COMPLEMENT ACTIVITY IN PNEUMONIA[1]

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In the evaluation of the immunity mechanism in man during pneumococcus pneumonia, complement studies have received little attention. Publications concerning the level of antibody in relation to recovery from pneumonia have been summarized up to 1939 by Heffron (1), and other studies have been published since that time (2 to 6). The few studies regarding complement activity in pneumonia which have been published present conflicting conclusions. Dick (7) studied 4 pneumonia patients and 2 controls and reported that complement activity increased during pneumonia and returned to normal after crisis. Veil and Buchholz (8) stated that complement activity did not decrease during pneumonia but presented no data. Robertson, Sia, and Cornwell (9) reported without specific data that "little evidence was obtained to show that the activating effect of fresh animal (including human) serum undergoes any significant alteration during the disease." Taplin (10) noted that 6 patients who failed to respond to adequate and specific serum therapy were found to be deficient in serum complement. Dingle (11) studied 73 patients with many diseases, 9 of whom had pneumonia. Only 2 of the 73 patients had diminished complement activity, and both of these were pneumonia patients.

It has been demonstrated *in vitro* by Robertson, Sia, and Cornwell (9) that the addition of fresh normal serum increased the pneumococcidal-promoting action of Type I antipneumococcus serum, and Ward and Enders (12) have reported that the opsonic effect of Type II type-specific antipneumococcus antibody is enhanced by the addition of complement, even though complement, in the absence of antibody, has no effect. Also, complement activity in the blood of normal individuals, as reported in the medical literature (9, 12 to 16, 17 to 21), is remarkably constant within a comparatively narrow range. These findings, particularly those of Taplin, suggested the need for further study of complement activity in pneumonia.

Accordingly, complement activity was studied in the blood serum of 75 patients admitted to the Albany Hospital from February 1940 to June 1941 for the treatment of pneumococcus pneumonia. This report presents the observations of that study with particular reference to outcome, pneumococcus type, bacteremia, serum administration, drug administration and serum sickness, although not every case was included in each series.

The diagnosis of pneumonia was confirmed in every case by an x-ray photograph of the chest and by demonstration of type-specific pneumococci on direct examination of the sputum (Neufeld).

METHODS

Blood specimens were collected, allowed to clot, centrifuged, and the sera pipetted off in the usual fashion. Sterile dry equipment was used throughout. The specimens were numbered and allowed to stand at refrigerator temperature overnight. They were then transferred, without any accompanying information other than the name of the patient and the date of bleeding, to the laboratory of Dr. Frank Maltaner[2] at the Division of Laboratories and Research of the New York State Department of Health. Specimens were kept at refrigerator temperature from the time of separation of the serum up to the time of testing, with the exception of the time required for transfer of the specimens to the laboratory. The distance between the hospital and the laboratory is but a few hundred yards, and it is believed that no appreciable effect on complement activity would be caused by the time required for that transfer.

The titrations were usually performed within forty-eight hours of the time that the blood was collected, although a few specimens were titrated as long as seventy-two hours after bleeding. Studies will be re-

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TABLE I

*Distribution of apparently healthy individuals according to complement activity**

Volume of serum*	Apparently healthy individuals
ml.	
0.0028–0.0029	1
0.0030–0.0039	4
0.0040–0.0049	23
0.0050–0.0059	19
0.0060–0.0067	7
Total	54

* Complement activity is inversely related to the volume of serum required to produce 50 per cent hemolysis in a standardized system. Therefore, an increase in volume indicates a decrease in complement activity, and a decrease in volume indicates an increase in complement activity.

ported elsewhere showing that the time interval between bleeding and testing was not a factor in the changes in complement activity discussed in this paper.

The titrations were performed according to the technic of Wadsworth, Maltaner and Maltaner (22), with adjustment of the dilutions to correct for the fact that complement activity of human serum is approximately half that of guinea pig serum (16, 23). The advantages of this method, the end point of which is the amount of serum required to effect 50 per cent hemolysis in a standardized system, as compared with those dependent upon the choice of the one tube in which hemolysis begins or ends, have been reported by Wadsworth, Maltaner and Maltaner (22). The chemical method of Heidelberger, recently published (24, 25), could not be used in a study of this nature because of the large amount of human serum required for that test.

Complement activity is reported in this study in terms of the volume of blood serum in milliliters required to produce 50 per cent hemolysis. Since the volume required is inversely related to complement activity, the larger the number of milliliters indicated, the lower the complement activity in the specimen of the blood serum, and vice versa.

Range of normal complement activity

The normal range of complement activity of human blood as determined by this method confirms the marked constancy of complement activity in normal individuals as measured by other methods (9, 12 to 16, 17 to 21).

Blood specimens were obtained from 54 apparently healthy individuals and the largest amount of serum required to effect 50 per cent hemolysis in this group was 0.0067 ml. (Table I). This will be considered as the lower limit of the range of normal complement activity for purposes of discussion in this paper and volumes of 0.0068 ml. or greater will be referred to in this study as indices of low complement activity.

Complement activity during pneumonia

Of the 71 pneumonia patients from whom blood specimens were collected on admission to the hospital, 12, or 16.9 per cent, were found to have low complement activity varying from 0.0069 ml. to complete failure of hemolysis in the largest amount of serum tested, *i.e.*, 0.2 ml. of undiluted serum. However, the specimens collected from these patients following recovery were found to have normal complement activity with a range of 0.0030 to 0.0051 ml. The percentage increase in complement activity on recovery was largest among those with the lowest complement activity at the time of admission to the hospital (Table III). There was no apparent trend in either direction among the 51 recovered cases with normal complement activity on admission to the hospital.

The case fatality rate among the 71 patients tested on admission to the hospital was 14.1 per cent. There were 8 deaths among the 59 patients

TABLE II

Distribution of pneumonia patients at time of admission to hospital and following recovery from pneumonia, according to complement activity and outcome*

Volume of serum*	Pneumonia patients		
	At time of admission		Following recovery
	Recovered	Died	
ml.			
Normal			
0.0023–0.0029	3	1	3
0.0030–0.0039	18	3	27
0.0040–0.0049	17	2	16
0.0050–0.0059	11	1	14
0.0060–0.0067	2	1	1
Total	51	8	61
Low			
0.0068–0.0069	1	1	
0.0070–0.0079	1		
0.0080–0.0089	1	1	
0.0090–0.0099			
0.0100–0.2000	7†		
Total	10	2	
Grand total	61	10	61

* See footnote on Table I.

† Specimens from two of these showed less than 50 per cent hemolysis with 0.2 ml. undiluted serum, the largest amount of serum tested.

with normal complement activity on admission, as compared with 2 deaths among the 12 cases with low complement activity on admission (Table II). It is therefore evident that complement activity at the time of admission to the hospital could not be used as an index of prognosis in this series of patients. However, it is important to note that complement activity was lower prior to death than at the time of admission to the hospital in 6 of the 7 patients on whom more than one determination was performed (Table IV). The results of the final tests indicated low complement activity in 6 of the 9 patients from whom specimens were collected within seventy-two hours of death.

All specimens of low activity at the time of admission to the hospital were obtained from patients with either Type I, III or VII pneumococcus infections (Table V). The sera of all of the 31 patients infected with types other than I, III and VII were within the normal range of complement activity at the time of admission to the hospital. The distribution of Type I patients was striking, since 9 of the 12 cases with low complement activity on admission were Type I cases, whereas only 12 of the 59 cases with normal complement activity on admission were found to be Type I infections.

Blood cultures were done on all patients at the time of admission to the hospital and no correlation could be found between the occurrence of bacteremia and the presence of low complement activity, since bacteremia occurred in 16 of the 59 patients with normal complement activity, and in 4 of the 12 patients with low complement activity.

Age and sex had no effect on complement activity in this group of patients, either on admission to the hospital or after recovery from the disease. These findings with respect to age are in agreement with those of Gunn (14) who reported that there were no differences in complement activity in normal individuals according to age.

The frequency of low complement activity among alcoholic patients at the time of admission to the hospital (3 in 20 patients) was similar to that among non-alcoholic patients (9 in 51 patients).

The occurrence of a diminished complement activity in the blood serum of certain pneumonia patients at the time of admission to the hospital is striking when compared with the marked uniformity of complement activity in normal individuals, but the significance of this change is not clear. A study of antigen, antibody and complement relationships in the blood of pneumonia patients may yield important information regarding the mechanism of recovery or death from

TABLE III

Complement activity of sera of pneumonia patients with low complement activity at time of admission to hospital and complement activity of same patients following recovery*

Case number	Volume of serum*		Percentage increase in volume* at time of admission
	During pneumonia at time of admission	Following recovery	
	ml.	*ml.*	
2	0.0069	0.0044	+ 56.8
59	0.0069	Died	
40	0.0083	Died	
64	0.0087	0.0036	+ 141.7
12	0.0077	0.0030	+ 156.7
66	0.0100	0.0038	+ 163.2
55	0.0110	0.0030	+ 266.7
39	0.0230	0.0031	+ 641.9
52	0.1000	0.0030	+3233.3
21	0.2000	0.0051	+3821.6
47	>0.2000†	0.0045	>+4344.4
15	>0.2000†	0.0031	>+6351.6

* See footnote on Table I.
† Less than 50 per cent hemolysis with 0.2 ml. undiluted serum, the largest amount tested.

TABLE IV

Complement activity of sera of pneumonia patients at time of admission to hospital and on final test before death*

Case number	At time of admission to hospital		Final test before death	
	Volume of serum*	Interval to death	Volume of serum*	Interval to death
	ml.	*days*	*ml.*	*days*
72	0.0028	1	0.0041	0
27†	0.0040	1	0.0040	1
45	0.0049	2	>0.2000‡||	2
40†	0.0083	2	0.0083	2
5	0.0060	3	0.0085	1
57	0.0030	5	0.0204§	3
33	0.0039	7	0.0030	3
60	0.0051	22	0.1778	0
17†	0.0036	30	0.0036	30
59	0.0069	33	0.0202	2

* See footnote on Table I.
† Only one test performed.
‡ Two specimens taken on the same day, one before and one following serum administration. The final specimen was taken two hours after serum administration (Table VI).
§ Specimen taken one day following serum administration (Table VI).
\|\| See footnote on Table III.

pneumonia. It would also be desirable to study complement activity in other diseases, especially during the days immediately preceding death, in order to discover whether the decrease in complement activity is a specific effect of the pneumonia or a general biological phenomenon which becomes manifest in many diseases in the period prior to death.

Complement activity after intravenous serotherapy and chemotherapy

The complement activities of specimens of blood serum taken immediately preceding and following intravenous administration of antipneumococcus horse serum, antipneumococcus rabbit serum, sodium sulfathiazole, and sodium sulfadiazine were compared.

There were decreases in complement activity after serum administration in 16 of 19 patients who received either horse or rabbit antipneumococcus serum (Table VI). These results are in marked contrast to those observed in patients who received approximately equal sized intravenous injections of 5 per cent solutions of sodium sulfathiazole or sodium sulfadiazine in distilled water. Seven of the 14 patients who received these drugs demonstrated decreases in complement activity (Table VII), but the degrees of change were less marked than among those patients who received serum (Table VI).

In this series of patients, there were no differences between the changes occurring after the injection of antipneumococcus horse and rabbit serum (Table VI). This differs from the results obtained *in vitro* by Zinsser and Parker (26), and confirmed with purified specific polysaccharides by Goodner and Horsfall (27), showing that antipneumococcus rabbit serum "under proper conditions" after union with pneumococcus antigen will fix complement, whereas antipneumococcus horse serum under the same circumstances will not do so.

TABLE V

Distribution of pneumonia patients at time of admission to hospital, according to complement activity and predominant type of pneumococcus

Pneumococcus type	Pneumonia patients		
	Total	Normal complement activity	Low complement activity
I	21	12	9
III	14	12	2
VII	5	4	1
All other	31	31	
Total	71	59	12

TABLE VI

Complement activity of sera of pneumonia patients immediately preceding and immediately following intravenous antipneumococcus serum administration*

Case number	Serum	Volume of serum*		Percentage increase or decrease in volume* following serum administration
		Preceding serum administration	Following serum administration	
		ml.	*ml.*	
36	Rabbit	0.0977	0.0091	− 90.7
42	Rabbit	0.0047	0.0042	− 10.6
58	Rabbit	0.0035	0.0035	No change
60	Horse	0.0051	0.0054	+ 5.9
48	Rabbit	0.0033	0.0035	+ 6.1
43	Horse	0.0065	0.0070	+ 7.7
54	Horse	0.0056	0.0061	+ 8.9
49	Rabbit	0.0057	0.0064	+ 12.3
63	Horse	0.0041	0.0050	+ 22.0
69	Rabbit	0.0055	0.0070	+ 27.3
68	Rabbit	0.0048	0.0062	+ 29.2
56	Rabbit	0.0056	0.0081	+ 44.6
75	Horse	0.0036	0.0056	+ 55.6
64	Horse	0.0087	0.0148	+ 70.1
66	Horse	0.0100	0.0186	+ 86.0
52	Horse	0.1000	>0.2000†	>+ 100.0
55	Rabbit	0.0110	0.0881	+ 700.9
45	Rabbit	0.0049	>0.2000†	>+3981.6
57	Rabbit	0.0030	0.1445	+4716.7

* See footnote on Table I.
† See footnote on Table III.

The decreases in complement activity following serum administration may possibly explain the occasional failure of huge amounts of antipneumococcus serum to control the disease in certain patients. Such failures occurred in the era prior to chemotherapy. This explanation is a likely one considering the experimental evidence of the enhancement of the opsonic and bactericidal effects of antipneumococcus serum by the addition of complement (9, 12). Moreover, the tendency of complement activity to decrease during the period prior to death (Table IV) may be one of the reasons for the relative failure of serotherapy when administered late in the course of the disease.

Complement activity during serum sickness

Serum sickness, for purposes of this study, is defined as a delayed reaction occurring at least one day following the administration of antipneumococcus serum and consisting of any one or a

TABLE VII

Complement activity of sera of pneumonia patients immediately preceding and immediately following intravenous drug administration*

Case number	Drug	Volume of serum*		Percentage increase or decrease in volume* following drug administration
		Preceding drug administration	Following drug administration	
		ml.	*ml.*	
74	S. Sd.	0.0033	0.0029	−12.1
56	S. Sth.	0.0040	0.0038	− 5.0
57	S. Sd.	0.0030	0.0029	− 3.3
73	S. Sd.	0.0043	0.0042	− 2.3
75	S. Sd.	0.0039	0.0039	No change
70	S. Sd.	0.0031	0.0031	No change
60	S. Sd.	0.0051	0.0051	No change
53	S. Sth.	0.0065	0.0068	+ 4.6
59	S. Sd.	0.0069	0.0075	+ 8.7
65	S. Sth.	0.0063	0.0070	+11.1
62	S. Sd.	0.0048	0.0054	+12.5
67	S. Sth.	0.0052	0.0063	+21.2
71	S. Sd.	0.0033	0.0041	+24.2
61	S. Sth.	0.0033	0.0041	+24.2

* See footnote on Table I.
S. Sth. = Sodium Sulfathiazole.
S. Sd. = Sodium Sulfadiazine.

combination of the following symptoms: urticaria, arthritis, and lymphadenopathy. These are frequently accompanied by fever, but in patients recovering from pneumonia it is not feasible to consider fever alone as diagnostic of serum sickness. Therefore, individuals presenting fever alone are not included in the serum sickness study. The severity of the disease varied from a few wheals lasting for a few hours to severe urticaria, arthritis, and lymphadenopathy lasting for six days. Only patients who recovered from pneumonia prior to the onset of serum sickness are included in Table VIII.

The blood specimens used in determining complement activity during serum sickness were taken during the first day of that disease and the results obtained were compared with those of blood specimens taken before serum sickness, during pneumonia at the time of admission to the hospital, and after serum sickness, following recovery from that disease.

One-half of the patients studied during serum sickness showed marked decreases in complement activity at that time (Table VIII). These changes could not be correlated with the nature or the severity of the symptoms presented, but further studies are being made in an attempt to discover why striking decreases in complement activity occurred in only half of these patients.

These results in patients with serum sickness are not inconsistent with the evidence of the literature: the decrease in complement activity in 4 animals with experimental serum sickness, as reported by Miura (28), and in the single human case, as reported by Francioni (29).

CONCLUSIONS

The blood serum of apparently healthy individuals had remarkably constant hemolytic complement activity within a comparatively narrow range.

At the time of admission to the hospital, the blood serum of one-sixth of the pneumonia patients studied had low complement activity. No correlation could be found between complement activity and age, sex, bacteremia or alcoholism.

No specimens of low complement activity were found on recovery from pneumonia.

Complement activity could not be used as an index of prognosis at the time of admission to the hospital in this series of pneumonia patients, but there was a tendency for complement activity to diminish in the period prior to death.

There was an unusual incidence of Type I infection among patients with low complement activity at the time of admission to the hospital.

TABLE VIII

Complement activity of sera of pneumonia patients preceding, during, and following serum sickness*

Case number	Volume of serum*		
	Preceding serum sickness	During serum sickness	Following serum sickness
	ml.	*ml.*	*ml.*
22	0.0032	0.0025	0.0039
14	0.0037	0.0026	0.0055
10	0.0046	0.0040	0.0036
36	0.0977	0.0042	Not done
21	0.2000	0.0049	0.0051
18	0.0034	0.0051	Not done
75	0.0039	0.0068	0.0038
68	0.0048	0.0068	0.0042
6	Not done	0.0120	0.0074
26	0.0041	0.0123	0.0053
30	0.0037	0.0200	0.0046
64	0.0087	0.0200	0.0036
25	0.0049	0.0400	0.0048
20	0.0054	0.1122	Not done
63	0.0041	>0.2000†	0.0030
66	0.0100	>0.2000†	0.0038

* See footnote on Table I.
† See footnote on Table III.

Complement activity of specimens of blood collected immediately following intravenous administration of antipneumococcus horse and rabbit serum was, in most cases, lower than the activity of specimens obtained from the same patients prior to serum administration. In contrast, the changes which occurred following the injection of sodium sulfathiazole and sodium sulfadiazine were not remarkable.

In one-half of the individuals studied, complement activity was lower during serum sickness than before or after serum sickness.

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