FURTHER LABORATORY STUDIES ON THE CLASSIFICATION OF PSITTACOSIS-LIKE AGENTS*

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In 1938 Francis and Magill (1) noted similarities among the meningopneumonitis, psittacosis, and lymphogranuloma venereum viruses, but failed to show that these agents were related. Since that time many reports (2–9) have appeared in the literature discussing the relationships of a group of viruses which resemble the agents of psittacosis and, like it, produce minute coccoid elementary bodies. This group of antigenically related agents includes strains isolated from human beings, birds, ferrets, and mice.

Although these viruses have been shown to have many common characteristics, certain differences and variations have been noted, not only in pathogenicity for animals and birds (3, 9, 15), but also in cross immunity tests within the group (7). Experiments on pathogenicity and cross immunity were, therefore, undertaken to determine the range and the possible significance of the variations among the viruses resembling psittacosis. These experiments were designed primarily to demonstrate differences between the virus isolated from certain cases of pneumonia (3) and the agent isolated by Pinkerton and Swank (2) and later by Meyer (9, 14) from pigeons and chickens. The virus from pigeons and chickens has been shown (9, 15) to differ somewhat from psittacosis virus. Differences between the "human pneumonitis" virus and psittacosis virus have already been described (3).

Materials and Methods

Strains of Virus.—As many distinct representatives of the group as could be obtained were included in the study. The following is a description of each strain:—

1. Strain S-F was isolated in 1940 from the spleen and lungs of two fatal cases of atypical pneumonia (3). Two other antigenically identical strains have been isolated from cases of pneumonia (7, 27).

2. Psittacosis strain 59 was isolated in 1941 from California shell parakeets in this laboratory. On the basis of its properties this strain was considered to be representative of the virus usually isolated from parrots and parakeets.

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3. Psittacosis (ornithosis) strain 134 was isolated in 1942 from California pigeons associated with a fatal case of atypical pneumonia, by Meyer and Eddie, Hooper Foundation for Medical Research, San Francisco. They kindly sent the strain to us. Meyer (8) designated these strains "ornithosis" to distinguish them from psittacosis of parrot origin. The term has been adopted in this report. Five other strains antigenically identical with this agent have been isolated by us from cases of atypical pneumonia (27).

4. Meningopneumonitis strain MP-F97 was isolated in 1934 from ferrets receiving human throat washings. This virus was kindly sent to us by Dr. Thomas Francis, Jr. Strain Cal 10 of the same virus, isolated in 1936 by Francis from ferrets receiving human throat washings, was sent to us through the courtesy of Dr. T. B. Turner (10).

5. Lymphogranuloma venereum strain LGV-St. was isolated in New Haven and kindly sent to us by Dr. Marion Howard of the Yale University School of Medicine.

6. Mouse pneumonia strain Greb was isolated from mice in 1941 at the Minnesota State Laboratory by Dr. Clara Nigg and kindly sent to us by her (11). Other strains very similar to this virus have been isolated in this laboratory and by others.

Animal Inoculation.—Pathogenicity tests were carried out in mice, hamsters, cotton rats, and guinea pigs. Mice were inoculated intranasally with 0.05 cc., intracerebrally with 0.03 cc., or intraperitoneally with 0.5 cc. to 1 cc. Cotton rats and hamsters received 0.4 cc. intranasally or 0.1 intracerebrally. Ten per cent suspensions of mouse brain passage were used except for strains LGV-St. and Greb when 10 per cent mouse lung was used.

Bird Inoculation.—Pathogenicity tests were done in pigeons, rice birds, and shell parakeets. All birds were obtained locally. The pigeons were inoculated with 0.15 cc. intracerebrally and the rice birds and parakeets were given 0.3 to 0.5 cc. intramuscularly in each pectoral muscle.

Latency of Infection.—Latency of infection was demonstrated in mice as follows: After one intraperitoneal inoculation the animals were sacrificed at the end of 21 days. The spleens and livers of at least 4 mice were ground together in a mortar and suspended in broth to make a 10 per cent suspension. Normal mice were inoculated intranasally and intracerebrally. Those inoculated by the intranasal route were killed in 7 days, and the lungs examined for macroscopic lesions. Impression smears were made from those showing consolidation and were stained by Macchiavello's method. The intracerebrally inoculated mice were observed for 14 days. If any died during this period impression smears were made of the brains and stained as above.

Tests for latent infections in birds were done as follows: At the end of the observation time the birds were autopsied and gross anatomical findings noted. The spleen, parts of the liver, kidneys, and testes or ovaries from each individual bird were ground in a mortar and made up to an approximately 10 per cent suspension. The rest of the procedure was the same as for latency in mice except that at least 2 serial passages were made, and in a number of cases as many as 5 and 6, before the birds were considered negative.

Cross Immunity Tests in Mice.—Mice were immunized or given latent infections by one, two, or three intraperitoneal inoculations of active virus. Mouse lung passage of each strain was employed in a 10^{-1} dilution for the strains S-F and ornithosis, 10^{-3} for meningopneumonitis, and 10^{-5} for psittacosis. Three weeks later the surviving

animals were tested intracerebrally for immunity, using 10 to 100,000 M.L.D. of mouse brain passage material containing homologous or heterologous strains. The titer of each virus was determined by intracerebral mouse titration at the time of the experiment. All animals and birds were kept in strict isolation according to the individual strains during the course of the pathogenicity and cross immunity experiments.

Complement Fixation Tests.—Antigens were prepared from meningopneumonitis strain Cal 10 propagated in the chorioallantoic membrane of 10 day old chick embryos and from lymphogranuloma venereum strain St. in the yolk sacs of 6 day old chick embryos according to the methods previously described (5). Normal control antigens were prepared from allantoic fluid and membranes and from yolk sacs. The antigens were all used untreated in the tests at the dilutions of 1:8 for Cal 10 and 1:6 for LGV as determined by titration. The tests were set up as for influenza (12).

TABLE I

Summary of Pathogenicity Tests for Certain Mammals

Strain of virus	Origin	Elementary bodies	Mice			Cotton rats	Hamsters		Guinea pigs	
			IN	IC	IP	IN	IN	IC	IC	IP
Ornithosis	Pigeon	+	+	+	±C	+	+			
Psittacosis	Parakeet or parrot	+	+	+	+C	+	+	±	±	0
MP-F97 (Cal 10)	Ferret	+	+	+	±C	+	+	+	±	±
S-F	Human	+	+	+C	0 NC	+	+	+	±	±
LGV	"	+	+	+C	0 NC	+	±	±		0
Greb	Mouse	+	+	0	0 NC	+	+			0

IN, intranasal; IC, intracerebral; IP, intraperitoneal. +, generally fatal; 0, no effect; ±, occasionally fatal. C, carrier stage; NC, no carrier stage.

Experimental Infections in Animals and Birds

Pathogenicity in Animals.—Six type strains were studied and compared on the basis of pathogenicity in animals. A summary of our own findings is tabulated in Table I. Intranasal inoculation of mice, cotton rats, and hamsters produced exudative pulmonary lesions, and usually death, in all animals. Each type injected intracerebrally caused paralysis and death in mice except strain Greb which produced only slight and transient signs of illness.

The greatest differences, and perhaps the most significant, are noted when pathogenicity and latency of infection are compared following intraperitoneal inoculation of mice. Psittacosis is the only strain which kills consistently and at high dilution in mice by the intraperitoneal route. Those which survive show long periods of latency lasting for months (26). Strains of ornithosis and meningopneumonitis prove fatal irregularly to white mice following intraperitoneal inoculation. Even repeated serial passage fails to enhance the pathogenicity to a degree comparable to psittacosis. However, latency of infection

is demonstrable up to at least 21 days by subinoculation. Meyer and Eddie's chicken virus (13) is similar in these respects. Pinkerton's strain of pigeon virus was reported (15) as negative intraperitoneally in mice. No data were available regarding the carrier stage.

Type strains S-F, LGV-St., and Greb gave negative results after inoculation by the intraperitoneal route. An occasional large spleen was noted following inoculations with strain S-F. Also, no latency of infection could be demonstrated after 3 weeks by passage of the livers and spleens of inoculated mice. These results confirm previous observations (3, 7).

Although type strain S-F did not produce the carrier state in mice following intraperitoneal inoculation, this virus could be recovered up to 21 days from the brains of mice inoculated intracerebrally. When mice were subinoculated they died and elementary bodies were demonstrated in the brains. Likewise, the persistence of strain LGV in the brains of mice after $3\frac{1}{2}$ months has been recorded by Jones, Rake, and McKee (22).

Psittacosis and the strains S-F and MP-F97 produced fever and sometimes death when inoculated into guinea pigs. When inoculated intraperitoneally, the strain S-F was relatively more virulent, often producing prolonged fever, emaciation, and death. Most strains of psittacosis are reported to have little effect in guinea pigs by the intraperitoneal route. Other investigators (1, 9, 16–21, 23, 25) have studied experimentally induced infections with the viruses of meningopneumonitis, ornithosis, and psittacosis in guinea pigs, rabbits, monkeys, and ferrets, but their findings in these animals do not serve to differentiate various strains.

Pathogenicity in Birds: Pigeons.—Three of the type strains were studied for virulence in pigeons inoculated intracerebrally.

Eight pigeons received ornithosis virus, 12 the "human" pneumonitis virus strain S-F, and 4 the virus of lymphogranuloma venereum. All of these birds were bled before inoculation and tested for antibodies by complement fixation (see later section). Five pigeons were kept as uninoculated controls. The strains ornithosis and S-F were mouse brain suspensions containing 100 M.L.D. in 0.03 cc. by intracerebral mouse titration. Strain LGV-St. was inoculated as a 10 per cent mouse lung suspension and had not been titrated. However, the control mice inoculated intranasally at the same time as the birds died in 3 days with four plus lung lesions.

Table II is a summary of the results in pigeons of inoculation with strains ornithosis, S-F, and LGV-St.

All 8 pigeons given strain ornithosis died between the 3rd and the 13th day after intracerebral injection. One bird dying on the 3rd day had a hemorrhage at the site of the inoculation. The brain smear was negative and no virus was recovered from this bird. Death may have been due to mechanical injury. Impression smears of the brains of the other 7 pigeons dying with this strain were positive for elementary bodies, and virus was demonstrated in five instances by inoculation of mice.

Out of a total of 12 pigeons receiving strain S-F, 9 were immature and 3 were mature. Five of the immature birds died between 8 and 27 days and 1 mature bird died on the 23rd day of observation. All direct smears of the pigeon brains were negative. The brain of each pigeon was passed serially in mice by both intranasal and intracerebral methods. The result was considered positive when elementary bodies were found in mouse lungs and brains. Virus was recovered from only 3 of the 6 pigeons which died. Of the 6 survivors receiving strain S-F only 1 was demonstrated by the usual procedure to be carrying the virus.

The birds were observed for neurological symptoms and in no instance were they seen in the group of pigeons inoculated with strain S-F. The usual findings were loss of appetite, ruffled feathers, progressive weakness, and finally death. Contrasted with this, those receiving strain ornithosis manifested marked neurological symptoms, the most prominent being opisthotonos (2, 15). The neck was drawn back and soon the bird was unable to stand. This condition was present in some birds for as long as 6 days, a pigeon in one case almost beating itself to death against the cage.

Four pigeons were injected intracerebrally with strain LGV-St. and observed for 16 days. All birds survived, and when the brain of each was examined for virus by intranasal and intracerebral mouse passage, negative results were obtained.

A simultaneous salmonella infection (*S. aertrycke*) was present in a number of the pigeons and made mouse passage difficult. Two of the uninoculated control pigeons which died were infected.

From the above findings it would seem that ornithosis virus is significantly more pathogenic for pigeons than the other two type strains studied.

Complement Fixation Tests on Experimental Pigeons.—Complement fixation tests were carried out on the pigeons. The results are shown in Table II. All pigeons were bled at the time of inoculation. Those dying had only the one specimen. The survivors were bled again before they were killed. One bird had antibodies to strain Cal 10 antigen prior to inoculation with type strain S-F. According to other authors (9, 15), the presence of complement-fixing antibodies did not necessarily render a pigeon immune to an intracerebral inoculation with ornithosis virus. Whether this lack of immunity in the presence of complement-fixing antibodies is applicable to strain S-F is difficult to conclude because of its low pathogenicity for pigeons.

Two other survivors in this group showed a definite increase in complement fixation titer. Neither of these birds was carrying latent infections of strain S-F. These results, however, indicate an antibody response resulting from the infection. A fourth surviving pigeon developed no antibodies, but virus was recovered. Meyer (8) reports that virus may be isolated from the tissues of immature birds, giving negative complement fixation reactions with psittacosis antigen.

One pigeon inoculated with strain LGV-St. showed a considerable rise in titer when tested with strain Cal 10 antigen. Three of 4 pigeons inoculated with the strain S-F showed a slight rise in antibodies to lymphogranuloma venereum antigen. These cross reactions contrast with the results of Eddie and Francis

TABLE II

Results of Intracerebral Inoculations of Pigeons with Strains Ornithosis, S-F, and LGV

Pigeon No.	Virus Inoculated	Age of bird	Death or survival in days	Neurological symptoms	Smear of brain	Mice inoculated*				Complement fixation‡	
						IN	Smear	IC	Smear	Cal 10 antigen	LGV antigen
1	Ornithosis	Immature	D3§	±	0	0	−	0	−	0/−	
2	"	"	D6‖	+	+	+	+	+	+	0/−	
3	"	"	D7‖	+	+	+	+	+	+	0/−	
4	"	"	D7	+	+	+	+	+	+	0/−	
5	"	"	D8	+	+	+	+	+	+	0/−	
6	"	"	D10	+	+	+	+	+	+	0/−	
7	"	"	D13	+	+	−	−	−	−	0/−	
8	"	"	D13	+	+	−	−	−	−	0/−	
9	S-F	"	D8	0	0	0	0	0	0	0/−	
10	"	"	D9	0	0	+	+	+	+	0/−	
11	"	"	D15	0	0	+	+	+	+	0/−	
12	"	"	D17‖	0	0	0	0	0	0	0/−	
13	"	"	D27	0	0	+	+	+	+	0/−	
14	"	"	S28	0	0	0	0	0	0	0/128	0/4
15	"	"	S28	0	0	0	0	0	0	0/32	0/4
16	"	"	S28	0	0	0	0	0	0	128/128	0/8
17	"	"	S28	0	0	+	+	+	+	0/0	0/0
18	"	Mature	D23	0	0	0	−	0	−	0/−	
19	"	"	S45	0	0	0	−	0	−	0/−	
20	"	"	S45	0	0	0	−	0	−	0/−	
21	LGV	Immature	S16	0		0		0		−	0/16
22	"	"	S16	0		0		0		0/512	0/32
23	"	"	S16	0		0		0		16/32	0/16
24	"	"	S16	0		0		0		0/4	0/0
25	Control	"	D14‖	0		0		0			
26	"	"	D28‖	0		0		0			
27	"	"	S28	0							
28	"	"	S28	0							
29	"	"	S28	0							

* Serial passage.

‡ Numerator is titer before inoculation; denominator is titer at the end of period of observation.

§ Hemorrhage at the site of inoculation.

‖ Salmonella infection.

(23), who reported that in a survey on pigeon bloods none of the birds reacted to strain LGV antigen. They suggested the use of pigeon sera as a differentiating point between the viruses of ornithosis and lymphogranuloma veneruem.

Rice Birds (Munia oryzivora).—Java rice birds have generally been considered highly susceptible to psittacosis (8). A series of these birds was, therefore, compared following intramuscular inoculation with the five type strains, orni-

TABLE III

Results of Intramuscular Inoculation of Java Rice Birds with Strains Ornithosis, MP-F97, S-F, LGV, and Greb

Rice bird No.	Virus inoculated	Age of bird	Death or survival in days	Hepatic necrosis	Enlarged spleen	Size	Smear	Mice inoculated			
								IN	Smear	IC	Smear
						mm.					
1	Ornithosis	Immature	D12	0	±	11 × 5	+	+	+		
2	"	"	D15	0	0	7 × 4	+	+	+		
3	"	"	D15	0	±	10 × 3.5	+	+	+		
4	"	"	D10	0	+	24 × 6	+	+	+	+	+
5	"	"	D13	0	+	12 × 5	+	+	+		
6	"	"	D17	0	±	11 × 3	+	+	+		
7	MP-F97	Mature	D9	0	0			+			
8	"	"	D13	+	+			+			
9	"	"	D14	±	+			+			
10	"	"	D15	0	+			+			
11	"	"	D13	0	+			+			
12	"	"	D14	+	+			+			
13	S-F	"	D8	0	0			+			
14	"	"	D13	0	0			+			
15	"	"	S28	0	+			0			
16	"	"	S28	0	+			0			
17	"	"	S28	0	+			0			
18	"	"	S28	±	+			0			
19	"	Immature	D13	0	+	16 × 5	+	+	+	+	+
20	"	"	D13	0	+	19 × 5	+	+	+	+	+
21	"	"	D14	±	+	15 × 5	+	+	+	+	+
22	"	"	D12	0	+	14 × 6	+	+	+	+	+
23	"	"	D14	+	+	16 × 6	+	+	+	+	+
24	"	"	D15	0	±	10 × 4	+	+	+	+	+
25	"	Mature	D10	0	+		+	+	+		
26	"	"	S28	0	+		0	0	0	0	0
27	LGV	Immature	S16	0	0			0		0	
28	"	"	S16	0	0			0		0	
29	"	"	S16	0	0			0		0	
30	"	"	S16	0	0			0		0	
31	"	"	S16	0	0			0		0	
32	Greb	"	S16	0	0			0		0	
33	"	"	S16	0	0			0		0	
34	"	"	S16	0	0			0		0	
35	"	"	S16	0	0			0		0	
36	"	"	S16	0	0			0		0	

thosis, MP-F97, S-F, LGV-St., and Greb. The results are summarized in Table III.

Of a group of 6 mature birds inoculated with strain MP-F97, and 6 immature birds with strain ornithosis, all died between the 9th and 17th days and virus was isolated

from each bird. Fourteen birds, 8 mature and 6 immature, were inoculated with strain S-F. Three mature and 6 immature birds died from the 12th to the 15th day. Virus was recovered from the tissues of all by the usual procedure. Elementary bodies were demonstrated in impression smears of spleens, livers, and peritoneal exudates of the birds and the lungs and brains of the mice. The recovered virus was determined to be strain S-F, the same as originally injected, by cross immunity tests with type strains S-F and ornithosis. In the 5 birds surviving 28 days, no virus was recovered from the livers or spleens. Of 5 immature birds receiving strain LGV-St. and 5 receiving strain Greb all survived 16 days and were negative on mouse passage.

TABLE IV

Results of Intramuscular Inoculations of Parakeets with Strains Ornithosis and S-F

Parakeet No.	Virus inoculated	Age of birds	Death or survival in days	Hepatic necrosis	Enlarged spleens	Size	Smear	Mice inoculated*			
								IN	Smear	IC	Smear
						mm.					
1	Ornithosis	Mature	D13	0	+	7 × 7	+				
2	"	Immature	D14	+	+	4 × 3	+				
3	"	Mature	D17	+	+	9 × 8	+				
4	"	Immature	D15	0	+	6.5 × 7	+				
5	"	"	D13	0	+	7 × 5	+				
6	"	Mature	D20	0	+	4 × 5	+	+	+	+	+
7	S-F	"	S28	0	0	2 × 2	−	0	0	0	0
8	"	Immature	D18	+	+	5 × 4	0	+	+	+	+
9	"	Mature	S28	0	+	4.5 × 5	−	+	+	+	+
10	"	"	S28	0	+	6 × 5	−	+	+	+	+
11	"	"	S28	0	0	2 × 2	−	0	0	0	0
12	"	Immature	S28	0	+	6 × 5	−	+	+	+	+

* Serial passages.

Smear, elementary bodies.

The results suggest a slight difference in the virulence of strains ornithosis, MP-F97, and S-F for rice birds. Mature birds seemed more resistant to strain S-F, and the survivors at the end of 28 days were negative for residual infection. According to Meyer (8) small inocula of psittacosis virus in rice birds may produce a latency for a period of 6 weeks.

Parakeets.—Many members of the Psittacidae family may be spontaneously infected with psittacosis virus (8). Among the representatives of the family is the shell parakeet. The results of intramuscular inoculation with two type strains, S-F and ornithosis, were compared in this bird. The dosage of virus in each group was 100 M.L.D. for mice. Table IV summarizes the findings in 12 birds.

The 6 parakeets injected with ornithosis virus died between the 13th and 20th days. Three were mature and 3 were immature. Immature birds, according to

Meyer (8), are more susceptible to infection than mature birds. However, no apparent difference was noted in this small series.

The usual size of the parakeet spleen is approximately 2 × 2 mm. All of this group exhibited splenic enlargement ranging from 4 × 3 mm. to 9 × 8 mm. Smears positive for elementary bodies were obtained in all cases either from livers, spleens, or peritoneal exudate. Two of the group had hepatic necrosis.

The series of 6 parakeets receiving strain S-F contained 2 immature and 4 mature birds. One immature died on the 18th day following 4 days of typical psittacosis symptoms, *e.g.*, sleepiness, ruffled feathers, and diarrhea with greenish stools. Autopsy showed hepatic necrosis and enlargement of the spleen to 5 × 4 mm. The smears of the organs were negative, but virus was recovered by intranasal and intracerebral inoculations in mice.

Of the 5 birds surviving for 28 days, 2 had no enlargement of the spleen and were normal otherwise. Repeated mouse passage failed to demonstrate the virus. One

TABLE V

Summary of Pathogenicity Tests for Birds

Virus	Origin	Pigeon			Parakeets				Rice birds			References
		IM	IC	Car	IM	Feeding	Exp	Car	IM	Feeding	Car	
Ornithosis	Pigeon	0	+	+	+	+	+	+	+	+	+	8, 9, 21
Pinkerton virus	"		+									2
Psittacosis	Parakeet or parrot		±	+	±	±	±	+	+	+	+	2, 8, 9, 15, 24
Meyer and Eddie chicken virus	Chicken	+	+		+				+			13, 14
MP-F97	Ferret		+	+	+			+	+		+	2, 3, 8, 9, 15
S-F	Human		±	±	±			±	±		0	3
LGV	"		0	0					0		0	
Greb	Mouse								0		0	

IM, intramuscular. Car, carrier state; Exp, exposure.

of these birds had been kept 18 days in the same glass jar with the bird that died. The remaining 3 survivors had splenic enlargement and latent infections, but showed no apparent signs of illness. All parakeets inoculated with type strain S-F were housed in the same isolation cubicle.

A summary of experimental results with eight strains tested in birds including our own data and additional published observations of others (2, 3, 8, 9, 13–15, 21, 24) is presented in Table V. The results presented in Tables II, III, and IV have shown that the type strain S-F differs from the type strain of ornithosis virus by its lower virulence in pigeons, rice birds, and parakeets when tested in birds of identical stock and source. The literature on ornithosis, meningopneumonitis, Pinkerton's pigeon virus, and Meyer and Eddie's chicken virus indicates that all of these strains are of high virulence for pigeons by the intracerebral route and for rice birds and parakeets by the intramuscular route.

The strain of psittacosis tested by Pinkerton and Moragues (15) was distinguished from their strain of ornithosis on the basis of the lower intracerebral virulence of the parrot strain in pigeons. Although most strains of psittacosis virus produce a fatal disease in rice birds, there are considerable variations in the susceptibility of various stocks of parakeets to strains of this agent.

Rice birds were apparently not infected with the virus of lymphogranuloma venereum or the mouse pneumonia virus strain Greb. The virus of lymphogranuloma venereum failed to produce demonstrable infection by the intracerebral route in pigeons.

TABLE VI

Intracerebral Immunity Tests with Strains S-F, Ornithosis, Psittacosis, and Meningopneumonitis

Immunizing virus	Route	Challenging virus															
		S-F				Ornithosis				Psittacosis				Meningopneumonitis			
		10–100 M.L.D.		10,000 M.L.D.		10 M.L.D.		100,000 M.L.D.		10 M.L.D.		10,000 M.L.D.		100 M.L.D.		10,000 M.L.D.	
		D*	P‡	D	P	D	P	D	P	D	P	D	P	D	P	D	P
S-F	1 × IP	0/31	0/31			19/24	5/5			20/25	3/5						
	2 or 3 × IP	0/23	0/23							3/6	3/3			11/14	3/3		
Ornithosis	1 × IP	0/7	0/7	1/7	0/6	0/7	0/7					1/6	0/5				
Psittacosis	1 × IP	1/9	0/8					4/6	0/2	0/5	0/5	1/4	0/3				
	2 × IP	2/16	0/14							0/5	1/5			4/10	4/6		
Meningopneumonitis	1 × IP	1/13	0/12			1/14	0/13			2/6	0/4					0/13	0/13
Controls	—	53/58	1/5	7/8	0/1	25/25	0/0	7/7	0/0	18/18	0/0	8/8	0/0	12/12	0/0	6/6	0/0

* D, deaths/mice tested.
‡ P, residual paralysis/mice surviving.

Cross Immunity Tests

Further studies were done on some of the type strains to determine if the variations noted in pathogenicity for animals and birds would be significant enough to be reflected in the cross immunity tests. Four type strains, S-F, ornithosis, psittacosis, and meningopneumonitis were selected for these experiments. Mice were tested for homologous and heterologous immunity with each member of the group. The results are presented in Table VI.

Irrespective of the number of intraperitoneal inoculations, strain S-F failed to produce any immunity against the other three type strains, but solid immunity was obtained with itself. Two type strains, ornithosis and meningopneumonitis, gave solid immunity both by heterologous and homologous tests

to all four viruses. Mice immunized with the strain of psittacosis exhibited very little immunity to the viruses of meningopneumonitis and ornithosis, but were completely immune to strain S-F and the homologous strain. Yanamura and Meyer (26) reported no immunity in mice vaccinated intraperitoneally with formalinized psittacosis virus and tested intracerebrally with the same virus. These results differ from our experiments in which active virus was used for immunization by the intraperitoneal route. The results with the active immunity test confirm the variations obtained with the type strains in experiments on pathogenicity in animals and birds and indicate that these differences are significant in classifying this group of viruses.

DISCUSSION

An attempt has been made to show that viruses which produce elementary bodies resembling those of psittacosis and cause pneumonia in mice and other animals following intranasal inoculation fall into several groups. This has been done by using type strains which appear to be representative of other antigenically identical strains within each group (7, 27). Undoubtedly, additional strains differing in antigenic composition or in other properties from those described in this paper will be found in the future. For this reason the classification must be considered tentative, but the experimental results at least serve to indicate the methods available for classification.

The virus of lymphogranuloma venereum and the mouse pneumonia virus (strain Greb) described by Nigg (11) are quite easily distinguished from the other viruses just considered by their properties in mice and birds and by antigenic differences (5, 6).

The viruses of psittacosis (parrot origin) and ornithosis (pigeon or chicken origin) have been differentiated on the basis of source (9, 13), intraperitoneal virulence in mice (2, 9, 13), and intracerebral virulence in pigeons (15). Our results also suggest that antigenic differences may be demonstrated by the intracerebral cross immunity test in mice. Except for source, the virus of meningopneumonitis is apparently identical with the virus of ornithosis (9, 15, 23).

It has been shown that the strain S-F from cases of human pneumonitis without known avian contact is not identical with meningopneumonitis virus (7) although this was at first believed to be the case. Differences of this agent from psittacosis virus have already been noted (3). Further evidence from pathogenicity tests in mice and birds and cross immunity tests in mice indicates differences between the strain S-F and ornithosis virus. The strain S-F may be distinguished from ornithosis by its lower virulence for birds and from psittacosis by its failure to kill mice or produce significant pathology when inoculated intraperitoneally. This strain also seems to have less tendency to latency in mice or birds than do the strains of avian origin. Furthermore, the

strain S-F is not antigenically identical with either ornithosis or psittacosis by the cross immunity method used in our laboratory.

From the experimental data it would appear that the ornithosis and meningopneumonitis viruses have a much broader antigenic structure since they produce complete immunity against themselves and all the other strains tested. The strain psittacosis contains an antigen which develops solid immunity for itself and type strain S-F, but very little for meningopneumonitis or ornithosis. Strain S-F has a still smaller range in that it immunizes only against itself by this method. This difference is not evident when the challenge inoculation is given by the intraperitoneal route. In a previous report (3) it was shown that mice receiving one intraperitoneal inoculation of strain S-F were immune to reinoculation by the same route with meningopneumonitis and psittacosis.

It does not seem logical to say that the differences in antigenic composition expressed by active cross immunity tests are wholly dependent on transient variations of one basic virus—psittacosis. Prolonged residence in one species can change the pathogenicity of a virus for another species, but concurrent changes in antigenic structure have not been demonstrated except perhaps in the sense of an evolutionary process.

SUMMARY

1. It was demonstrated by pathogenicity and latency tests in mammals and birds that variations exist among a group of viruses, isolated from several sources and producing coccoid elementary bodies resembling those of psittacosis.

2. Active cross immunity tests emphasized these differences and confirmed their significance.

3. On the basis of the present experimental evidence the psittacosis-like viruses causing atypical pneumonia in man may be classified into three groups: psittacosis, ornithosis, and human pneumonitis (strain S-F) of undetermined origin.

BIBLIOGRAPHY

1. Francis, T., Jr., and Magill, T. P., *J. Exp. Med.*, 1938, **68,** 147.
2. Pinkerton, H., and Swank, R. L., *Proc. Soc. Exp. Biol. and Med.*, **45,** 704.
3. Eaton, M. D., Beck, M. D., and Pearson, H. E., *J. Exp. Med.*, 1941, **73,** 641.
4. Rake, G., Eaton, M. D., and Shaffer, M. F., *Proc. Soc. Exp. Biol. and Med.*, 1941, **48,** 528.
5. Eaton, M. D., Martin, W. P., and Beck, M. D., *J. Exp. Med.*, 1942, **75,** 21.
6. Eaton, M. D., and Corey, M., *Proc. Soc. Exp. Biol. and Med.*, 1942, **51,** 165.
7. Beck, M. D., and Eaton, M. D., *J. Infect. Dis.*, 1942, **71,** 97.
8. Meyer, K. F., *Medicine*, 1942, **21,** 175.
9. Meyer, K. F., Eddie, B., and Yanamura, H. Y., *Proc. Soc. Exp. Biol. and Med.*, 1942, **49,** 609.
10. Turner, T. B., and Fleming, W. L., *J. Exp. Med.*, 1939, **70,** 629.

11. Nigg, C., *Science*, 1942, **95,** 49.
12. Eaton, M. D., and Rickard, E. R., *Am. J. Hyg.*, Section B, 1941, **33,** 23.
13. Meyer, K. R., and Eddie, B., *Proc. Soc. Exp. Biol. and Med.*, 1942, **49,** 522.
14. Meyer, K. F., *Schweiz. med. woch.*, 1941, **71,** 1377.
15. Pinkerton, H., and Moragues, V., *J. Exp. Med.*, 1942, **75,** 575.
16. Rivers, T. M., and Berry, G. P., *J. Exp. Med.*, 1935, **61,** 205.
17. Rivers, T. M., Berry, G. P., and Rhoads, C. P., *J. Am. Med. Assn.*, 1930, **95,** 579.
18. Rivers, T. M., and Berry, G. P., *Proc. Soc. Exp. Biol. and Med.*, 1930, **27,** 802.
19. Lazarus, A. S., and Meyer, K. F., *J. Bact.*, 1939, **38,** 171.
20. Rivers, T. M., and Berry, G. P., *J. Exp. Med.*, 1931, **54,** 105, 119.
21. Meyer, K. F., and Eddie, B., *Proc. Soc. Exp. Biol. and Med.*, 1933, **30,** 483.
22. Jones, H. P., Rake, G., and McKee, C. M., *Proc. Soc. Exp. Biol. and Med.*, 1941, **48,** 318.
23. Eddie, B., and Francis, T., Jr., *Proc. Soc. Exp. Biol. and Med.*, 1942, **50,** 291.
24. Tomlinson, T. H., *Pub. Health Rep., U. S. P. H S.*, 1941, **56,** 1073.
25. Bedson, S. P., *Brit. J. Exp. Path.*, 1933, **14,** 162.
26. Yanamura, H. Y., and Meyer, K. F., *J. Immunol.*, 1942, **44,** 195.
27. Meiklejohn, G., Beck, M. D., and Eaton, M. D., *J. Clin. Inv.*, in press.