has been identified as a protoporphyrin. It is this pigment and not blood that appears in choline-induced chromodacryorrhea.

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Serological Differentiation of Primary Atypical Pneumonia from Virus Pneumonia of the Psittacosis Group.*

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Despite the widespread occurrence of agents of the psittacosis-lymphogranuloma group in birds and mammals, there is now definite evidence that few cases of virus pneumonia are caused by psittacosis or related agents, while many atypical pneumonias may be caused by a new filterable virus recently transmitted to chick embryos, cotton rats, and hamsters.[1] Eaton and Corey,[2] using complement fixation tests with the virus of meningopneumonitis propagated in allantoic fluid and the virus of lymphogranuloma venereum grown in yolk sacs of chick embryos, found that among 70 patients with pneumonitis of unknown etiology, 10 to 15% showed an increase in complement-fixing antibodies for the psittacosis group. Isolation of a psittacosis-like virus was successful in only 10 of over 250 specimens of sputum or lung inoculated intranasally into mice or cotton rats.[2,3] In a series of 45 cases Smadel,[4] using agar slant tissue cultures of psittacosis virus as antigen, demonstrated a significant rise in complement-fixing antibodies in 10 cases, or about 22%. The virus was isolated from 2 of these patients. Workers in Francis' laboratory,[5] using an antigen from mouse lung infected with meningopneumonitis virus and refined by differential centrifugation, obtained a significant increase in titer (fourfold or greater) in only 1 case among 57 cases of atypical pneumonia. In the same laboratory, psittacosis-like viruses were isolated from the sputum or throat washings of 5 of 20 patients by inoculation of Java rice birds and subsequent intranasal passage in mice or by passage in mice alone. The relation of these findings to the etiology of atypical pneumonia was not clear, since none of the 5 patients had any increase in complement-fixation titer for meningopneumonitis associated with their illness. In a recent article Dingle[6] states that in the army the diagnosis of psittacosis or ornithosis was not once established in a series of more than 500 patients with atypical pneumonia and other respiratory infections. The method of testing was not stated.

During an epidemic of 19 cases of pneumonitis in the bayou region of Louisiana, a virus similar to the "psittacine group" was isolated from the throat washings of 3 of 4 patients studied and from the lungs at autopsy of 2 of these patients.[7] This outbreak seems to have been quite different in its general characteristics from primary atypical pneumonia. In other publications since 1940 psitttacosis-like viruses were identified as the

* The studies and observations on which this paper is based were supported by the International Health Division of The Rockefeller Foundation, in cooperation with the California State Department of Public Health.

1 Eaton, M. D., Meiklejohn, G., and van Herick, W., *J. Exp. Med.*, 1944, **79**, 649; *J. Exp. Med.*, 1945, **82**, 317, **329**.

2 Eaton, M. D., and Corey, M., PROC. SOC. EXP. BIOL. AND MED., 1942, **51**, 165.

3 Meiklejohn, G., Beck, M. D., and Eaton, M. D., *J. Clin. Invest.*, 1944, **23**, 167.

4 Smadel, J. E., *J. Clin. Invest.*, 1943, **22**, 57.

5 Francis, T., Jr., quoted by Dingle, J. H. *et al.*, *Am. J. Hygiene*, 1944, **39**, 269.

6 Dingle, J. H., *Bull. N. Y. Acad. Med.*, 1945, **21**, 235.

7 Olson, B. J., and Larson, C. L., *Pub. Health Rep.*, 1944, **59**, 1373.

causative agents in small groups of 2 to 6 cases, and where large numbers of cases of atypical pneumonia were analyzed only single blood specimens were tested by complement fixation. Additional papers contain details of the isolation of psittacosis-like viruses from birds or mammals without convincing evidence of the etiological relation of these agents to virus pneumonia in man.

The results of complement fixation tests with certain antigens prepared from rodent lungs indicate that the test for the psittacosis group in primary atypical pneumonia may not always be specific. Thomas and his associates[8] obtained in 14 of 35 cases of atypical pneumonia an increase in complement-fixing antibodies to suspensions of mouse lungs infected with the pneumonia virus of mice,[9] and sera of 5 of the patients also reacted with dissimilar antigens prepared from the viruses of influenza, cat pneumonitis, and meningopneumonitis. In our laboratory,[1,10] increases in complement-fixing antibodies were observed in 8 of 23 cases of atypical pneumonia with an antigen prepared from the sedimentable tissue components of hamster lungs infected with a virus isolated from cotton rats which is unrelated to the virus of atypical pneumonia or to any of the agents mentioned above.

Because of the occurrence of these apparently non-specific reactions in some cases of atypical pneumonia, differentiation of psittacosis and ornithosis from primary atypical pneumonia by the complement fixation test alone is somewhat uncertain. The development of a neutralization test with the virus recently isolated from patients with atypical pneumonia[1] facilitates such differentiation, as is shown in the present paper.

Materials and Methods. Complement fixation tests for the psittacosis group were performed with allantoic fluid antigens of the virus of meningopneumonitis and yolk sac antigens of the virus of lymphogranuloma venereum as previously described.[11] One modification was introduced; the yolk sac antigen was extracted 3 times with ⅔ volume of ether, the ether being pipetted off after centrifugation.

Neutralization tests with the virus of primary atypical pneumonia (strain De) were done according to the published method[1] using serial dilutions of serum and 10% suspensions of chick embryo lung, trachea and amniotic membrane containing the virus. Titers were recorded as the highest final dilution of serum which completely prevented the appearance of pulmonary lesions in hamsters inoculated intranasally with the serum-virus mixtures, when 75 to 100% of the animals inoculated with virus plus normal horse serum had lung lesions.

Agglutination tests were done with the indifferent streptococcus No. 344 isolated by Thomas and his associates,[12] using the method described in the original report.

Cold agglutination tests were done with 1% suspensions of type O human erythrocytes, according to the technic previously described.[13] In the tests for complement fixation, streptococcal agglutination, and cold agglutination the titers were expressed in terms of the original serum dilutions before adding the reagents. In the agglutination tests titers less than 10 were considered not significant and were recorded as zero.

Results. Table I contains the data derived from complement fixation, neutralization, and agglutination tests with sera of 11 patients with primary atypical pneumonia, all of whom showed increases in neutralizing antibodies to the virus of atypical pneumonia, as indicated in the third column of the table. The virus was demonstrated in the sputum of 3 of these patients by inoculation of gradocol filtrates into the amnion of chick embryos[1] and subsequent passage to hamsters. Cold agglutinins

8 Thomas, L., Curnen, E. C., Mirick, G. S., Zeigler, J. E., and Horsfall, F. L., Jr., PROC. SOC. EXP. BIOL. AND MED., 1943, **52**, 121.

9 Horsfall, F. L., Jr., and Hahn, R. G., *J. Exp. Med.*, 1940, **71**, 391.

10 Eaton, M. D., unpublished observations.

11 Eaton, M. D., Martin, W. P., and Beck, M. D., *J. Exp. Med.*, 1942, **75**, 21.

12 Thomas, L., Mirick, G. S., Curnen, E. C., Zeigler, J. E., and Horsfall, F. L., Jr., *Science*, 1943, **98**, 566.

13 Meiklejohn, G., PROC. SOC. EXP. BIOL. AND MED., 1943, **54**, 181.

TABLE I.
Cases of Primary Atypical Pneumonia with Increases in Virus-Neutralizing Antibodies.

Case initials	Serum, days after onset	Serological titers: Neutralization virus of atypical pneumonia	Agglutination streptococcus No. 344†	Cold agglutination†	Complement fixation psittacosis group‡
Ta	4	2	0	10	4a
	31	8	0	80	4a
Ha	7	<2	0	10	8a
	20	4	10	320	8a
Yo*	4	<2	—	0	—
	25	64	40	640	4b§
Ex	2	4	0	—	32a
	30	32	0	—	32a
Mu*	4	<2	0	0	—
	18	8	10	1280	0b§
Bu*	4	<2	0	0	0a
	25	32	0	160	0ab§
Hr	6	<4	10	0	0a
	12	16	40	160	0a
Bt	5	<2	0	10	0a
	17	8	80	80	0ab
Do	5	<4	0	10	—
	25	16	20	160	0b§
Sc	9	4	20	10	0a
	19	16	20	80	0a
Pr	6	<2	0	10	0a
	26	8	0	40	0a

* Virus of primary atypical pneumonia isolated from sputum.
† 0 = titers of less than 10. — = not done.
‡ a = complement fixation with group antigen lymphogranuloma venereum.
b = complement fixation with group antigen meningopneumonitis.
§ Developed nonspecific complement fixation with hamster lung (see text).

appeared in 10 of the sera and 7 had agglutinins for the indifferent streptococcus in titers ranging from 10 to 80. It may be seen in the last column of Table I that no change in complement-fixing antibodies for the psittacosis group was found in 8 cases. In the remaining 3, only the convalescent sera were tested and all had titers below a significant level. Sera of four of the patients listed in Table I, Yo, Mu, Bu, and Do, gave nonspecific complement fixation with the sedimentable components† of normal hamster lung, and also of hamster lung infected with the agent W2 from cotton rats.[1] In these 4 cases the reaction went to a higher titer in the convalescent serum than in the acute phase serum.

In Table II are presented the serological results in 10 cases of virus pneumonia in each of which the cause was attributable to one of the strains belonging to the psittacosis group. From the sputum of 4 of these patients a psittacosis-like virus was isolated. As discussed in a previous report[3] the virus in cases Bc and Pr was identified as the SF strain, in case Cr as a strain of meningopneumonitis, and in case Sp as a strain of pigeon ornithosis. In 8 cases an increase in complement-fixing antibodies to the psittacosis group was demonstrated, as shown in the last column of the table. In the case Bc a rise in antibodies had been demonstrated with serum specimens taken on the 8th and 17th day of illness,[3] but these were no longer available at the time of the present study. In the remaining 2 cases only convalescent specimens were available. The patient Mg was a nurse who was presumably infected with the SF strain at the same time as 2 other nurses from whom this strain

† This material was obtained by centrifuging suspensions of lung first at 2,000 R.P.M. for 20 minutes in a horizontal head, then centrifuging the supernatant at 5,000 R.P.M. for one hour in an angle head. The 5,000 sediment after washing was used for complement fixation at 4°C for 18 hours.

TABLE II.
Cases of Virus Pneumonia with Increases in Complement-Fixing Antibodies to the Psittacosis Group.

Case initials	Serum, days after onset	Serological titers: Neutralization virus of atypical pneumonia	Agglutination streptococcus No. 344†	Cold agglutination†	Complement fixation psittacosis group‡
Wi	2	8	0	0	8ab
	13	8	0	0	32ab
Ro	7	128	20	0	4a
	17	128	20	0	64a
Ho	6	<4	0	0	4a
	30	<4	0	0	64a
Bc*	pre	16	0	—	—
	8 mo.	16	0	—	16ab§
Cr*	pre	4	—	—	0b
	30	4	0	—	32ab
Pr*	pre	—	—	—	4b
	21	<4	0	—	32ab
Me	8	<4	0	—	4ab
	45	<4	0	—	16ab
St	9	--	—	—	8ab
	41	<4	0	—	32ab
Sp*	23	<4	0	—	32b
Mg	30	<4	0	—	32b

* Virus of psittacosis group isolated from sputum.
† 0 = titer of less than 10.
‡ a Complement fixation with group antigen lymphogranuloma venereum.
b Complement fixation with group antigen meningopneumonitis.
§ Increase in complement fixation titer was demonstrated with intermediate serum specimens.

was isolated,[14] but the only laboratory evidence of infection in case Mg was the high complement fixation titer. In those cases in which antigens from both meningopneumonitis and lymphogranuloma vereum were used the titers of a given serum with each antigen were the same or differed by only a twofold dilution.

In 6 of the cases in Table II paired serum specimens were available for neutralization tests with the virus of atypical pneumonia and no increase in antibodies to this virus was found. In the remaining cases the titers of the convalescent specimens were less than 4, indicating that the illness caused no significant response of neutralizing antibodies. The sera from 5 patients, Bc, Pr, Me, St, and Mg, had been stored at 4°C for 3 to 4 years at the time the neutralization tests were done. It is known, however, that the virus neutralizing antibody withstands storage for at least 2½ years at 4°C, and antibodies without change in titer were found in one 4-year-old serum (Bc in Table II).

[14] Eaton, M. D., Beck, M. D., and Pearson, H. E., *J. Exp. Med.*, 1941, **73**, 641.

Agglutinins for the indifferent streptococcus No. 344 were found in one of the 10 cases presented in Table II. In this case (Ro) there was no change in the agglutination titer and the initial level of neutralizing antibodies for the virus of atypical pneumonia was high. It is possible, therefore, that this patient, who was a nurse, may have been infected with the virus of atypical pneumonia some weeks or months before the illness which produced the rise in psittacosis-group antibodies. No cold agglutinins were found in the sera of three patients from whom fresh specimens were available for testing.

Discussion. From the available data it appears that the complement fixation test is a reasonably reliable indicator of infection with a virus of the psittacosis group when a definite increase in antibodies associated with illness is demonstrated and when precautions are taken to exclude the fixation with normal or pathological tissue components such as those found in rodent lungs infected with unrelated agents.[1,8,10] The cold agglutination and the non-specific complement fixation may be considered as serological reactions of a hetero-

genetic nature which frequently occur with primary atypical pneumonia, but are not necessarily limited to this disease. Many patients with atypical pneumonia show an increase in virus neutralizing antibodies without development of cold agglutinins or the other reactions. Evidence for the specificity of the virus neutralization in atypical pneumonia has been presented in this and other publications.[1] Agglutination of the indifferent streptococcus seems to occur with greater frequency in primary atypical pneumonia than in virus pneumonias of the psittacosis group. This finding suggests a possible specific association, at least in certain cases, of the streptococcus and virus in the pathogenesis of the disease.[15]

Data presented elsewhere[1] indicate that the virus of atypical pneumonia isolated and propagated in chick embryos is distinct in respect to host range, pathogenicity, and other properties from viruses of the psittacosis-lymphogranuloma group. The results presented in this paper show that these agents are also antigenically distinct as judged by complement fixation and neutralization tests on human sera.

Summary. In 11 cases of primary atypical pneumonia with increases in neutralizing antibodies for the virus propagated in chick embryos no significant development of complement-fixing antibodies for agents of the psittacosis group were found. Ten of the patients had cold hemagglutinins and 7 showed significant titers of agglutinins for the indifferent streptococcus No. 344. In an additional 10 cases of virus pneumonia where the presence of an agent of the psittacosis group was demonstrated by virus isolation or complement fixation, no significant development of neutralizing antibodies for the virus of atypical pneumonia occurred. The serum of one of the latter 10 patients showed streptococcal agglutination at a dilution of 1:20 without change in titer.

Dr. Gordon Meiklejohn collected many of the specimens and compiled much of the clinical data. The assistance of Miss M. Dorthy Beck, Miss Marilla Corey, Miss V. Lee Hanford, Miss Marjorie Hunt, and Mr. William van Herick is gratefully acknowledged.

[15] Thomas, L., Mirick, G. S., Curnen, E. C., Ziegler, J. E., Jr., and Horsfall, F. L., Jr., *J. Clin. Invest.*, 1945, **24**, 227.

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Experimental Intestinal Myiasis in Man.*

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The possibility of a true intestinal myiasis has been much discussed.

It was demonstrated that, when swallowed, the maggots of many different species may cause violent abdominal distress, vomiting, diarrhea and even systemic reactions. It is not quite clear however if these disturbances are caused by the accidental presence of dipterous larvæ and will clear up after their rapid elimination, or if some species of maggots can adapt themselves to intestinal environments, survive for a certain time, even multiply and cause a true intestinal myiasis.

A few observations seem to indicate that such possibility exists. Herms and Gilbert[1] described a case in which a woman apparently suffered from intestinal myiasis that had extended for many years, Thebauld[2] recovered living dipterous larvæ from the stool of a child suffering from typhoidal affection. In

* This study was made possible through the facilities of the Governmental Medical Services of the Belgian Congo.

[1] Herms, W. B., and Gilbert, Q. O., *Ann. Int. Med.*, 1933, **6**, 941.

[2] Thebauld, V., *Arch. Parasit.*, 1901, **4**, 353.