiltered throat washings by means of serial intranasal passage in the mongoose. Moreover, when filtered throat washings were inoculated directly onto the chorio-allantoic membrane of the developing chick embryo, the virus was found to be present in the embryos through at least 30 serial passages.

It seems evident that this virus was not isolated from the mongoose itself since multiple serial passages of suspensions of normal mongoose lungs failed to produce significant pulmonary lesions in the mongoose.

Previous reports (3, 5) on the clinical characteristics of acute pneumonitis have emphasized the contagious nature of this disease and have pointed out that many persons who were in contact with ill patients subsequently developed the illness. The disease produced in the mongoose by the virus isolated from throat washings of patients with acute pneumonitis retained this characteristic. Normal mongooses placed in contact with infected mongooses developed pulmonary lesions entirely comparable to those produced by direct inoculation of the virus.

Mongooses infected by the virus were found to develop specific neutralizing antibodies in their serum during convalescence. Similarly human beings with acute pneumonitis produced antibodies against this virus, since serum obtained from them during convalescence neutralized the virus whereas serum taken during the acute phase of the disease did not. This evidence indicates that these patients were actually infected with this virus and strongly suggests that the virus was etiologically related to the disease itself.

It should be emphasized that the throat washings and sera were obtained from patients in three widely separated localities in New York State. Since the convalescent sera obtained in one area were capable of neutralizing the strains of the virus isolated from patients in another area, it seems evident that the clinical syndromes observed in these different localities had a common etiology.

Cross neutralization tests with specific antisera against this virus and the PR8 strain of influenza A virus indicated that these two viruses did not possess a common antigenic component. This result might have been anticipated since, as has previously been shown (17), acute pneumonitis does not produce an increase in neutralizing antibodies against influenza A virus.

The fact that of all species tested only the mongoose was susceptible to infection by this virus differentiates it from the other pneumotropic viruses. The laborious neutralization technique which seemed necessary and the very large number of mongooses which would have been required made it impractical to attempt specific immunological differentiation from many other viruses. Nonetheless, the evidence obtained suggests that this virus is dissimilar to meningopneumonitis virus (18), psittacosis virus (19), Rift Valley fever virus (20), and lymphocytic choriomeningitis virus (15). Moreover, this virus does not appear to have any properties in common with those of the agent which was described by Stokes, Kenney, and Shaw (6).

SUMMARY

1. A virus capable of producing pulmonary consolidation in the wild mongoose (*Herpestes griseus*) has been isolated from throat washings obtained from four patients with a clinical syndrome termed acute pneumonitis.

2. The virus was not pathogenic for ferrets, mice, guinea pigs, rabbits, monkeys, voles, hamsters, deer mice, skunks, opossums, or woodchucks.

3. The virus was filterable through Berkefeld V and N candles, was not inactivated by glycerin or by freezing and drying in vacuum, and was propagated for at least 30 serial passages on the chorio-allantoic membrane of the developing chick embryo.

4. Normal mongooses placed in contact with infected mongooses developed pulmonary consolidation.

5. The virus was neutralized by the serum of mongooses convalescent from the infection but was not neutralized by normal mongoose serum.

6. Serum of human beings convalescent from acute pneumonitis also neutralized the virus, but serum obtained from the same individuals during the acute phase of the disease failed to do so.

7. The evidence so far obtained strongly suggests that this virus is the cause of acute pneumonitis in human beings. It differs from other viruses known to cause infections of the respiratory tract in man.

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