A VIRUS FROM CASES OF ATYPICAL PNEUMONIA

RELATION TO THE VIRUSES OF MENINGOPNEUMONITIS AND PSITTACOSIS*

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The isolation of three different etiologic agents from atypical bronchopneumonias has recently been reported. Dyer, Topping, and Bengston (1) have described an outbreak of pneumonitis apparently resulting from laboratory infections in which the rickettsiae of American Q fever were shown to be the cause. Weir and Horsfall (2) have reported infection of the mongoose with a filterable agent from cases of atypical pneumonia. In 1939, Stokes, Kenney, and Shaw (3) isolated from the nasopharyngeal washings of a patient with bronchopneumonia, by inoculation of ferrets and mice, a virus which produced pneumonia and encephalitis in experimental animals.

In describing the clinical manifestations of atypical bronchopneumonias, some authors (4, 6) have considered psittacosis in the differential diagnosis. A comparison of the cases described by Reimann (4), Kneeland and Smetana (6), and Longcope (7) with cases of recognized psittacosis (8) in some instances reveals clinical similarities. However, the cases described by other authors (1, 2, 16) were milder and showed less similarity to psittacosis. Apparently transmission of the infectious agent from one human being to another occurs quite readily under certain conditions. In some outbreaks several cases have occurred in a series indicating repeated direct human-to-human infections.

This paper will describe the isolation of a psittacosis-like virus from four cases of atypical pneumonia. The properties of this agent differed in certain respects from those of the ordinary strains of psittacosis. The new strain of virus is antigenically related to, but not identical with, the strain of virus isolated by Francis and Magill (5) and named by them the virus of meningopneumonitis. Both of these strains are also antigenically related to psittacosis virus from parrots.

Materials and Methods

The six cases studied were all connected epidemiologically. A brief summary is given to make clear the sources of material. Case 1 entered a hospital in San Francisco on Mar. 8, 1940, after having been ill for approximately 1 week with an influenza-like disease.

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Examination revealed a bronchopneumonia with pleurisy and effusion. On Mar. 18, the patient died. The cause of death was given as influenzal bronchopneumonia. No autopsy was performed. Contact with parrots or other psittacine birds could not be demonstrated by questioning the family of the patient.[1] Cases 2, 3, and 4 were nurses who took care of case 1 and contracted a similar illness with pneumonia. All became ill between Mar. 25 and 27, and two died on Apr. 7. Specimens of lung, liver, and spleen obtained at autopsy from cases 2 and 3 were sent to our laboratory.[2] Case 4 recovered after a severe illness. Cases 5 and 6 were laboratory workers who contracted a disease similar to that of the previous cases, apparently as a result of infection with the agent isolated from the lungs of cases 2 and 3. Case 5 became ill on Aug. 11, 1940, and recovered after an illness lasting approximately 3 weeks. Case 6 became ill on Aug. 16 and recovered after a prolonged illness of about 6 weeks. Throat washings, sputum, and blood from these two cases were studied.

In all six patients the disease was characterized by an onset with influenza-like symptoms lasting 2 to 6 days accompanied by gastrointestinal complaints, severe headache, and in one case signs of meningeal irritation. All cases developed a bronchopneumonia of varying extent which was not detected until several days after the onset of the illness. The temperature was high and usually continuous and the pulse relatively slow. One case had an intermittent fever, then an afebrile period followed by a mild relapse and later by a migrating arthritis. The white blood count was normal or only slightly elevated. Sputum examinations revealed no significant bacteria. Clinically the disease was similar to many of the cases of atypical pneumonia described in the literature (4, 6, 7).

Animal Inoculation.—Mice and Syrian hamsters (*Cricetus auratus*) were inoculated intranasally, intracerebrally, and intraperitoneally; guinea pigs intracerebrally and intraperitoneally; rabbits intraperitoneally; and Java ricebirds (*Munia oryzivora*) intramuscularly. Intracerebral and intranasal inoculations were done under ether anesthesia.

Complement Fixation Tests.—Antigen was prepared from the infected lungs of mice by grinding with alundum and saline. The resultant suspension was centrifuged at 1,500 R.P.M. to remove large particles. It was used in the tests unheated at a concentration of 2 per cent wet mouse lung. Normal control antigen was similarly prepared from normal mouse lungs. The test in other details was identical with that used for influenza.

Isolation of Virus by Intranasal Inoculation of Mice

Intranasal inoculation of twelve mice with unfiltered suspension of lung obtained at autopsy from cases 2 and 3 resulted in no apparent illness, but when the animals were sacrificed on the 7th day many small round bluish grey focal lesions were found in the lungs of all mice. The appearance of these lesions in tissue section under low and high magnification is shown in Figs. 1 and 2. No similar lesions were seen in the lungs of mice which had been inoculated with various other materials or in normal

[1] Epidemiological investigations were done under the direction of Dr. J. C. Geiger, director of public health for the city of San Francisco.

[2] Through the kindness of Dr. A. M. Moody and Dr. W. T. Cummings of the Southern Pacific Hospital.

mice. The lesions were entirely different from those produced by the viruses of influenza and mouse pneumonia.

Culture of the original human material showed a *Staphylococcus aureus* in the lungs of one case and nothing in the other. Cultures from the mouse lungs gave no growth on the ordinary bacteriological culture media. Further passage from the lungs of the first mice increased the virulence of the

TABLE I

Results of Inoculating Mice with Material from Four Cases of Atypical Pneumonia

Case No.	Material	Day of illness	Mice inoculated intranasally	Mice inoculated intraperitoneally	Interval of intraperitoneal passage
					days
2	Lung	13th	Pos. 1*	Neg. 3	7, 5, 19
	Liver	13th	Neg. 2	—	
	Spleen	13th	Neg. 2	—	
3	Lung	14th	Pos. 1†	Neg. 3	7, 5, 19
	Liver	14th	Neg. 3	—	
	Spleen	14th	Pos. 2	—	
5	Throat washing	2nd	Neg. 4	—	
	Blood	5th	Neg. 3	Doubtful 3‡	7, 20, 21
	Sputum	10th	Pos. 2	Neg. 4	7, 7, 8, 14
	Sputum	13th	Pos. 2	Doubtful 2‡	8, 20, 33
6	Throat washing	2nd	Neg. 4	—	
	Sputum	8th	Pos. 1	Neg. 4	7, 7, 7, 21
	Sputum	12th	Pos. 1	Neg. 3	21, 21, 21
	Sputum	36th	Neg. 1	—	

Explanation of columns 4 and 5: Pos. 1 means definite pathology seen on first and subsequent passages; Neg. 4 means no pathology or symptoms in mice after four passages.

* Repeated twice with original material, positive both times on first passage.

† Repeated twice with original material, positive once on first passage.

‡ Intranasal passage of livers and spleens of mice showing peritoneal exudate gave no lung lesions.

infectious agent until on the fourth passage the mice developed sticky eyes, ruffled fur, bubbling respiration, and died within 2 to 3 days.

A similar result was obtained with sputum taken on the 10th and 13th days of illness from case 5 and on the 8th and 12th days of illness from case 6. No significant bacteria were found. The results of mouse inoculation are summarized in Table I.

The same material which produced lung lesions in the first or second passage after intranasal inoculation of mice failed to produce any significant illness or pathology after intraperitoneal inoculation. Repeated passage

of suspensions of livers and spleens of mice by the intraperitoneal route at various intervals of time had no effect except that when passage was done at an interval of about 20 days, enlarged spleens and slight peritoneal exudates were noted in some of the mice. However, further passages did not increase the virulence of the agent and subinoculation of liver and spleen suspensions into mice by the intranasal route did not produce characteristic lung lesions.

As shown in Table I, negative results were obtained by intranasal inoculation of mice with suspensions of liver from two cases, spleen from one case, blood from one case, and throat washings taken on the 2nd day of illness from two cases. From the spleen of one case virus was isolated.

Properties of the Virus

The viruses isolated from cases 2 and 3 were apparently identical and have been studied so far in the greatest detail. In subsequent sections of this paper this strain will be designated by the letters S-F. A strain of meningopneumonitis virus sent us by Dr. Thomas Francis, Jr., for comparison with our strain will be designated M.P.-F97.

In impression smears from the lungs of mice dying 2 to 3 days after intranasal inoculation with strains S-F or M.P.-F97, elementary bodies which were stained red by Machiavello's method were seen. The bodies were very similar in size and appearance to the Levinthal-Coles-Lillie bodies of psittacosis. They were also seen occasionally in peritoneal and meningeal exudates after intracerebral or intraperitoneal inoculation. Similar bodies were not seen in the lungs of normal mice nor in mice inoculated with the viruses of influenza or mouse pneumonia.

After centrifugation of suspensions of infected mouse lung containing the strain S-F at 5,000 R.P.M. for 1 hour, practically all of the infectious material was sedimented. Attempts to filter the agent through Berkefeld V and Seitz filters have so far been unsuccessful. The large particle size of the virus was indicated by these experiments.

Pathogenicity for Animals

Mice. Intranasal Inoculation.—With the strain S-F, the appearance of lung lesions in mice depended upon the stage of the disease and the amount of virus inoculated. Dilutions of 10^{-3} to 10^{-4} did not produce a uniformly fatal disease and small round grey foci were seen. With larger doses the foci became confluent and solid or patchy greyish pink lesions were produced. In mice dying acutely after intranasal inoculation of 10 per cent lung suspensions, the lungs were completely consolidated, deep red, and very edematous. There was a small amount of sticky pleural exudate. The various lesions observed were not dissimilar from those seen in mice inoculated intranasally with parrot

strains of psittacosis virus (9, 10). The virus has been carried through fifteen intranasal passages in mice without apparent change.

Intracerebral Inoculation.—After the strain S-F had been carried for four passages by intranasal inoculation, mice were inoculated intracerebrally with a bacteriologically sterile lung suspension. After a few intracerebral passages a dilution of 10^{-2} of mouse brain was uniformly fatal to mice in 4 days. Higher dilutions up to 10^{-5} killed part of the mice in 6 to 10 days with paralysis of varying degree preceding death.

TABLE II

Lesions and Survival of Virus in Mice after Intraperitoneal Inoculation

Mice inoculated intraperitoneally with strain	Dilution	Killed	Peritoneal exudate	Enlargement of spleen and liver	Result of subinoculation of livers and spleens to mice	
					Intranasally*	Intracerebrally*
		day				
S-F	10^{-1}	3	+ watery	0	++++, +++, ++	—
S-F	10^{-1}	6	+ sticky	0	++, +, ±	—
S-F	10^{-1}	9	0	0	±, 0, 0	S, S, S
S-F	10^{-1}	12	0	0	0, 0, 0	S, S, S
S-F	10^{-1}	18	0	0	0, 0, 0	9, S, S
S-F	10^{-1}	25	0	0	0, 0, 0	S, S, S
S-F	10^{-1}	30	0	0	0, 0, 0	S, S, S
M.P.-F97	10^{-2}	3	+++ sticky	0	7, 7, +++	—
M.P.-F97	10^{-2}	6	++ fibrin	±	5, 5, 7	—
M.P.-F97	10^{-2}	9	++ fibrin	+	9, +++, ++	6, 6, 7P
M.P.-F97	10^{-2}	12	+++ fibrin and ascites	+	8, 10, 10	5P, 5, 7
M.P.-F97	10^{-2}	18	+ fibrin	±	9, 9, 10	4, 4, 4
M.P.-F97	10^{-2}	25	±	±	7, 9, ++++	5, 5, 7P
M.P.-F97	10^{-2}	30	+	±	++++, ++++, +++	5, 5, 5

* Explanation of symbols: Figures in 6th and 7th column represent day of death of mice. Plus signs under intranasal inoculation indicate lung lesions in mice which survived 10 days. P after day of death signifies paralysis of hind legs observed before death. S means mice survived without symptoms. 0 means survival without lesions.

The strains M.P.-F97 and S-F both produced an identical type of pneumonia without encephalitis or meningitis after intranasal inoculation and both gave meningitis and paralysis of the hind legs without pneumonia after intracerebral inoculation.

Intraperitoneal Inoculation.—Over one hundred mice were inoculated intraperitoneally with 10 per cent lung or brain suspensions of the strain S-F from the later passages which were highly virulent by the intranasal or intracerebral routes. Of the few animals which died, none showed definite lesions in the abdominal organs or peritoneal exudate and the deaths were considered to be non-specific.

Definite differences between the strains S-F and M.P.-F97 were observed after intraperitoneal inoculation of mice. Thirty mice were inoculated intraperitoneally with each strain and killed at various intervals. The results presented in Table II show that the strain S-F produced only a slight peritoneal exudate during the first week, but nothing

later except occasionally a slightly enlarged spleen. During the first 6 days some of the mice appeared ill and subinoculation of the livers and spleens by the intranasal route produced lung lesions characteristic of the virus, but after 9 days the virus was no longer detectable by intranasal or intracerebral inoculation. On the other hand, the strain M.P.-F97 produced illness or death with a heavy peritoneal exudate which was later followed by a deposit of fibrin, enlargement of the liver and spleen, and development of ascites. A carrier state was set up in mice which recovered from the acute infection and virus was detectable by intranasal or intracerebral subinoculation of liver and spleen for as long as 30 days. Paralysis was seen in about 5 per cent of mice inoculated intraperitoneally with either strain.

Intraperitoneal passages with the strain S-F were started by inoculation of lung suspension from the 8th, 10th, and 13th intranasal passages. The virus was then carried by intraperitoneal passage of liver and spleen suspensions at intervals of 7 days for eight passages. Although the virulence of the agent was not appreciably increased, intranasal inoculation of suspensions of liver and spleen from the last passage produced characteristic lesions in the lungs of mice, indicating that the virus was successfully carried by this method.

Subcutaneous Inoculation.—Mice inoculated subcutaneously with the strain S-F showed no definite lesions except a slight enlargement of the regional nodes. This is in contrast to the marked induration and suppuration which follows subcutaneous inoculation of the strain M.P.-F97.

Guinea Pigs. Intracerebral Inoculation.—The strain S-F, inoculated intracerebrally, caused fever, ruffled fur, emaciation, and sometimes death. A meningitis with hyperemia of the brain, and occasionally small pneumonic patches in the lungs, were noted at autopsy. There were no pathological changes in the liver and spleen.

Intraperitoneal Inoculation.—The strain S-F produced, in guinea pigs inoculated intraperitoneally, a prolonged febrile illness lasting 8 to 14 days with extreme emaciation and temperatures ranging from 104°F. to 105.3°F. Some animals died in 7 to 14 days.

Passage of the virus intraperitoneally in guinea pigs resulted in a decrease in virulence during the first few passages.

Lesions observed in animals inoculated by this route included a fibrinous peritoneal exudate, hyperemia of the peritoneal surfaces, sometimes hepatic necrosis, patchy lesions in the lungs, and slight enlargement of the spleen. These lesions tended to regress after about 2 weeks and in animals sacrificed after 6 weeks the abdominal organs were negative, but grey patches probably representing healed lesions were seen in the lungs. Virus was recovered from the lungs, liver, and spleen of animals in the acute stages of the disease by intranasal inoculation of mice.

Inoculation of the strain M.P.-F97 into guinea pigs by the intraperitoneal route resulted in a mild transient illness. No lesions were seen in the abdominal organs except a slight watery peritoneal exudate and reddening of the peritoneal surfaces. From these results it appears that the strain S-F is much more virulent for guinea pigs than the strain M.P.-F97.

Syrian Hamsters. Intranasal Injection.—Strains S-F and M.P.-F97, intranasally injected, produced in Syrian hamsters a pneumonia similar to that observed in mice. Elementary bodies were abundant in smears of the lungs stained by Machiavello's method.

Intracerebral Injection.—The two strains injected intracerebrally resulted in an illness manifested by rough fur, weakness, ataxia, coma, and death in 3 to 7 days. No definite paralysis was noted. The principal lesion was a meningitis.

Microscopic Pathology

Study of sections of the lungs and brains of animals receiving the strains S-F and M.P.-F97 intranasally and intracerebrally revealed pathological changes identical with those described by Francis and Magill (5). (See Figs. 1 and 2.)

The livers of a number of mice receiving the two strains intraperitoneally were examined microscopically. The cells in certain areas of the livers appeared vacuolated and infiltration with leucocytes was observed. Occasionally, only small nests of mononuclear cells were seen. In the livers of mice receiving the strain S-F, areas of degeneration were less marked and less frequent than in the mice inoculated with the strain M.P.-F97, and the watery peritoneal exudate contained a moderate number of large mononuclear cells and lymphocytes, but elementary bodies were seldom demonstrated. The spleens showed no striking pathology.

Pathogenicity for Java Ricebirds

The purpose of these experiments was to determine if the strains under investigation showed the same degree of infectiousness for Java ricebirds as do the strains of psittacosis virus from parrots and other psittacine birds (15).

Java ricebirds were inoculated in the pectoral muscle with 10 per cent mouse brain and mouse lung suspensions containing the strains S-F and M.P.-F97. Mice inoculated intranasally at the same time died with typical lung lesions. The results are presented in Table III. Two of the ricebirds (Nos. 1 and 2) which received the strain S-F died in 8 and 14 days respectively without definite pathology except for the presence of moderate diarrhea in one bird and some fluid in the lungs and pleural cavity of the other. Virus was demonstrated in the organs of these birds by intranasal inoculation of suspensions of lung, liver, and spleen into mice. Mice inoculated intraperitoneally remained well. Four of the ricebirds inoculated with the strain S-F survived for 28 days, at which time they were killed and autopsied. Moderately enlarged spleens were seen in all four birds, and one (No. 6) had questionable lesions in the liver and lungs. The presence of active virus could not be demonstrated by subinoculation of livers, lungs, and spleens into mice.

The ricebirds receiving the strain M.P.-F97 died in 9 to 15 days. Nos. 8 to 12 inclusive had hepatic necrosis, enlarged spleens, patches of consolidation in the lungs, and diarrhea. In the organs of all birds, virus was readily demonstrated by subinoculation of mice by the intranasal and intraperitoneal routes.

Serological Tests

Sera obtained from cases 4, 5, and 6 were tested by complement fixation against the strains S-F and M.P.-F97. From the results in Table IV, it

can be seen that an increase in complement-fixing antibodies during illness to both strains was demonstrated in serum specimens taken from cases

TABLE III

Results of Intramuscular Inoculation of Java Ricebirds with Strains S-F and M.P.-F97

Ricebird No.	Inoculated intramuscularly with	Death or survival	Hepatic necrosis	Enlarged spleen	Lung lesions	Diarrhea	Intranasally infected mice*	Intraperitoneally infected mice*
		day						
1	S-F	8	0	0	0	±	2	0
2	S-F	13	0	0	±	0	6	0
3	S-F	s28	0	+	0	0	0	0
4	S-F	s28	0	+	0	0	0	0
5	S-F	s28	0	+	0	0	0	0
6	S-F	s28	±	+	±	0	0	0
7	M.P.-F97	9	0	0	0	+	4	5
8	M.P.-F97	13	+	+	+	±	6	3
9	M.P.-F97	14	±	++	0	0	6	3
10	M.P.-F97	15	0	++	0	0	6	3
11	M.P.-F97	13	0	++	0	0	4	2†
12	M.P.-F97	14	+	++	+	+	4	2†

* Figures denote average day of death of mice with typical lesions after inoculation with suspensions of lung, liver, and spleen from ricebirds. 0 = survival without lesions.

† Colon bacillus peritonitis.

TABLE IV

Complement Fixation Tests

Case No.	Day of illness	Titer with antigen from strain*	
		S-F	M.P.-F97
4	45th	1:16	1:32
5	Pre	0	0
	21st	1:16	1:16
6	8th	1:32	1:16
	17th	1:128	1:128
E	Normal	0	0
H	Normal	1:8	1:4
P	Normal	0	0
S	Normal	0	0
Q	Normal	1:4	0

* Strains S-F and M.P. were used as saline suspensions of consolidated mouse lung.

5 and 6. Other normal human sera showed either no fixation at 1:4 or titers between 1:4 and 1:8 with the strains S-F and M.P.-F97. None of the sera fixed complement with normal mouse lung.

The serum from case 4 was first tested by complement fixation with heated psittacosis tissue culture antigen by Dr. K. F. Meyer and Miss B. Eddie (11) at the laboratories of The George Williams Hooper Foundation. This serum gave a high titer with psittacosis antigen and Dr. Meyer very kindly sent us a portion of it which was tested with the results shown in Table IV. Subsequently the serum specimens from cases 5 and 6 were also tested in Dr. Meyer's laboratory. These tests showed an increase in the titer of complement-fixing antibodies with the psittacosis antigen, similar to that observed with the strains S-F and M.P.-F97.

Production of complement-fixing antibodies in guinea pigs and mice with the strain S-F was irregular. However, the serum of animals immune to the strain M.P.-F97, although failing to give definite fixation with unheated mouse lung antigens from this strain, did give titers of 1:16 or above with the heated antigen from psittacosis virus in tissue culture. Studies on this phase of the problem have not yet been completed.

No satisfactory method has yet been developed of demonstrating in the serum of immune animals neutralizing antibodies to the strain S-F. The method used by Francis and Magill (5) could not be applied to our strain because of its low virulence for mice by the intraperitoneal route.

Active Immunity

Homologous Immunity.—Mice which had received a single intraperitoneal inoculation with the strain S-F were not immune to infection by the intranasal route, but did resist infection by the intracerebral route. This is in agreement with the results of Francis and Magill (5). A single intranasal dose of virus sufficient to produce small lung lesions without killing any mice (about 1/10 M.L.D.) did not produce definite immunity to reinoculation with approximately 10 M.L.D. by the same route. Similarly, single subfatal doses of virus given intracerebrally often failed to produce solid immunity to intracerebral inoculation of 10 M.L.D. However, larger amounts of virus inoculated intranasally, although fatal to some of the mice, usually produced solid immunity in the survivors to reinoculation by the same route. When multiple inoculations were done, starting with 1/10 M.L.D. and increasing the amount ten times at each successive injection, a much more solid immunity was produced.

Guinea pigs which had recovered from intraperitoneal infection with the strain S-F showed no rise in temperature or other signs of illness after a second intraperitoneal inoculation with this strain.

Cross-Immunity.—Although it was difficult to produce cross-immunity to M.P.-F97 with the strain S-F by intranasal inoculation, the converse was not true. Mice receiving one subfatal dose of the strain M.P.-F97 intranasally were definitely immune to the strain S-F when tested 1 month later. Mice which had received two intranasal inoculations with the strain S-F did not resist infection by the intranasal route with 100 M.L.D. of the strain M.P.-F97, although animals from this same group were immune to the homologous strain.

Similar results were obtained when the cross-immunity tests in mice were done by intracerebral and intraperitoneal inoculation as shown in Table V. Animals inoculated by either route with the strain S-F were solidly immune to this strain inoculated intracerebrally, but succumbed to intracerebral inoculation with the strain M.P.-F97. Mice inoculated intracerebrally or intraperitoneally with the strain M.P.-F97 were immune to intracerebral inoculation of the strains S-F and M.P.-F97.

Mice immunized by the intraperitoneal route with the strains S-F and M.P.-F97 survived intraperitoneal inoculation with 1 M.L.D. of the strain M.P.-F97 (10 per cent mouse brain suspension). Mice of this same group were tested by Dr. Meyer with one of his strains of psittacosis virus and

TABLE V

Cross Immunity Tests by Intracerebral and Intraperitoneal Inoculation

Immunized with	Route	Intracerebral test with*		Intraperitoneal test with*	
		S-F 10 M.L.D.	M.P.-F97 10 M.L.D.	M.P.-F97 1 M.L.D.	Psittacosis† 1,000 M.L.D.
S-F	Intracerebral	0/3	11/14	—	—
S-F	Intraperitoneal	0/11	14/14	1/7	0/10
M.P.-F97	Intracerebral	0/14	0/4	—	—
M.P.-F97	Intraperitoneal	0/3	0/14	0/6	0/8
Psittacosis (inactive)	Intraperitoneal	—	—	3/19	0/10
0	(Control)	14/16	14/14	11/13	10/10

* Numerator is number of mice dead, denominator is number tested.

† A typical strain of psittacosis virus at a dilution of 10^{-4} was used in these tests.

were found to be protected against death when inoculated intraperitoneally with approximately 1,000 M.L.D. (last column, Table V). Mice immunized with inactive psittacosis virus in Dr. Meyer's laboratory and tested by us were found to be immune to 1 M.L.D. of the strain M.P.-F97 inoculated intraperitoneally.

DISCUSSION

The antigenic relationship of the strains S-F and M.P.-F97 to each other and to psittacosis virus is clearly indicated by the results of complement fixation and active immunity tests in mice. These findings do not necessarily indicate an identical origin for all strains. For example, the viruses of influenza A and swine influenza are antigenically related, but the former is transmitted from one human being to another while the latter is infectious primarily for swine.

The results presented in Table I strongly suggest that the strains isolated by us came from human material since in three out of four cases positive results were obtained in all of the mice of the first passage. This view is supported by the demonstration of an increase in complement-fixing antibodies in cases 5 and 6 (Table IV). This increase was associated with an illness similar to that of cases 2 and 3 and apparently resulted from laboratory infection. A similar strain of virus was isolated from the sputum. The more remote origins of the strain S-F are obscure. History of contact with psittacine birds within a period of 1 month before onset was not obtained in any case.

On the basis of the results published by Francis and Magill (5), it appears that the strain M.P.-F97 and other antigenically related strains of the virus of meningopneumonitis isolated by these authors came either from ferrets or from the throats of human beings with a disease clinically resembling epidemic influenza.[3] The failure to demonstrate neutralizing antibodies to the virus in the serum of the human cases does not constitute decisive evidence of the non-human origin of the virus, because it is known that infection with the virus of psittacosis, to which the virus of meningopneumonitis is antigenically related, does not regularly stimulate the production of neutralizing antibodies (12).

The possibility that the strain S-F came from a source other than psittacine birds is suggested by certain peculiarities of the strain namely, (*a*) its low virulence in the peritoneum of mice as compared with its relatively high virulence in the lungs or brain, (*b*) the relatively low virulence for Java ricebirds which readily become infected with psittacosis virus by contact with diseased parrots, (*c*) the failure of the strain to produce a carrier state in mice and birds which recover. With respect to these three properties, the strain M.P.-F97 appears to be more closely related to true psittacosis virus than the strain S-F. Both strains seem to have a relatively high pneumotropism. Hornus (9) has shown that psittacosis virus is not modified in its intraperitoneal virulence for mice by repeated intranasal passage. Paralysis in mice after intracerebral or intraperitoneal inoculation is caused not only by these two strains, but also occasionally by strains of psittacosis virus (8) from parrots.

It is possible that an as yet unknown vector may be responsible for the spread of these atypical strains of virus. Bedson (13) has recently described

[3] Since this paper was prepared for publication Dr. Francis has informed us that a majority of the cases studied in the epidemic referred to showed an increase in neutralizing antibodies to the virus of influenza B (17).

the infection of human beings with psittacosis virus from fulmar petrels, a previously unsuspected source. Pinkerton and Swank (14) have reported the isolation of a psittacosis-like virus from thiamin-deficient pigeons. This agent was pathogenic for mice by intracerebral inoculation, but intraperitoneal or subcutaneous inoculation was without effect. Finally, it is possible that one or both of the agents which we have studied are actually representatives of a group of psittacosis-like viruses that have become adapted to man with an increased infectiousness for the respiratory tract and a diminished virulence for birds and for mice inoculated by the intraperitoneal route.

SUMMARY

From the lungs of two fatal cases and from the sputum of two non-fatal cases of atypical bronchopneumonia, a psittacosis-like virus was isolated by direct intranasal inoculation of mice. Intraperitoneal injection of the same human material into mice gave negative results.

The virus has a relatively high virulence for mice by intranasal or intracerebral inoculation, but does not kill after intraperitoneal inoculation.

Its virulence for Java ricebirds is low and it fails to produce a carrier state in mice and birds.

Two cases showed an increase in complement-fixing antibodies to the new virus and to psittacosis.

The virus is antigenically related to the viruses of meningopneumonitis and psittacosis by complement fixation and by active immunity tests in mice.

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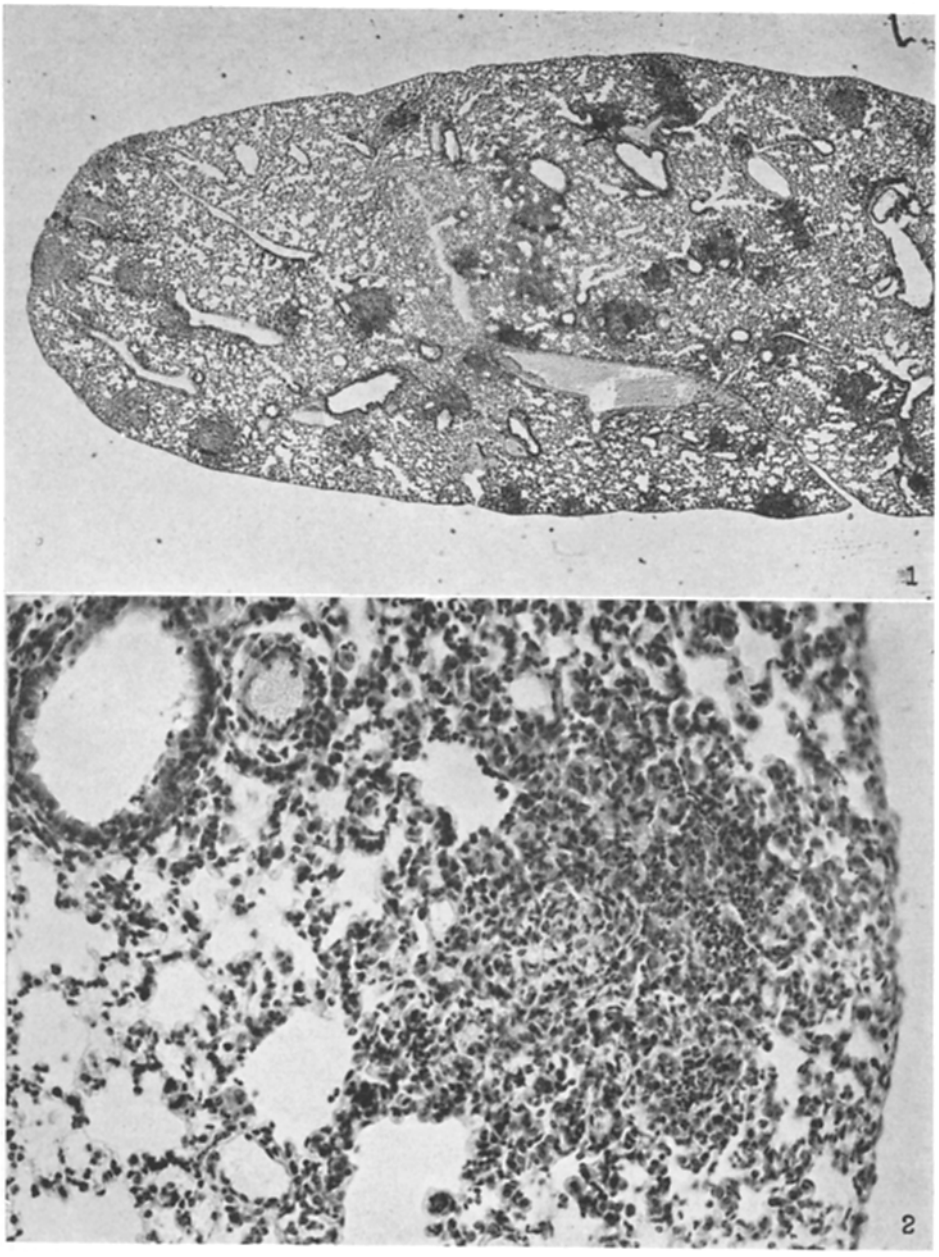
EXPLANATION OF PLATE 32

FIG. 1. Section of mouse lung taken from second intranasal passage of lung material from case 2. Hematoxylin and eosin. There are many small localized areas of infiltration which appear darker than the surrounding lung tissue. × 22.

FIG. 2. Same, showing detail of one of the foci. In the center is a dense accumulation of mononuclear cells with a few small pockets of polymorphonuclear leucocytes. The walls of the adjacent alveoli are swollen and there is some fluid in the air spaces. There is also some perivascular infiltration, but no striking change in the columnar epithelium of the bronchiole. × 251.



(Eaton *et al.*: Virus from cases of atypical pneumonia)