and Enzmann[2] found that the enzyme exerted a strong damaging effect on the ova themselves. The effect would seem to be one of destruction rather than disaggregation, and the data available do not warrant the conclusion that proteolytic enzymes play any part in the phenomenon here studied as it occurs in the oviduct. On the other hand, it seems quite plausible that motion of the cilia of the oviduct epithelium has a complementary action.

Summary. Crude or highly purified preparations known to be very rich in hyaluronidase, such as extracts from rattlesnake venom, leech tissues, and testicle, have a very pronounced effect in dispersing the follicular cells surrounding the ova of mice. It appears very probable that hyaluronidase itself, which plays such an important part in infection, is also responsible for this effect, an indispensable step in fertilization. Final conclusions cannot be reached until the enzyme is obtained in pure state.

P.S. When the manuscript of this paper was ready for publication McClean and Rowlands[4] reported the results of experiments similar to ours but carried out on rat ova and reached the same conclusions as we have.

[4] McClean, D., and Rowlands, L. W., *Nature*, 1942, **150**, 627.

14052

Complement Fixation with Dissimilar Antigens in Primary Atypical Pneumonia.

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In complement-fixation tests with sera from patients with primary atypical pneumonia of unknown etiology, it was found unexpectedly that the convalescent serum from a number of patients reacted positively in high dilution with various and apparently unrelated antigens. Since this peculiar and as yet unexplained property can lead to difficulty in the interpretation of the results of complement-fixation tests with sera obtained from patients with this clinical syndrome the phenomenon has been studied.

Materials and Methods. Specimens of serum were obtained from patients acutely ill with primary atypical pneumonia in the Rockefeller Hospital. Additional specimens of serum were obtained from these patients throughout the course of the illness and during convalescence. The sera were stored at 4°C.

Mouse lung antigens. Antigens were prepared from the lungs of normal albino Swiss mice and from the lungs of similar mice which had been infected with one or another of the following viruses: a. pneumonia virus of mice, Horsfall and Hahn;[1] b. influenza A virus, PR8 strain; c. cat pneumonitis virus, Baker;[2] and d. meningo-pneumonitis virus.[3] Mice infected with agents a, b, and d were killed usually on the fifth day after intranasal inoculation, while mice infected with agent c were killed on the second day. The lungs were removed aseptically, ground with abrasive and suspended in a final concentration of 2% by wet weight in 0.85% NaCl buffered at pH 7.2. The suspension was centrifuged at 1500 RPM for 10 minutes and the supernate withdrawn and used as antigen. When parallel tests were done with different antigens they were all

Note: The Bureau of Medicine and Surgery does not necessarily undertake to endorse views or opinions which are expressed in this paper.

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

[2] Baker, J. A., *Science*, 1942, **96**, 455.

[3] Francis, T., Jr., and Magill, T. P., *J. Exp. Med.*, 1938, **68**, 147.

prepared on the same day from mouse lungs which either had been freshly removed or stored as intact specimens at −70°C for some days.

Other tissue antigens. Antigens were prepared also in a manner identical with that described above from the following individual normal tissues: mouse liver, mouse spleen, rabbit lung, rabbit liver, rabbit spleen, rabbit kidney, guinea pig lung, hooded rat lung, beef heart, human lung, and chick-embryo yolk sac.

Complement-fixation test. The human sera were inactivated at 56°C for 30 minutes and serial two-fold dilutions in 0.85% NaCl were made. To 0.2 ml of a given serum dilution was added 0.2 ml of a particular antigen, and 0.5 ml of 0.85% NaCl containing 2 units of complement. The pooled serum from 8 or more guinea pigs was used as a source of complement. The mixture was incubated at 37.5°C in a water bath for 2 hours, after which there was added 0.7 ml of sensitized sheep cells, consisting of 0.5 ml of 2% sheep cells and 0.2 ml of 0.85% NaCl containing 2 units of amboceptor. The mixture was again incubated in the water bath for 30 minutes and the degree of hemolysis was then estimated. Complement-fixation titers were expressed in terms of the highest serum dilution in which little or no hemolysis occurred. Serum dilutions refer only to the initial dilution before the addition of other reagents. Hemolytic and anticomplementary controls were included in each test. All reagents were tested for anticomplementary activity in twice the highest concentration used. Acute phase and convalescent sera from individual patients were always tested at the same time.

Results. In numerous experiments with infected mouse lung antigens it was found that the convalescent sera from certain patients with primary atypical pneumonia fixed complement in equally high titer irrespective of the nature of virus used to infect the mice.

Acute phase and convalescent sera from 35 patients with primary atypical pneumonia were tested against antigen prepared from the lungs of mice infected with pneumonia virus of mice[1] which will hereafter be referred to as PVM antigen. The results of these tests are shown in Table I. It will be observed that the complement-fixation titers of the convalescent sera against this antigen were strikingly higher than were the titers of the acute phase sera. Of the former sera 20 possessed titers of 1:16 or higher, whereas only 4 of the latter sera fixed complement in comparable dilutions. In 14 of the 35 cases an increase of 4-fold or more in the titer of the convalescent serum as compared with that of the acute phase serum was demonstrated.

Acute phase and convalescent sera from 8 patients with influenza A, 6 patients with pneumococcus pneumonia, 6 patients with scarlet fever and 3 patients with psittacosis were also tested against PVM antigen. The results of these tests as well as those with sera from patients with primary atypical pneumonia are shown in Table II. It will be noted that among the sera obtained from 23 patients with various acute infectious diseases other than primary atypical pneumonia no significant increase in complement fixing titer against this antigen was observed during convalescence.

From the 14 patients with primary atypical pneumonia showing a 4-fold or greater increase in complement-fixing titer against PVM antigen 5 were selected and their sera retested simultaneously against 6 different antigens. The individual antigens were prepared from normal mouse lungs, the lungs of mice infected

TABLE I.

Results of Complement-Fixation Tests Using Antigen Prepared from the Lungs of Mice Infected with Pneumonia Virus of Mice Against Acute Phase and Convalescent Sera from 35 Patients with Primary Atypical Pneumonia.

Serum	Days after onset of illness	Complement fixing titer of serum: 0	1:4	1:8	1:16	1:32	1:64	1:128	1:256
		Number of patients with indicated titers							
Acute phase	1 to 7	17	7	7	2	2	0	0	0
Convalescent	10 " 42	5	7	3	9	4	4	2	1

TABLE II.
Results of Comparison of Complement-Fixing Titers of Acute Phase and Convalescent Sera from a Number of Acute Infectious Diseases Against Antigen Prepared from Lungs of Mice Infected with Pneumonia Virus of Mice.

Acute infectious disease	No. of cases tested	No. of cases which showed indicated change in complement-fixing titer of convalescent serum compared with acute phase serum		
		No change	Two-fold increase	Four-fold or greater increase
Primary atypical pneumonia	35	10	11	14
Influenza A	8	8	0	0
Pneumococcus pneumonia	6	6	0	0
Scarlet fever	6	6	0	0
Psittacosis	3	3	0	0

with the following viruses: pneumonia virus of mice, influenza A virus (PR8 strain), meningo-pneumonitis virus, and cat pneumonitis virus; and the yolk sac of normal chick embryos. The results are shown in Table III. It will be seen that with but one exception there was observed a significant increase in the complement-fixing titers of all these convalescent sera against each of the antigens prepared from infected mouse lungs irrespective of the nature of the virus used to infect the mice. It will also be observed that in 4 instances a significant increase in complement-fixing titer of the convalescent serum was demonstrated when normal mouse lung antigen was employed, while in 2 instances a similar result was obtained with normal yolk sac antigen. It should be noted that on the whole titers were lower with normal than with infected mouse lung antigens.

When antigens were prepared from various tissues other than mouse lung and were tested against acute phase and convalescent sera from patients with primary atypical pneumonia similar results were obtained. However, in general the increases in complement-fixing titer of the convalescent sera were neither so marked nor so frequently encountered as they were with mouse lung antigens. Two patients (B and L in Table III) whose sera were tested against normal human lung antigen showed an increase in titer from 0 to

TABLE III.
Results of Complement Fixation Tests with Acute Phase and Convalescent Sera from Selected Patients with Primary Atypical Pneumonia and 6 Different Antigens.

Primary atypical pneumonia Case	Day after onset on which serum was obtained	Complement-fixing titer of serum against indicated antigen					
		Mouse lung					Normal chick embryo Yolk sac
		Normal	PVM[1]	PR8[2]	MP[3]	Cat Pn[4]	
L	3	0	1-4	1-16	0	1-4	0
	13	1-32	1-64	1-128	1-32	1-128	1-8
B	3	1-4	1-4	1-8	1-4	1-8	0
	13	1-4	1-16	1-32	1-8	1-32	0
P	3	0	0	1-4	0	1-4	0
	14	1-16	1-16	1-32	1-16	1-16	1-16
Br.	8	0	0	1-4	0	0	0
	22	1-8	1-8	1-16	1-8	1-8	0
F	3	0	0	0	0	—	—
	42	1-32	1-256	1-128	1-64	1-128	—

[1] Pneumonia virus of mice.
[2] Influenza A virus (PR8 strain).
[3] Meningo-pneumonitis virus.
[4] Cat pneumonitis virus.

1:8 and 0 to 1:32 in their acute phase and convalescent sera respectively. Guinea pig, rabbit, and rat lung antigens showed similar changes in the titer of the convalescent serum in 2 other instances. Rabbit liver, spleen and kidney and beef heart antigens were tested against acute phase and convalescent sera from numerous patients. In most instances no change in titer against these antigens was demonstrated during convalescence although at times there was evidence of fixation of complement in dilutions of 1:4 or 1:8 in the convalescent serum. The most striking change was exhibited by the convalescent serum from patient F (Table III) which possessed a titer of 1:256 against each of these antigens.

All of the convalescent sera which were found to react positively with mouse lung antigens were tested for the presence of sheep cell hemolysins by the usual method with negative results in each instance. Moreover, none of these sera fixed complement in the presence of psittacosis virus tissue culture antigen[4] or lymphocytic choriomeningitis virus soluble antigen.[5] It should be emphasized that both these antigens were prepared by technics which removed the greater part of sedimentable tissue components. Four of the convalescent sera which reacted positively with mouse lung antigens were found to be negative in Wassermann tests.

It was found that the time of the appearance of the capacity to fix complement with different antigens varied in different cases. In those instances in which the reaction was found it was usually present at the end of the second week of illness and was invariably demonstrated by the end of the third week. In one case serum taken on the 7th day gave a titer of 1:8 against infected mouse lung antigen while another serum obtained on the 14th day possessed a titer of 1:32. In 3 cases it was found that sera obtained 4 weeks after onset gave lower titers than did sera from the same patients taken during the second week of illness. In one (F in Table III) the titer decreased from 1:256 in the fifth week to 1:64 8 weeks after onset. So far complete disappearance of the property with time has not been observed although sera obtained later than the eighth week have not been tested. Sera stored for 2 months in the icebox showed no loss in titer against mouse lung antigen.

The capacity of convalescent sera to fix complement with different antigens was abolished or markedly reduced by two procedures: absorption of the sera with a suspension of normal mouse lungs, or heating the sera at 65°C for 30 minutes. One volume of serum was mixed with 3 volumes of a 10% suspension of normal mouse lungs prepared in a manner identical to that used for antigens, incubated at 37.5°C for 2 hours and then kept at 4°C overnight. The mixture was then centrifuged at 12,000 RPM for 30 minutes and the supernatant material was tested against normal mouse lung antigen. A portion of the same serum mixed with an equivalent amount of 0.85% NaCl was treated in an identical manner. It was found that the control serum had a complement-fixing titer against normal mouse lung antigen of 1:32, whereas the absorbed serum had a titer of but 1:4.

Aliquots of a pool of convalescent sera each of which had been shown to react positively with different antigens were heated for 30 minutes at 56°C, 60°C, 65°C and 70°C respectively. Each sample was then tested against 4 different infected mouse lung antigens similar to those shown in Table III. It was found that the complement-fixing titer against each antigen was reduced by 4-fold or more as a result of heating the serum at 60°C and that all reactivity under the conditions of this test was abolished when the serum had been heated at 65°C.

The nature of the component of the different antigens which is responsible for the positive reactions with convalescent serum from patients with primary atypical pneumonia is unknown. However, there is evidence which suggests that it may be associated with some rather large particles in the tissue suspensions. Antigens, whether prepared from normal or infected mouse lungs, were most reactive when centrifuged least. Centrifugation of normal mouse lung antigen at 3,000 RPM for 30 minutes caused a 4-fold reduction in its reac-

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

[5] Smadel, J. E., Baird, R. D., and Wall, M. J., *J. Exp. Med.*, 1939, **70**, 53.

tivity as compared with a control antigen. Centrifugation at 12,000 RPM removed all of the reacting material from the supernatant fluid. The sediment when resuspended in 0.85% NaCl was found to possess a reactivity almost identical with that of the original suspension. Effective antigens were found to be unstable and to diminish rapidly in reactivity on standing. Antigens which had been stored overnight at 4°C were frequently found to be distinctly anticomplementary.

Discussion. The fact that patients convalescent from primary atypical pneumonia often develop in their serum the capacity to fix complement with a number of different antigens seriously complicates the interpretation of complement-fixing tests in this syndrome. This is particularly true when relatively crude tissue suspensions are employed as a source of antigen. Similar reactions were not observed when convalescent sera from certain other acute infectious diseases, either of bacterial or virus etiology, were tested. Since in primary atypical pneumonia positive reactions could in some instances be demonstrated with antigens prepared from a variety of organs obtained from a number of unrelated species it is not apparent what component common to the antigens used might explain the observed phenomenon. The available evidence indicates that it may be possible in one or both of two ways to circumvent the practical difficulties imposed by this property of certain convalescent sera. First, the sera may be rendered non-reactive by heating at 65°C for 30 minutes, a procedure which has been shown by Casals and Palacios[6] to be of importance in carrying out complement-fixation tests against neurotropic virus antigens. Second, the antigens to be tested may be prepared relatively free of easily sedimentable tissue components, a procedure which has been found to be of value in eliminating non-specific reactions in complement-fixation tests with a number of virus antigens.

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[6] Casals, J., and Palacios, R., *J. Exp. Med.*, 1941, **74**, 409.

14053

Two New Salmonella Types with Similar Somatic Antigens.*

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A. *Salmonella florida.* The only culture of *S. florida* thus far recognized was isolated by Mrs. Mildred Galton from the feces of a patient affected with a febrile disease which was accompanied by diarrhea. It was sent to the writers for serological examination. The organism possessed the cultural and biochemical characteristics of the Salmonella. Acid and gas were produced from glucose, xylose, rhamnose, maltose, trehalose, and sorbitol. Lactose, sucrose, inositol, adonitol, and salicin were not fermented. Dextro-tartrate, citrate, and mucate were attacked but levo-tartrate and meso-tartrate were unaffected. The bacillus produced large amounts of H_2S but did not form indol nor liquefy gelatin.

Alcohol-treated suspensions of the organism were agglutinated to the titer of *S. onderstepoort* O serum [(I), VI, XIV, XXV] and to a lesser degree by *S. carrau* O serum (VI, XIV, XXIV). When tested with absorbed serums the organism was found to possess antigen

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